Identification of mechanical parameters to be used as a firmness standard on quality evaluations of stored blueberry

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy in Plant Science

at Massey University, Manawatu, New Zealand

Rivera Smith, Sebastian

2022

Abstract

Blueberry firmness is considered a relevant quality variable influencing consumer acceptability of fresh blueberries. However, the blueberry supply chain and research community have not yet adopted a standard method to measure firmness on postharvest quality evaluations.

This thesis has focused on characterising the mechanical properties of blueberry 'Nui' and 'Rahi' as influenced by different factors such as storage relative humidity (i.e., fruit water loss), controlled atmosphere and harvest maturity. The mechanical parameters were obtained by using the instrumental methods of texture profile analysis (TPA) equipped with a flat plate and the penetration test equipped with a 0.39 mm round tip diameter needle probe. Mechanical parameters of hardness slope (BHS, also known as chord stiffness) of TPA and displacement at skin break (DSk) of the penetration test can be used to track water loss changes in stored blueberries. The DSk and BHS can also accurately detect quality changes induced by controlled atmosphere storage. In addition, BHS can detect maturity differences in stored blueberries, but the force at skin break (FSk) provides better detection of maturity differences at harvest evaluations.

To demonstrate the relevance of chord stiffness evaluations at a commercial level, sensory evaluation of texture of hand-touch firmness using a formal sensory panel setting and trained assessors was related to instrumental mechanical parameters. Chord stiffness measured as BHS using a flat plate compression and skin break slope (SSk) measured using a needle probe were strongly related to consumer sensory perception of hand-touch firmness. A blueberry batch with an average BHS ≤0.47 kN m^{-1} or SSk ≤0.13 kN m^{-1} was associated with a very high likelihood of unmarketable berries (i.e., berries are 'soft' or 'very soft').

In summary, BHS was an informative parameter of blueberry quality across factors inducing the textural changes and providing commercially relevant information about consumer acceptability. These results can assist the development of a standard instrumental method to measure postharvest firmness on blueberry quality evaluations for research and commercial purposes. Further studies should focus on validating the feasibility of BHS to determine blueberry quality across other sources of textural variation, such as calcium and ethylene-related treatments. In addition, threshold values for mechanical parameters related to consumer acceptance (sensory analysis) may be identified across an extensive range of blueberry genotypes and using other sensory descriptors also relevant to the consumers, such as crispness. Finally, this research identifies alternative areas for further studies, such as the blueberry firming (an increase of firmness during storage) occurring consistently on blueberries 'Nui' stored under high RH in regular air or a controlled atmosphere of 5 kPa CO_2 + 4 kPa O_2.

Acknowledgments

The author of this thesis acknowledges:

School of Agriculture and Environment, Massey University, New Zealand, for the financial support to pursuing PhD studies at Massey University.

Agencia Nacional de Investigacion y Desarollo (ANID), Chilean Government, for the financial support to pursuing PhD studies at Massey University.

Gourmet Blueberries Ltd. for providing the blueberries used in this thesis.

Research supervision panel, Prof Andrew East, Dr Huub Kerckhoffs, Dr Svetla Sofkova-Bobcheva and Dan Hutchins for their mentoring and assistance.

Micaela Reyes, Talon Sneddon, Peter Jeffery, and Sue Nicholson for the help provided in the experimental setup and evaluations in Postharvest Laboratory, Massey University.

Francisca Ducommun for the help in organising the plant selection and fruit sampling at the commercial plantation.

Prof Joan Hort for the assistance and supervision of sensory evaluation methods used.

Dr Francesco Cappai and Dr Lara Giongo for their contribution to the literature review.

Former and current members of the Massey Postharvest Group. Prof Julian Heyes, Dr Abdul Jabbar, Dr Sunny George Gwanpua, Dr Mo Li, Dr Jamal Olatunji, Dr Jaqueline Oseko, Dr Gabe Redding, Nicolas Tapia, Leo Lai, Zoe Yang, Praveen Kumar, Talon Sneddon, Abdulquadri Alaka, Yujie Han, Carlos Lopez, Raquel Lopez, Jack Hosking, Josephine Longuet-Higgins, and Magdalena Urbanska for their support and scientific discussion.

My beloved parents and family for their patience and support.

Table of contents

List of Tables

List of Figures

Mechanical parameters. Abbreviations, Interpretation and, units

Mechanical parameter	Abbreviation	Interpretation	Unit
Texture profile analysis (TPA)			
Hardness	BH	Maximum force necessary to attain a given target compression distance (mm) or deformation strain (%).	N
Hardness slope	BHS	Linear rate of force (N) increment by deformation distance (mm) between trigger force and maximum force at deformation strain (%).	kN m^{-1}
Resilience	BR	Success in regaining the original berry height during withdrawal of the first compression.	-
Cohesiveness	BCo	Strength of the internal bonds comprising the berry body.	-
Springiness	BSp	How well the berry springs back after the first compression force is removed.	-
Apparent modulus of elasticity	E	Resistance to deformation of a viscoelastic material.	MPa
Chord stiffness	CS	Slope of the chord drawn between any two specific points on the force-deformation curve.	KN m^{-1}
Penetration test			
Force at skin break	FSk	Penetration force required to pierce the skin.	N
Displacement at skin break	DSk	Probe displacement at epidermis rupture.	mm
Skin break slope	SSk	Slope of the chord between trigger force and FSk.	kN m^{-1}
Skin break work energy	WSk	Work energy that is needed to break the epidermal skin.	J

Introduction

Fruit firmness in plant science is referred to the textural or mechanical attribute that can denote differences in maturity and quality (Timm et al., 1996; Abbott, 1999; Lu and Abbott, 2004). For fresh blueberries, firmness has been linked with maturity (Vicente et al., 2007; Moggia et al., 2016), quality (Vilela et al., 2016) and consumer purchasing decisions (Yue and Wang, 2017). For example, firmer blueberries are preferred by consumers (Ehlenfeldt and Martin, 2002), and the predominance of 'soft' blueberries in a batch can lead to consumer rejection as a fresh food product (Beaudry et al., 1998; Nunes et al., 2004; Schotsmans et al., 2007; Rodriguez and Zoffoli, 2016).

Blueberry firmness methods can vary between literature references and commercial levels. Firmness methods previously described have been conducted by subjective evaluations such as sensory hand-touch firmness (Sanford, 1991; Miller et al., 1993; Schotsmans et al., 2007: Rodriguez and Zoffoli, 2016) or instrumental objective evaluations such as compression (Ballinger et al., 1973; Slaughter and Rohrbach, 1985; Timm et al., 1996; Prussia et al. 2006; Giongo et al., 2022), penetration (Forney et al., 2003; Silva et al., 2005; Giongo et al., 2013; 2022) or a combination of different mechanical tests (Giongo et al., 2021). However, neither of these methods had been yet identified as a universal standard to measure blueberry firmness in quality evaluations conducted by the research community or the commercial supply chain.

As a result of the lack of a standard method to assess firmness, different mechanical methods have previously been used to evaluate the effect of the same postharvest practices/technologies on blueberry quality. For example, research studies conducted to determine the benefits and risks of firmness quality of controlled atmosphere storage (i.e., variation of atmospheric CO_2 and O_2 composition) have used different methods. Compression by a 25-60 mm diameter flat plate (Allan-Wojtas et al., 2001; Harb and Streif, 2004; Schotsmans et al., 2007; Cantin et al., 2012; Paniagua et al., 2014; Falagan et al., 2020), compression by a small diameter (2-4 mm) flat surface cylindrical probe (Alsmairat et al., 2011: Chiabrando and Giacalone, 2011; Rodriguez and

Zoffoli, 2016), penetration tests (Duarte et al., 2009; Concha-Meyer et al., 2015) or a combination of compression and penetration methods (Forney et al., 2003).

The use of different methods to assess firmness in blueberry quality evaluations leads to the following problems:

(1) Firmness data obtained using different mechanical tests (e.g., penetration, compression) and operational settings (e.g., test speed, probe size and shape) cannot be confidently compared (Feng et al., 2011). Hence, the longevity of the data and the cross-validation between the research community and the commercial industry can be at risk.

(2) Suppose the instrumental firmness evaluation method selected in an experimental design is not a confident indicator of blueberry quality. In that case, the study of the effect of a certain blueberry management/technology can be risked as induced by methodological error.

(3) Suppose the instrumental firmness evaluation method selected to assist commercial grading operations is not an indicator of consumer acceptance. In that case, a less profitable decision can be incorrectly selected. For example, the industry can incorrectly destinate a blueberry batch as a processed food system when the firmness is good enough to be accepted by the consumer as a fresh blueberry.

Considering the above-mentioned problems, a universal standard method of blueberry firmness is required to generate consistent and comparable data at the research and commercial levels. The firmness standard is also needed to represent consumer judgment, allowing firmness thresholds related to the consumer's sensory perception (e.g., 'soft' vs 'firm' blueberries). The author of this thesis identifies the standardisation of blueberry firmness evaluation procedures as a research opportunity worth to be explored.

Examples of the benefits when using a standard method to evaluate firmness can be described from other fruit industries such as kiwifruit. The penetration test is the universally accepted standard firmness method for kiwifruit. It is conducted using a non-deformable convex tip probe of 7.9 mm diameter penetrating to a depth of 8 mm of fruit flesh (Harker et al., 1996; Feng et al., 2011). This penetration test has allowed standardising firmness ranges associated with kiwifruit quality and consumer acceptance. A consumer will accept a kiwifruit as ready to be eaten (eating ripe) when firmness is between 5 N and 10 N (Feng et al., 2011). Firmness values higher than the eating ripeness range (>15 N) may be a kiwifruit too firm to be pleasantly chewed in the mouth. Whilst firmness below the eating ripeness range (<4 N) may indicate an overripe (senescent) or very soft kiwifruit. In both cases, a very firm or very soft kiwifruit can lead to consumer rejection as a fresh food product.

On the one hand, the author of this thesis does not reject the premise that different mechanical parameters can provide similar information for firmness on blueberry quality evaluations or sensory perception. On the other hand, the author may not discard the fact that even if the mechanical parameter can provide information about the blueberry quality, the method can vary in its sensitivity to determine the firmness. Hence, two different mechanical methods will be used in this PhD Thesis:

(1) A double compression test (named texture profile analysis, TPA) will be conducted with a flat plate probe of 25 mm diameter. TPA is considered an imitative test of the repeated bite of food, and the test consists of two compression cycles to a predefined fruit deformation (strain %) distance (Pons and Fiszman, 1996). Multiple mechanical properties can be calculated using TPA. The most relevant for solid food (blueberries) are hardness (N), resilience (-), cohesiveness (-), and springiness (-). In addition, the first compression cycle of TPA can be used to measure the chord stiffness (or hardness slope, kN m^{-1}) and the apparent modulus of elasticity (kPa) (Prussia et al., 2006).

(2) A penetration test will be conducted with a 0.39 mm round tip diameter needle probe. Mechanical parameters of a penetration test are obtained by combining compression and shear force and are often related to the moment when the blueberry skin breaks or is pierced. Output parameters obtained from the penetration test are the force at skin

break (N), skin break slope (kN m^{-1}), displacement at skin break (SSk) and skin break work-energy (J).

In addition to instrumental mechanical parameters, textural properties were sensory evaluated using the hand-feel firmness method. The hand-touch firmness is often assessed by squeezing a blueberry between the thumb and index fingers and rated based on hand-feel intensity (Nunes et al., 2004). This method was selected due to its relevance and the fact that it is being used in commercial supply chains worldwide (Beaudry et al., 1998; Schotsmans et al., 2007; Cantin et al., 2012; Rodriguez and Zoffoli, 2016). Hand-touch firmness is a key representation of consumers' judgment of fresh blueberry texture when the fruit is hand-manipulated before consumption (from package to mouth).

Blueberry firmness has been previously reported to be influenced by different factors. Consequently, the universal firmness standard needs to be informative, independent of the factors (e.g., storage relative humidity, harvest maturity, controlled atmosphere) or mechanism (i.e., turgor loss, cell wall degradation) inducing different textures of blueberry. The author of this thesis has selected water loss (Paniagua et al., 2013), controlled atmosphere (Schotsmans et al., 2007; Paniagua et al., 2014) and harvest maturity (Moggia et al., 2016; Moggia et al., 2018) to generate different firmness values (data dispersion) in the experimental designs. In addition, this thesis used different blueberry genotypes, such as highbush blueberry 'Nui' and Rabitteye 'Rahi' and 'Centurion'. All fruit was harvested by Massey University personnel from the same commercial orchard (Gourmet blueberries) located in Hawke's Bay, New Zealand.

Thesis objectives

The main objective of this thesis is to identify a mechanical test that can be used to measure firmness on postharvest quality evaluations and be related to consumer acceptance.

The specific objectives of this thesis are:

(1) Identify the most relevant mechanical parameters related to storage relative humidity (water loss) of 'Nui' and 'Rahi' blueberries.
(2) Identify the most relevant mechanical parameters related to controlled atmosphere storage technology of 'Nui' and 'Rahi' blueberries.
(3) Identify the mechanical parameters that best describe the harvest maturity that are relevant at the commercial level of 'Nui' and 'Rahi' blueberries.
(4) Determine the relationship between instrumental mechanical parameters and sensory evaluation of texture (i.e., hand-touch firmness) on 'Centurion' blueberry.

This thesis intends to contribute to developing a standard to measure blueberry firmness that can be universally used on quality evaluations conducted by the research community or the commercial supply chain (i.e., quality checkpoints). In addition, the identified firmness method is expected to be an instrumental mechanical test that can be easily implemented to be measured in commercial blueberry quality checkpoints.

Although other studies have approached the lack of a standard firmness method on blueberries, the novelty of this thesis is the evaluation of the same mechanical tests (TPA and penetration) as influenced by various factors or mechanisms of texture induction. In addition, this thesis has used a trained sensory panel setting to determine the relationship between instrumental mechanical parameters and sensory hand-touch firmness (consumer acceptance), which was not explored by previous studies.

Thesis structure

This thesis contains one literature review chapter, three experimental research chapters and one overall discussion chapter.

Chapter 1. Blueberry firmness - A review of the textural and mechanical properties used in quality evaluations

This chapter corresponds to the literature review and will be used to Identify the textural/mechanical methods previously used on blueberries and the factors that can be used to manipulate blueberry textural properties. In addition, the review explores *state of art* and prerequisites for generating a universal standard to measure blueberry firmness on quality evaluations.

Two experts on blueberry breeding and phenotyping (including blueberry textural and mechanical evaluations) were invited to collaborate on this review. Dr Lara Giongo (Fondazione Edmund Mach, Italy) and Dr Francesco Cappai (Ohalo Genetics, United States of America). The author of this thesis wrote >90 % of the literature review.

Chapter 2. Influence of water loss on mechanical properties of stored blueberries

This research chapter is associated with objective 1 and was set to identify the mechanical parameters that can be used to track firmness/quality changes as induced by water loss. In addition, this chapter provides information on operational settings to conduct the double compression test (texture profile analysis).

Chapter 3. Influence of harvest maturity and storage technology on mechanical properties of blueberries

This research chapter was associated with objectives 1, 2 and 3. Consequently, it was devised to identify mechanical parameters related to harvest maturity and storage technologies, including relative humidity management and controlled atmosphere storage (atmospheric modification of CO_2 and O_2).

Chapter 4. Instrumental mechanical parameters related to hand-touch firmness measures of blueberries

This research chapter is associated with objectives 1 and 4. This chapter was set to study the relationship between instrumental mechanical parameters and sensory texture (hand-touch firmness) on blueberries with different water loss levels. In addition, this chapter has developed silicone spheroids (imitating real blueberries) of different hand-touch firmness intensities to calibrate assessor performance on the sensory evaluations. The design of the sensory evaluation methods was assisted and supervised by Dr Joanne Hort (Food Experience and Sensory Testing Laboratory, Massey University).

Chapter 5. Overall discussion and future research directions

This chapter will provide an overview of the main results obtained from the three research chapters. In addition, the chapter will discuss the prerequisites and future work directions toward developing a standard method to measure firmness on postharvest quality evaluations of fresh blueberries.

Most of the content and results presented in Chapters 1, 2 and 3 were used to write research articles published in Elsevier Journals.

(1) Rivera, S., Giongo, L., Cappai, F., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A. 2022. Blueberry firmness – A review of mechanical and textural properties used on quality evaluations. Postharvest Biol. Technol. 192, 1112016. https://doi.org/10.1016/j.postharvbio.2022.112016.

(2) Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2021a. Influence of water loss on mechanical properties of stored blueberries. Postharvest Biol. Technol. 176. https://doi.org/10.1016/j.postharvbio.2021.111498

(3) Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2021b. Data of texture profile analysis performed by different input settings on stored 'Nui' and 'Rahi' blueberries. Data in Brief. 38, 107313. https://doi.org/10.1016/j.dib.2021.107313

(4) Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2022. Influence of harvest maturity and postharvest technologies on mechanical properties of blueberries. Postharvest Biol. Technol. 191, 111961. https://doi.org/10.1016/j.postharvbio.2022.111961

The author of this thesis has requested Copyright permission from Elsevier to publish the content of these publications in open access of the Massey University (NZ) repository. Elsevier Researcher Support has indicated that as the first author of all publications, the author of this thesis retains the right to reuse the content in a PhD thesis, and no formal permission is required. However, the final published format of these articles cannot be attached to this thesis. In addition, this thesis provides additional information not included in the original published versions.

References

Abbott, H.A., 1999. Quality measurements of fruits and vegetables. Postharvest Biol. Technol. 15, 207-225. https://doi.org/10.1016/S0925-5214(98)00086-6

Allan-Wojtas, P.M., Forney, C.F., Carbyn, S.E., Nicholas, K.U.K.G., 2001. Microstructural indicators of quality related characteristics of blueberries - An integrated approach. Lebensm.-Wiss. U.-Technol. 34, 23-32. https://doi.org/10.1006/fstl.2000.0738

Alsmairat, N., Contreras, C., Hancock, J., Callow, P., Beaudry, R., 2011. Use of combinations of commercially relevant O2 and CO2 partial pressures to evaluate the sensitivity of nine highbush blueberry fruit cultivars to controlled atmospheres. HortScience 46(1), 74-79. https://doi.org/10.21273/HORTSCI.46.1.74

Ballinger, W.E., Kushman, L.J., Hamann, D.D., 1973. Factors affecting the firmness of highbush blueberries. J. Amer. Soc. Hort. Sci. 98(6), 583-587.

Beaudry, R.M., Moogia, C., Retamales, J.B., Hancock, J.F., 1998. Quality of ‘Ivanhoe’ and ‘Bluecrop’ blueberry fruit transported by air and sea from Chile to North America. HortScience 33(2), 313-317.

Cantin, C.M., Minas, I.S., Goulas, V., Jimenez, M., Manganaris, G.A., Michailides, T.J., Crisosto, C.H., 2012. Sulfur dioxide fumigation alone or in combination with CO_2-enriched atmosphere extends the market life of Highbush blueberry fruit. Postharvest Biol. Tec. 67, 84-91. https://doi.org/10.1016/j.postharvbio.2011.12.006

Chiabrando, V., Giacalone, G., 2011. Shelf-life extension of Highbush blueberry using 1-methylcyclopropene stored under air and controlled atmosphere. Food Chem. 126, 1812-1816. https://doi.org/10.1016/j.foodchem.2010.12.032

Concha-Meyer, A., Eifert, J.D., Williams, R.C., Marcy, J.E., Welbaum, G.E., 2015. Shelf-life determination of fresh blueberries (Vaccinium corymbosum) stored under controlled atmosphere and ozone. Inter. J. Food Sci. 2015, 164143. https://doi.org/10.1155/2015/164143

Duarte, C., Guerra, M., Daniel, P., Camelo, A.L., Yommi, A., 2009. Quality changes of Highbush blueberries fruit stored in CA with different CO_2 levels. J. Food Sci. 74, 154-159. https://doi.org/10.1111/j.1750-3841.2009.01118.x

Ehlenfeldt, M.K., Martin, R.B., 2002. A survey of fruit firmness in Highbush blueberry and species-introgressed blueberry cultivars. Hortscience 37, 386-389. https://doi.org/10.21273/HORTSCI.37.2.386

Falagan, N., Miclo, T., Terry, L.A., 2020. Graduated controlled atmosphere: a novel approach to increase 'Duke' blueberry storage life. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.00221

Feng, J., McGlone, A., Tanner, D., White, A., Olsson, S., Petley, M. and Woolf, I., 2011. Effect of penetration speed on flesh firmness measured in stored kiwifruit. Postharvest Biol. Technol. 61, 29-34. https://doi.org/10.1016/j.postharvbio.2011.01.014

Forney, C.F., Jordan, M.A., Nicholas, K.U.K.G., 2003. Effect of CO_2 on physical, chemical, and quality changes in 'Burlington' blueberries. Acta Horticul. 600, 587-593.

Giongo, L., Poncetta, P., Loretti, P., Costa, F., 2013. Texture profiling of blueberries (*Vaccinium* spp.) during fruit development, ripening and storage. Postharvest Biol. Tec. 76, 34-39. https://doi.org/10.1016/j.postharvbio.2012.09.004

Giongo, L., Ajelli, M., Pottorff, M., Perkins-Veazie, Iorizzo, M., 2022. Comparative multi-parameters approach to dissect texture subcomponents of highbush blueberry cultivars at harvest and postharvest. Postharvest Biol. Technol. 183, 111696. https://doi.org/10.1016/j.postharvbio.2021.11696.

Harb, J.Y., Streif, J., 2004. Controlled atmosphere storage of highbush blueberries cv 'Duke'. Eur. J. Hort. Sci. 69(2), 66-72.

Harker, F.R., Maindonald, J.H., Jackson, P.J. 1996. Penetrometer measurement of apple and kiwifruit firmness: operator and instrument differences. J. Amer. Soc. Hort. Sci. 121(5):927-936.

Lu, R., Abbot, J.A., 2004. Force/Deformation techniques for measuring texture, In: Kilcast, D. (Ed.), Texture in food Volume 2, Solid foods. Woodhead Publishing Limited, England.

Miller, W.R., McDonald, R.E., Cracker, T.E., 1993. Quality of two Florida blueberry cultivars after packaging and storage. HortScience 28(2), 144-147. https://doi.org/10.21273/HORTSCI.28.2.144

Moggia, C., Graell, J., Lara, I., Schmeda-Hirschmann, G., Thomas-Valdes, S., Lobos, G.A., 2016. Fruit characteristics and cuticle triterpenes as related to postharvest quality of highbush blueberries. Sci. Hortic. 211, 449-457. http://dx.doi.org/10.1016/j.scienta.2016.09.018

Moggia, C., Gonzalez, C., Lobos, G. A., Bravo, C., Valdes, M., Lara, I., Graell, J., 2018. Changes in quality and maturity of 'Duke' and 'Brigitta' blueberries during fruit development: postharvest implications. Acta Horticul. 1194, 1495-1501. https://doi.org/10.17660/ActaHortic.2018.1194.209

Nunes, M.C., Emond, J.P., Brecht, J.K., 2004. Quality curves for highbush blueberries as a function of the storage temperature. Proceedings of the Ninth North American Blueberry Research. Small Fruits Rev., 423-438. https://doi.or/10.13000/J301v03n03_10

Paniagua, A.C., East, A.R., Hindmarsh, J.P., Heyes, J.A., 2013. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. Postharvest Biol. Tec. 79, 13-19. https://doi.org/10.1016/j.postharvbio.2012.12.016

Paniagua, A.C., East, A.R., Heyes, J.A., 2014. Interaction of temperature control deficiencies and atmosphere conditions during blueberry storage on quality outcomes. Postharvest Biol. Tec. 95, 50-59. https://doi.org/10.1016/j.postharvbio.2014.04.006

Pons, M., Fiszman, S.M., 1996. Instrumental Texture profile analysis with particular reference to gelled systems. J. Texture Stud. 27, 597-624. https://doi.org/10.1111/j.1745-4603.1996.tb00996.x

Prussia, S.E., Tetteh, M.K., Verma, B.P., NeSmith, D.S., 2006. Apparent modulus of elasticity from FirmTech 2 firmness measurements of blueberries. Trans. ASABE 49, 113-121. https://doi.org/10.13031/2013.20219

Rodriguez, J., Zoffoli, J.P., 2016. Effect of sulphur dioxide and modified atmosphere packaging on blueberry postharvest quality. Postharvest Biol. Technol. 117, 230-238. https://doi.org/10.1016/j.postharvbio.2016.03.008

Sanford, K.A., Lidster, P.D., McRae, K.B., Jackson, E.D., Lawrence, R.A., Stark, R., Prange, R.K., 1991. Lowbush blueberry quality changes in response to mechanical damage and storage temperature. J. Amer. Soc. Hort. Sci. 116(1), 47-51. https://doi.org/10.21273/JASHS.116.1.47

Schotsmans, W., Molan, A., MacKay, B., 2007. Controlled atmosphere storage of rabbiteye blueberries enhances postharvest quality aspects. Postharvest Biol. Technol. 44(3), 277-285. https://doi.org/10.1016/j.postharvbio.2006.12.009

Silva, J.L., Marroquin, E., Matta, F.B., Garner, J.O., Stojanovic, J., 2005. Physicochemical, carbohydrate and sensory characteristics of highbush and rabbiteye blueberry cultivars. J. Sci. Food Agr. 85(11), 1815-1821. https://doi.org/10.1002/jsfa.2083

Slaughter, D.C., Rohrbach, R.P., 1985. Developing a blueberry firmness standard. Transactions of the ASAE 28(3), 986-992. https://doi.org/10.13031/2013.32374

Timm, E.J., Brown, G.K., Armstrong, P.R., Beaudry, R.M., Shirazi, A., 1996. Portable instrument for measuring firmness of cherries and berries. Appl. Eng. Agric. 12, 71-77. https://doi.org/10.13031/2013.25441

Vicente, A.R., Ortugno, C., Rosli, H., Powell, A.L.T., Greve, L.C., Labavitch, J.M., 2007. Temporal sequence of cell wall disassembly events in developing fruits 2 Analysis of blueberry (Vaccinium species). J. Agric. Food Chem. 55, 4125-4130. https://doi.org/10.1021/jf063548j.

Vilela, A., Goncalves, B., Ribeiro, C., Fonseca, A.T., Correia, S., Fernandes, H., Ferreira, S., Bacelar, E., Silva, A.P., 2016. Study of textural, chemical, color and sensory properties of organic blueberries harvested in two distinct years: A chemometric approach. J. Texture Stud. 47, 199-207. https://doi.org/10.1111/jtxs.12173.

Yue, C., Wang, J., 2017. Consumer preferences for fresh blueberry attributes. Acta Hortic. 1800, 1-8. https://doi.org/10.17660/ActaHortic.2017.1180.1

Chapter 1. Blueberry firmness - A review of the textural and mechanical properties used in quality evaluations

This literature review was devised to identify instrumental mechanical parameters and sensory methodologies previously used to evaluate blueberry texture when characterising the fruit quality of blueberries. In addition, this review will identify the most critical preharvest and postharvest factors which can influence blueberry firmness. Consequently, the information provided in this review will be used in the research chapters of this thesis to:

(1) Select the sources of variance (postharvest factors) that can be used to generate fresh blueberries with different textural properties.

(2) To learn about how informative and suitable to implement different instrumental mechanical and sensory methods previously used on blueberries. Consequently, to guide the selection of instrumental and sensory firmness methods used in this thesis.

(3) To understand the prerequisites to identify/develop a standard method to measure firmness on quality evaluations of blueberries.

This review was written following the guide of authors of the Elsevier (Amsterdam, The Netherlands) journal, Postharvest Biology and Technology. The article was written in collaboration with two international co-authors (not part of this thesis committee), Dr Francesco Cappai (Ohalo Genetics, USA) and Dr Lara Giongo (Fondazione Edmund Mach, Italy). However, the author of this thesis performed the main literature review, conceptualization, investigation, visualizations, and writing > 90 % of the original draft.

The published version of the literature review is:

Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2022. Blueberry firmness – A review of mechanical and textural used on quality evaluations. Postharvest Biol. Technol. 192, 112016. https://doi.org/10.1016/j.postharvbio.2022.112016.

AUTHORS AND AFFILIATIONS

Sebastian Rivera[a,b], Lara Giongo[c], Francesco Cappai[d], Huub Kerckhoffs[b,e]; Svetla Sofkova-Bobcheva[b], Daniel Hutchins[f], Andrew East[a]

[a] Massey Agrifood Digital Lab, Massey University, Palmerston North, New Zealand
[b] School of Agriculture and Environment, Massey University, Palmerston North, New Zealand
[c] Fondazione Edmund Mach I-38010, S. Michele all'Adige (TN)-Via E. Mach, Italy
[d] Running Tide Technologies, Portland, Maine, USA
[e] StartAfresh, Mt. Maunganui, New Zealand
[f] Gourmet Blueberries, NZ Gourmet Ltd, Auckland, New Zealand

KEYWORDS

Vaccinium spp; compression test; penetration test; Texture Profile Analysis; penetration test.

ABSTRACT

Firmness is an important parameter for fresh blueberries as it influences the quality perceived by consumers and postharvest storage potential. However, the blueberry research community has not yet identified a universal standard method that can evaluate firmness for quality purposes. Different mechanical tests have been considered, offering different perspectives on this quality trait. This review summarises the most common methods previously used to evaluate textural and mechanical properties of fresh blueberries as influenced by pre- and postharvest factors. In addition, this review intends to assist the blueberry research community and commercial supply chain when selecting suitable methods to measure blueberry firmness as a fruit quality response. Different research initiatives to develop, optimize or standardise instrumental methods to assess blueberry firmness and relate to consumer sensory perception are reviewed. Mechanical parameters obtained by compression tests are the most previously used techniques to evaluate the influence of genotype, maturity, calcium, and postharvest management on blueberry firmness or to relate to sensory descriptors. However, standardising operational settings (e.g., compression distance, loading speed, and calculation procedures) is required to make results comparable across data collection conditions. Whether other mechanical test methods such as penetration or a combination of tests can better characterise blueberry quality or the relationship with consumer acceptance remains unknown and is worth studying.

1.1. Introduction

Fruit firmness in plant science is often referred to as the textural or mechanical attributes that can denote differences in the fruit maturity or quality of horticultural commodities (Timm et al., 1996; Abbott, 1999; Lu and Abbott, 2004; Musacchi and Serra, 2018). As a result, fruit firmness can have variable descriptions or interpretations depending on the textural or mechanical evaluation protocols. For most fruit commodities, firmness is referred to as the force (measured in Newtons, N) required to penetrate a fruit using a non-deformable probe (Lu and Abbott, 2004). However, this definition applies universally to firm flesh fruit such as apples, kiwifruit, and peaches. In the case of small soft berries, firmness is often evaluated based on the deformation of the berry when applying a fixed load (Lu and Abbott, 2004).

Several methodologies have been reported to evaluate blueberry firmness. These vary from simple sensory methods, such as squeezing or rolling a blueberry between thumb and index fingers (Sanford et al., 1991; Miller et al., 1993; Nunes et al., 2004; Rodriguez and Zoffoli, 2016), to sophisticated mechanical methods such as the compression test, which requires the use of automatic equipment such as a texture analyser or Instron universal testing machine (Donahue and Work 1998; Chiabrando et al., 2009; Giongo et al., 2013). Several factors drive the decision of which method or instrumental test (e.g., penetration or compression test) to use when a research experiment is designed. A relevant factor is the availability of the testing machine (e.g., texture analyzer, FirmTech 2) and type of probe (e.g., compression plates, needle probe), and how easy it is to implement and conduct the test and analyse the data.

Among the factors influencing blueberry firmness, genotype, maturity, calcium management, and postharvest storage conditions have been identified as the most relevant. However, different methods have been used to characterise the effect of pre- and postharvest factors on textural quality. To further understand and compare the impact of these factors, it is essential to standardise the quality evaluation methods. During the history of research studies on blueberries, different studies have contributed to providing insights that can assist the development of a firmness standard.

Blueberry quality and storage potential can be affected by bruising damage (i.e., flesh browning) induced by machine harvesting or impact damage when dropped in a packing line (Ballinger et al., 1973; Sanford et al., 1991; Xu et al., 2015; Moggia et al., 2017). However, the advanced requirements for mechanical methods to model the mechanical damage resistance of blueberries are beyond the scope of this literature review.

This literature review will summarise:

(1) The methodologies previously used to assess fresh blueberry textural quality and instrumental mechanical parameters.

(2) The pre- and postharvest factors which most affect blueberry texture and mechanical parameters.

(3) The history and state of the art on the development and optimisation of mechanical methods to evaluate firmness and consumer preferences of blueberry.

This review provides a started point to design the methodologies used on this thesis. In addition, the author hope that this review will offer a comprehensive summary of the research in this field to date and help the blueberry community to adopt suitable methods to measure blueberry firmness as a quality parameter for different purposes (breeding, pre and postharvest treatment evaluations, and commercial quality assessment).

1.2. Methods to evaluate blueberry firmness

In food science, the terms "texture" and "material properties" have different meanings and should not be used interchangeably. Texture relates to sensorial descriptors of structural, mechanical, and surface properties of food when perceived by a human when consuming (chewing, touching, and hearing) food such as fruit (Szczesniak, 2002; Kemp et al., 2009). In comparison, mechanical properties are related to parameters measured by an instrumental machine, which generally records food sample changes in shape or size when an external loading (force) is applied (Szczesniak, 1963). Hereafter, terms related to "texture" will be used when referring to sensory perception by a human subject, and "mechanical or material" attributes will be used to denote the parameters assessed by an instrumental device and calculated by a specific physical model (Chen, 2020).

This section will summarise sensory and instrumental mechanical methods used to characterise firmness as a blueberry quality response. The most used instruments and mechanical methods in blueberry research studies, such as uniaxial compression, penetration, and texture profile analysis, will be described based on probes used to conduct the tests and mechanical parameters that each mechanical test can measure. In addition, we present strengths, weaknesses, and recommendations for different instrumental used to characterise the mechanical parameters of blueberry for research or commercial purposes. Finally, studies exploring the use of true non-destructive techniques that can be used to predict/evaluate mechanical parameters of blueberry are reviewed.

1.2.1. Sensory methods

Sensory analysis of blueberry quality can be performed considering two assessment systems: hand-feel touch perception and mouthfeel (**Table 1.1**). Hand-feel touch evaluations are often conducted by gentle squeezing (or rolling) between the index and thumb fingers and scored from soft to firm (Sanford et al., 1991; Miller et al., 1993; Nunes et al., 2004; Rivera et al., 2013; Rodriguez and Zoffoli, 2016; Moggia et al., 2022). The commercial blueberry supply chain had mainly used hand-touch firmness to assess blueberry textural quality when instrumental devices were unavailable or when the texture was required to be evaluated by a low-cost and rapid methodology (Beaudry et al., 1998; Schotsmans et al., 2007; Cantin et al., 2012; Nunes, 2015; Rodriguez and Zoffoli, 2016; Moggia et al., 2022). However, tactile evaluation of berries can be inaccurate and inconsistent, and the assessor's judgment can vastly influence the results (Slaughter and Rohrbach, 1985; Schotsmans et al., 2007). The use of assessors with enough training and experience is recommended to decrease variability when conducting hand-feel evaluations. However, growers and exporters have an ongoing interest in having an objective (instrumental) method to determine fresh blueberry firmness (Moggia et al., 2022).

Sensory evaluation conducted by mouthfeel or chewing is often performed by objective sensory techniques and sensory panels with a large population (e.g., >30) of previously trained or untrained consumers (Donahue et al., 2000; Saftner et al., 2008; Lobos et al., 2014; Gilbert et al., 2015) or experienced trained panellists (Blaker et al., 2014) with lower numbers of panellists. The evaluated attributes of the oral mastication test are obtained by scoring the sensations experienced at different stages of the mastication process (**Table 1.1**).

Table 1.1. Sensory attributes previously reported for blueberries.

Sensory system	Attribute	Description	Score	Reference
Hand-feel	Hand-touch firmness	Resistance force to berry deformation upon finger touch pressure	Soft to Firm	Sanford et al., 1991; Miller et al., 1993; Beaudry et al., 1998; Nunes et al., 2004; Schotsmans et al., 2007; Nunes and Emond, 2007; Rivera et al., 2013; Nunes, 2015; Rodriguez and Zoffoli, 2016; Ktenioudaki et al., 2021; Moggia et al., 2022
Mouthfeel	Firmness OR texture during chewing	Force required to break or fracture the blueberry sample between molars	Soft to Firm	Ballinger et al., 1973; Rosenfeld et al., 1999; Saftner et al., 2008; Blaker et al., 2014; Lobos et al., 2014; Vilela et al., 2016
	Crispness OR bursting energy	Force and sound as the berry breaks or fractures during the first or second chew	Mushy to Crisp/crunchy or rigid	Rosenfeld et al., 1999; Saftner et al., 2008; Blaker et al., 2014; Vilela et al., 2016
	Juiciness OR succulence	Quantity of juice released from the flesh when chewed	Not juicy to Juicy	Rosenfeld et al., 1999; Saftner et al., 2008; Blaker et al., 2014; Vilela et al., 2016
	Graininess	Texture given by stone cells or seeds	Smooth to grainy	Blaker et al., 2014
	Mealiness	Pasty or dry feeling in the mouth	Not mealy to Mealy	Blaker et al., 2014
	Skin toughness	Amount of residual skin during chewing after the flesh is gone	Tender to tough skin	Silva et al., 2005; Saftner et al., 2008; Blaker et al., 2014
	Texture liking	Overall texture liking	-100 (greatest disliking) to 100 (greatest liking)	Gilbert et al., 2015

1.2.2. Instrumental mechanical methods

Among mechanical methods used for previous research on blueberry quality, the compression test is the most reported method to measure mechanical parameters (**Table 1.2**). Other mechanical tests have also been conducted, including penetration (puncture), impact, and shear tests (**Table 1.2**). In addition, recently, a double compression test, knowns as texture profile analysis, has been used in blueberry research due to its ability (but questionable) to imitate sensory descriptors (Chiabrando et al., 2009; Xie et al., 2018; Li et al., 2021; Olmedo et al., 2021; Giongo et al., 2022). Several studies have considered a combination of different mechanical tests to assess blueberry quality (Silva et al., 2005; Blaker et al., 2014; Vance et al., 2017; Giongo et al., 2022).

Mechanical tests conducted on blueberries show a viscoelastic behaviour under mechanical loading, and hence mechanical properties of blueberries are mainly evaluated as a function of force, deformation, and time (Abbott, 1999). Consequently, when reporting the method, operational settings such as crosshead test-speed and maximum loading to a specific berry deformation (or penetration distance) must be clearly defined.

Table 1.2. Mechanical parameters for each instrumental test, previously evaluated on blueberries.

Instrumental test	Mechanical parameter	Unit	Indicator	Reference
Compression test (**Fig.1.1 A,C,D**)	Maximum force	N	Maximum force by using a target compression distance or deformation strain	Ferraz et al., 2001; Schotsmans et al., 2007; Paniagua et al., 2013; Vilela et al., 2016; Scheidt and Silva, 2018; Moggia et al., 2022
	Rupture point	N	Maximum force to berry rupture (berry releasing juice)	Silva et al., 2005
	Chord stiffness, loading slope, OR FirmTech firmness	N mm^{-1}	Rate of force increment by deformation distance. Calculated as the slope of the chord drawn between any specific points on the force-deformation curve	Slaughter and Rohrbach, 1985; Rohrbach and Mainland, 1993; Timm et al., 1996; Ehlenfeldt and Martin, 2002; NeSmith et al., 2005; Saftner et al., 2008; Li et al., 2011; Leiva-Valenzuela et al., 2013; Blaker et al., 2014; Moggia et al., 2017; Vance et al., 2017; Cappai et al., 2018; Moggia et al., 2022
	Apparent modulus of elasticity	MPa	Deformation behaviour of a viscoelastic material	Prussia et al., 2006; Donahue and Work, 1998; Giongo et al., 2022
Penetration (puncture) test (**Fig. 1.1 B**)	Skin break force OR skin toughness	N	Penetration force required to pierce the skin	Forney et al., 2003; Silva et al., 2005; Duarte et al., 2009; Giongo et al., 2013; Concha-Meyer et al., 2015; Vance et al., 2017; Jaramillo-Sánchez et al., 2019; Jaramillo-Sánchez et al., 2021; Hu et al., 2021; Giongo et al., 2022;
	Distance at the skin rupture point	mm or strain %	Probe displacement at epidermis rupture	Giongo et al., 2013; Jaramillo-Sánchez et al., 2019; Jaramillo-Sánchez et al., 2021; Giongo et al., 2022
	Skin break work energy	J	Work energy that is needed to break the epidermal skin	Jaramillo-Sánchez et al., 2019; Jaramillo-Sánchez et al., 2021; Giongo et al., 2022
	Stiffness	N mm^{-1} OR N $\%^{-1}$	The slope of the initial linear portion of the force-deformation curve	Giongo et al., 2013; Jaramillo-Sánchez et al., 2019; Jaramillo-Sánchez et al., 2021; Giongo et al., 2022
Texture profile analysis (**Fig. 1.1 C**)	Hardness		Maximum force during the first compression cycle	Chiabrando et al., 2009; Xie et al., 2018; Li et al., 2021; Olmedo et al., 2021; Giongo et al., 2022
	Resilience	Ratio (-)	Success in regaining the original berry height during withdrawal of the first compression. Calculated as A1w/A1 in **Fig. 1.1 C**	Chiabrando et al., 2009; Xie et al., 2018; Giongo et al., 2022
	Cohesiveness	Ratio (-)	Strength of the internal bond comprising the berry body. Calculated as (A2+A2w)/(A1+A1w) in **Fig. 1.1 C**	Chiabrando et al., 2009; Xie et al., 2018; Li et al., 2021; Giongo et al., 2022
	Gumminess	N	Force necessary to chew a semisolid until ready for swallowing. Calculated as the multiplication of hardness and cohesiveness	Chiabrando et al., 2009; Giongo et al., 2022
	Chewiness	J	Energy needed to chew a solid food until ready for swallowing. Calculated as the multiplication of hardness, cohesiveness, and d2 (**Fig. 1.1 C**)	Chiabrando et al., 2009; Li et al., 2021; Giongo et al., 2022.
	Springiness 1	Ratio (-)	How well the berry springs back after the first compression force is removed. Calculated as d2/d1 from **Fig. 1.1 C**	Xie et al., 2018; Li et al., 2021; Giongo et al., 2022
	Springiness 2	mm	Distance recovered by the sample between the end of the first bite and the start of the second bite. Calculated as d2 from **Fig. 1.1 C**	Chiabrando et al., 2009; Giongo et al., 2022
Impact force response (Drop test)	Contact time	s	Duration of the impact when the berry is dropped from a fixed height	Patel et al., 1993
Shear test	Shear force	N g^{-1}	Maximum force peak, often related to the skin break	Makus and Morris, 1993; Silva et al., 2005
	Shear energy	J	Indicator of the internal strength of bonds of the berry	Silva et al., 2005
Laser air-Puff	Laser air-puff firmness index	kPa mm^{-1}	Air tank pressure divided by the maximum deformation displacement	Li et al., 2011
	Springiness index	Ratio (-)	Indicator of the elasticity of the berry.	

1.2.2.1. Compression test

Uniaxial compression tests often use the induction of a predefined deformation (i.e., change in length) of blueberry samples by applying a loading perpendicular to the sample equatorial plane (i.e., normal stress direction) using a non-deformable probe of known dimension, which is moving at a constant crosshead speed.

The data is recorded in a force (y-axis) and deformation (x-axis) graph (**Fig. 1.1 A, D**). The force-deformation curve often initiates when the compression probe contacts the blueberry and exceeds a minimum trigger force (e.g., 0.05 N). This trigger force is predefined by the operator and regulated by the equipment accuracy (Abbott, 1999; ASAE, 2008). During the test, force is recorded as the berry is deformed by the probe descending (downstroke) until reaching a target deformation distance (mm) or strain (% of deformation as a proportion of the initial fruit height), which is predetermined by the operator or the testing machine.

When performing a compression test (single or double compression), blueberries are often oriented with the stem-calyx axis perpendicular to the compression probe, and hence the deformation is conducted equatorially (Ballinger et al., 1973; Rohrbach and Mainland, 1993; Donahue and Work, 1998; Ferraz et al., 2001; Ehlenfeldt and Martin, 2002; Saftner et al., 2008; Ochmian, 2012; Leiva-Valenzuela et al., 2013; Blaker et al., 2014; Paniagua et al., 2014) Conversely, compression has also been performed by applying a load to the base of the berry (stem end to blossom end) (Ferraz et al., 2001; Ochmian, 2012). However, Ferraz et al. (2001) demonstrated that equatorial compression produces smoother and more consistent force-deformation curves than compression from stem-end to blossom-end of the berry.

Alternatively, a compression test has also been conducted by recording the maximum force to 30 mm compression of a group of blueberries (i.e., 30 g) placed in a plastic beaker or cylinder of defined diameter (Sanford et al., 1991; Nunes, 2015; Ktenioudaki et al., 2021). This kind of compression test, where a group of berries are compressed in bulk (and consequently mass data is collected), represents a viable option when data collection speed is prioritised. However, this technique is also likely to introduce considerable inconsistencies, such as variations in the contact area between berries interacting during the test. Hence, it is unclear whether this simultaneous compression of a group of berries generates data that accurately tracks blueberry quality and consumer acceptability.

The results from a compression test are often an approximately linear increase of force as the berry is deformed to ~25 % strain of its equatorial diameter (Ballinger et al., 1973; Rohrbach and Mainland, 1993).

When berries are compressed beyond 25 % of their initial diameter, the increase in force (as the probe compresses the berry) can be halted when the berry rupture point is achieved, which is abruptly followed by a continuous decrease of force (**Fig. 1.1 A)**. The rupture point in the force-deformation curve can also be accompanied by a visible failure of the berry structure (ASAE, 2008). For example, Silva et al. (2005) evaluated the maximum compression force as the point the blueberry began releasing juice.

Blueberry compression is usually performed to a small target deformation (e.g., ≤ 2 mm), which does not produce a visible rupture point, and consequently, the test has been referred to as non-destructive compression (Timm et al., 1996; Ferraz et al., 2001; Chiabrando and Giacalone, 2011; Falagan et al., 2020). An advantage of non-destructive methodologies is that it allows tracking quality changes of the same fruit throughout postharvest storage. However, the compression test can only be considered a valid non-destructive methodology when fruit cellular structure is unaffected. Even a gentle plate compression to 1.5 N (e.g., 1-2 mm deformation) can induce micro-cracks in the blueberry microscopic structure (Allan-Wojtas et al., 2001). Consequently, multiple rounds of berry compression might affect the results, most likely actively reducing the firmness of the fruit. Further research is required to test whether multiple compressions (temporally dispersed) at small compression forces (e.g., < 0.5 N) can skew data in the resulting datasets.

1.2.2.1.1. Probes used for compression test

Different probe types have been used to conduct compression tests and can be divided into flat surface plates of high contact area (e.g., > 15 mm dimeter plate) or cylindrical probes of contact area smaller than the blueberry diameter (e.g., 2-4 mm area).

Flat surface plates are standard compression probes that can be used to conduct parallel plate contact compression (ASAE, 2008). A common requirement is that the plate should be larger than the fruit surface contact area, facilitating the evaluation of compression mechanics of the whole fruit (Harker et al., 1997). Reported dimensions of the compression plates used on blueberries often vary between 15 mm and 75 mm in diameter (Prussia et al., 2006; Schotsmans et al., 2007; Rivera et al., 2013; Paniagua et al., 2013; Cantin et al., 2012; Vilela et al., 2016; Moggia et al., 2017; Lobos et al., 2018; Falagan et al., 2020; Giongo et al., 2022). A compression plate is also the main probe used when mechanical parameters are analysed by a FirmTech compression machine (Prussia et al., 2006). However, plates have also been used in the texture analyser (Schotsmans et al., 2007; Paniagua et al., 2013; Cantin et al., 2012; Rivera et al., 2013; Vilela et al., 2016; Hu et al., 2015); Instron universal testing machines (Donahue and Work, 1998; Ferraz et al., 2001); and Ametek force gauge (Rohrbach and Mainland, 1993).

Cylindrical probes are often of small diameter (2-4 mm) with a flat or domed tip (Blaker et al., 2014; Chiabrando and Giacalone, 2011; Concha-Meyer et al., 2015; Rodriguez and Zoffoli, 2016; Scheidt and Silva, 2018; Ortiz et al., 2018; Manzi and Lado, 2019; Moggia et al., 2022). Small cylindrical probes have been used on various testing machines, including texture analyser (Vicente et al., 2007; Angeletti et al., 2010; Ortiz e al., 2018; Manzi and Lado, 2019), Instron universal testing machines (Blaker et al., 2014); and durometers (Chiabrando and Giacalone, 2011; Rodriguez and Zoffoli, 2016; Moggia et al., 2022).

Compression test performed using a small cylindrical probe (< 4 mm) can potentially puncture the blueberry skin, penetrating all the way to the flesh tissue. Under this condition, the method should be considered as a penetration (or puncture) test rather than compression.

Consequently, research studies should explicitly declare if the skin is punctured or not when a small cylindrical probe is used. An example of this would be the penetration method reported by Giongo et al. (2013; 2022) or the compression test reported by Moggia et al. (2022).

Small cylindrical probes generate significantly different results from assays that employ compression plates. Small probes provide a smaller contact area than plate probes; hence, smaller forces are achieved compared with a compression plate executed to the same target deformation distance (e.g., 1 or 2 mm). In addition, for compression using a plate probe, the area of contact with a spherical fruit surface (i.e., blueberry), may increase as deformation distance increases. While, when using small cylindrical probes, the area of contact with fruit surface may not be influenced by the deformation distance (Moggia et al., 2022). Consequently, these differences can influence subsequent quantification of mechanical parameters and hence interpretation of the result.

1.2.2.1.2. Mechanical parameters from the compression test

Mathematical processing of the compression test data described in the force-deformation graph enables simultaneous calculation of different mechanical parameters and subsequent adimensional indexes. The most frequently reported parameters from compression tests conducted on blueberry are the maximum force (N) and slope of force-deformation downstroke compression plot, also knowns as chord stiffness (kN m^{-1}) (**Table 1.2; Fig. 1.1 A**). Other mechanical parameters, such as apparent modulus of elasticity (Pa), have also been reported (**Table 2**).

The maximum force (N) is often calculated as the force achieved at a predefined deformation distance (mm) or strain (%) of equatorial berry diameter (**Fig. 1.1 A**). However, if the deformation distance exceeds maximum resistance of berry cellular structure, maximum force may be measured at the deformation distance equivalent to the rupture point (N) (ASAE, 2008; **Fig. 1.1 A**).

Chord stiffness, loading slope, and FirmTech firmness (kN m^{-1}) refer to the same mechanical parameter, as the force rate increases as the blueberry is deformed. It is calculated as the straight-line slope drawn between two specific points on the force-deformation curve (Eq. 1.1, Slaughter and Rohrbach, 1985).

$$CS = \frac{F_m - F_0}{\mathrm{D}} \qquad \textbf{(Eq. 1.1)}$$

Where Fm and F_0 are the maximum and minimum force (N), respectively, and D is the deformation (mm) of a blueberry which is achieved between the minimum and maximum force.

The most common procedure considers the use of a minimum force equal to the trigger force and the maximum force achieved at a predefined small deformation (< 25 % strain) (Ballinger et al., 1973; Slaughter and Rohrbach, 1985). Alternatively, when using the FirmTech compression device, firmness is calculated as the loading slope between a minimum and maximum force thresholds predefined by the operator (**Fig. 1.1 D**). However, it is important to keep in mind that the threshold minimum and maximum force selected for the calculation of the loading slope can influence the output result (Prussia et al., 2006). Hence it is recommended that operational settings are reported in research publications, and over the course of time, and standard predetermined settings are used across research studies and instruments to enable data standardization. As an example, studies conducted by Moggia et al. (2016; 2017; 2018; 2022), Arrington and DeVetter (2017), and Lobos et al. (2018; 2021) a have used FirmTech operation settings previously reported by Ehlenfeldt and Martin (2002) of 0.15 N and 1.96 N as minimum and maximum force thresholds. Consequently, it is recommended to use the same operational settings as other previous studies when chord stiffness data require to be compared.

The apparent modulus of elasticity (Pa) is a direct descriptor of the elastic properties or the deformation behaviour of fruit commodities (Prussia et al., 2006; ASAE, 2008; Giongo et al., 2013). Modulus of elasticity is routinely determined as the ratio of stress (force per cross-sectional area of the probe) to strain (% or mm) and should only be measured considering the non-destructive elastic portion of the force-deformation curve (Abbott, 1999).

Assuming that the fruit is viscoelastic, and the compression deformations are small (<25 % berry diameter), for fruit materials of convex shape (i.e., blueberry) compressed using parallel plate contact, the apparent modulus of elasticity can be determined using Eq. 1.2, which is based on Hertz contact theory (Donahue and Work 1998; Pussia et al., 2006; ASAE, 2008).

$$E = \frac{0.338\,F\,(1-\mu^2)}{D^{3/2}} \times \left[\mathrm{K_U} \times \left(\frac{1}{\mathrm{R_U}} + \frac{1}{\mathrm{R'_U}}\right)^{1/3} + \mathrm{K_L} \times \left(\frac{1}{\mathrm{R_L}} + \frac{1}{\mathrm{R'_L}}\right)^{1/3} \right]^{3/2} \qquad \text{(Eq. 1.2)}$$

Where *E* is the apparent modulus of elasticity (Pa); F is the force (N) measured by the load cell on the testing machine; D is the blueberry deformation (m) at the given force; R_U and R'_U are the minimum and maximum, respectively, radii of curvature (m) at the upper point of contact (plate probe); R_L and R'_L are the minimum and maximum, respectively, radii of curvature (m) at the lower point of contact (platform support). K_U and K_L are constants calculated as 1.351 for contact angle to plate probe of 90° (ASAE, 2008). μ is the Poisson's ratio (dimensionless), which measures the deformation of the food material in the perpendicular direction to the uniaxial compression. Poisson ratio is assumed for blueberries to be a value close to 0.4 (Prussia et al., 2006). Alternatively, Poisson's ratio can be further calculated considering calculation procedures described by Lu and Abbott (2004) or Sirisomboon et al. (2012).

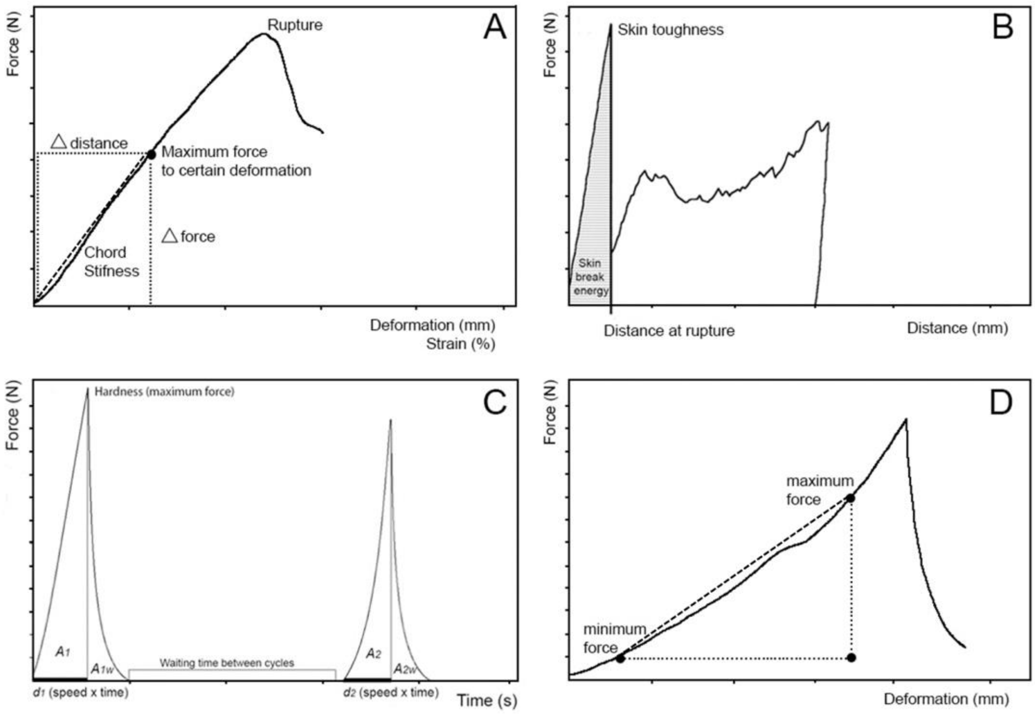


Fig. 1.1. Graphical representation of mechanical tests performed using a Texture Analyzer (A-C) and FirmTech compression device (D). A. Single compression test to 50 % deformation of the equatorial diameter of 'Rahi' blueberry, using a compression plate of 25 mm diameter. In this example, the rupture point occurred at approximately 35 % strain (5 mm deformation). B. Penetration test performed using a 2 mm needle probe to 25 % deformation of blueberry 'Rahi'. In this example, the needle probe breaks the skin at approximately 4.0 % strain (0.6 mm deformation). C. Texture profile analysis to 15 % deformation on blueberry 'Rahi'. See Table 1 to further calculation procedures of the mechanical parameters obtained from the double compression graph. D. Compression test recorded by a FirmTech 2 device using a compression plate. FirmTech firmness is calculated as the slope of the force-deformation between the minimum and maximum force thresholds. Fig 1.1 D was adapted from Donahue et al. (2000).

1.2.2.2. Penetration test

A penetration (puncture) test is a destructive method that combines stresses achieved by force of compression (normal direction) and shear (tangential direction) as recorded by the downstroke movement and penetration of a non-deformable probe of predefined shape and dimension into a target depth at the fruit equator (Harker et al. 1997; Lu and Abbott, 2004). For most of the firm fleshed fruits such as apple, kiwifruit, and peach, a penetration test is a standard firmness method universally used for quality evaluations (Harker et al., 1997; Abbot, 1999). In this case, the test involves penetration of a cylindrical probe into the flesh of a peeled fruit (Harker et al., 1997; Abbott, 1999). However, on soft fleshed fruits, the penetration test is commonly conducted on fruits with intact peel, and hence the probe must break the skin before penetrating the flesh tissue. This procedure has been reported for grapes (Letaief et al., 2008), raspberries (Giongo et al., 2019), and blueberries (Forney et al., 2003; Duarte et al., 2009; Giongo et al., 2013; Concha-Meyer et al., 2015; Vance et al. 2017; Giongo et al., 2022).

As in the case of the compression test, the assessor should perform a penetration test using predefined operational settings of trigger force, test speed, and target penetration distance. The resulting data is recorded in a force (y-axis) and distance (x-axis) graph (**Fig. 1.1 B**).

1.2.2.2.1. Probes used on penetration test

Probes previously used to collect penetration parameters of blueberries include needle probes such as the P/2N (Stable Micros Systems, UK); or the 1.8 mm maximum diameter (item 320398, ZwickRoell, Italy) reported by Giongo et al. (2022). Other probes for penetration test include cylindrical flat end probes of 2 mm (Silva et al., 2005; Concha-Meyer et al., 2015; Giongo et al., 2022), 3 mm (Jaramillo-Sanchez et al., 2019), or 4 mm diameter (Giongo et al., 2013; Giongo et al., 2022).

When using needle probes, test machines with high resolution and accuracy at relatively low force are required to measure mechanical properties as the skin breakpoint is usually achieved below 1 N of force. This includes testing devices such as Texture Analysers with a 5 kg load cell (Giongo et al., 2022), and less sophisticated Wagner force gauges (Forney et al., 2003; Vance et al., 2017).

1.2.2.2.2. Mechanical parameters for the penetration test

Previously reported mechanical parameters for a penetration test are often related to the moment when the blueberry skin breaks (or is pierced). For example, force at skin break or skin toughness (N), skin break distance (mm or %), and skin break energy (J) are often reported (**Table 1.2**).

Skin break force is the most reported mechanical parameter on blueberries (**Table 1.2**). This parameter is detected on the standard force-distance curve, as the force achieved just before an abrupt and constant decrease in force coincides with the point of visible penetration of the skin by a non-deformable probe (**Fig. 1.1 B**).

Although skin break force is measured at the point of skin rupture, resistance of fruit flesh may also contribute to the loading stress, and hence this parameter does not only describe the force resistance of the isolated skin. Silva et al. (2005) reported an average of 45 % less force on five blueberry genotypes when the skin was pierced from inside to outside the berry, compared to the force achieved by rupturing the skin from outside to inside. Other important parameters obtained from the penetration test included the distance at skin break, the skin break work energy and stiffness (**Table 1.2**). In addition, the penetration test conducted using a needle probe can be used to provide additional mechanical parameters related to the characterization of different tissues layers of blueberry, such as epidermis, hypodermis, and parenchyma (Giongo et al., 2022).

1.2.2.3. Texture Profile Analysis

A particular example of a mechanical test that attempts to estimate food's sensory descriptors instrumentally is the texture profile analysis (TPA). This test imitates oral chewing by performing two consecutive compressions of the food sample using a flat rigid plate (Pons and Fiszman, 1996). Data is obtained from the force (y-axis) and time (x-axis) curves (**Fig. 1.1 C**). To accurately measure the mechanical parameters of the force-time graph, a constant crosshead speed must be set for the downstroke and the upstroke of both cycles. Additional settings predefined by the operator include the compression target strain (% of deformation related to initial fruit length) and the waiting time between compression cycles influence the results. TPA has been used on diverse fruit produce, including pomes (Guine, 2013), drupes (Contador et al., 2016), and small berries such as grapes (Letaief et al., 2008), raspberries (Giongo et al., 2019), and blueberries (**Table 1.2**).

Adaptations or optimisations have been proposed to the original TPA test through the short history of its use on blueberries.

(1) Hardness, chewiness, springiness, resilience, cohesiveness, and gumminess are the most common TPA descriptors utilised for blueberries (**Table 1.2**). However, to be very strict with the original TPA definitions, the descriptor of gumminess should not be reported for blueberries because it is only defined for semisolid foods (Pons and Fiszman, 1996).

(2) Standard size samples are recommended when using TPA (Pons and Fiszman, 1996). However, preparing even-size samples using cork borers and knives as done in other fruit such as peach (Contador et al., 2016) and melon (Lazaro and de Lorenzo, 2015) is impractical for blueberry due to the small fruit size and relatively soft flesh texture. Consequently, the test has previously been performed using a whole intact berry (Chiabrando et al., 2009; Xie et al., 2018; Li et al., 2021; Olmedo et al., 2021; Giongo et al., 2022). One of the main challenges of using the whole berry is the variability in sample dimensions which influences the force quantification. Fruit with the same material properties can produce different hardness (at a predefined deformation strain %) if the sample size varies greatly (Trinh and Glasgow, 2012). Hence, when conducting TPA, the selection of blueberries in the same range of equatorial diameter is recommended. In addition, the chord stiffness (Rohrbach and Mainland, 1993) and modulus of elasticity (Trinh and Glasgow, 2012) have been preferred to hardness.

(3) Input operational settings of compression speed, strain distance, and time duration between compression cycles can influence TPA parameters such as hardness, cohesiveness, springiness, and chewiness (Alvarez et al., 2002; Rosenthal, 2010; Madieta et al., 2011). For blueberries, TPA has been previously conducted by selecting any of two compression strain

distances, 15 % (Li et al., 2021) or 30 % (Chiabrando et al., 2009; Xie et al. 2018; Giongo et al., 2022).

1.2.2.4. Other mechanical tests

Other tests used to characterise mechanical parameters but not extensively used by the research community when evaluating blueberry quality are the impact response analysis (drop test), the shear test and laser air-puff.

The impact response analysis considers that differences in fruit bounce can be used to measure firmness (Patel et al., 1993; McGlone and Schaare, 1998). A soft blueberry of equal mass will have a more prolonged contact duration (s) than a firm berry when bouncing after it is dropped from a predetermined height (Patel et al., 1993). The impact response has provided the basis for commercial grading systems, such as the SoftSort grader described for kiwifruit (McGlone and Schaare, 1998).

The shear test determines the shear force and energy when cutting a solid food sample into pieces using a sharp knife (Lu and Abbott, 2004; Silva et al., 2005). Although this test is more recommended on muscle foods (i.e., meats) rather than fruit (Lu and Abbott, 2004), the test has previously been conducted on blueberries (Makus and Morris, 1993; Silva et al., 2005). Two mechanical parameters, shear force and maximum energy, have been previously reported on different cultivars (**Table 2**). For calculating the shear force, Makus and Morris (1993) performed the shearing test on a group of 150 g of fruit using a TP-1A texture press (Food Technology Corp., USA). Silva et al. (2005) executed the test on a group of 50 berries using a Kramer shear press (Food Texture Corp, USA). In this last study, the sheer force provided similar cultivar differentiation compared to the maximum force by a penetration test (Silva et al., 2005).

A very innovative test to evaluate firmness as a non-contact mechanical method is the laser air-puff method developed by the University of Georgia, USA (Li et al., 2011). The method is based on blueberry deformation, generated by a brief puff of pressurised air and measured using a laser displacement sensor. The test is based on the concept that a softer fruit would have higher deformation. This method can obtain two parameters on blueberries, the laser air-puff firmness index and the springiness index (**Table 1.2**).

1.2.3. Testing machines

Texture analysers (e.g., Stable Micro System, UK or Zwick Roell, Italy), FirmTech 2 (Bioworks, USA), Universal Testing Machine (Instron, USA), force gauges (e.g., Ametek, USA; Wagner, USA) and durometers (e.g., Durofel®, France) are the most common testing machines used to assess mechanical properties of blueberry.

The Stable texture analyser, Instron universal testing machines, and FirmTech 2 compression devices used in blueberry research often follow the procedures recommended by the ASABE Standards of "*compression test of food materials of convex shape*" (ASAE, 2008). The most important recommendations can be summarised as follows:

(1) Suitable testing machine equipment records the change in deformation as a function of the load applied to the berry.

(2) The load should be recorded with an accuracy of ±1 % of the maximum expected value.

(3) The equipment should allow setting a constant compression rate (crosshead speed).

(4) A hardened metal plate with a smooth surface should be used to support the sample.

Adaptions to this last recommended formality (4) have also been reported, especially when using parallel plate compression. Due to the oblate spheroid shape and the small contact area for blueberry, balancing and restricting the movement of the berry prior to and during compression is important to ensure accurate mechanical measurements. To avoid the balancing movement, berries can be held over a small flat metal washer ring of 7-10 mm internal diameter using an Instron universal testing machine (Ballinger et al., 1973) or Stable texture analyser (Paniagua et al., 2013). For the FirmTech compression device, the design considers an adaptation of the platform support where berries are held during compression. A turntable with 25 shallow depressions of 10 mm diameter and 2 mm depth enables support for each of 25 berries during the automated compression procedure (Prussia et al., 2006; Mitcham et al., 1998).

Although the different testing machines can be used to calculate the same mechanical parameter (e.g., FirmTech firmness compared to Instron chord stiffness), the equipment varies in precision, resolution, operation ease, and cost. In addition, FirmTech, Stable texture analyser, and Instron universal testing machine can perform fully automatic tests, while analog and electronic durometers and force gauges often require human interaction with the fruit sample during the assessment.

The recommendation of when to use each instrumental device will depend on the objective of the data collection and the portability of the device. A stable texture analyser or Instron universal testing machine is recommended when data must be obtained with a high precision, resolution, and accuracy of operational settings (e.g., constant loading rate). These conditions allow assumptions that most of the data variation is attributed to natural berry to berry variation rather than an instrumental error (Slaughter and Rohrbach, 1985). In addition, when using a texture analyser or a Instron universal testing machine, a vast range of mechanical tests can be conducted and parameters extracted, which is facilitated by the relative ease of procedures related to changing the operational settings (e.g., number of compression cycles, crosshead speeds, and target modes) and hardware components (e.g., probe type, load cell capacity).

On the other hand, when mechanical tests are used to evaluate mechanical parameters under commercial quality control operations, where mass data collection may be required, trade-offs between accuracy, speed of data collection, portability, and cost may be necessary. Texture analysers and Instron universal testing machines might not be ideal due to the slow operational speed (limiting the sample size), lack of portability, and the high investment and operational cost. Considering the instrumental options available in the market and previously reported for blueberry studies, the Wagner force gauge (Forney et al., 2003; Vance et al., 2017), Durofel durometer (Chiabrando et al., 2009; Rodriguez and Zoffoli, 2016), Penefel durometer (Moggia et al., 2022), and Ametek force gauge (Sanford, et al., 1991; Patel et al., 1993; Rohrbach and Mainland, 1993) are portable instruments that provide lower cost, higher speed, and higher throughput solutions to collect information. The FirmTech compression device seems to offer a compromise between all the parameters mentioned above and can be used in commercial evaluations (Prussia et al., 2006; Moggia et al., 2022). A positive feature of FirmTech compared to other testing machines is that it provides a fully automatic operation for each evaluation batch of up to 25 berries in its unique designed turntable, which can further reduce the labour cost of operations (Mitcham et al., 1998; Lu and Abbott, 2004; Prussia et al., 2006). In addition, Moggia et al. (2022) demonstrated that different FirmTech 2 devices might provide comparable results when devices are properly calibrated, and the same operational parameters are used.

On the other hand, commercial orientated equipment can have significant limitations. The main limitations for durometer (i.e., Durofel) and force gauges are related to operating procedures. The compression procedures often required the assessor to hold the fruit sample with one hand and perform the compression movement with the other hand holding the probe device. Hence, data error can be induced by different operators and by inaccuracies of the same operator (Patel et al., 1993). Consequently, intense training and experience are prerequisites for the operators to regulate the consistency of handheld device operation to eliminate potential errors introduced from variation in the compression angle, sample deformation distance, speed of movement, and the force applied by the assessor's fingers when holding the berry (Mitcham et al., 1998). An alternative solution to the force gauges is to mount this equipment on a motorised or mechanical stand to facilitate the standardisation of operation procedures (Rohrbach and Mainland, 1993).

An additional limitation of force gauges and durometers is that both instruments only report a single mechanical parameter. The maximum force to a compression distance (e.g., hardness to 2 mm) is provided when using a Shore durometer (Alsmairat et al., 2011) or Ametek force gauge (Patel et al., 1993), and the force to penetrate the skin (skin toughness) is found when using a Wagner gauge (Forney et al., 2003; Vance et al., 2017). Alternatively, Rohrbach and Mainland (1993) estimated the blueberry stiffness using an Ametek force transducer, knowing the compression displacement.

For durometers, an additional limitation is associated with the data unit, which is reported as a non-standard unit of force. For example, Shore durometer units range from 0 to 100 (Alsmairat et al., 2011), and the Durofel Index ranges from 1 to 60 (Chiabrando et al., 2009; Rodriguez and Zoffoli, 2016). For the Durofel, a linear regression model (r^2 = 0.97) can be used to transform the Durofel Index into Newtons (N), as proposed by Rodriguez and Zoffoli (2016).

The main limitations of the FirmTech 2 compression device can be related to its portability (a computer is required) and the mechanical test capability. The FirmTech firmness (g mm^{-1}) is the only mechanical property automatically calculated from the force-deformation curve (Donahue et al., 2000; Prussia et al., 2006; Moggia et al., 2017).

1.2.4. Non-destructive techniques

Automatic real-time inspection and grading of blueberry quality to facilitate sorting undesired soft berries is an important and growing interest for the commercial blueberry supply chain. The selection of a true non-destructive (non-invasive) technique may facilitate this commercial requirement. In addition, blueberries can experience firming or softening throughout storage as influenced by postharvest storage management of relative humidity (Paniagua et al., 2013). Consequently, a non-invasive firmness method would benefit the research community in studying mechanical changes of the same berry as influenced by postharvest management.

Non-invasive methods previously used in blueberries include electronic detectors of aromatic volatiles (Simon et al., 1996), and optical techniques such as hyperspectral microscope imaging (Park et al., 2022), optical coherence tomography (Li et al., 2021), and near-infrared (NIR) hyperspectral evaluation (Leiva-Valenzuela et al., 2013; Leiva-Valenzuela et al., 2014; Hu et al. 2016). These techniques usually do not measure firmness directly, and consequently, output data is first required to be related to mechanical parameters to provide a measure of firmness (**Table 1.3**).

Hyperspectral techniques have been the most studied non-destructive methods to assess blueberry mechanical parameters, producing acceptable prediction performance (r = 0.6-0.9) across studies (**Table 1.3**). Hyperspectral imaging of blueberries has previously been conducted with three different sensing configurations, including reflectance (Leiva-Valenzuela et al., 2013; Leiva-Valenzuela et al., 2014, transmittance (Leiva-Valenzuela et al., 2014), or interactance (Hu et al. 2016). However, Leiva-Valenzuela et al. (2014) indicated that reflectance sensing mode results in a better prediction of blueberry chord stiffness than transmittance and may be easier to implement in commercial operations. On the other hand, Hu et al. (2016) have demonstrated that hyperspectral imaging in interactance mode can be used to predict mechanical parameters of the TPA and penetration test (**Table 1.3**).

A limitation of the experimental design of previously conducted studies in hyperspectral imaging is the lack of a strong and consistent manipulation of pre- or postharvest factors (e.g., genotype differences, harvest maturity, storage relative humidity management) to generate berries with different mechanical properties (**Table 1.3**). Consequently, the studies may have considered a relatively small range of mechanical parameter distribution (Leiva-Valenzuela et al., 2014), and the previously reported performances do not assure that the firmness prediction can be reproduced when any other pre- or postharvest factor influences mechanical properties.

In addition, hyperspectral imaging can generate models subject to overfitting, *i.e.,* the model developed might only be deployable on a limited number of very similar conditions (Hu et al., 2016). However, changes in blueberry mechanical parameters can be attributed to different causes (e.g., water loss, cell wall degradation, presence of stone cells) that can potentially affect the spectra profiling of blueberries, biasing the results. Hence, further study of hyperspectral imaging considering different causes of mechanical changes may be required. As an example, other studies on non-destructive techniques have considered a clear strategy to manipulate mechanical parameters. Simon et al (1996) used an electronic sniffer to study 'Bluecrop' of different maturity, and Li et al (2021) used optical coherence tomography to study 'Centurion' stored in different relative humidity environments that produced different water loss levels.

Table 1.3. Use of non-invasive techniques to predict instrumental mechanical methods previously reported for blueberry

Texture variation method	Non-destructive method		Mechanical method		Prediction performance (R)[a]	Reference
	Technique	Parameter	Machine, test	Parameter		
'Bluecrop' blueberry harvested at five maturities	Electronic sniffer	Aromatic volatiles	Force gauge, plate compression	Force at rupture point	0.62	Simon et al., 1996
Commercial highbush blueberry stored for 3-14 d at 4 °C	Hyperspectral imaging	Reflectance sensing between 500 and 1000 nm	Texture analyser, plate compression	Chord stiffness	0.83-0.87	Leiva-Valenzuela et al., 2013
Commercial Rabbiteye blueberry stored for 3-14 d at 4 °C	Hyperspectral imaging	Reflectance sensing between 400 and 1000 nm	Texture Analyser, plate compression	Chord stiffness	0.77	Leiva-Valenzuela et al., 2014
		Transmittance sensing between 563 and 939 nm		Chord stiffness	0.59	
Two batches of commercial imported blueberry from Latin America and stored for 6 d at 4 °C	Hyperspectral imaging	Interactance sensing between 675 and 1000 nm	Texture analyser, texture profile analysis	Hardness	0.77	Hu et al., 2016
				Springiness	0.84	
				Cohesiveness	0.91	
				Resilience	0.86	
			Texture analyser, penetration test	Skin toughness	0.71	Hu et al., 2016
				Distance at skin break	0.65	
				Chord stiffness	0.58	
				Final force	0.62	
'Centurion' blueberry stored on four RHs at 4 °C	Optical coherence tomography	Cell thickness of first visible layer	Texture analyser, texture profile analysis	Hardness slope	-0.72	Li et al., 2021
				Cohesiveness,	-0.80	
				Springiness	0.88	
Blueberry from two regions stored for 1-5 d at 20 °C	Hyperspectral microscope imaging	Average Intensity at 530 nm of parenchyma cell-wall	Texture analyser, penetration test	Maximum force	0.93	Park et al., 2022

[a] Correlation coefficient

1.3. Factors affecting textural and mechanical parameters

Firmness can impact blueberry consumption by influencing consumer preferences (Blaker et al., 2014), potential postharvest storage life (Hancock et al., 2008; Giongo et al., 2013, Giongo et al., 2022), and the likelihood of bruising due to mechanical impact damage (Moggia et al., 2017). Therefore, studying the pre- and postharvest factors influencing blueberry firmness is critical to assisting marketability and quality improvements of blueberries. This section reviews the most studied factors influencing changes in mechanical parameters, including genotype, berry maturity management, calcium applications, and postharvest technologies (i.e., time, temperature, relative humidity, and controlled atmospheres). To identify the most widespread mechanical methods used to evaluate the effect of these factors on blueberries, a total of 60 references were grouped by the mechanical test method (**Fig. 1.2 A**) and type of equipment used (**Fig. 1.2 B**).

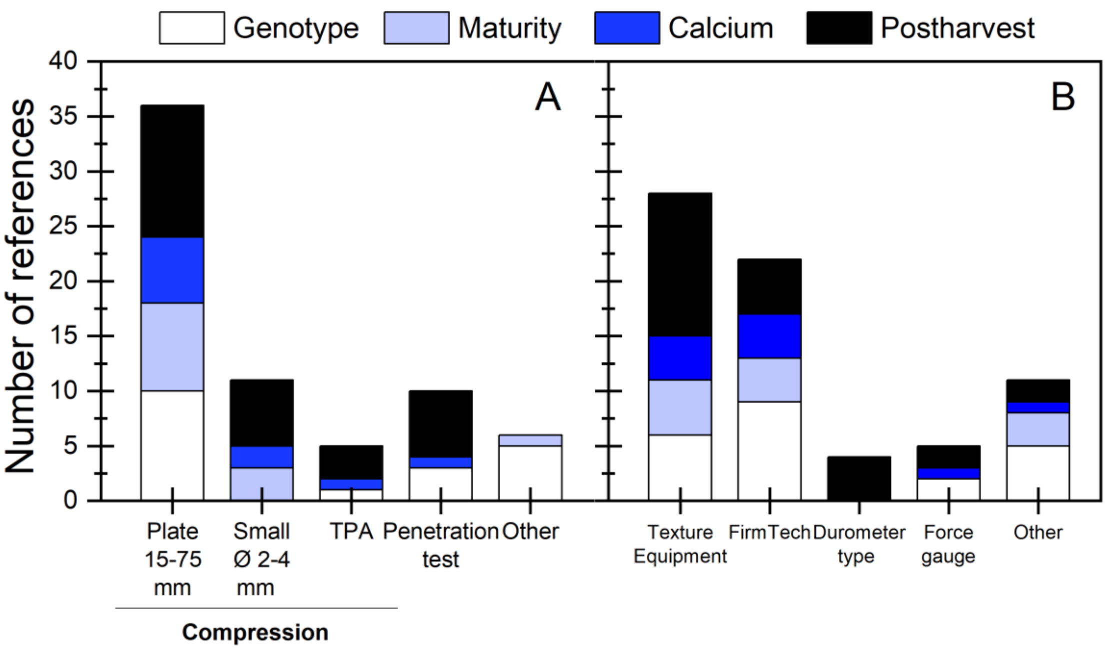


Fig. 1. 2. Mechanical test methods (A) and equipment types (B) that have previously been used to evaluate the influence of pre- and postharvest factors on the mechanical parameters of blueberry. Other test methods (in A) include shear test, impact response analysis and laser-air puff. Texture equipment (in B) includes texture analysers and Instron universal testing machines. This figure was constructed using 60 previously reported studies. For details of each reference, see **Supplementary Table 1.1**.

1.3.1. Blueberry genotype

Blueberries belong to the *Ericaceae* family and *Vaccinium* genus. The most cultivated blueberry types worldwide are highbush blueberry (including *V. corymbosum* L. and interspecific hybrids of *Vaccinium* genus) and rabbiteye blueberry (*Vaccinium ashei* Reade; syn. *V. virgatum* Ait.). For each blueberry type, a long list of cultivars has been reported, and this list is expected to increase due to the ongoing expansion of blueberry breeding programs worldwide (Lobos et al., 2015; Cappai et al., 2018). Along with the increase in available cultivars, improvement in blueberry textural characteristics is expected. Considering data from 1915 to 2015, Cappai et al. (2018) described a positive linear relationship between FirmTech firmness and the time (year) that the cultivar was released.

Firmness is a genetically controlled trait (Cappai et al., 2018; 2020); hence, the genotype of a given blueberry is a factor that will impact firmness. Differences in texture and mechanical parameters have previously been described for different genotypes. These differences are potentially associated with microstructural characteristics, including cell size, degree of cell-to-cell contact and air space, thickening of the parenchyma cell wall, and the number of stone cells (Allan-Wojtas, 2001). In addition, differences in mechanical properties of blueberry genotypes have also been related to the cell wall chemical composition (Silva et al., 2005). Conversely, Blaker and Olmstead (2015) found that the differences in quantitative cell wall material evaluations did not associate with textural differences when comparing standard texture genotypes with the genotypes displaying a crisp sensory texture (see *1.4.2.1 blueberry crispness* for further description of crisp texture).

Sensory and instrumental methods have been considered to characterise texture differences of blueberry genotypes. Sensory attributes obtained by oral evaluation (**Table 1.1**) have been commonly used to assess genotypic differences (Donahue et al., 2000; Saftner et al., 2008; Blaker et al., 2014; Vilela et al., 2016), and plate compression is the most used instrumental test

(**Fig. 1.2 A**). In addition, the FirmTech is the most common instrument used due to the rapid and automatic evaluation of multiple blueberry samples (Cappai et al., 2018).

When measuring the firmness of multiple genotypes, the reported coefficient of variation of average firmness data obtained by FirmTech was approximately 15 % (Ehlenfeldt and Martin, 2002; NeSmith et al., 2005; Saftner et al., 2008; Blaker et al., 2014). Chord stiffness determined using an Instron universal testing machine or a texture analyser also revealed similar levels of variation between cultivars (Rohrbach and Mainland, 1993; Giongo et al., 2022).

Other tests have also been used to detect genotypic differences. A penetration test has provided differences between highbush and rabbiteye blueberry types, with the cultivars of rabbiteye having higher skin toughness (Silva et al., 2005). Giongo et al. (2022) characterised mechanical differences between commercially available and advanced selection genotypes using a multi-parameter approach that included penetration tests with flat probes of 2 mm, 4 mm, and a needle probe along with texture profile analysis.

The use of alternative methods, such as the shear test (Silva et al., 2005; Makus and Morris, 1993), impact response analysis (Patel et al., 1993; Simon et al., 1996), and laser air-puff parameters (Li et al., 2001), have also identified differences between blueberry cultivars.

1.3.2. Berry development and harvest maturity

Blueberry maturity at harvest can affect the eating experience of consumers. Appearance (i.e., surface colour and size), flavour (i.e., ratio of total soluble solids to acidity), and softening (i.e., firmness) have been reported to change during the late maturity development of blueberries attached to the plant (Moggia et al., 2018). Consequently, blueberries are harvested when these characteristics maximise the eating experience, which is often related to 100 % surface blue colour berry or full maturity (Forney, 2009; Moggia et al., 2018).

Some developmental changes, such as external fruit colour, and berry size, can be easily measured. However, more sophisticated evaluation techniques are required to detect changes in flavour (increased total soluble solids and decreased acidity) and changes in texture (Giongo et al., 2013; Moggia et al., 2018). Blueberry flavour can be partly characterised using the universal instrumental methods of refractometry and titration to analyse the soluble solids and acidity, respectively (Moggia et al., 2018). However, when textural changes are evaluated, different techniques can be used. Biochemical analysis of cell wall modifications, including pectin and hemicellulose, can be performed to detect softening (Proctor and Miesle, 1991; Vicente et al., 2007; Chea et al., 2019). However, these methods are highly sophisticated and cannot be used on routine quality evaluations. Consequently, a mechanical test may be more appropriate.

The compression test has been the preferred method to evaluate mechanical parameters during blueberry growth and development (**Fig. 1.2 A**). Consequently, the parameters of maximum force to a predefined deformation (Vicente et al., 2007; Hancock et al., 2008; Chea et al., 2019; Lin et al., 2020), and chord stiffness (Ballinger et al., 1973; Timm et al., 1996) or FirmTech firmness (Moggia et al., 2016; 2018) have previously been used to assess maturity changes.

Significant softening changes during fruit development were previously described. A higher softening rate was observed at the early ripening stages, specifically when the berry surface turned green to red (Ballinger et al., 1973; Vicente et al., 2007; Moggia et al., 2018; Chea et al., 2019). A second important softening has been described between surface colour change from 50 % pink to 100 % blue surface colour and continues to occur if berries are kept for a longer period on the plant and hence become overmature (Moggia et al., 2018). Consequently, even an outstanding firmness genotype harvested at an overmature stage can present a significant prevalence of soft berries, affecting the average firmness of the harvested batch (Lobos et al., 2018; Moggia et al., 2018).

Only one article reports an alternative method to evaluate blueberry maturity, which used the impact response analysis (Patel et al., 1993). The contact time increased as the maturity stage of ’Centurion’ increased from green to overripe blue fruit, indicating that the advanced maturity berry may be softer.

1.3.3. Calcium management

Calcium plays an essential role in fruit ripening and quality, including regulating metabolism, physiological disorders, and maintaining cell structural integrity (Ferguson, 1984). However, this review will only focus on the calcium effect on textural or mechanical properties.

Calcium content associated with cell wall pectin polysaccharides was observed to affect maximum compression force (i.e. hardness) of blueberry ‘Emerald’ (firm) and ’Jewel‘ (softer) during postharvest storage (Olmedo et al., 2021). This relationship may be explained by the effect of calcium on the binding of unesterified pectin and the consequent reduction of cell wall degradation (Angeletti et al., 2010; Olmedo et al., 2021)

The compression test has previously been the preferred test to study the effect of calcium on firmness (**Fig. 1.2 A**). Chord stiffness (Hanson, 1995) or Firmtech firmness (Ochmian, 2012; Vance et al., 2017; Arrington and DeVetter, 2017; Lobos et al., 2021) are the most reported mechanical parameters. However, the use of maximum force to compression has also been reported (Stuckrath et al., 2008; Angeletti et al., 2010). In addition, studies have considered other evaluation methods such as skin toughness by penetration test (Vance et al., 2017) and texture profile analysis (Olmedo et al., 2021).

One of the most remarkable works on the effect of calcium on blueberry firmness was conducted by Angeletti et al. (2010). The study reported a positive impact of soil calcium (Gypsum, $CaSO_4$) on fruit cell wall calcium content and maximum force to 6 mm compression by a 3 mm diameter flat probe. However, the effect of calcium on mechanical parameters has been highly controversial, mainly when calcium is applied directly to leaves and fruit (foliar application). On the one hand, positive effects on mechanical parameters of using foliar calcium application have previously been reported (Stuckrath et al., 2008; Ochmian, 2012; Lobos, 2021).

On the other hand, no effect of calcium application was observed (Hanson, 1995; Vance et al., 2017; Arrington and DeVetter, 2017; Manzi and Lado, 2019). Lobos et al. (2021) reported that foliar calcium should be applied at early stages (e.g., fruit set to 16 d after) to affect fruit calcium content and FirmTech firmness.

1.3.4. Postharvest management

1.3.4.1. Temperature

Correct temperature management is the most important postharvest technology for blueberries to decrease fruit metabolism and extend storage life due to water loss and rots reduction. The recommended postharvest temperature to store blueberry is around 0 °C (Nunes et al., 2004; Forney, 2009).

The influence of temperature management after harvest on mechanical parameters has mainly been studied using compression tests with variable equipment choices, including Ametek gauge (Sanford et al., 1991), FirmTech (Tetteh et al., 2004; NeSmith et al., 2005, Cappai et al. 2020), and Stable texture analyser (Paniagua et al., 2014). The penetration test has also been considered (Concha-Meyer et al., 2015). In addition, sensorial attributes by mouthfeel (Rosenfeld et al., 1999) and hand-feel (Sanford et al., 1991; Nunes et al., 2004) have previously been used to evaluate the influence of storage temperature.

In general terms, better mechanical parameters are obtained when postharvest fruit temperature is managed below 10 °C (Sanford et al., 1991; Tetteh et al., 2004; NeSmith et al., 2005). However, Forney (2009) reported a higher chord stiffness on blueberries stored at 10 °C than 0 °C when the water vapour pressure deficit was the same at both temperatures (i.e., 0.212 kPa). Similarly, Paniagua et al. (2014) observed higher maximum force to 1 mm compression when blueberries were stored at 4 °C compared to 1 °C, and the water loss was less than 1.5 % at both storage temperatures.

1.3.4.2. Relative humidity (water loss)

As it is affected by the storage temperature and relative humidity, the water vapour pressure deficit is the main environmental driving force leading to blueberry transpiration (water loss) during postharvest (Paniagua et al., 2013). Consequently, high relative humidity (>95 % RH) complementing cool storage is the most recommended strategy to retain a low water loss rate (Paniagua et al., 2013; Moggia et al., 2016).

Water loss affects the fresh net weight of a blueberry package (i.e., clamshell) and also induces other quality issues such as expression of shrinkage, shrivel and softening (Nunes and Emond, 2007; Paniagua et al., 2013; Moggia et al., 2016). The effect of water loss on blueberry softening is mostly explained by decreasing the internal turgor pressure (Giongo et al., 2013; Paniagua et al., 2013). In addition, cellular morphology, such as the thickness of the most external cellular layers, has also been related to changes in water loss and mechanical parameters (Li et al., 2021).

The relationship between water loss increase and mechanical changes has mainly been evaluated using a single or double compression test (Ferraz et al., 2000; Paniagua et al., 2013; Moggia et al., 2016; Li et al., 2021).

On the other hand, if cumulative water loss is minimum (e.g., < 1-2 %) during storage, a firming effect has been detected when evaluating the maximum force to 1 mm deformation (Paniagua et al., 2013).

1.3.4.3. Postharvest technologies used to control rot

Blueberry rots are one of the most critical threats to blueberry quality as they can greatly impact the market life of fresh blueberries (Forney, 2009). Reductions in rot prevalence have been described when using postharvest technologies, including controlled atmosphere (CA) storage (Forney et al., 2003; Harb and Streif, 2004; Alsmairat et al., 2011; Cantin et al., 2012; Rodriguez and Zoffoli, 2016; Falagan et al., 2020), sulfur dioxide (SO_2) (Cantin et al., 2012; Rivera et al., 2013; Saito et al., 2020), ozone (Jaramillo-Sanchez et al., 2019), atmospheric cold plasma (ACP) treatment (Hu et al., 2021), and UV-C light irradiation (Jaramillo-Sanchez et al., 2021).

To test the effect of CA or SO_2 on mechanical parameters, compression test methods have been previously conducted using texture analysers (Schotsmans et al., 2007; Cantin et al., 2012; Rivera t al., 2013; Paniagua et al., 2014; Falagan et al., 2020), the FirmTech (Forney et al., 2003; Harb and Streif, 2004), the Shore durometer type 00 (Alsmairat et al., 2011), or the Durofel (Chiabrando and Giacalone, 2011; Rodriguez and Zoffoli, 2016). In addition, the penetration test has also been considered to assess the effect of CA (Forney et al., 2003; Duarte et al., 2009; Concha-Meyer et al., 2015). The effect of ozone (Concha-Meyer et al., 2015; Jaramillo-Sanchez et al., 2019), cold plasma (Hu et al., 2021), and UV-C light (Jaramillo-Sanchez et al., 2021) have been studied using the penetration test alone (**Fig. 1.2 A**).

Among the postharvest technologies, storage in CA with high CO_2 (typically > 10 kPa), SO_2 fumigation, or prolonged ACP exposition (i.e., 20 min) has previously been reported to produce adverse effects on mechanical parameters, depending on the cultivar and the storage duration (Allan-Wojtas et al., 2001; Forney et al., 2003; Schotsmans et al., 2007; Duarte et al., 2009; Cantin et al., 2012; Rivera et al., 2013; Roguiez and Zoffoli, 2016; Hu et al., 2021). Conversely, the use of CA had also shown positive retention or increase of mechanical properties of stored blueberries when $O_2 + CO_2$ was set at 20 kPa + 10 kPa (Paniagua et al., 2014), or 5 kPa + 10 kPa (Falagan et al., 2020).

1.4. Standardisation of firmness evaluation

This section will summarise the studies contributing to the optimisation of mechanical methods to evaluate blueberry firmness (**Table 1.4**). This section also will describe the previously reported relationship between sensory textural and mechanical parameters (**Table 1.5**). The sensory descriptor of crispness will also be addressed due to its importance in influencing consumer acceptability (Blaker et al., 2014). Finally, a discussion about using texture profile analysis to imitate sensory descriptors of blueberry is provided.

1.4.1. Research contributions to development or optimization of mechanical methods

As previously described in this review, the instrumental firmness of blueberries has been described using different mechanical parameters: the most recurrent, the maximum force to a predefined compression, and the chord stiffness. However, these parameters have been routinely evaluated using different operational conditions and testing machines.

The main disadvantage of using different methods to measure blueberry firmness is the impedance to comparing and validating results collected either in the commercial blueberry supply chain or within the research community. For example, in reviewing the firmness differences of blueberry genotypes, Cappai et al. (2018) only included the data of the studies that used the FirmTech firmness. This decision was because data obtained using different operational conditions and probe types are not comparable. In addition, more standardised FirmTech firmness data for blueberry genotypes were available than other mechanical methods. This example demonstrates that firmness data's longevity can be at risk if the data cannot be easily used again in the future, or the methods cannot be replicated accurately.

The standardisation of firmness for blueberries will require the selection of a standard mechanical parameter and operational settings such as crosshead speed, target distance, and probe type. The apple is an example of a fruit with a global standard description of firmness commonly used across research studies and commercial evaluations. Apple firmness is usually measured as the maximum penetration force of the peeled flesh at an equatorial position, using predefined operational settings of penetration distance (7.9 mm depth) and probe type (11.0 mm Magness-Taylor probe) (Musacchi and Serra, 2018). This firmness standardisation has facilitated the provision of consistent quality data between growers and seasons and enabled optimisation of breeding program outcomes and development of suitable pre-and postharvest management, such as harvest timing and storage technologies (Abbott, 1999; Lu and Abbott, 2004; Musacchi and Serra, 2018). Hence, having a standard methodology will shorten the developmental time of new technologies. New management practices can be adopted as lessons can be extracted from data produced globally.

Several studies have provided valuable contributions to the development and improvement of mechanical methods or instruments to evaluate blueberry firmness. The most significant contributions are summarised in **Table 1.4**, with the main inferences being summarised as:

(1) Different mechanical tests and testing machines (prototypes or commercially available) have been developed and evaluated.

(2) The mechanical parameters of compression tests of maximum force and chord stiffness (or FirmTech firmness) were the most evaluated, modelled or optimised parameter.

(3) Operational settings such as compression distance and fruit position during compression can produce different results.

(4) To develop a firmness standard, the method must be informative of the quality independent of the factors or mechanisms inducing firmness differences.

(5) A sample size ≥ 30 berries may be used to evaluate the chord stiffness or the FirmTech firmness with minimum error.

(6) To date, there is not a universally accepted standard for measuring blueberry firmness as a quality response.

Table 1.4. Summary of the studies contributing on the development or optimization of mechanical methods or instruments to evaluate blueberry firmness.

Mechanical test	Instrument	Main contributions	Reference
Compression	Instron universal testing machine	Use of chord stiffness (g cm^{-1}) to measure blueberry firmness	Ballinger, 1973
Compression	Instron universal testing machine and a blueberry compression instrument (BCI)	Firmness can be determined by measuring the chord stiffness (N m^{-1}) to 25 % deformation using the Instron universal testing machine. The BCI allows measuring the time to reach 2 mm deformation that can be used to evaluate firmness. A sample size of approximately 28 berries estimates the population mean by a small deviation	Slaughter and Rohrbach, 1985
Compression	Ametek force transducer	Optimisation of an Ametek device to be used as a low-cost evaluation method of blueberry stiffness	Rohrbach and Mainland, 1993
Compression	Portable firmness measuring device	Development and evaluation of a compression device for cherries and berries. This prototype provided the basis for the commercial FirmTech 2 (Lu and Abbott, 2004)	Timm et al. 1996
Compression	Instron universal testing machine	Maximum force (N) to < 1 mm deformation of the berry equator obtained by four compression cycles produced smother and consistent force-deformation curves	Ferraz et al., 2000
Compression	FirmTech 2	Methodology to calculate the Apparent Modulus of Elasticity (Pa) using a FirmTech 2	Prussia et al., 2006
Compression	FirmTech 2	Modelling of the rate of FirmTech firmness (g mm^{-1}) reduction as influenced by temperature and time	Tetteh et al., 2004
Compression	FirmTech 2	FirmTech firmness ranges to categorise blueberries as soft (<1.6 N mm^{-1}), moderate (1.6-1.8 N mm^{-1}) and firm (1.81-2.0 N mm^{-1})	Moggia et al., 2017
Compression	FirmTech 2	A sample size > 25 berries allows a good estimation of the FirmTech firmness (g mm^{-1}) when evaluating different blueberry genotypes (e.g., phenotyping)	Cappai et al., 2018
Compression	FirmTech 2 durometers	Relationship between FirmTech and durometers output data is not strong, especially when blueberries are classified as firm to touch	Moggia et al., 2022
Penetration	Wagner Gram Dial GDK 50	First study reporting the use of skin toughness (N) to evaluate blueberry quality	Forney et al., 2003
Penetration	Stable texture analyzer	Estimation of a Storage Index to compute the changes of mechanical parameters during the storage time	Giongo et al., 2013
Texture profile analysis (TPA)	Stable texture analyzer	First study exploring the use Texture profile analysis or double compression on blueberries	Chiabrando et al., 2010
Compression and Penetration	Zwick Roell texture analyser	The most complete profile of mechanical parameters on different blueberry genotypes evaluated up to date	Giongo et al., 2022
Impact Response Analysis	BerryBounce	Development and evaluation of a rapid instrumental device (BerryBounce) to assess firmness (i.e., contact time) of raspberries and blueberries	Patel et al., 1993
Non-mechanical contact deformation	Laser air-puff	Evaluation of a novel instrument to assess the laser air-puff firmness index and springiness index of blueberries	Li et al., 2011

1.4.2. Relationship between textural and mechanical properties

Due to the subjectivity of human senses, the food industry prefers instrumental methods rather than sensory descriptors to assess the quality of their products. However, the chosen instrumental method should best represent human sensation and food preferences to rely on the mechanical technique as a form of a reliable measure of quality. Consequently, analysis of the relationship between large and trained sensory panels and instrumental parameters may be used to facilitate the standardisation of cost-effective instrumental methods (Kemp et al., 2009).

Correlations between sensory descriptors of food oral processing and instrumental methods have previously been described on blueberries, with correlation coefficients ranging from weak (r = 0.33) to strong (r = 0.86) (**Table 1.5**). The variability between the coefficients can be observed even for the same combination of instrumental and sensory evaluation methods. For example, sensory score during chewing was poor (Saftner et al., 2008; Lobos et al., 2014) or strong (Ballinger et al., 1973; Blaker et al., 2014) correlated with instrumental chord stiffness (**Table 1.5**). We hypothesize that reported differences could mainly be attributed to the blueberries samples (e.g., genotypes) and the subjectivity of the sensory methods used in each research study.

Instrumental mechanical parameters used to correlate with sensory descriptors mainly utilise a compression test (**Table 1.5**). Consequently, knowledge of whether other mechanical test methods, such as needle penetration and impact response, have a better relationship with sensory evaluation remains unknown. In addition, to assist blueberry breeding programs, commercial grading operations, and quality control, it would be beneficial to determine quantitative threshold ranges of instrumental parameters that can be used as a ‘rule of thumb’ when related to consumer preferences. Furthermore, as much as the texture variability increases in terms of genotypes expression, a wider range of descriptors should be set to dissect texture complexity more precisely.

Table 1.5. Correlation coefficient (r) between instrumental mechanical parameters and sensory texture previously reported in blueberry studies.

Instrumental analysis		Sensory analysis		Correlation coefficient (r)	Reference
Machine, test	**Parameter**	**System**	**Parameter**		
Instron universal testing machine, plate compression	Chord stiffness	Mouthfeel	Texture during chewing	0.7 - 0.81	Ballinger et al., 1973
FirmTech 2 plate compression	Chord stiffness	Mouthfeel	Juiciness	0.48	Saftner et al., 2008
			Crispness	0.44	
			Texture during chewing	0.33	
FirmTech 2, plate compression	Chord stiffness	Mouthfeel	Crispness	0.81	Blaker et al., 2014
			Firmness during chewing	0.85	
			Skin toughness	0.75	
Instron universal testing machine, puncture compression	Bioyield	Mouthfeel	Crispness	0.86	Blaker et al., 2014
			Firmness during chewing	0.82	
			Skin toughness	0.78	
FirmTech 2, plate compression	Chord stiffness	Mouthfeel	Texture during chewing	0.38	Lobos et al., 2014
Texture analyser, plate compression[a]	Maximum force	Hand-feel	Finger pressure firmness	0.34	Nunes, 2015
Texture analyser, plate compression	Maximum force	Mouthfeel	Juiciness (succulence)	0.54	Vilela et al., 2016

[a] This test was the only study conducted by simultaneous compression of a group of blueberries (30 g) rather than by individual berry.

It is quite possible for some mechanical parameters that changes in a specific measured range are inconsequential for the consumer experience, while other measured ranges are critical sensory experience indicators. As an example, for kiwifruit, significant softening occurs between harvest (approximately 60-80 N) to when they are considered edible (approximate at 6-8 N) (McAtee et al., 2015). However, improvements in firmness in the 60 N - 10 N range are irrelevant for consumer acceptability because the fruit is not edible in this range. Conversely, kiwifruit can also be considered oversoft at approximately 5 N, and hence the detection of a 6 N firmness from a 4 N firmness fruit can be regarded as commercially very important. Similar quantification of how soft is too soft or, conversely, how a firm blueberry is firm enough for consumer acceptance is required for the blueberry supply chain.

A complicating and interesting factor in the consumption of blueberries is that due to the small size and ease of hand manipulation, blueberries are often handled (from clamshell to mouth) immediately prior to consumption. Hence, hand-feel touch may also be an essential instance in the judgement of blueberry quality (**Table 1.1**). In addition, hand-touch firmness has been reported to be used in commercial operations (Schotsmans et al., 2007; Cantin et al., 2012; Rodriguez and Zoffoli, 2016). The relationship between hand-touch firmness by sensory panellists and instrumental parameters seems like an opportunity to identify instrumental methods to assess blueberry quality related to consumer acceptability. Among the studies reported in this review, only Nunes (2015) reported a correlation coefficient (r = 0.34) between hand-touch firmness and maximum force to simultaneous compression of a group of berries (**Table 1.5**). However, the study was conducted using trained individuals rather than a formal sensory panel setting. Whether this reported relationship can be improved by considering sensory panels or other instrumental mechanical methods remains to be studied.

Another interesting point about blueberries compared to other fruits such as apples and kiwifruit is that they can be purchased in bulk and potentially consumed in multiple numbers (2-5 fruit at once) rather than as individual fruits. This fact raises questions about how to handle, analyse, and interpret the firmness data when blueberries are manipulated and consumed as a group (i.e., 2-5):

(1) Does the presence of a single soft berry out of a group (i.e., 2-5) negatively impact consumer acceptance?

(2) Is firmness to be averaged, or should the distribution of firmness in a specific range (e.g., first quartile range) be considered more useful for consumer acceptance?

Consequently, it is worth studying if the mechanical test that allows analysing a group of blueberries simultaneously, such as the Kramer shear test (Makus and Morris, 1993; Silva et al. 2005) and compression of a group (i.e., 30 g) of blueberries (Sanford, 1991; Nunes, 2015; Ktenioudaki et al., 2021), are better representative of sensory properties of bulk blueberry consumption or manipulation in hand.

This review has provided a summary of true non-destructive (non-invasive) techniques related to mechanical parameters (**Table 1.3**). Up to date, studies in non-invasive methods have not considered the statistical relation to sensory texture, which is required to understand the strengths of these techniques to segregate blueberries based on consumer sensory acceptability.

1.5. Conclusions and future research opportunities

Although the research community and commercial supply chain do not have a universal standard method to characterise blueberry firmness, mechanical parameters obtained from a compression test have provided quality characterisation as influenced by pre and postharvest factors. However, important variability in the reported methods, which affect the results, including the testing machine, probe type, target deformation, and calculation procedures from the compression-deformation curve, were found in this review. Consequently, the standardisation of operational settings should be considered, as described by different studies presented in **Table 1.4**. In addition, we cannot ignore the fact that the evaluation methods may need to vary if the data is collected for research or commercial purposes, as each of these settings has different constraints that require different decisions to balance ease of data capture, cost, and accuracy of the resulting data.

Mechanical tests other than compression tests have also been used on blueberries, with the penetration test being the second most reported. This test has provided differentiation of berries as influenced by genotype differences (Giongo et al., 2022), storage time (Giongo et al., 2013), and postharvest controlled atmosphere conditions (Forney et al., 2003). However, whether the penetration test can provide better results than the compression test to evaluate quality changes remains to be studied. In addition, this review cannot discard the fact that determining a standard for quality characterisation may require a combination of mechanical test methods as described by Giongo et al. (2022).

True non-destructive methods (non-invasive) have shown promising results relating to blueberry mechanical parameters. Future studies will hopefully further explore how different causes of mechanical changes (e.g., water loss, cell wall properties) can affect instrumental data to ensure that the correlative results produced to date are reproducible across different scenarios.

The relationship of collected instrumental mechanical parameters to sensory texture scores should dictate the value of the collected data. These relationships have to date only been determined considering sensory parameters by mastication. However, hand-touch firmness seems more suitable to imitate how consumers potentially first interact with, and hence infer about, blueberry quality before being consumed (i.e., compression between fingers). Whether the mechanical methods and non-destructive methods described in this review relate to hand-touch remains unknown and is worth investigating.

1.6. Acknowledgment

Sebastian Rivera acknowledges the School of Agriculture and Environment, Massey University (NZ), and Agencia Nacional Nacional de Investigacion y Desarollo (ANID), Chile for the financial support to pursuing Ph.D. studies at Massey University.

1.7. Author Statement

Sebastian Rivera: Conceptualization, Visualization, Writing - Original draft; **Lara Giongo**: Conceptualization, Writing - Review & Editing**; Francesco Cappai:** Conceptualization, Writing - Review & Editing**; Huub Kerckhoffs**: Writing - Review & Editing; **Svetla Sofkova-Bobcheva**: Writing - Review & Editing; **Dan Hutchins**: Writing - Review and Editing; **Andrew East**: Conceptualization, Supervision, Writing - Review and Editing.

1.8. References

Abbott, H.A., 1999. Quality measurements of fruits and vegetables. Postharvest Biol. Technol. 15, 207-225. https://doi.org/10.1016/S0925-5214(98)00086-6

Allan-Wojtas, P.M., Forney, C.F., Carbyn, S.E., Nicholas, K.U.K.G., 2001. Microstructural indicators of quality related characteristics of blueberries - An integrated approach. Lebensm.-Wiss. U.-Technol. 34, 23-32. https://doi.org/10.1006/fstl.2000.0738

Alvarez, M.D., Canet, W., Lopez, M. 2002. Influence of deformation rate and degree of compression on textural parameters of potato and apple tissues in texture profile analysis. Eur. Food Res. Technol., 215, 13-20. https://doi.org/10.1007/s00217-002-0515-0

Alsmairat, N., Contreras, C., Hancock, J., Callow, P., Beaudry, R., 2011. Use of combinations of commercially relevant O2 and CO2 partial pressures to evaluate the sensitivity of nine highbush blueberry fruit cultivars to controlled atmospheres. HortScience 46(1), 74-79. https://doi.org/10.21273/HORTSCI.46.1.74

Angeletti, P., Castagnasso, H., Miceli, E., Terminiello, L., Concellon, A., Chaves, A., Vicente, A.R., 2010. Effect of preharvest calcium applications on postharvest quality, softening and cell wall degradation of two blueberry (*Vaccinium corymbosum*) varieties. Postharvest Biol. Technol. 58, 98-103. https://doi.org/10.1016/j.postharvbio.2010.05.015

Arrington, M., DeVetter, L., 2017. Foliar applications of calcium and boron do not increase fruit set or yield in northern highbush blueberry (*Vaccinium corymbosum*). HortScience 52, 1259-1264. https://doi.org/10.21273/HORTSCI12207-17

ASAE, 2008. Compression test of food material of convex shape. ASABE Standards S368.4., 1-8.

Ballinger, W.E., Kushman, L.J., Hamann, D.D., 1973. Factors affecting the firmness of highbush blueberries. J. Amer. Soc. Hort. Sci. 98(6), 583-587.

Blaker, K.M., Plotto, A., Baldwin, E.A., Olmstead, J.W., 2014. Correlation between sensory and instrumental measurements of standard and crisp-texture southern Highbush blueberries (*Vaccinium corymbosum* L. interspecific hybrids). J. Sci. Food Agric. 94, 2785-2793. https://doi.org/10.1002/jsfa.6626

Blaker, K.M., Olmstead, W., 2015. Cell wall composition of the skin and flesh tissue of crisp and standard texture southern highbush blueberry genotypes. Journal of Berry Research 5, 9-15.

Beaudry, R.M., Moogia, C., Retamales, J.B., Hancock, J.F., 1998. Quality of 'Ivanhoe' and 'Bluecrop' blueberry fruit transported by air and sea from Chile to North America. HortScience 33(2), 313-317.

Cantin, C.M., Minas, I.S., Goulas, V., Jimenez, M., Manganaris, G.A., Michailides, T.J., Crisosto, C.H., 2012. Sulfur dioxide fumigation alone or in combination with CO_2-enriched atmosphere extends the market life of Highbush blueberry fruit. Postharvest Biol. Technol. 67, 84-91. https://doi.org/10.1016/j.postharvbio.2011.12.006

Cappai, F., Benevenuto, J., Ferrao, L.F.V., Munoz, P., 2018. Molecular and genetic bases of fruit firmness variation in blueberry - A review. Agronomy 8, 174. https://doi.org/10.3390/agronomy8090174

Cappai, F., Amadeu, R.R., Benevenuto, J., Cullen, R., Garcia, A., Grossman, A., Ferrao, L.F.V., Munoz, P., 2020. High-resolution linkage map and QTL analyses of fruit firmness in autotetraploid blueberry. Front. Plant Sci. 11, 562171. https://doi.org/10.3389/fpls.2020.562171

Chea, S., Yu, D., Park, J., Oh, H.D., Chung, S.W., Lee, H.J., 2019. Fruit softening correlates with enzymatic and compositional changes in fruit cell wall during ripening in 'Bluecrop' highbush blueberries. Sci. Horticul. 245, 163-170. https://doi.org/10.1016/j.scienta.2018.10.019

Chen, J. 2020. It is important to differentiate sensory property from the material property. Trends Food Sci. Technol. 96, 268-270. https://doi.org/10.1016/j.tifs.2019.12.014

Chiabrando, V., Giacalone, G., Rolle, L., 2009. Mechanical behaviour and quality traits of Highbush blueberry during postharvest storage. J. Sci. Food Agric. 89, 989-992. https://doi.org/10.1002/jsfa.3544

Chiabrando, V., Giacalone, G., 2011. Shelf-life extension of Highbush blueberry using 1-methylcyclopropene stored under air and controlled atmosphere. Food Chem. 126, 1812-1816. https://doi.org/10.1016/j.foodchem.2010.12.032

Concha-Meyer, A., Eifert, J.D., Williams, R.C., Marcy, J.E., Welbaum, G.E., 2015. Shelf-life determination of fresh blueberries (Vaccinium corymbosum) stored under controlled atmosphere and ozone. Inter. J. Food Sci. 2015, 164143. https://doi.org/10.1155/2015/164143

Contador, L., Diaz, M., Hernandez, E., Shinya, P., Infante, R., 2016. The relationship between instrumental tests and sensory determinations of peach and nectarine texture. Eur. J. Hortic. Sci. 81, 189-196. http://dx.doi.org/10.17660/eJHS.2016/81.4.1

Donahue, D.W., Work, T.M., 1998. Sensory and textural evaluation of Maine wild blueberries for the fresh pack market. J. Texture Stud. 29, 305- 312. https://doi.org/10.1111/j.1745-4603.1998.tb00172.x

Donahue, D.W., Benoit, P.W., Lagasse, B.J., Buss, W.R., 2000. Consumer and instrumental evaluation of Maine wild blueberries for the fresh pack market. Postharvest Biol. Technol. 19, 221-228. https://doi.org/10.1016/S0925-5214(00)00101-0

Duarte, C., Guerra, M., Daniel, P., Camelo, A.L., Yommi, A., 2009. Quality changes of Highbush blueberries fruit stored in CA with different CO_2 levels. J. Food Sci. 74, 154-159. https://doi.org/10.1111/j.1750-3841.2009.01118.x

Ehlenfeldt, M.K., Martin, R.B., 2002. A survey of fruit firmness in Highbush blueberry and species-introgressed blueberry cultivars. Hortscience 37, 386-389. https://doi.org/10.21273/HORTSCI.37.2.386

Falagan, N., Miclo, T., Terry, L.A., 2020. Graduated controlled atmosphere: a novel approach to increase 'Duke' blueberry storage life. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.00221

Ferguson I.B., 1984. Calcium in plant senescence and fruit ripening. Plant Cell Environ. 7,477–489. https://doi.org/10.1111/j.1365-3040.1984.tb01438.x

Ferraz, A.C.O., Sargent, S.A., Fox, A.J., 2000. Delays to cooling and postharvest quality of fresh Florida blueberries. Proc. Fla. State Hortic. Soc. 113, 268-271.

Ferraz, A.C., Sargent, S.A., Fox, A.J., 2001. A nondestructive method for measuring firmness of blueberry fruit. Proc. Fla. State Hortic. Soc. 114, 138-142.

Forney, C.F., Jordan, M.A., Nicholas, K.U.K.G., 2003. Effect of CO_2 on physical, chemical, and quality changes in 'Burlington' blueberries. Acta Hortic. 600, 587-593.

Forney, C.F., 2009. Postharvest issues in blueberry and cranberry and methods to improve market-life. Acta Hortic. 810 (2), 785-798. https://doi.org/10.17660/ActaHortic.2009.810.105

Giacosa, S., Zeppa, G., Baiano, A., Segade, S.R., Gerbi, V., Rolle, L., 2015. Assessment of sensory firmness and crunchiness of table grapes by acoustic and mechanical properties. Aus. J. Grape Wine Res. 21, 213-225. https://doi.org/10.1111/ajgw.12126

Gilbert, J.L., Guthart, M.J., Gezan, S.A., Pisaroglo de Carvalho, M., Schwieterman, M.L., Colquhoun, T.A, Bartoshuk, L.M., Sims, C.A., Clark, D.G., Olmstead, J.W., 2015. Identifying breeding priorities for blueberry flavour using biochemical, sensory, and genotype by environment analyses. Plos One 10(9), e0138494. https://doi.org/10.1371/journal.pone.013849

Gilbert, J.L., Olmstead, J.W., Colquhoun, T.A,, Levin, L.A., Clark, D.G., Moskowitz, H.R., 2014. Consumer-assisted selection of blueberry quality traits. HortScience 49(7), 864-873. https://doi.org/10.21273/HORTSCI.49.7.864

Giongo, L., Ajelli, M., Poncetta, P., Ramos-Garcia, M., Sambo, P., Farneti, B., 2019. Raspberry texture mechanical profiling during fruit ripening and storage. Postharvest Biol. Technol. 149, 177-186. https://doi.org/10.1016/j.postharvbio.2018.11.021

Giongo, L., Poncetta, P., Loretti, P., Costa, F., 2013. Texture profiling of blueberries (*Vaccinium* spp.) during fruit development, ripening and storage. Postharvest Biol. Technol. 76, 34-39. https://doi.org/10.1016/j.postharvbio.2012.09.004

Giongo, L., Ajelli, M., Pottorff, M., Perkins-Veazie, Iorizzo, M., 2022. Comparative multi-parameters approach to dissect texture subcomponents of highbush blueberry cultivars at harvest and postharvest. Postharvest Biol. Technol. 183, 111696. https://doi.org/10.1016/j.postharvbio.2021.11696

Guine, R.P.F., 2013. Variation of textural attributes of S. Bartolomeu pears at maturation, storage, and drying. Int. J. Food Proper. 16, 180-192. https://doi.org/10.1080/10942912.2010.535191

Hampson, C., Stanich, K., McKenzie, D.L., Herbert, L., Lu, R., Li., J., Cliff, M.A., 2014. Determining the optimum firmness for sweet cherries using Just-About-Right sensory methodology. Postharvest Biol. Technol. 91, 104-111. http://dx.doi.org/10.1016/j.postharvbio.2013.12.022

Hancock, J., Callow, P., Serce, S., Hanson, E., Beaudry, R., 2008. Effect of cultivar, controlled atmosphere storage, and fruit ripeness on the long-term storage of highbush blueberries. HortTechnology 18(2), 199-205. https://doi.org/10.21273/HORTTECH.18.2.199

Hanson E.J., 1995. Preharvest calcium sprays do not improve highbush blueberry (Vaccinium corymbosum L.) quality. HortScience 30,977–978. https://doi.org/10.21273/HORTSCI.30.5.977

Harb, J.Y., Streif, J., 2004. Controlled atmosphere storage of highbush blueberries cv 'Duke'. Eur. J. Hort. Sci, 69(2), 66-72.

Harker. F.R., Redgwell, R.J., Hallet, I.C., Murray, S.H., Carter, G., 1997. Chapter 2. Texture of Fresh Fruit. In Horticultural Reviews 20. Janick, J. (Ed). Wiley and Sons, Inc. 121-224. https://doi.org/10.1002/9780470650646.ch2

Hu, M.H., Dong, Q.L., Liu, B.L., Opara, U.L., Chen, L., 2015. Estimating blueberry mechanical properties based on random frog-selected hyperspectral data. Postharvest Biol. Technol. 106, 1-10. https://doi.org/10.1016/j.postharvbio.2015.03.014

Hu, M.H., Dong, Q.L., Liu, B.L. Opara, U.L., 2016. Prediction of mechanical properties of blueberry using hyperspectral interactance imaging. Postharvest. Biol. Technol. 115, 122-131. https://doi.org/10.1016/j.postharvbio.2015.11.021

Hu, X., Sun, H., Yang, X., Cui, D. Wang, Y., Zhuang, J., Wang, X., Ma, R., Jiao, Z., 2021. Potential use of atmospheric cold plasma for postharvest preservation of blueberries. Postharvest Biol. Technol. 179, 111564. https://doi.org/10.1016/j.postharvbio.2021.111564

Jaramillo-Sánchez, G., Contigiani, E.V., Castro, M.A., Hodara, K., Alzamora, S.M., Garcia-Loredo, A., Nieto, A.B., 2019. Freshness maintenance of blueberries (*Vaccinium corymbosum* L.) during postharvest using ozone in aqueous phase: Microbiological, structure, and mechanical issues. Food Bioprocess Tech. 12, 2136-2147. https://doi.org/10.1007/s11947-019-02358-z

Jaramillo-Sánchez, G., Contigiani, E.V., Coronel, M.B., Alzamora, S.M., Garcia-Loredo, A., Nieto, A.B., 2021. Study of UV-C treatments on Postharvest life of blueberries O'Neal and correlation between structure and quality parameters. Heliyon 7, e07190. https://doi.org/10.1016/j.heliyon.2021.e07190

Kemp, S.E., Hollowood, T., Hort, J., 2009. Sensory evaluation: a practical handbook. Wiley-Blackwell, John Willey & Sons Ltd, United Kingdom.

Ktenioudaki, A., O'Donnell, C.P., Emond, J.P., Nascimento Nunes, M.C., 2021. Blueberry supply chain: critical steps impacting ruit quality and application of a boosted regression tree model to predict weight loss. Postharvest Biol. Technol. 179, 111590. https://doi.org/10.1016/j.postharvbio.2021.111590

Lazaro, A., de Lorenzo, C., 2015. Texture analysis in melon landraces through instrumental and sensory methods. Int. J. Food Proper. 18, 1575-1583. https://doi.org/10.1080/10942912.2014.923441

Letaief, H., Rolle, L., Gerbi, V., 2008. Mechanical behavior of winegrapes under compression tests. Am. J. Enol. Viticult. 59, 323-329. https://www.ajevonline.org/content/59/3/323.article-info

Leiva-Valenzuela, G.A., Lu, R., Aguilera, J.M., 2013. Prediction of firmness and soluble solids content of blueberries using hyperspectral reflectance imaging. J. Food Eng. 115, 91-98. http://dx.doi.org/10.1016/j.jfoodeng.2012.10.001

Leiva-Valenzuela, G.A., Lu, R., Aguilera, J.M., 2014. Assessment of internal quality of blueberries using hyperspectral transmittance and reflectance images with whole spectra or selected wavelengths. Innov. Food Sci. Emer. Technol. 24, 2-13. http://dx.doi.org/10.1016/j.ifset.2014.02.006

Li, C.Y., Luo, J.W., MacLean, D., 2011. A novel instrument to delineate varietal and harvest effects on blueberry fruit texture during storage. J. Sci. Food Agric. 91, 1653-1658. https://doi.org/10.1002/jsfa.4362

Li, M., Rivera, S., Franklin, D., Nowak, E., Hallet, I., Kolenderska, S., Urbanska, M., Vanholsbeeck, F. East, A., 2021. Use of optical coherence tomography and light microscopy for characterisation of mechanical properties and cellular level responses of 'Centurion' blueberries during weight loss. J. Food Eng. 303, 110596. 10.1016/j.jfoodeng.2021.110596

Lin, Y., Huang, G., Zhang, Q., Wang, Y., Dia, V.P., Meng, X., 2020. Ripening affects the physicochemical properties, phytochemicals, and antioxidant capacities of two blueberry cultivars. Postharvest Biol. Technol. 162, 111097. https://doi.org/10.1016/j.postharvbio.2019.111097

Lobos, G.A., Callow, P., Hancock, J.F., 2014. The effect of delaying harvest date on fruit quality and storage of late highbush blueberry cultivars (*Vaccinium corymbosum* L.). Postharvest Biol. Technol. 87, 133-139. https://doi.org/10.1016/j.postharvbio.2013.08.001

Lobos, G.A., Hancock, J.F., 2015. Breeding Blueberries for a changing global environment: A review. Front. Plant Sci., 6, 1-14. https://doi.org/10.3389/fpls.2015.00782

Lobos, G.A., Bravo, C., Valdes, M., Graell, J., Ayala, I.L., Beaudry, R.M., Moggia, C., 2018. Within-plant variability in blueberry (*Vaccinium corymbosum* L): maturity at harvest and position within the canopy influence fruit firmness at harvest and postharvest. Postharvest Biol. Technol. 146, 26-35. https://doi.org/10.1016/j.postharvbio.2018.08.004

Lobos, T.E., Retamales, J.B., Luengo Escobar, A., Hanson, E.J., 2021. Timing of foliar calcium sprays improves fruit frimness and antioxidants in "Liberty" blueberries. J. Soil Sci. Plant Nutr., 21, 426-436. https://doi.org/10.1007/s42729-020-00371-2

Lu, R., Abbot, J.A., 2004. Force/Deformation techniques for measuring texture, In: Kilcast, D. (Ed.), Texture in food Volume 2, Solid foods. Woodhead Publishing Limited, England.

Madieta, E., Symoneaux, R., Mehinagic, E., 2011. Textural properties of fruit affected by experimental conditions in TPA tests: an RSM approach. Int. J. Food Sci. Technol. 46, 1044-1052. https://doi.org/10.1111/j.1365-2621.2011.02606.x

Makus, D.J, Morris, J.R., 1993.A comparison of fruit of highbush and rabbiteye blueberry cultivars. J. Food Quality 16 (6), 417-428. https://doi.org/10.1111/j.1745-4557.1993.tb00268.x

Manzi, M., Lado, J., 2019. Foliar applications of calcium do not impact on fruit and leaf nutrient concentration or quality of 'O'Neal' blueberry. J. Hortic. Sci. Biotechnol. 94,676–684. https://doi.org/10.1080/14620316.2019.1577185

McAtee, P.A., Richardson, A.C., Nieuwenhuizen, N.J., Gunaseelan, K., Hoong, L., Chen, X., Atkinson, R.G., Burdon, J.N., David, K.M., Schaffer, R.J., 2015. The hybrid non-ethylene and ethylene ripening response in kiwifruit (*Actinidia chinensis*) is associated with differential regulation of MADS-box transcription factors. MBC. Plant Biol. 15:304. https://doi.org/10.1186/s12870-015-0697-9

McGlone, V.A., Schaare, P.N., 1998. Design and performance of a fruit firmness grader. Acta Hortic. 464, 417-422. https://doi.org/10.17660/ActaHortic.1998.464.64

Moggia, C., Graell, J., Lara, I., Schmeda-Hirschmann, G., Thomas-Valdes, S., Lobos, G.A., 2016. Fruit characteristics and cuticle triterpenes as related to postharvest quality of highbush blueberries. Sci. Hortic. 211, 449-457. http://dx.doi.org/10.1016/j.scienta.2016.09.018

Moggia, C., Graell, J., Lara, I., Gonzalez, G., Lobos, G.A., 2017. Firmness at harvest impacts postharvest fruit softening and internal browning development in mechanically damaged and non-damaged Highbush blueberries (*Vaccinium corymbosum* L.). Front. Plant Sci. 8. https://doi.org/ 10.3389/fpls.2017.00535

Moggia, C., Gonzalez, C., Lobos, G. A., Bravo, C., Valdes, M., Lara, I., Graell, J., 2018. Changes in quality and maturity of 'Duke' and 'Brigitta' blueberries during fruit development: postharvest implications. Acta Horticul. 1194, 1495-1501. https://doi.org/10.17660/ActaHortic.2018.1194.209

Moggia, C., Valdes, Y., Arancibia, A., Valdes, M., Radrigan, C., Icaza, G., Beaudry, R., Lobos, G. 2022. A comparison of firmness assessment instruments for fresh blueberry fruit. HortTechnology 32(2), 172-181. https://doi.org/10.21273/HORTTECH04960-21

Miller, W.R., McDonald, R.E., Cracker, T.E., 1993. Quality of two Florida blueberry cultivars after packaging and storage. HortScience 28(2), 144-147. https://doi.org/10.21273/HORTSCI.28.2.144

Mitcham, E.j., Clayton, M., Biasi, W.V., 1998. Comparison of devices for measuring cherry fruit firmness. HortScience 33(4), 723-727. https://doi.org/10.21273/HORTSCI.33.4.723

Musacchi, S., Serra, S., 2018. Apple fruit quality: Overview on pre-harvest factors. Sci. Hortic. 234, 409-430. https://doi.org/10.1016/j.scienta.2017.12.057

NeSmith, D.S., Nunez-Barrios, A., Prussia, S.E., Aggarwal, D., 2005. Postharvest berry quality of six rabbiteye blueberry cultivars in response to temperature. J. Amer. Pomol. Soc. 59(1), 13-17. https://doi.org/10.21273/HORTSCI.46.9.1278

Nunes, M.C., Emond, J.P., Brecht, J.K., 2004. Quality curves for highbush blueberries as a function of the storage temperature. Proceedings of the Ninth North American Blueberry Research. Small Fruits Rev. 423-438. https://doi.or/10.13000/J301v03n03_10

Nunes, M.C. Emond, J.P., 2007. Relationship between weight loss and visual quality of fruits and vegetables. Proc. Fla. State Hort. Soc. 120, 235-245.

Nunes, M.C., 2015. Correlation between subjective quality and physicochemical attributes of fresh fruits and vegetables. Postharvest Biol. Technol. 107, 43-54. http://dx.doi.org/10.1016/j.postharvbio.2015.05.001

Ochmian I., 2012. The impact of foliar application of calcium fertilizers on the quality of highbush blueberry fruits belonging to the “Duke” cultivar. Not. Bot. Horti. Agrobot. Cluj-Napoca 40,163–169. https://doi.org/10.15835/nbha4028058

Olmedo, P., Zepeda, B., Rojas, B., Silva-Sanzana, C., Delgado-Rioseco, J., Fernandez, K., Balic, I, Arriagada, C., Moreno, A., Defilippi, B.G., Campos-Vargas, R., 2021. Cell wall calcium and hemicellulose have a role in the fruit firmness during storage of blueberry (Vaccinium spp). Plants 10, 553, 1-13. https://doi.org.10.3390/plants10030553

Ortiz, C.M., Franceschinis, F., Grozeff, G.E.G., Chan, H., Labavitch, J.M., Crisosto, C., Vicente, A.R., 2018. Pre-treatment with 1-methylcyclopropene alleviates methyl bromide-induced internal breakdown, softening and cell wall degradation in blueberry. Postharvest Biol. Technol. 146, 90-98. https://doi.org/10.1016/j.postharvbio.2018.08.018

Paniagua, A.C., East, A.R., Hindmarsh, J.P., Heyes, J.A., 2013. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. Postharvest Biol. Technol. 79, 13-19. https://doi.org/10.1016/j.postharvbio.2012.12.016

Paniagua, A.C., East, A.R., Heyes, J.A., 2014. Interaction of temperature control deficiencies and atmosphere conditions during blueberry storage on quality outcomes. Postharvest Biol. Technol. 95, 50-59. https://doi.org/10.1016/j.postharvbio.2014.04.006

Park, B., Shin, T.S., Cho, J.S., Lim, J.H., Park, K.J. 2022.Characterizing hyperspectral microscope imagery for classification of blueberry firmness with deep learning methods. Agronomy 12, 85. https://doi.org/10.3390/agronomy12010085

Patel, N., McGlone, A., Schaare, P., Hall, H., 1993. "BerryBounce": a technique for the rapid and non-destructive measurement of firmness in small fruit. Acta Hortic. 352, 189-198. https://doi.org/10.17660/ActaHortic.1993.352.26

Piazza, L., Giovenzana, V., 2015. Instrumental acoustic-mechanical measures of crispness in apples. Food Res. Int. 69, 209-215. http://dx.doi.org/10.1016/j.foodres.2014.12.041

Pons, M., Fiszman, S.M., 1996. Instrumental Texture profile analysis with particular reference to gelled systems. J. Texture Stud. 27, 597-624. https://doi.org/10.1111/j.1745-4603.1996.tb00996.x

Proctor, A., Miesle, T.J., 1991. Polygalacturonase and pectinmethylesterase activities in developing highbush blueberries. HortScience, 26(5), 579-581. https://doi.org/10.21273/HORTSCI.26.5.579

Prussia, S.E., Tetteh, M.K., Verma, B.P., NeSmith, D.S., 2006. Apparent modulus of elasticity from FirmTech 2 firmness measurements of blueberries. Trans. ASABE 49, 113-121. https://doi.org/10.13031/2013.20219

Rivera, S.A., Zoffoli, J.P., Latorre, B.A., 2013. Determination of optimal sulfur dioxide time and concentration product for postharvest control of gray mold of blueberry fruit. Postharvest Biol. Technol. 83, 40-46. https://doi.org/10.1016/j.postharvbio.2013.03.007

Rodriguez, J., Zoffoli, J.P., 2016. Effect of sulphur dioxide and modified atmosphere packaging on blueberry postharvest quality. Postharvest Biol. Technol. 117, 230-238. https://doi.org/10.1016/j.postharvbio.2016.03.008

Rohrbach, R.P., Mainland, C.M., 1993. Accurate low-cost measurement of blueberry firmness for research workers. Acta Hortic. 338-353. https://doi.org/10.17660/ActaHortic.1993.346.47

Rosenfeld, H.J., Meberg, K.R., Haffner, K., Sundell, H.A., 1999. MAP of highbush blueberries: sensory quality in relation to storage temperature, film type and initial high oxygen atmosphere. Postharvest Biol. Technol. 16, 27-36. https://doi.org/10.1016/S0925-5214(98)00102-1

Rosenthal, A.J., 2010. Texture profile analysis - How important are the parameters. J. Texture Stud. 41, 672-684. https://doi.org/10.1111/j.1745-4603.2010.00248.x

Saftner, R., Polashock, J., Ehlenfeldt, M., Vinyard, B., 2008. Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. Postharvest Biol. Technol. 49, 19-26. https://doi.org/10.1016/j.postharvbio.2008.01.008

Saito, S. Obenland, D., Xiao, C.L., 2020. Influence of sulfur dioxide -emitting polyethylene packaging on blueberry decay and quality during extended storage. Postharvest Biol. Technol. 160, 111045. https://doi.org/10.1016/j.postharvbio.2019.111045

Sanford, K.A., Lidster, P.D., McRae, K.B., Jackson, E.D., Lawrence, R.A., Stark, R., Prange, R.K., 1991. Lowbush blueberry quality changes in response to mechanical damage and storage temperature. J. Amer. Soc. Hort. Sci. 116(1), 47-51. https://doi.org/10.21273/JASHS.116.1.47

Scheidt, T.B., Silva, F.V.M., 2018. High-pressure processing and storage of blueberries: effect on fruit hardness. High Press. Res. 38, 80-89. https://doi.org/10.1080/08957959.2017.1402895

Schotsmans, W., Molan, A., MacKay, B., 2007. Controlled atmosphere storage of rabbiteye blueberries enhances postharvest quality aspects. Postharvest Biol. Technol., 44(3), 277-285. https://doi.org/10.1016/j.postharvbio.2006.12.009

Silva, J.L., Marroquin, E., Matta, F.B., Garner, J.O., Stojanovic, J., 2005. Physicochemical, carbohydrate and sensory characteristics of highbush and rabbiteye blueberry cultivars. J. Sci. Food Agr. 85(11), 1815-1821. https://doi.org/10.1002/jsfa.2083

Simon, J.E., Hetzroni, A., Bordelon, B., Miles, G.E., Charles, D., 1996. Electronic sensing of aromatic volátiles for quality sorting of blueberries. J. Food Sci. 61 (5), 967-970.

Sirisomboon, P., Tanaka, M., Kojima, T., Panmanas, S., 2012. Evaluation of tomato textural mechanical properties. J. Food Eng. 111, 618-624. https://doi.org/10.1016/j.jfoodeng.2012.03.007

Slaughter, D.C., Rohrbach, R.P., 1985. Developing a blueberry firmness standard. Trans. ASAE 28(3), 986-992. https://doi.org/10.13031/2013.32374

Stuckrath, R., Quevedo, R., Fuente, L., Hernandez, A., Sepulveda, V., 2008. Effect of foliar application of calcium on the quality of blueberry fruit. J. Plant Nutr. 31,1299-1312. https://doi.org/10.1080/01904160802135076

Szczesniak, A.S., 1963. Classification of textural characteristics. J. Food Sci. 28 (4), 385-389. https://doi.org/10.1111/j.1365-2621.1963.tb00215.x

Szczesniak, A.S., 2002. Texture is a sensory property. Food Qual. Pref. 13 (4), 215-225. https://doi.org/10.1016/S0950-3293(01)00039-8

Tetteh, M.K., Prussia, S.E., NeSmith, D.S., Verma, B.P., 2004. Modelling blueberry firmness and mass loss during cooling delays and storage. Trans. Am. Soc. Agric. Engin. 47, 1121-1127.

Timm, E.J., Brown, G.K., Armstrong, P.R., Beaudry, R.M., Shirazi, A., 1996. Portable instrument for measuring firmness of cherries and berries. Appl. Eng. Agric. 12, 71-77. https://doi.org/10.13031/2013.25441

Trinh, K.T., Glasgow, S., 2012. On the Texture profile analysis test, Chemeca 2012: Quality of life through chemical engineering: 23-26 September 2012, Wellington, New Zealand.

Vance, A.J., Jones, P., Strik, B.C., 2017. Foliar calcium applications do not improve quality or shelf life of strawberry, raspberry, blackberry, or blueberry fruit. Hortscience 52, 382-387. https://doi.org/10.21273/HORTSCI11612-16

Vicente, A.R., Ortugno, C., Rosli, H., Powell, A.L.T., Greve, L.C., Labavitch, J.M., 2007. Temporal sequence of cell wall disassembly events in developing fruits 2 Analysis of blueberry (Vaccinium species). J. Agric. Food Chem. 55, 4125-4130. https://doi.org/10.1021/jf063548j

Vilela, A., Goncalves, B., Ribeiro, C., Fonseca, A.T., Correia, S., Fernandes, H., Ferreira, S., Bacelar, E., Silva, A.P., 2016. Study of textural, chemical, color and sensory properties of organic blueberries harvested in two distinct years: A chemometric approach. J. Texture Stud. 47, 199-207. https://doi.org/10.1111/jtxs.12173

Xie, G.F., Wang, X.H., Wei, K.H., Wang, R., Cao, S., Ji, N., Yang, X.Z., 2018. Effects of 1-methylcyclopropene on texture properties of Rabbiteye blueberry during long-term storage and simulated transportation. Food Sci. Tech. Campinas 38, 188-192. https://doi.org/10.1590/1678-457x.21816

Xu, R., Takeda, F., Krewer, G., Li, C., 2015. Measure of mechanical impacts in commercial blueberry packaging lines and potential damage to blueberry fruit. Postharvest Biol. Technol., 110, 103-113. https://doi.org/10.1016/j.postharvbio.2015.07.013

1.9. Appendices Chapter 1

1.9.1. Supplementary tables

Supplementary Table 1.1. Literature references that were used to create Fig. 1.2. References are separated by the source of variance (genotype, maturity, calcium application and postharvest technologies), mechanical test methods and equipment type used. Note that some references are allocated in more than a single category of the source of variance, mechanical method, or equipment used.

Source of variance	Mechanical test methods				
	Plate probe of 15-75 mm compression	Small diameter probe of 2-4 mm compression	Texture profile analysis (TPA)	Penetration test	Other
Genotype	Slaughter and Rohrbach, 1985; Donahue et al., 2000; Allan-Wojtas et al., 2001; Ehlenfeldt and Martin, 2002; Silva et al., 2005; Saftner et al., 2008; Blaker et al., 2014; Vilela et al., 2016; Moggia et al., 2017; Cappai et al., 2018	NR	Giongo et al., 2022	Silva et al., 2005; Giongo et al., 2013; Giongo et al., 2022;	Makus and Morris, 1993; Patel et al., 1993; Silva et al., 2005; Li et al., 2011; Simon et al., 1996
Maturity	Ballinger et al., 1973; Proctor and Miesle, 1991; Timm et al., 1996; Hancock et al., 2008; Lobos et al., 2014: Moggia et al., 2016; Lobos et al., 2018; Moggia et al., 2018	Vicente et al., 2007; Chea et al., 2019; Lin et al., 2020	NR	NR	Patel et al., 1993.
Calcium	Hanson, 1995; Stuckrath et al., 2008; Ochmian, 2012; Vance et al., 2017; Arrington and Wasko DeVetter, 2017; Lobos et al., 2021	Angeletti et al., 2010; Manzi and Lado, 2019	Olmedo et al., 2021	Vance et al., 2017	NR
Postharvest[b]	Sanford et al., 1991; Teeth et al., 2004; Forney et al., 2003; Harb and Streif, 2004; NeSmith et al., 2005; Schotsmans et al., 2007; Cantin et al., 2012; Rivera et al., 2013; Paniagua et al., 2013; Paniagua et al., 2014; Falagan et al., 2020; Saito et al., 2020	Chiabrando et al., 2009; Duarte et al., 2009; Alsmairat et al., 2011; Chiabrando and Giacalone, 2011; Chen et al., 2015; Rodriguez and Zoffoli, 2016	Chiabrando et al., 2009; Xie et al., 2018; Li et al., 2021	Forney et al., 2003; Concha-Meyer et al., 2015; Jaramillo-Sánchez et al., 2019; Jaramillo-Sanchez et al., 2021; Hu et al., 2021	NR

Source of variance	Equipment type				
	Texture Equipment	FirmTech	Durometer	Force Gauge	Other
Genotype	Slaughter and Rohrbach, 1985; Silva et al., 2005; Giongo et al., 2013; Blaker et al., 2014; Vilela et al., 2016; Giongo et al., 2022	Donahue et al., 2000; Allan-Wojtas et al., 2001; Ehlenfeldt and Martin, 2002; Saftner et al., 2008; Li et al., 2011; Makus and Morris, 1993; Blaker et al., 2014; Moggia et al., 2017; Cappai et al., 2018	NR	Patel et al., 1993; Silva et al., 2005	Slaughter and Rohrbach, 1985; Patel et al., 1993; Makus and Morris, 1993; Simon et al., 1996; Li et al., 2011
Maturity	Ballinger et al., 1973; Proctor and Miesle, 1991; Vicente et al., 2007; Chea et al., 2019; Lin et al., 2020	Lobos et al., 2014; Moggia et al., 2016; Lobos et al., 2018; Moggia et al., 2018	NR	NR	Proctor and Miesle, 1991; Timm et al., 1996; Hancock et al., 2008
Calcium	Stuckart et al., 2008; Angeletti et al., 2010; Manzi and Lado, 2019; Olmedo et al., 2021	Ochmian, 2012; Arrington and Wasko DeVetter, 2017; Vance et al., 2017; Lobos et al., 2021	NR	Vance et al., 2017	Hanson, 1995
Postharvest[b]	Schotsmans et al., 2007; Chiabrando et al., 2009; Cantin et al., 2012; Rivera et al., 2013; Paniagua et al., 2013; Paniagua et al., 2014; Concha-Meyer et al., 2015; Chen et al., 2015; Xie et al., 2018; Jaramillo-Sánchez et al., 2019; Falagan et al., 2020; Li et al., 2021; Jaramillo-Sánchez et al., 2021	Teeth et al., 2004; Forney et al., 2003; Harb and Streif, 2004; NeSmith et al., 2005; Saito et al., 2020	Chiabrando et al., 2009; Alsmairat et al., 2011; Chiabrando and Giacalone, 2011; Rodriguez and Zoffoli, 2016	Sanford et al., 1991; Forney et al., 2003	Duarte et al., 2009; Hu et al., 2021

[a] NR = not previously reported

[b]Postharvest Technologies includes temperature and relative humidity management, controlled atmosphere, modified atmosphere packaging, sulphur dioxide, and ozone.

1.9.2. Statement of contribution Chapter 1

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Sebastian Anibal Rivera Smith
Name/title of Primary Supervisor:	Dr. Svetla Sofkova-Bobcheva

In which chapter is the manuscript /published work: Chapter 1

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Rivera, S., Giongo, L., Cappai, F., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A. 2022. Blueberry firmness – A review of mechanical and textural properties used on quality evaluations. Postharvest Biol. Technol. 192, 1112016. https://doi.org/10.1016/j.postharvbio.2022.112016

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Sebastian Rivera Smith
Date:	04-Aug-2022
Primary Supervisor's Signature:	Svetla Sofkova-Bobcheva
Date:	8-Aug-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

GRS Version 5 – 13 December 2019
DRC 19/09/10

Chapter 2. Influence of water loss on mechanical properties of stored blueberries

This research chapter was devised to identify the better mechanical parameters to track postharvest changes (i.e., softening and firming) as influenced by water loss throughout postharvest storage. Blueberry instrumental mechanical parameters were measured using the methods of double compression (texture profile analysis, TPA) and penetration test. Both methods were identified in **Chapter 1** as a common instrumental test previously reported on blueberries (**Table 1.2**.). Storage relative humidity treatments were used to manipulate fruit water loss and consequently mechanical parameters of 'Nui' and 'Rahi' blueberries. Storage relative humidity was selected based on **Chapter 1** as a critical factor influencing blueberry firmness (**Fig 1.1**.).

Most of the results presented in **Chapter 2** were used to write two research articles:

(1) Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2021. Influence of water loss on mechanical properties of stored blueberries. Postharvest Biology and Technology 176. https://doi.org/10.1016/j.postharvbio.2021.111498

(2) Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2021. Data of texture profile analysis performed by different input settings on stored 'Nui' and 'Rahi' blueberries. Data in Brief. 38, 107313. https://doi.org/10.1016/j.dib.2021.107313

The author of this thesis has worked for both articles in the conceptualization, research methodology, formal analysis and investigation, paper visualizations, and writing > 90 % of the original draft.

AUTHORS AND AFFILIATIONS

Sebastian Rivera[a,d], Huub Kerckhoffs[b], Svetla Sofkova-Bobcheva[a], Dan Hutchins[c], Andrew East[d]

[a] School of Agriculture and Environment, Massey University, Palmerston North, New Zealand
[b] Ministry for Primary Industries, Wellington, New Zealand
[c] Gourmet Blueberries, NZ Gourmet Ltd, Auckland, New Zealand
[d] Massey Agrifood Digital Lab, Massey University, Palmerston North, New Zealand

KEYWORDS

Vaccinium spp; storage relative humidity; postharvest quality; Texture Profile Analysis; penetration test.

ABSTRACT

Moisture loss is considered a main cause of blueberry softening during postharvest storage. However, the causal relationship between softening and water loss has only previously been described by force to 1 mm compression. This study was performed to identify suitable instrumental tests that allow the separation of blueberries with different water loss values during storage. Mechanical properties were measured by double compression (texture profile analysis) and penetration test. Variability on blueberry mechanical properties was created by regulating storage relative humidity and consequently water loss. As water loss increases during storage, the hardness slope (slope of a straight line drawn between the trigger force of 0.06 N and the force at 15 % strain) obtained by the compression test reduces, and the displacement at berry skin break is obtained by the penetration test, increases. Therefore, these parameters can be potentially used to quantify mechanical changes in stored blueberries.

2.1. Introduction

Blueberry texture is considered an important economic trait in the fresh market, as excessive softening could negatively affect consumer acceptance (Ehlenfeldt and Martin, 2002). In a survey performed in 2011 in the USA, berries that are “flavorless”, “rotten”, “dry or shrivelled” and “too soft” were indicated by consumers as the most common reasons for dissatisfaction after blueberry purchase (Daniels et al., 2018). Consequently, blueberry breeding programs are particularly interested in the accurate evaluation of mechanical properties and blueberries with a low softening rate through postharvest operations of packing, storage, and transportation (Cappai et al., 2018). Under these postharvest operations, moisture loss could be considered the most influential factor regulating blueberry softening and shrivelled symptoms (Paniagua et al., 2013).

Given the high importance of texture on stored blueberries, a standard methodology with objective quantification of mechanical properties that allows comparison between different blueberry populations would assist scientific developments and quality improvements. In other fruit industries such as apple and kiwifruit, standard firmness measurements are used to facilitate interpretation of results and longevity of the data across the global research community. For example, in the apple industry, the maximum force of flesh penetration to a depth of 7.9 mm using an 11 mm Effegi probe is the standard for firmness evaluation (Musacchi and Serra, 2018). However, firmness is generally evaluated subjectively using a berry squeeze (or rolling) between fingers within the blueberry supply chain. This methodology is consequently highly influenced by the judgment of the assessor, and hence ambiguity remains in the reported results (Slaughter and Rohrbach, 1985).

Characterisation of mechanical properties of viscoelastic food should be performed by a force, time, and displacement (Lu and Abbott, 2004). Consequently, several instrumental methods have been proposed to quantify mechanical properties in blueberry. Equipment such as BioWork FirmTech, Instron universal testing machine, and texture analyser has been frequently utilised by researchers as efficient pieces of equipment for characterising blueberry quality at harvest and during storage (**Table 2.1**). However, only a few studies on blueberry postharvest considered the evaluation of more than a single mechanical property (Donahue and Work, 1998; Chiabrando et al., 2009; Giongo et al., 2013; Hu et al., 2015; Jaramillo-Sánchez et al., 2019). For example, Paniagua et al. (2013) accounted a single mechanical parameter to establish the relationship between postharvest water loss and softening, the maximum force to 1 mm deformation. To further understand the quality behaviour of stored blueberries, it would be interesting to evaluate the influence of water loss on several mechanical properties.

Texture profile analysis (TPA) is considered a well-established method for assessing food products (Meullenet et al., 1997). Consisting of double compression in a reciprocating motion to a given strain using a flat surface probe, TPA aims to imitate the repeated bite on a food product (Pons and Fiszman, 1996). From the obtained double compression graph, multiple mechanical properties can be calculated, such as hardness, resilience, cohesiveness, springiness, gumminess, chewiness, adhesiveness, and fracturability (Pons and Fiszman, 1996; Meullenet et al., 1997). Consequently, TPA was used to characterize the quality of fresh bananas (Chauhan et al., 2006), grapes (Letaief et al., 2008; Segade et al., 2013; Giacosa et al., 2014), pears (Guine, 2013), melons (Lazaro and de Lorenzo, 2015), peaches (Contador et al., 2016), strawberries (Kartal et al., 2012), raspberries (Giongo et al., 2019), and blueberries (Chiabarando et al., 2009; Hu et al., 2015; Xie et al., 2018). However, when studying the changes of TPA attributes during storage of 'Bluecrop' and 'Coville', Chiabrando et al. (2009) did not account for the effect of different storage relative humidity levels. Moreover, methodological and interpretation flaws observed through the historical use of TPA in food (Pons and Fiszman, 1996; Trinh and Glasgow, 2012; Peleg, 2019) should be revisited for blueberries.

(1) The interpretation of TPA results could be affected by methodological settings such as probe type, deformation distance (strain %), the time between compression cycles, specimen temperature, test and post-test speed, and the calculation procedures for each mechanical property (Rosenthal, 2010; Madieta et al., 2011; Trinh and Glasgow, 2012; Nishinari et al., 2013; Peleg, 2019).

(2) The original recommendation for TPA, was the use of samples of the same height and an area at least that of the plunger base (Pons and Fiszman, 1996). This recommendation is important for interpreting maximum force to certain deformation (hardness) results (Trinh and Glasgow, 2012). Nevertheless, for small soft berries (i.e., blueberries), a similar specimen size is not representative, especially when blueberries are assessed over time, and water loss affects fruit shrinkage (Paniagua et al., 2013). To facilitate the interpretation of results on variable sample size, researchers have pointed out recommendations such as the calculation of the mean

chord stiffness (Rohrbach and Mainland, 1993) and Young's modulus of elasticity (Thrin and Glasgow, 2012).

(3) TPA descriptor of gumminess is not applicable for blueberries because gumminess is defined only for semisolid food (Szczesniak, 1963; Pons and Fiszman, 1996).

(4) TPA descriptor of chewiness is defined for solid foods (Szczesniak, 1963), and can be calculated for blueberries. However, the physical process associated with food chewiness is better imitated through the calculation of the parameter rate of breakdown than using the chewiness from TPA. Moreover, the rate of breakdown should consider more than two compression cycles for an accurate calculation (Trinh and Glasgow, 2012).

(5) TPA descriptor of adhesiveness should be zero for most fruit products (Pons and Fiszman, 1996), and hence is not applicable in blueberries.

In summary, evaluation and standardization of TPA operational conditions and calculation procedures are required for a reliable characterisation of blueberries under different water loss conditions.

Overall, blueberry mechanical properties results could be influenced by the structural properties of the epidermis and flesh (Allan-Wojtas et al., 2001). Further assessment of fruit epidermis's influence on the fruit's mechanical properties can be achieved by a penetration test (Letaief et al., 2008; Sirisomboon et al., 2012; Jaramillo-Sanchez et al., 2019). Outcomes from the penetration (or puncture) test are the results of the sum of compression and shearing force of a food sample (Lu and Abbott, 2004). The penetration test has been satisfactorily used to characterise mechanical properties in other fresh produce such as grapes (Letaief et al., 2008), tomatoes (Sirisomboon et al., 2012), and raspberries (Giongo et al., 2019).

Table 2.1. Instrument and methodologies used for characterization of blueberry mechanical properties.

Instrument	Method	Mechanical parameter	Reference
BerryBounce	Fruit drop from a fixed height (i.e., 30 mm) onto a force sensor (force sensing resistor)	Contact time on force sensor (s)	Patel et al., 1993
Force gauge (AMETEK)	Force required to compress the berry on some fraction of diameter (i.e., 20 % or 25 %)	Mean force (N) Stiffness or slope of force/deformation (N m^{-1})	Patel et al., 1993 Rohrbach and Mainland, 1993
Portable firmness measuring instrument	Compression between two parallel flat plates to maximum force threshold (i.e., 1.5 or 2 N)	Slope of force/deformation (N m^{-1}) Deformation to peak force (mm)	Timm et al., 1996 Hancock et al., 2008
FirmTech (BioWorks)	Single compression with a 3 mm, 15 mm, 25 mm or 30 mm probe to fixed maximum force (e.g., 200 gf)	Slope of force/deformation (gf m^{-1}) between min (e.g. 15 gf) and maximum force (e.g. 200 gf)	Allan-Wojtas et al., 2001; Ehlenfeldt and Martin, 2002; Tetteh et al., 2004; Saftner et al., 2008; Li et al., 2011; Ochmian, 2012; Blaker et al., 2014; Moggia et al., 2017a; Vance et al., 2017
		Apparent modulus of elasticity (Pa)	Prussia et al., 2006
Durofel® (Copa Technologies)	Single compression with a cylindrical plunger of 3 mm diameter	Firmness (Durofel scale of 1 (soft) to 60 (firm); Newtons= 0.47 + 0.077 Durofel reading (Rodriguez et al., 2016)	Chiabrando et al., 2009; Chiabrando and Giacalone, 2011; Rodriguez et al., 2016
Effegi penetrometer	Single penetration compression with a cylindrical plunger of 2 mm diameter	Force at skin break (g)	Duarte et al., 2009
Testing machine (Instron Corporation)	Compression between two parallel flat plates to 25 % of berry diameter	Chord stiffness (N m^{-1})	Slaughter and Rohrbach, 1985; Rohrbach and Mainland, 1993
	Single compression between two parallel flat plates to berry rupture	Maximum force (N), Deformation (mm), apparent modulus of elasticity (Pa)	Donahue and Work, 1998
	Compression between two parallel flat plates to ≤1.25 mm of berry equatorial diameter	Maximum force (N)	Ferraz et al., 2001
	Single penetration compression using a 3 mm or 4 mm penetration probe	Bioyield force (N)	Blaker et al., 2014
	Single penetration compression to 1 cm using a flat-end cylindrical probe of 3 mm.	Maximum force to skin rupture (N) Displacement at skin rupture (mm) Skin break work energy (J) Stiffness (N m^{-1})	Jaramillo-Sánchez et al., 2019
TA.XT texture analyser (Stable Micro Systems)	Single penetration compression to 4 mm or 6 mm deformation with a 3 mm diameter cylindrical probe	Maximum force (N)	Angeletti et al., 2010 Ortiz et al., 2018
	Single penetration compression to 90 % deformation with a 4 mm diameter flat probe	Maximum Force (N), Maximum force strain (%), Final force (N) Area (N %), Force linear distance (-), Young's module (N %)	Giongo et al., 2013
	Single compression to 1 mm or 4 mm deformation with a 25 mm diameter cylindrical probe	Maximum force (N)	Paniagua et al., 2013; Cantin et al., 2012.
	Single compression to 2 mm deformation with a 60 mm diameter plate probe	Maximum force (N)	Schotsmans et al., 2007
	Single compression to 3 mm or 8 mm deformation with a 75 mm diameter plate probe	Maximum force (N)	Rivera et al., 2013; Vilela et al., 2016
	Texture profile analysis (TPA) to 30 % deformation and 10 s between cycles with a 35 mm diameter cylindrical probe	Hardness (N), springiness (mm), cohesiveness (-), chewiness (J), gumminess (N), resilience (-)	Chiabrando et al., 2009
	TPA to 30 % deformation and 10 s between cycles with a 50 mm diameter plate probe	Hardness (N), springiness (mm), cohesiveness (-), chewiness (J), gumminess (N), resilience (-)	Hu et al., 2015
	TPA to 30 % deformation with a 36 mm cylindrical flat probe	Hardness (N), springiness (mm), cohesiveness (-), chewiness (J), gumminess (N), resilience (-)	Xie et al., 2018
	TPA to 75 % deformation and 5 s between cycles with a 35 mm diameter plate probe	Maximum force on the first compression (N)	Liu et al., 2019
Laser air-puff firmness tester, equipped with a LB041 Laser displacement sensor (Keyence)	Fruit surface deformation by the air-puff	Firmness index (Pa m^{-1}) Springiness index (-)	Li et al., 2011
CT3 texture analyser (Brookfield)	Compression to 4 mm deformation with a 4 mm diameter flat cylindrical probe	Hardness (N)	Scheidt and Silva, 2018
Correx 50-g tension gauge (Wagner Instruments)	Penetration force required to pierce the skin with a blunt needle	Skin toughness (N)	Vance et al., 2017

This study was conducted to identify a suitable test that will enable the separation of blueberries with different mechanical properties induced by water loss in the postharvest environment. This study investigates:

(1) The relationship among mechanical properties obtained by texture profile analysis (TPA) and penetration test on stored blueberries.

(2) The identification of the most relevant mechanical properties related to water loss of stored blueberries.

With these investigations, progress towards recommending the most useful mechanical evaluations for blueberry are made.

2.2. Material and methods

2.2.1. Blueberry material

Highbush 'Nui' (*Vaccinium corymbosum*) and rabbiteye 'Rahi' (*Vaccinium ashei*) were hand-harvested at commercial maturity (100 % blue surface colour) from a commercial orchard located at Hawke's Bay, New Zealand. Blueberries were placed into 125 g plastic clamshells and immediately transported for 3 h in a polystyrene cooler box at 17.8 ± 1.4 °C and 70.0 ± 6.6 % relative humidity to the postharvest lab, Massey University (Palmerston North, NZ).

2.2.2. Fruit quality evaluation

At harvest time, fruit size was determined in 45 healthy berries by measuring equatorial diameter with a digital calliper (IP54, Shahe, China). Maturity was characterised by measuring total soluble solids and acidity on six juice samples of 10 berries each. Total soluble solids (TSS, %) were measured in a 0.5 mL juice sample using a digital refractometer (PR 32α, Atago, Tokyo, Japan). Acidity (% citric acid equivalent) was determined by titration with NaOH (0.1 N) until pH 8.2, considering 1 g of juice sample diluted in 50 mL of reverse osmosis water. A digital pH-meter (TitroLine easy, SI Analytics, Mainz, Germany) was used for this purpose. Results were expressed as grams of citric acid equivalent for 0.1 kg of fresh weight, using a milliequivalent factor for citric acid of 0.064.

2.2.3. Storage relative humidity treatments

Three to six hours after harvest, healthy berries of 'Nui' and 'Rahi' were randomly distributed in 60 individual fabric mesh bags of ~90 cm^3 (24 berries for each mesh bag). Each mesh bag was individually weighed on a digital balance (PR50003DR, Mettler Toledo, USA) at 20 °C and placed inside one of each glass jar (0.58 L) containing 48 extra berries.

To induce different water loss rates during storage, the glass jars were randomly assigned to each combination of four storage times (7 d, 14 d, 21 d, or 28 d) and five airflows (0 mL s^{-1}, 0.25 mL s^{-1}, 0.5 mL s^{-1}, 1 mL s^{-1}, or 2 mL s^{-1}) at 4.0 ± 0.7 °C in triplicate. Each airflow generates a relative humidity range because of the equilibrium relative humidity, which is obtained by the balance of the moisture released by the fruit and the dry air (RH: 16.2 ± 1.0 %) entering the jar (Paniagua et al., 2013).

Airflow through each glass jar was designed following the methodology presented by Paniagua et al. (2013). Jars were closed with a modified lid containing two rubber septa. One rubber septum was used for dry air input (inlet) and another for air output (outlet), allowing the release of dry air from the jar to the storage room environment. Airflow through each jar was regulated using a needle valve placed in a manifold and connected to the jar by tubing. Airflows were checked at the outlet every two days using a gas flow meter (ADM 1000, Agilent Technologies, CA, USA). For the airflow of 0 mL s^{-1}, two holes of 4 mm located on the jar lid were left open to allow air refresh by diffusion. A dual temperature and RH logger (iButton®, Maxim Integrated, CA, USA) was attached halfway inside each jar to record the resulting temperature and RH every 800 s. Five storage relative humidities of 96.1 ± 1.4 %, 92.0 ± 1.9 %, 89.0 ± 0.9 %, 75.2 ± 5.5 %, 53.7 ± 5.5 % for 'Nui', and 97.4 ± 1.3 %, 93.8 ± 2.4 %, 90.2 ± 2.0 %, 78.4 ± 5.8 %, and 59.7 ± 8.6 % for 'Rahi' were created by the controlled airflows of 0 mL s^{-1}, 0.25 mL s^{-1}, 0.5 mL s^{-}

^{1}, 1 mL s^{-1}, or 2 mL s^{-1}, respectively. The raw data of the relative humidity (%) recorded throughout the storage period for each airflow treatment of 'Nui' and 'Rahi' is presented and discussed in the appendices of this chapter (see *2.9.1. Relative humidity recorded by i-Buttons loggers*).

To determine the water loss through storage, 15 mesh bags were removed from each glass jar for each storage time (7 d, 14 d, 21 d, and 28 d) and were weighed again. Using Eq. 2.1., the cumulative water loss (WL) of the 24 berries on each mesh bag was determined for each storage time**.**

$$WL\ (\%) = \frac{Wi - Wf}{Wi} \times\ 100 \qquad \textbf{(Eq. 2.1)}$$

Where Wi is the initial weight, and Wf is the weight after each storage time.

From each of the 15 mesh bags (3 replications x 5 airflow treatments) removed at each storage time, 20 healthy berries with the absence sign of mould growth or decay lesions were selected. Each group of 20 berries was randomly divided into two groups of ten berries each and assigned to either TPA or penetration test. Equatorial diameter and mechanical properties were assessed at 20 °C pulp temperature, two hours after berries were removed from the 4 °C room.

2.2.4. Mechanical properties of texture profile analysis

A texture analyser (TA.XT plus, Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell and a 25 mm cylindrical flat-ended probe (P/25, Stable Micro Systems) was used for texture profile analysis (TPA). During the TPA test, the blueberries were placed with the stem-calyx axis oriented parallel to the surface over a flat metal ring of 10 mm internal diameter and 1 mm height. The starting position of the compression probe was 21 mm height from the platform surface. The probe starts moving at a pre-test speed of 1.6 mm s^{-1} until touching the berry (trigger force = 0.06 N). After the trigger force is achieved, the probe starts the first compression (downstroke) at a test speed of 0.8 mm s^{-1} until the target strain (15 % of berry equatorial height) is completed. The probe ascends (upstroke) at the test speed of 0.8 mm s^{-1} to the position where the probe previously recognised the berry height (trigger force). The first compression cycle is completed, and the probe waits for 10 s at this position. After the waiting time is completed, the probe starts the second compression cycle, descending at the same test speed to the same target distance as the first compression. Finally, the probe ascends at a post-test speed of 0.8 mm s^{-1} to the starting position. All acquisitions were made with a resolution of 250 points per second.

Texture profile analysis descriptors of berry hardness (N), resilience (-), cohesiveness (-), and springiness (-) were estimated from the double compression graph (**Fig. 2.1 A**) using Exponent software (Version 6.1.14.0, Stable Micro Systems). Calculation and interpretation of these mechanical properties were based on reported methodologies (**Table 2.2**).

Additional mechanical properties calculated from the double compression graph (**Fig. 2.1 A**) were hardness slope and apparent modulus of elasticity (**Table 2.2**). Hardness slope (kN m^{-1}) was calculated as a straight slope line drawn between trigger force (i.e., 0.06 N) and the maximum force at 15 % strain. Hardness slope can be considered equivalent to blueberry chord stiffness, defined as the slope line drawn between any two specified points on the force/deformation curve (Slaughter and Rohrbach, 1985; Rohrbach and Mainland, 1993).

The apparent modulus of elasticity (*E,* Pa) was calculated following the equation for parallel plate contact of spherical samples of viscoelastic behaviour (**Eq. 2.2**) provided by Prussia et al. (2006).

$$E = \frac{0.338\,F\,(1-\mu^2)}{D^{3/2}} \times \left[\mathrm{K_U} \times \left(\frac{1}{\mathrm{R_U}} + \frac{1}{\mathrm{R'_U}}\right)^{1/3} + \mathrm{K_L} \times \left(\frac{1}{\mathrm{R_L}} + \frac{1}{\mathrm{R'_L}}\right)^{1/3}\right]^{3/2} \quad \textbf{(Eq. 2.2)}$$

Where *E* is the apparent modulus of elasticity (Pa); F is the force (N) at maximum berry deformation (i.e., 15 % strain); D is the probe displacement (m) at maximum deformation distance; μ is the blueberry Poisson's ratio (dimensionless); R_U and R'_U are the minimum and maximum, respectively, radii of curvature (m) of convex shape object at the upper point of contact (plate probe); R_L and R'_L are the minimum and maximum, respectively, radii of curvature

(m) of convex shape object at the lower point of contact (platform support). K_U and K_L are constants calculated from the geometry of the upper and lower point of contact, respectively. A Poisson's ratio (μ) of 0.4 was assumed for blueberries (Prussia et al. 2006). Assumptions for spherical objects were considered. Thus, R_U, R'_U, R_L, R'_L were equal to the radii of curvature (R) and K_U=K_L=1.351 (Prussia et al., 2006). Radii of curvature were calculated for each berry by the equatorial diameter obtained from texture analysis readings.

The utilized TPA operational settings of 15 % strain and 10 s of time duration between both compression cycles were selected from preliminary studies performed using 'Nui' and 'Rahi' blueberries. The resulted data is presented in a Data in Brief article titled "Data of texture profile analysis performed by different input settings on stored 'Nui' and 'Rahi' blueberries" (Rivera et al., 2021*,* see **Appendix 2.9.2**). Results showed target strain of 15 % allowed better ability than 30 % strain on separating hardness, hardness slope, and the apparent modulus of elasticity of blueberries stored in four relative humidity (**Supplementary Fig. 2.2**). These results were more evident in 'Rahi' than 'Nui' blueberry. Regarding the combination between strain and time duration as TPA settings. For 'Nui', 30 % strain and 2 s between cycles showed the best ability to separate the cohesiveness of different relative humidity levels. Still, no differences among operational settings were observed for springiness (**Supplementary Fig. 2.3**). However, the combination of 15 % strain and 10 s allowed a better separation of four storage relative humidity levels for mechanical properties of springiness and cohesiveness of 'Rahi' blueberry (**Supplementary Fig. 2.2**). Consequently, 15 % strain and 10 s duration were selected for TPA operational settings.

2.2.5. Mechanical properties of the penetration test

The penetration test was performed using a TA.XT plus texture analyser equipped with a 5 kg load cell and a needle probe (SMS P/2N, Stable Micro Systems) with a round tip of 0.39 mm diameter and 1.96 mm at maximum basal diameter. Fruit penetration was performed at the berry equator to a distance of 30 % strain of each berry equatorial diameter. Pre-test and test speeds were set at 5 mm s^{-1} and 1 mm s^{-1}, respectively, considering a trigger test force of 0.01 N. During the penetration test, the blueberries were placed over a flat metal ring of 10 mm internal diameter with the stem-calyx axis oriented parallel to the surface. All acquisitions were made with a frequency of 250 points per second. For each berry, maximum resistance of the berry skin to the penetration of the needle probe (force at berry skin break, N), displacement at berry skin break (mm), and berry skin break work energy (mJ) were estimated from the obtained curve (**Fig. 2.1 B**). Skin break work energy was determined as the area under the force/deformation curve between trigger force (i.e., 0.01 N) and the force to berry skin break.

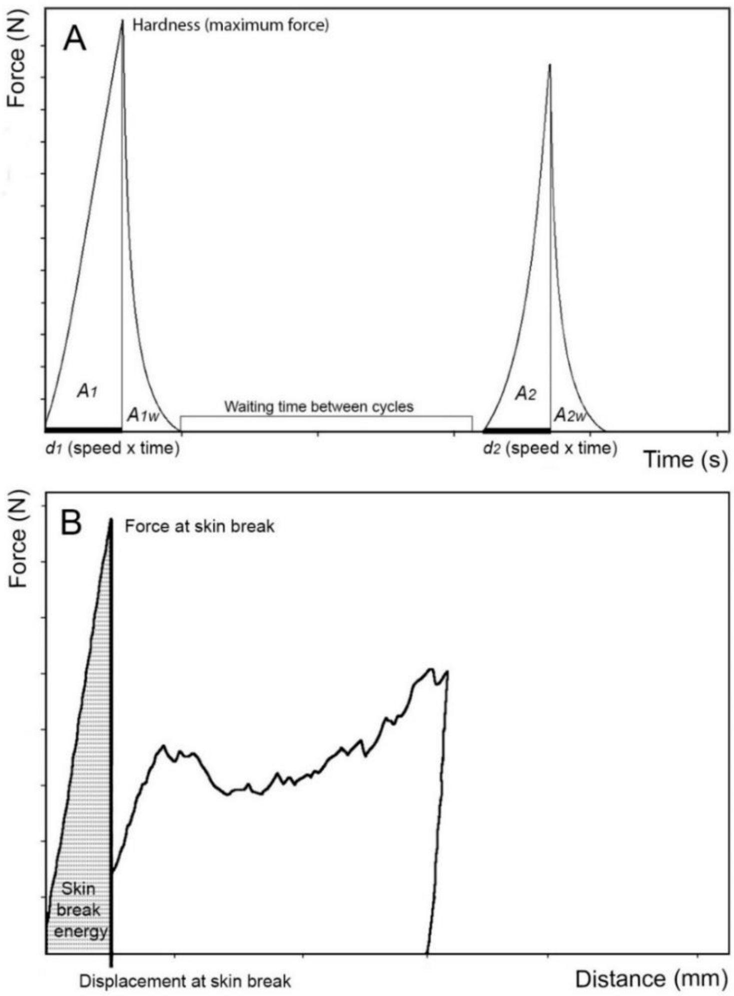


Fig. 2.1. Graphic representation of texture profile analysis (A) and penetration test (B). In Plot A, the speed is referred to the used test speed of 0.8 mm s^{-1}. See Table 2.2 for further definitions of mechanical properties obtained from the double compression graph (A).

Table 2.2. Mechanical properties obtained from texture profile analysis. Units, interpretation, and calculation from the double compression graph.

Mechanical parameter	Unit	Interpretation	Calculation procedure	Curve portion	Reference
Hardness (BH)	N	Force necessary to attain a given deformation (strain %)	Peak force of the first compression cycle to achieve selected strain distance.	First compression cycle	Chiabrando et al., 2009
Distance 1 (d_1)	mm	Travel distance of the probe set by the user on first compression (i.e. strain %)	Descend distance between berry trigger force (0.06 N) and maximum compression force during the first compression cycle (i.e. BH).	First compression cycle	Trinh and Glasgow, 2012; Hu et al., 2015
Resilience (BR)	Ratio (-)	Success in regaining the original berry height during withdrawal of the first compression	Area during withdrawal (upstroke) of first compression cycle (A_{1w}) divided by the downstroke area of first compression (A_1).	First compression cycle	Chiabrando et al., 2009
Cohesiveness (BCo)	Ratio (-)	Strength of the internal bonds comprising the berry body	Area of work during second compression cycle ($A_2 + A_{2w}$) divided by area of work during first compression cycle ($A_1 + A_{1w}$).	First and second compression cycle	Chiabrando et al., 2009
Distance 2 (d_2)	mm	Berry compression distance during the second compression cycle	Descend distance between berry touch and maximum compression force during the second compression cycle.	Second compression cycle	Trinh and Glasgow, 2012; Hu et al., 2015
Springiness (BSp)	Ratio (-)	How well the berry springs back after the first compression force is removed	d_2/d_1	First and second compression cycle	Trinh and Glasgow, 2012; Hu et al., 2015
Hardness slope (BHS)	kN m^{-1}	Rate of force increment by deformation distance	Slope of a straight line drawn between trigger force (0.06 N) and maximum force (i.e. BH) at d_1.	First compression cycle	Slaughter and Rohrbach, 1985
Apparent modulus of elasticity (E)	MPa	Resistant to deformation of a viscoelastic material (i.e., blueberry).	**Eq. 2.1**	First compression cycle	Prussia et al., 2006

2.2.6. Statistical analysis

For each cultivar, twenty storage conditions obtained by the combination of four storage times and five storage relative humidity were distributed in a complete randomised design in triplicate. One glass jar containing 72 berries was considered as the experimental unit.

The influence of storage time and relative humidity on fruit water loss was analysed using a general linear model (GLM) in ANOVA. Means were separated using LSD Fisher (least significant difference test) at *P*-value < 0.05.

Pearson's correlation coefficient (r) matrix was constructed to determine the linear association between response variables of water loss, berry diameter, and nine mechanical properties (six mechanical properties obtained from TPA and three mechanical properties of penetration test). Sixty observations obtained from the different storage conditions (five storage relative humidity levels for each of four storage times in triplicate) were considered for each blueberry cultivar.

A biplot of principal component analysis (PCA) was constructed to visualize the relationship between response variables (loading plot) and the storage conditions (score plot). PCA was performed using the correlation matrix (rescaled values) of the response variables of berry diameter, fruit water loss, four mechanical properties obtained from TPA, and three mechanical properties of penetration test. The response variables were obtained from sixty (60) observations for each blueberry cultivar. To identify response variables with the largest effect on each principal component and the direction of each variable on the two first components, the loading plot was constructed using the coefficients of each variable for the first two components.

A backward elimination regression analysis was performed to identify the most relevant mechanical properties (independent variables) explaining the water loss changes (dependent variable) during storage. The initial model considered all the mechanical properties (candidate variables) related to water loss variability, as identified from PCA and correlation analysis. The least significant mechanical properties were eliminated using an alpha value to remove of 5 % (*P*-value < 0.05). The coefficient values of the model equation and the variance inflation factor were presented for each mechanical property. Variance inflation factor (VIF) indicates the level of collinearity between the independent variables, and a high VIF is related to higher collinearity with the other variables included in the model. Simple linear regression between water loss (Y-axis) and each mechanical property (x-axis) selected from the backward elimination regression was also performed. For each regression model, the adjusted determination coefficient (R^2 adj) and root mean square error (RMSE) were reported. For each blueberry cultivar, 'Nui' and 'Rahi', sixty observations were considered, and each of the observations is the average of ten readings (ten berries). The assumption of the linear relationship between the independent and dependent variables was corroborated by inspecting the plot obtained between predicted values (X-axis) and residuals (Y-axis). Normality of the residuals was verified by visual inspection of the normal Q-Q plot, and homoscedasticity by inspecting the plot obtained between predicted values (X-axis) and studentized residuals (Y-axis).

The ability of mechanical properties to differentiate berry softening and firming during postharvest storage was evaluated by the mechanical properties selected from the best fit model. An ANOVA was performed on selected mechanical properties obtained at harvest time and after 7 d, 14 d, 21 d, and 28 d of the storage in the highest (96-97 %) and lowest (54-60 %) storage relative humidity. Dunnett's test (*P-value* < 0.05) was used as a mean comparison method, considering the harvest time (0 days) data as a control.

Statistical analysis was performed using statistical software InfoStat 2020 (Universidad Nacional de Córdoba, Argentina), and figures were plotted (except for the principal component analysis) using Origin 2019b (OriginLab Corporation, MA, USA).

2.3. Results

2.3.1. Fruit maturity and quality at harvest

Blueberry quality at harvest was characterised by equatorial diameter, total soluble solids (TSS), titratable acidity (TA) and TSS:TA ratio (**Table 2.3**). The equatorial diameter at harvest time ranged from 13.9 mm to 17.7 mm for 'Nui' and 13.1 mm to 17.7 mm for 'Rahi'. The TSS:TA ratio ranged between 6.9 and 9.6 for 'Nui' and 14.2 and 21.3 for 'Rahi'. Consequently, the coefficient of variation (CV) was lower than 6.5 % and 13.5 % for berry diameter and TSS:TA, respectively. Fruit sampling was performed on roughly homogeneous fruit size and maturity (**Table 2.3**).

Blueberries 'Rahi' showed a 2-fold higher TSS:TA ratio than 'Nui', mainly attributed to the higher acidity of 'Nui' (**Table 2.3**). The TSS:TA ratio is considered a good indicator of fruit maturity, and a higher TSS:TA ratio could be related to more advanced maturity (Moggia et al., 2017a). However, the use of a single numerical range of TSS: TA as a standard of harvest maturity is not suitable because TSS:TA ranges can vary by cultivar (Saftner et al., 2008; Moggia et al., 2017a) and year (Moggia et al., 2017a).

Table 2.3. Fruit quality at harvest day for 'Nui' and 'Rahi' blueberries sampled at 100 % blue surface colour.

Cultivar	Harvest date	Equatorial diameter		TSS[c]	TA[c]	TSS:TA [c]	
		(mm) [a]	CV (%)[b]	(%)	(% citric acid)	(-)[d]	CV (%)[b]
'Nui'	December 11th, 2018	15.6 ± 1.0	6.2	10.8 ± 0.3	1.4 ± 0.12	7.9 ± 0.9	11.4
'Rahi'	February 2nd, 2019	16.7 ± 1.0	6.0	11.1 ± 0.6	0.62 ± 0.06	18.1 ± 2.4	13.3

[a] Mean ± standard deviation (n = 45 berries for 'Nui' and 'Rahi').
[b] CV = coefficient of variation (%) calculated as, $(\frac{standard\ deviation}{mean}) \times 100$.
[c] TSS = total soluble solids; TA = titratable acidity, citric acid equivalent; TSS:TA = ratio between TSS and TA.
[d] mean ± standard deviation (n = 6 samples of 10 berries each).

2.3.2. Postharvest water loss

As expected, low storage relative humidity (RH, 54-75 % for 'Nui', and 60-78 % for 'Rahi') was obtained by the higher airflow level (1-2 mL s^{-1}). Meanwhile, commercially recommended RH (89-97 %) was obtained with lower airflows (≤ 0.5 mL s^{-1}). Water loss was influenced by the combination of storage RH and time on both cultivars (**Fig. 2.2 A**, **B**). The highest cumulated water loss for 'Nui' (16.7 %) and 'Rahi' (13.4 %) resulted in berries stored at deficient RH for the maximum storage time (28 d). Meanwhile, the lowest water loss was 1.32 % for 'Nui' and 0.57 % for 'Rahi' stored at 96-97 % RH for 28 d.

Increases in water loss as influenced by storage time and RH agreed with reported results for 'Centurion' blueberries (Paniagua et al., 2013). A maximum water loss of 15 % was observed in 'Centurion' by Paniagua et al. (2013) and Franklin (2019). However, the same storage time and airflow resulted in considerably less water loss in this study by using 'Nui' (12 %, **Fig. 2.2 A**) and 'Rahi' (10 %, **Fig. 2.2 B**). For the utilised airflow experimental technique, the resulting storage RH and water loss differences between cultivars may be attributed to differences in berry water vapour permeance (WVP) and vapour pressure deficit that is influenced by equilibrium relative humidity created inside the jar. Steady-state relative humidity inside the jar is determined by the airflow level, berry surface area, the number of berries, and WVP (Paniagua et al., 2013). Blueberry genotype traits such as cuticle properties and stem scar size can affect WVP (Moggia et al. 2017b).

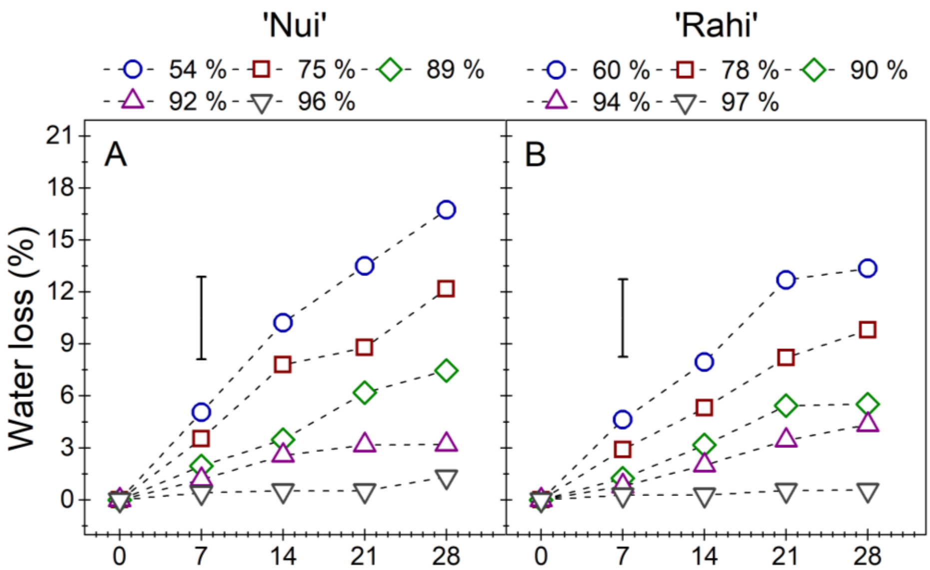


Fig. 2.2. Average water loss (%) of 'Nui' (A-B) and 'Rahi' (C-D) blueberries stored for 7 d, 14 d, 21 d, and 28 d at 4 °C, on storage relative humidity of 54 %, 75 %, 89 %, 92 % and 96 % for 'Nui', and 60 %, 78 %, 90 %, 94 %, and 97 % for 'Rahi'. Dots= mean of three replications of 24 berries each. Bars = represent the least significant difference (LSD) at *P*-value < 0.05 level for the postharvest condition (storage relative humidity and time), as determined by the LSD-Fisher test.

2.3.3. Postharvest mechanical parameters

The raw data obtained on ‘Nui’ and ‘Rahi’ blueberries assessed by texture profile analyses (TPA) and penetration tests are exemplified for each storage RH level (**Fig 2.3**). Differences in raw mechanical data as related to the RH were visualised on TPA and penetration tests. For TPA, blueberries in low humidity (< 80 % RH) displayed an evident higher maximum force (N) to reach 15% of berry deformation (on both compression cycles) when compared to blueberries stored in high RH (> 95 % RH) for ‘Nui’ (**Fig 2.3 A)** and ‘Rahi’ **(Fig 2.3 B**).

For the penetration test, differences in the force-deformation plots were visualised for displacement distance (mm) at berry rupture (skin break). Blueberries stored on low RH displayed a higher displacement at skin break in ‘Nui’ (**Fig 2.3 C)** and ‘Rahi’ (**Fig 2.3 D)**. Interestingly, although there were evident differences in distance to skin rupture, no differences were y visualised for the maximum force needed to produce skin rupture (force at skin break) (**Fig 2.3 C, D)**.

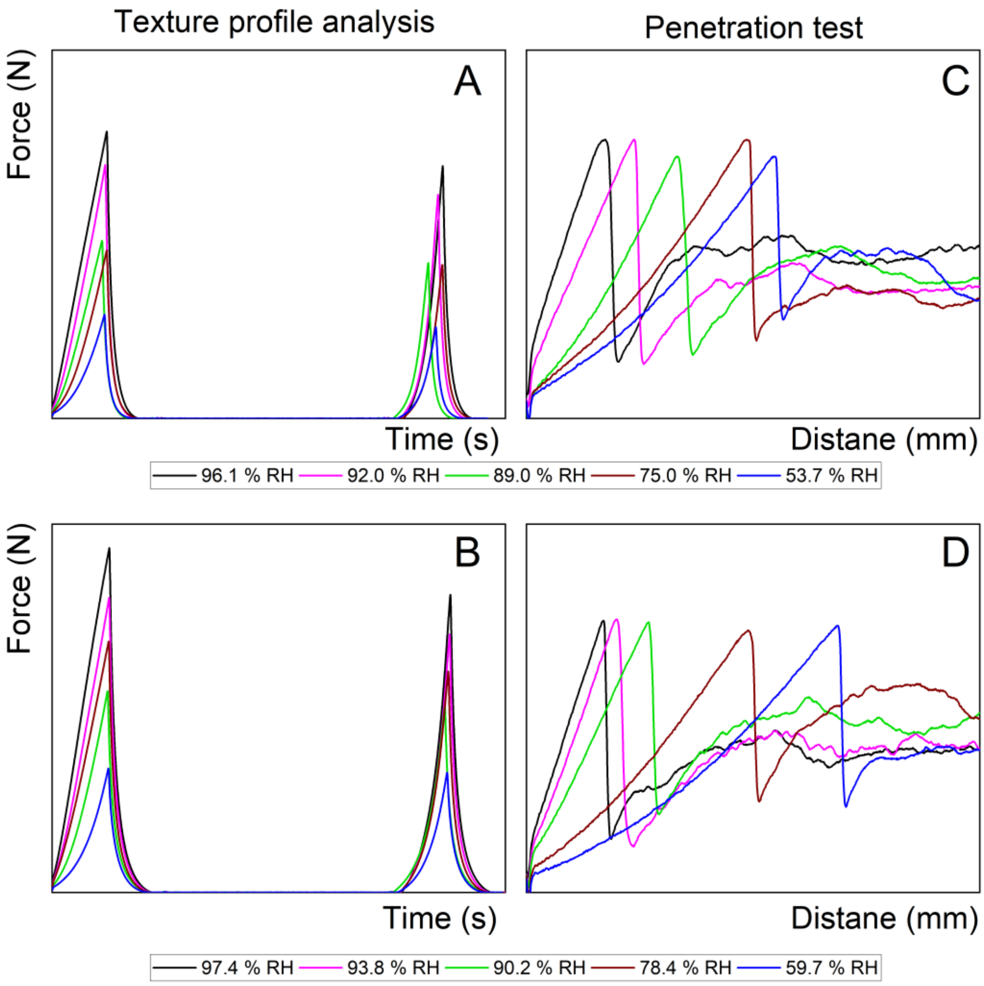


Fig. 2.3. Examples of graphical representation of the raw data provided by texture analyser using texture profile analysis TPA (A, B) and penetration test (C, D) on 'Nui' and 'Rahi' stored on each of five relative humidity (RH) level for 28 d at 4.6 °C. Each plot is the resulting mechanical graph of a single berry that better represents the mean for each storage RH. The raw data were obtained from Exponent Software (Version 6.1.14.0, Stable Micro Systems) and plotted on Origin 2019b (OriginLab Corporation, MA, USA). For further explanation of the mechanical parameters obtained from TPA and penetration plots, see **Fig. 1.2.**

2.3.4. Relationship between postharvest variables and storage conditions

To visualize the relationships between blueberries stored under different conditions (score plot) and the response variables (loading plot), a principal component analysis (PCA) was conducted for each cultivar (**Fig. 2.4**).

For the mechanical properties showing a robust correlation (r > ±0.95), only one mechanical property was considered on PCA. This decision was applied for the correlations between hardness and hardness slope, hardness and apparent modulus of elasticity; hardness slope and apparent modulus of elasticity; and cohesiveness and resilience (**Table 2.4** and **2.5**). Consequently, hardness, apparent modulus of elasticity, and cohesiveness were not included in PCA.

Cumulative explanation of data variation accounted by first and second principal components (PCs), were 89.4 % for 'Nui' and 84.5 % for 'Rahi'. The first two components accounted for most of the data variation, and hence no other PCs are presented. Differences between 'Nui' and 'Rahi' were small **(Fig. 2.4)**.

Loading plots (**Fig. 2.4 A, B**) showed that PC 1 is mostly associated with postharvest variables of hardness slope (BHS), resilience (BR), displacement at skin break (DSk), skin break energy (WSk), and water loss (WL). Meanwhile, PC 2 was mainly associated with force to skin break (FSk).

Score plots (**Fig. 2.4 C, D**) showed that blueberries stored on contrasting RHs (i.e., 54-60 % compared with 96-97 %) were positioned on opposite quadrants of PC1. Consequently, berries stored in commercial deficient RH were associated with higher results for resilience, displacement at skin break, skin break energy and water loss, and lower results for hardness slope and berry diameter (**Fig. 2.4**).

The relationships between postharvest variables visualized on the loading plot of PC 1 and 2 (**Fig. 2.4 A, B**), were quantified by presenting the Pearson's correlation matrix for 'Nui' (**Table 2.4**) and 'Rahi' (**Table 2.5**). Fruit water loss was positively and strongly correlated with mechanical properties of resilience, displacement at berry skin break, and skin break work energy. Contrarily, water loss was negatively and strongly correlated with hardness slope and berry diameter (**Table 2.4** and **Table 2.5**). As storage water loss increases, hardness slope and diameter decrease, while resilience, displacement at berry skin break, and skin break work energy increase. Berry springiness was moderately correlated (r = 0.45 for 'Nui' and r = 0.57 for

'Rahi') with water loss changes. Force at skin break was negligibly correlated with water loss and any other mechanical property, except for skin break energy (**Fig. 2.4 A, B**).

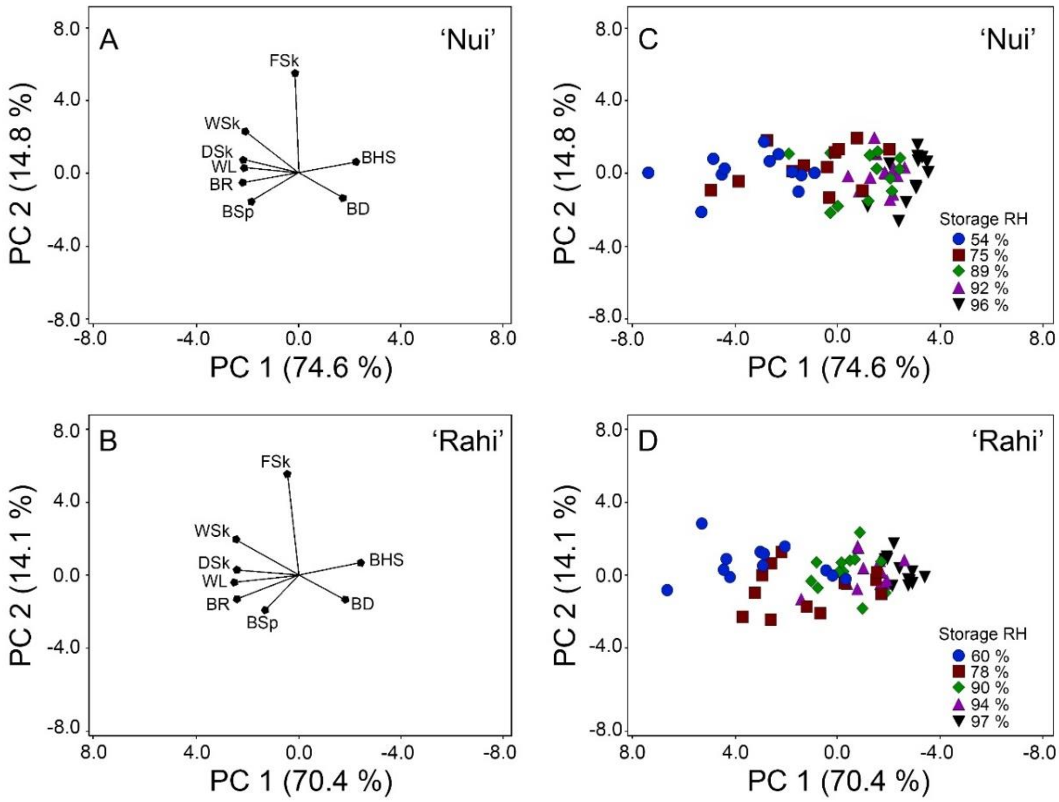


Fig. 2.4. Loading (A and B) and score (C and D) plots of principal component (PC) 1 and 2, representing the relationship between eight postharvest response variables of 'Nui' (A) and 'Rahi' (B) blueberries stored in five relative humidity level for 7 d, 14 d, 21 d, and 28 d at 4 °C. BR = resilience (-), BSp = springiness (-), BHS = hardness slope (kN m^{-1}), FSk = force at berry skin break (N), DSk = displacement at berry skin break (mm), WSk = skin break work energy (mJ), WL = water loss (%), and BD = berry diameter (mm). For the score plots (C and D), each dot (n = 60) considers the average of 10 berries for mechanical properties, 24 berries for water loss, and 20 berries for berry diameter.

Table 2.4. Pearson correlation between postharvest response variables of 'Nui' blueberries stored for 7 d, 14 d, 21 d, and 28 d at 4.6 °C in five storage relative humidity of 54 %, 75 %, 89 %, 92 % and 96 %.

Postharvest parameter	BH	BR	BCo	BSp	BHS	*E*	FSk	DSk	WSk	BD	WL
Hardness (BH)		<0.001[b]	<0.001	<0.001	<0.001	<0.001	0.895	<0.001	<0.001	<0.001	<0.001
Resilience (BR)	-0.96[a]		<0.001	<0.001	<0.001	<0.001	0.694	<0.001	<0.001	<0.001	<0.001
Cohesiveness (BCo)	-0.97	0.99		<0.001	<0.001	<0.001	0.604	<0.001	<0.001	<0.001	<0.001
Springiness (BSp)	-0.75	0.81	0.83		<0.001	<0.001	0.196	<0.001	<0.001	<0.001	<0.001
Hardness slope (BHS)	0.99	-0.97	-0.98	-0.75		<0.001	0.763	<0.001	<0.001	<0.001	<0.001
Apparent modulus (*E*)	0.96	-0.95	-0.97	-0.74	0.99		0.628	<0.001	<0.001	<0.001	<0.001
Force at skin break (FSk)	0.02	-0.05	-0.07	-0.17	0.04	0.06		0.888	0.035	0.18	0.92
Displacement at skin break (DSk)	-0.91	0.87	0.89	0.62	-0.91	-0.90	0.02		<0.001	<0.001	<0.001
Skin break work energy (WSk)	-0.88	0.84	0.85	0.58	-0.88	-0.85	0.27	0.96		<0.001	<0.001
Berry diameter (BD)	0.85	-0.81	-0.81	-0.62	0.81	0.75	-0.18	-0.86	-0.88		<0.001
Water loss (WL)	-0.93	0.88	0.90	0.57	-0.93	-0.91	0.01	0.96	0.92	-0.82	

[a] Pearson correlation coefficient (r). Values in red represent negligible correlation (*P*-value > 0.05), in black weak to moderate correlation (r = 0.3 - 0.79), in green strong correlations (r = 0.8 - 0.95) and in blue very strong correlation (r > 0.95).
[b] *P*-value
n= 60

Table 2.5. Pearson correlation between postharvest response variables of 'Rahi' blueberries stored for 7 d, 14 d, 21 d, and 28 d at 4.6 °C in five storage relative humidity of 60 %, 78 %, 90 %, 94 %, and 97 %.

Postharvest parameter	BH	BR	BCo	BSp	BHS	*E*	FSk	DSk	WSk	BD	WL
Hardness (BH)		<0.001[b]	<0.001	<0.001	<0.001	<0.001	0.328	<0.001	<0.001	<0.001	<0.001
Resilience (BR)	-0.93[a]		<0.001	<0.001	<0.001	<0.001	0.585	<0.001	<0.001	<0.001	<0.001
Cohesiveness (BCo)	-0.93	0.99		<0.001	<0.001	<0.001	0.579	<0.001	<0.001	<0.001	<0.001
Springiness (BSp)	-0.44	0.64	0.67		<0.001	0.003	0.968	0.002	0.002	<0.001	<0.001
Hardness slope (BHS)	0.99	-0.93	-0.93	-0.42		<0.001	0.466	<0.001	<0.001	<0.001	<0.001
Apparent modulus (*E*)	0.96	-0.90	-0.90	-0.38	0.98		0.759	<0.001	<0.001	<0.001	<0.001
Force at skin break (FSk)	-0.13	0.07	0.07	-0.01	-0.10	-0.04		0.254	<0.001	0.03	0.51
Displacement at skin break (DSk)	-0.89	0.86	0.89	0.39	-0.89	-0.86	0.15		<0.001	<0.001	<0.001
Skin break work energy (WSk)	-0.86	0.82	0.84	0.39	-0.85	-0.81	0.42	0.95		<0.001	<0.001
Berry diameter (BD)	0.86	-0.79	-0.79	-0.39	0.82	0.74	-0.28	-0.77	-0.78		<0.001
Water loss (WL)	-0.94	0.90	0.93	0.45	-0.94	-0.91	0.09	0.96	0.91	-0.80	

[a] Pearson correlation coefficient (r). Values in red represent negligible correlation (*P*-value > 0.05), in black weak to moderate correlation (r = 0.3 - 0.79), in green strong correlations (r = 0.80 - 0.95) and in blue very strong correlation (r > 0.95).
[b] *P*- value
n= 60

2.3.5. Identification of the relevant mechanical properties for water loss changes

The mechanical properties of hardness slope, resilience, displacement at berry skin break, and skin break energy were used as candidate independent variables for modelling the water loss (dependent variable) during storage of 'Nui' and 'Rahi' (**Table 2.6**). This classification of variables, mechanical properties as independent variables and water loss as the dependent variable, was performed to detect mechanical properties related to water loss changes but may not represent the logical sequence of natural events.

For both cultivars, hardness slope and displacement at berry skin break were kept in the best fit model obtained by backward elimination regression. Consequently, these regression models showed the lowest root mean square error (RMSE), and the highest adjusted coefficient of determination (**Table 2.6**). Variance inflation factor (VIF) was lower in the best fit model, indicating lower collinearity between variables after removing berry resilience and skin break energy (**Table 2.6**). Simple linear regression models also showed a strong coefficient of determination by using displacement at skin break (R^2 = 0.92 - 0.93). Therefore, a robust explanation of water loss variability can be obtained by using displacement at berry skin break alone, but better when complemented with hardness slope (**Table 2.6**).

Table 2.6. Linear regression analysis for water loss changes (dependent variable) and mechanical properties (independent variable) of hardness slope (BHS), resilience (BR), displacement at berry skin break (DSk), and skin break work energy (WSk). Sixty (60) observations were used for each regression model.

Models	All candidates (initial)			Backward elimination			Simple (BHS)		Simple (DSk)	
'Nui'	**Coef.**	**SE[a]**	**VIF[b]**	**Coef.**	**SE[a]**	**VIF[b]**	**Coef.**	**SE[a]**	**Coef.**	**SE[a]**
BHS (kN m⁻¹)	-6.14^{*c}	2.33	24.0	-4.24***	1.17	6.1	-13.40***	0.71	-	-
BR (-)	-20.28	21.31	16.7	-	-	-	-	-	-	-
DSk (mm)	8.14***	1.53	18.8	7.44***	0.87	6.1	-	-	10.33***	0.39
WSk (mJ)	-5.99	9.45	13.1	-	-	-	-	-	-	-
Constant	9.48	8.03	-	2.0	2.51		22.87***	0.95	-7.0***	0.50
R^2 adj[d]		0.94			0.94		0.86		0.92	
RMSE[e]		1.74			1.59		3.48		1.87	
'Rahi'	**Coef.**	**SE[a]**	**VIF[b]**	**Coef.**	**SE[a]**	**VIF[b]**	**Coef.**	**SE[a]**	**Coef.**	**SE[a]**
BHS (kN m⁻¹)	-6.40***	1.20	9.5	-6.29***	0.86	4.9	-14.68***	0.68	-	-
BR (-)	-1.91	12.04	7.7	-	-	-	-	-	-	-
DSk (mm)	7.49***	1.04	15.6	6.38***	0.58	4.9	-	-	10.18***	0.36
WSk (mJ)	-8.74	6.81	11.1	-	-	-	-	-	-	-
Constant	7.65	4.90	-	6.76***	1.70	-	24.41***	0.93	-5.51***	0.39
R^2 adj[d]		0.96			0.96		0.89		0.93	
RMSE[e]		0.82			0.73		2.07		1.29	

[a] SE = standard error
[b] VIF = Variance inflation factor
[c] **P*-value <0.05, ***P*-value <0.01 ****P*-value <0.001
[d] R^2 adj= adjusted coefficient of determination.
[e] RMSE = Root Mean Square Error.

The mechanical properties of hardness slope and displacement to berry skin break were tested on the ability to show softening as induced by low relative humidity and firming, by high RH (Panigua et al., 2013). Blueberry softening was detected on both cultivars by a decrease of the hardness slope and an increase of the displacement at berry skin stored at low RH (**Fig. 2.5**). Blueberry firming at high RH storage was only observed in 'Nui' as measured by a higher hardness slope. Firming was not detected by using displacement to skin break (**Fig. 2.5 A, B**).

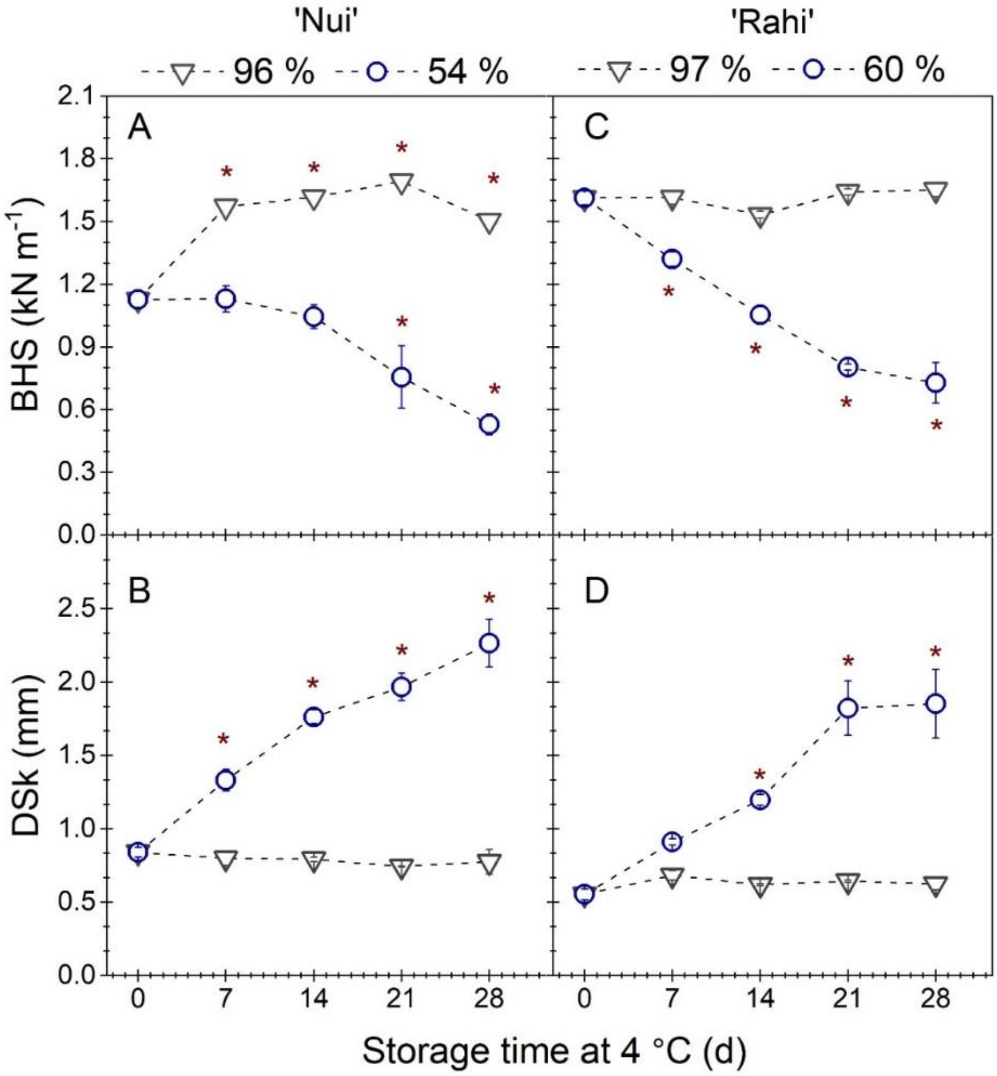


Fig. 2.5. Variation of average hardness slope (BHS, A and C) and displacement at berry skin break (DSk, C and F) of 'Nui' (A-B) and 'Rahi' (C-D) as influenced by the postharvest condition of storage time (0 d, 7 d, 14 d, 21 d, and 28 d at 4.6 °C) and storage relative humidity (54-60 % and 96-97 %). Dots with the asterisk symbol (*) indicate a significant difference (*P*-value < 0.05) in comparison to mechanical properties at harvest time (0 d), according to Dunnett's test. Dots = mean of three replications of 10 berries each (n = 30 berries). Bars = standard error of the mean of three replications.

2.4. Discussion

2.4.1. Texture profile analysis as influenced by water loss

The utilised airflow experimental design proposed by Paniagua et al. (2013) resulted in water loss ranges that include those generally observed under commercial conditions (e.g., 5 – 8 %)

and were related to the variation of mechanical properties results during storage (**Fig. 2.4 and 2.5**).

Texture profile analysis parameters of hardness (N) and hardness slope (kN m^{-1}) were strongly related to water loss during storage (**Table 2.4 and Table 2.5**). In agreement with our study, reduction of the force to a given deformation (i.e. hardness) when storage weight loss was higher than 3 % has previously been reported on highbush cultivars 'Bluecrop' (Angeletti et al., 2010; Liu et al., 2019), 'Brilliant' (Chen et al., 2015), 'O'Neal' (Angeletti et al., 2010), 'Sierra' (Liu et al., 2019), and 'Star' (Cantin et al., 2012), and on rabbiteye blueberries, 'Bonita' (Ferraz et al., 2000) and 'Centurion' (Panigua et al., 2013; Franklin, 2019). Similarly, Moggia et al. (2016) observed higher FirmTech firmness in 'Duke' and 'Brigitta' stored in bag conditions (high relative humidity). Moggia et al. (2017b) also reported a higher reduction of FirmTech firmness on blueberry germplasm lines showing higher weight loss.

Ballinger et al. (1973) and Rohrbach and Mainland (1993) previously described that the compression test of blueberries often generates in the force-deformation plot an approximately linear increase of force (N) as the berry is deformed (mm) up to ~25 % strain. This observation was coincident with force-deformation plots of 'Nui' and 'Rahi' blueberries stored in high RH (**Fig. 2.3**). However, when blueberries were stored at low RH, the force-deformation of TPA showed a less linear (curved upward) shape during first downstroke compression cycle on 'Nui' (**Fig. 2.3 A)** and 'Rahi' **(Fig 2.3 B**). Similarly, a less linear pattern of force-deformation was observed on the compression portion (before skin break) of the penetration test of 'Nui' and 'Rahi' blueberries stored in low humidity **(Fig 2.3 C, D).**

The curved portion of the force-deformation plot of blueberries stored at low RH (or with high water loss) may indicate less elastic deformation as low turgor pressure may be expected on those blueberries. Lower elastic deformation was previously described on tomatoes with low turgor pressure (Jackman et al. 1992).

As described in **Chapter 1**, the FirmTech compression devise is one of the most used at the commercial and research level. FimTech firmness readings can be considered equivalent to the hardness slope evaluated in our study and can potentially be used to differentiate berries stored in different relative humidity. However, as hardness slope considered the slope between trigger force (0.06 N) and maximum force at the given compression displacement (15 % strain) by using a texture analyser, and FirmTech firmness uses the slope of force/deformation graph between a selected minimum (e.g., 0.15 N) and maximum force (e.g., 2 N), further study is needed.

The need for comparison of Firmtech firmness and hardness slope is particularly relevant when considering that the force-deformation graphs obtained by the texture analyser (at a compression rate of 1 mm s^{-1}) do not always follow a linear increase in force as the berry is deformed (**Fig. 2.3 A, B**). However, FirmTech is generally conducted using a faster compression speed (e.g., 7 mm s^{-1} reported by Prussia et al. (2006) or 16 mm s^{-1} by Lobos et al. (2018)). Different test speeds can generate differences in the shape of the force-deformation curve as the rate of change in the contact area (between fruit and probe) depends on the deformation rate when using a compression plate. In addition, higher maximum force is expected with higher compression speed (Rosenthal, 2010). Hence, whether the force-deformation curve obtained by FirmTech using blueberries stored in different RHs provide a similar force-deformation shape as observed in this chapter using a texture analyser (**Fig 2.3**) remains to be studied.

Strong correlations among TPA parameters were also observed (**Table 2.4** and **Table 2.5**). In agreement with our results, a positive correlation between cohesiveness and resilience (Hu et al., 2015; Xie et al., 2018), and a negative correlation between cohesiveness and hardness have previously been observed in stored blueberries (Hu et al., 2015). We hypothesized the negative relationship between hardness and cohesiveness could be explained by the calculation procedures (**Fig. 2.1** and **Table 2.2**). A berry with a higher hardness implies a higher force to be applied during the first downstroke compression, increasing the chances of disruption of the berry cellular structure. Consequently, less recovery for the second compression cycle could be expected (**Fig. 1**). Rosenthal (2010) proposed the same explanation for the observed reduction of cohesiveness as the deformation target increases on experimental gel cylinders. It would be interesting to compare the effect of storage RH treatments on blueberry cohesiveness, considering a fixed target force instead of a fixed target strain.

The apparent modulus of elasticity (*E*, MPa) represented the resistance to the deformation of viscoelastic materials (Prussia et al., 2006) and was very strongly correlated with hardness and hardness slope (**Table 2.4 and 2.5**). Consequently, *E* can also be considered for studying water loss changes in blueberries. However, to obtain the apparent modulus of elasticity (*E*), more complex calculation procedures than hardness slope is required. This complexity was simplified by assumptions such as the Poisson's ratio which was assumed to be 0.4 (Prussia et al., 2006). However, blueberry Poisson's ratio can be calculated by measuring the fruit moisture content (Sirisomboon et al., 2012). Additionally, our work utilised a flat metal ring to support the berry during compression, which could affect the calculation of radii of curvature (R_L and R'_L) and K_L constant at the lower point of contact (**Eq. 2.2**).

Texture profile analysis is considered an imitative test of the repeated bite of food. However, the given compression displacement (15 % strain) and time duration between compression cycles (10 s) do not mimic human mastication. During mastication, the jaw bite usually generates a severe berry fracture (e.g., 75 % strain), and the human jaw works at an average rate of 42 bites per minute (Rosenthal, 2010). Selected deformation distance is likely to be more reproducible of the berry reaction to human touch (manipulation in hand and between fingers). Blueberry squeeze between fingers is a decisive instance of inferring texture quality based on experience (Slaughter and Rohrbach, 1985).

2.4.2. Penetration test as influenced by water loss

Storage water loss was strongly explained by displacement to skin break (penetration test) alone or in combination with hardness slope (**Table 2.6**). Displacement to skin break (DSk) represents the compression displacement of the needle probe to reach maximum force causing the skin to break. Interestingly, the maximum force causing skin break (FSk) was not related to water loss changes during storage (**Fig. 2.4**). Jaramillo-Sanchez et al. (2019), using a 3 mm flat-end probe in 'O'Neal' blueberries, observed that the force to skin break remained unaffected through storage time, while the displacement at skin break increased. However, this increase was not statistically significant, and the maximum weight loss was considerably less (~2 %) than applied in our study. Giongo et al. (2013), using a penetration test with a 4 mm flat-end probe on different cultivars, observed an increase in the maximum force strain (%) after 60 d of storage at 4 °C and 85 % relative humidity. This behaviour was also observed on wine grapes by Bellincontro et al. (2009); as weight loss increases from 0 to 20 %, an increase of grape deformation (mm) by flat plate compression to 1 N force was obtained.

4.2.3. Blueberry genotype differences

Blueberry cultivar has been identified as important source of variability of mechanical properties evaluated at harvest time (Giongo et al., 2013), 1-2 d after harvest (Ehlenfeldt and Martin, 2002; Saftner et al., 2008; Blaker et al., 2014), or up to 6 - 8 weeks of storage (Hancock et al. 2008; Giongo et al., 2013). In our work, 'Nui' showed a lower hardness slope (1.13 kN m^{-1}) than 'Rahi' (1.62 kN m^{-1}) at harvest time (**Fig. 2.5 A, C**). In partial agreement, Ehlenfeldt and Martin (2002) observed higher average FirmTech firmness on *V. ashei* (rabbiteye blueberries) compared to cultivars derived from *V. corymbosum* (northern highbush) and *V. darrowi*. Cappai et al. (2018) reviewed the phenotype variation on blueberry firmness and observed Southern Highbush selections (a higher percentage of V. darrowi and V. ashei ancestry) tend to be firmer than northern highbush. However, higher firmness values among cultivars were better related to the year the cultivars were released; hence, modern cultivars are firmer. However, the cultivars used in our study, 'Nui' and 'Rahi', were released in New Zealand in similar years, 1987 for 'Nui' and 1991 for 'Rahi' (Patel and George, 1997).

Another different behaviour between cultivars was 'Nui' showed firming, as measured by an increment of hardness slope in high relative humidity storage, and 'Rahi' did not. Consequently, under low water loss conditions, 'Nui' reached similar hardness slope values to 'Rahi' (**Fig. 2.5 A, C**). As other rabbiteye blueberries such as 'Centurion' and 'Maru' has shown firming (Schotsmans et al., 2007; Paniagua et al., 2013; Paniagua et al., 2014), further studies are needed to conclude the influence of the blueberry genotype on hardness slope increase under low water loss (<1.5 %).

Increased water loss influenced the mechanical properties of 'Nui' and 'Rahi' in a similar way (**Table 2.6**). As cumulated water loss varied from 2 to 8 % (commercially relevant range), an equal increment of displacement at skin break of ~0.59 mm was estimated for 'Nui' and 'Rahi' (**Table 2.6**). Giongo et al. (2013) described a similar behaviour by observing a similar storage index (as measured by variation of the force strain) of 'Misty' (highbush hybrid) compared to 'Powderblue' (rabbiteye). Still, both cultivars, 'Misty' and 'Powderblue', showed a greater variation in the force strain than the highbush cultivars 'Draper', 'Ozarkblue', and 'Aurora' (Giongo et al., 2013). However, Giongo et al. (2013) did not report the cumulated water loss during storage for each cultivar. For further studies of the influence of the genotype on the causal relationship between water loss and mechanical properties, cultivars with higher contrast in storage indexes must be selected.

2.5. Conclusions and recommendations

This work demonstrates water loss induces changes in mechanical properties obtained by compression and penetration tests, and mechanical properties are strongly correlated with each other.

This work in providing multiple mechanical properties can support commercial production and breeding improvements by informing mechanical tests for stored blueberries. The key findings of this study are:

(1) Fruit softening induced by water loss through storage can be detected by different methodologies, including double compression (Texture Profile Analysis) and Penetration test.
(2) Water loss changes in stored blueberries were strongly related to the displacement at berry skin break by penetration test and the slope of the force/deformation between the trigger force (0.06 N) and force to 15 % by a flat plate compression test.
(3) The maximum force needed to break (penetrate) the berry skin with a needle probe does not provide a description of the relationship between water loss and softening.
(4) Fruit firming as induced by low water loss could be detected on 'Nui' blueberries by using hardness slope (or chord stiffness).

Further knowledge is needed to standardize a test for evaluating mechanical properties and identify threshold values defining consumer acceptance. Questions that remain to be answered include:

(1) Determination of data consistency across sources of heterogeneity for mechanical properties such as harvest maturity (Slaughter and Rohrbach, 1985; Patel et al., 1993; Timm et al. 1996; Hancock et al., 2008; Moggia et al., 2017a; Lobos et al., 2018), calcium management (Angeletti et al., 2010; Ochmian 2012), and commercial postharvest technologies such as controlled atmosphere (Schotsmans et al., 2007; Duarte et al., 2009; Cantin et al., 2012; Paniagua et al., 2014).
(2) The identification of potential sources of methodology bias, such as fruit size variability.
(3) Sensory evaluation is a robust indicator of quality acceptance by consumers. The relationship between mechanical properties and sensory descriptors by a trained panel will facilitate industry improvements. Given the experimental methodologies utilised, the evaluation of sensory firmness by manipulation between fingers appears to be a more logical interpretation than the evaluation of texture descriptors by mastication.

2.6. Acknowledgment

The authors acknowledge Gourmet Blueberries Ltd. for providing 'Nui' and 'Rahi' blueberries; Francisca Ducommun for the help provide in organising the plant selection and fruit sampling at the commercial plantation; Talon Sneddon and Sue Nicholson for the help provided at the experimental setup and during the evaluations in Postharvest Laboratory, Massey University. Sebastian Rivera acknowledges the School of Agriculture and Environment, Massey University (NZ), and Agencia Nacional Nacional de Investigacion y Desarollo (ANID), Chile for the financial support to pursuing Ph.D. studies at Massey University.

2.7. Author Statement

Sebastian Rivera: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing - Original draft; **Huub Kerckhoffs**: Conceptualization, Supervision, Writing - Review and Editing; **Svetla Sofkova-Bobcheva**: Supervision, Writing - Review and Editing; **Dan Hutchins**: Resources, Supervision, Writing - Review and Editing; **Andrew East**: Conceptualization, Methodology, Resources, Supervision, Writing - Review and Editing.

2.8. References

Allan-Wojtas, P.M., Forney, C.F., Carbyn, S.E., Nicholas, K.U.K.G., 2001. Microstructural indicators of quality related characteristics of blueberries - An integrated approach. Lebensm.-Wiss. U.-Technol. 34, 23-32. https://doi.org/10.1006/fstl.2000.0738

Angeletti, P., Castagnasso, H., Miceli, E., Terminiello, L., Concellon, A., Chaves, A., Vicente, A.R., 2010. Effect of preharvest calcium applications on postharvest quality, softening and cell wall degradation of two blueberry (*Vaccinium corymbosum*) varieties. Postharvest Biol. Technol. 58, 98-103. https://doi.org/10.1016/j.postharvbio.2010.05.015

Bellincontro, A., Nicoletti, I., Valentini, M., Tomas, A., De Santis, D., Corradini, D., Mencarelli, F., 2009. Integration of nondestructive techniques with destructive analyses to study postharvest water stress of winegrapes. Am. J. Enol. Viticult. 60, 57-65.

Blaker, K.M., Plotto, A., Baldwin, E.A., Olmstead, J.W., 2014. Correlation between sensory and instrumental measurements of standard and crisp-texture southern Highbush blueberries (*Vaccinium corymbosum* L. interspecific hybrids). J. Sci. Food Agric. 94, 2785-2793. https://doi.org/10.1002/jsfa.6626

Cantin, C.M., Minas, I.S., Goulas, V., Jimenez, M., Manganaris, G.A., Michailides, T.J., Crisosto, C.H., 2012. Sulfur dioxide fumigation alone or in combination with CO_2-enriched atmosphere extends the market life of Highbush blueberry fruit. Postharvest Biol. Technol. 67, 84-91. https://doi.org/10.1016/j.postharvbio.2011.12.006

Cappai, F., Benevenuto, J., Ferrao, L.F.V., Munoz, P., 2018. Molecular and genetic bases of fruit firmness variation in blueberry - A review. Agronomy 8, 174. https://doi.org/10.3390/agronomy8090174
Chauhan, O.P., Raju, P.S., Dasgupta, D.K., Bawa, A.S., 2006. Instrumental textural changes in banana (var. Pachbale) during ripening under active and passive modified atmosphere. Int. J. Food Proper. 9, 237-253. https://doi.org/10.1080/10942910600596282
Chen, H.J., Cao, S.F., Fang, X.J., Mu, H.L., Yang, H.L., Wang, X., Xu, Q.Q., Gao, H.Y., 2015. Changes in fruit firmness, cell wall composition and cell wall degrading enzymes in postharvest blueberries during storage. Scientia Hortic. 188, 44-48. https://doi.org/10.1016/j.scienta.2015.03.018
Chiabrando, V., Giacalone, G., Rolle, L., 2009. Mechanical behaviour and quality traits of Highbush blueberry during postharvest storage. J. Sci. Food Agric. 89, 989-992. https://doi.org/10.1002/jsfa.3544
Chiabrando, V., Giacalone, G., 2011. Shelf-life extension of Highbush blueberry using 1-methylcyclopropene stored under air and controlled atmosphere. Food Chem. 126, 1812-1816. https://doi.org/10.1016/j.foodchem.2010.12.032
Contador, L., Diaz, M., Hernandez, E., Shinya, P., Infante, R., 2016. The relationship between instrumental tests and sensory determinations of peach and nectarine texture. Eur. J. Hortic. Sci. 81, 189-196. http://dx.doi.org/10.17660/eJHS.2016/81.4.1
Daniels, M., Simonne, A.H., Jensen, S., Diehl, D., Shelnutt, K.P., Bruhn, C., Brecht, J.K., Wysocki, A.F., Cook, R., Mitcham, E., 2018. Consumer perceptions of quality and price of high-value specialty crops in supermarkets in the United States: lessons for producers, handlers and educators. Acta Hortic. 1213, 21-30. https://doi.org/10.17660/ActaHortic.2018.1213.3
Donahue, D.W., Work, T.M., 1998. Sensory and textural evaluation of Maine wild blueberries for the fresh pack market. J. Texture Stud. 29, 305- 312. https://doi.org/10.1111/j.1745-4603.1998.tb00172.x
Donahue, D.W., Benoit, P.W., Lagasse, B.J., Buss, W.R., 2000. Consumer and instrumental evaluation of Maine wild blueberries for the fresh pack market. Postharvest Biol. Technol. 19, 221-228. https://doi.org/10.1016/S0925-5214(00)00101-0
Duarte, C., Guerra, M., Daniel, P., Camelo, A.L., Yommi, A., 2009. Quality changes of Highbush blueberries fruit stored in CA with different CO_2 levels. J. Food Sci. 74, 154-159. https://doi.org/10.1111/j.1750-3841.2009.01118.x
Ehlenfeldt, M.K., Martin, R.B., 2002. A survey of fruit firmness in Highbush blueberry and species-introgressed blueberry cultivars. Hortscience 37, 386-389. https://doi.org/10.21273/HORTSCI.37.2.386
El Agamy, S.Z.A., Aly, M.M., Biggs, R.H., 1982. Fruit maturity as related to ethylene in Delite blueberry. Proc. Fla. State Hortic. Soc. 95, 245-246.
Ferraz, A.C.O., Sargent, S.A., Fox, A.J., 2000. Delays to cooling and postharvest quality of fresh Florida blueberries. Proc. Fla State Hortic. Soc. 113, 268-271.
Ferraz, A.C., Sargent, S.A., Fox, A.J., 2001. A nondestructive method for measuring firmness of blueberry fruit. Proceedings of the Florida State Horticultural Society 114, 138-142.
Franklin, D.K. 2019. Characterising texture and cellular level responses of 'Centurion' blueberries during storage in different weight loss conditions. Master of Food Technology Thesis. Massey University, Albany, New Zealand.
Forney, C.F., 2009. Postharvest issues in blueberry and cranberry and methods to improve market-life. Acta Hortic., 785-798. https://doi.org/10.17660/ActaHortic.2009.810.105
Giacosa, S., Torchio, F., Segade, S.R., Giust, M., Tomasi, D., Gerbi, V., Rolle, L., 2014. Selection of a mechanical property for flesh firmness of table grapes in accordance with an OIV ampelographic descriptor. Am. J. Enol. Viticult. 65, 206-214. https://doi.org/10.5344/ajev.2014.13115

Giongo, L., Ajelli, M., Poncetta, P., Ramos-Garcia, M., Sambo, P., Farneti, B., 2019. Raspberry texture mechanical profiling during fruit ripening and storage. Postharvest Biol. Tec. 149, 177-186. https://doi.org/10.1016/j.postharvbio.2018.11.021

Giongo, L., Poncetta, P., Loretti, P., Costa, F., 2013. Texture profiling of blueberries (*Vaccinium* spp.) during fruit development, ripening and storage. Postharvest Biol. Technol. 76, 34-39. https://doi.org/10.1016/j.postharvbio.2012.09.004

Guine, R.P.F., 2013. Variation of textural attributes of S. Bartolomeu pears at maturation, storage, and drying. Int. J. Food Proper. 16, 180-192. https://doi.org/10.1080/10942912.2010.535191

Hancock, J., Callow, P., Serce, S., Hanson, E., Beaudry, R., 2008. Effect of cultivar, controlled atmosphere storage, and fruit ripeness on the long-term storage of highbush blueberries. HortTechnology 18, 199-205. https://doi.org/10.21273/HORTTECH.18.2.199

Hu, M.H., Dong, Q.L., Liu, B.L., Opara, U.L., Chen, L., 2015. Estimating blueberry mechanical properties based on random frog-selected hyperspectral data. Postharvest Biol. Technol. 106, 1-10. https://doi.org/10.1016/j.postharvbio.2015.03.014

Jackman, R.L., Marangoni, A.G., Stanley. D.W. 1992. The effect of turgor pressure on puncture and viscoelastic properties of tomato tissue. J. Texture Stud. 23, 475-489. https://doi.org/10.1111/j.1745-4603.1992.tb00036.x

Jaramillo-Sanchez, G., Contigiani, E.V., Castro, M.A., Hodara, K., Alzamora, S.M., Loredo, A.G., Nieto, A.B., 2019. Freshness maintenance of blueberries (*Vaccinium corymbosum* L.) during postharvest using ozone in aqueous phase: Microbiological, structure, and mechanical issues. Food Bioprocess Tech. 12, 2136-2147. https://doi.org/10.1007/s11947-019-02358-z

Kartal, S., Aday, M.S., Caner, C., 2012. Use of microperforated films and oxygen scavengers to maintain storage stability of fresh strawberries. Postharvest Biol. Technol. 71, 32-40. https://doi.org/10.1016/j.postharvbio.2012.04.009

Lazaro, A., de Lorenzo, C., 2015. Texture analysis in melon landraces through instrumental and sensory methods. Int. J. Food Proper. 18, 1575-1583. https://doi.org/10.1080/10942912.2014.923441

Letaief, H., Rolle, L., Gerbi, V., 2008. Mechanical behavior of winegrapes under compression tests. Am. J. Enol. Viticult. 59, 323-329.

Li, C.Y., Luo, J.W., MacLean, D., 2011. A novel instrument to delineate varietal and harvest effects on blueberry fruit texture during storage. J. Sci. Food Agric. 91, 1653-1658. https://doi.org/10.1002/jsfa.4362

Liu, B.H., Wang, K.F., Shu, X.G., Liang, J., Fan, X.L., Sun, L., 2019. Changes in fruit firmness, quality traits and cell wall constituents of two Highbush blueberries (*Vaccinium corymbosum* L.) during postharvest cold storage. Acta Hortic. 246, 557-562. https://doi.org/10.1016/j.scienta.2018.11.042

Lobos, G.A., Bravo, C., Valdes, M., Graell, J., Ayala, I.L., Beaudry, R.M., Moggia, C., 2018. Within-plant variability in blueberry (*Vaccinium corymbosum* L): maturity at harvest and position within the canopy influence fruit firmness at harvest and postharvest. Postharvest Biol. Technol. 146, 26-35. https://doi.org/10.1016/j.postharvbio.2018.08.004

Lu, R., Abbot, J.A., 2004. Force/Deformation techniques for measuring texture, In: Kilcast, D. (Ed.), Texture in food Volume 2, Solid foods. Woodhead Publishing Limited, England.

Madieta, E., Symoneaux, R., Mehinagic, E., 2011. Textural properties of fruit affected by experimental conditions in TPA tests: an RSM approach. Int. J. Food Sci. Technol. 46, 1044-1052. https://doi.org/10.1111/j.1365-2621.2011.02606.x

Meullenet, J.F.C., Carpenter, J.A., Lyon, B.G., Lyon, C.E., 1997. Bi-cyclical instrument for assessing texture profile parameters and its relationship to sensory evaluation of texture. J. Texture Stud. 28, 101-118. https://doi.org/10.1111/j.1745-4603.1997.tb00104.x

Moggia, C., Graell, J., Lara, I., Schmeda-Hirschmann, G., Thomas-Valdes, S., Lobos, G.A., 2016. Fruit characteristics and cuticle triterpenes as related to Postharvest quality of highbush blueberries. Sci. Hortic. 211, 449-457.

Moggia, C., Graell, J., Lara, I., Gonzalez, G., Lobos, G.A., 2017a. Firmness at harvest impacts postharvest fruit softening and internal browning development in mechanically damaged and non-damaged Highbush blueberries (*Vaccinium corymbosum* L.). Front. Plant Sci. 8. https://doi.org/ 10.3389/fpls.2017.00535

Moggia, C., Beaudry, R.M., Retamales, J.B., Lobos, G.A., 2017b. Variation in the impact of stem scar and cuticle on water loss in Highbush blueberry fruit argue for the use of water permeance as a selection criterion in breeding. Postharvest Biol. Technol. 132, 88-96. https://doi.org/10.1016/j.postharvbio.2017.05.019

Moggia, C., González, C., Lobos, G.A., Bravo, C., Valdés, M., Lara, I. and Graell, J., 2018. Changes in quality and maturity of 'Duke' and 'Brigitta' blueberries during fruit development: postharvest implications. Acta Hortic. 1194, 1495-1501. https://doi.org/10.17660/ActaHortic.2018.1194.209

Musacchi, S., Serra, S., 2018. Apple fruit quality: Overview on pre-harvest factors. Sci. Hortic. 234, 409-430. https://doi.org/10.1016/j.scienta.2017.12.057

Nishinari, K., Kohyama, K., Kumagai, H., Funami, T., Bourne, M.C., 2013. Parameters of Texture Profile Analysis. Food Sci. Technol. Res. 19, 519-521. https://doi.org/10.3136/fstr.19.519.

Ochmian, I., 2012. The Impact of foliar application of calcium fertilizers on the quality of Highbush blueberry fruits belonging to the 'Duke' cultivar. Not. Bot. Horti. Agrobo 40(2), 163-169. https://doi.org/10.15835/nbha4028058

Ortiz, C.M., Franceschinis, F., Grozeff, G.E.G., Chan, H., Labavitch, J.M., Crisosto, C., Vicente, A.R., 2018. Pre-treatment with 1-methylcyclopropene alleviates methyl bromide-induced internal breakdown, softening and cell wall degradation in blueberry. Postharvest Biol. Technol. 146, 90-98. https://doi.org/10.1016/j.postharvbio.2018.08.018

Paniagua, A.C., East, A.R., Hindmarsh, J.P., Heyes, J.A., 2013. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. Postharvest Biol. Technol. 79, 13-19. https://doi.org/10.1016/j.postharvbio.2012.12.016

Paniagua, A.C., East, A.R., Heyes, J.A., 2014. Interaction of temperature control deficiencies and atmosphere conditions during blueberry storage on quality outcomes. Postharvest Biol. Technol. 95, 50-59. https://doi.org/10.1016/j.postharvbio.2014.04.006

Patel, N., George, J.P., 1997. Progress with breeding blueberries in New Zealand. Acta Hortic. 446, 149-154. https://doi.org/10.17660/ActaHortic.1997.446.21

Patel, N., McGlone, A., Schaare, P., Hall, H., 1993. "BerryBounce": a technique for the rapid and non-destructive measurement of firmness in small fruit. Acta Hortic. 352, 189-198. https://doi.org/10.17660/ActaHortic.1993.352.26

Peleg, M. 2019. The instrumental texture profile analysis revisited. J. Texture Stud. 50:362–368. https://doi.org/10.1111/jtxs.12392

Prussia, S.E., Tetteh, M.K., Verma, B.P., NeSmith, D.S., 2006. Apparent modulus of elasticity from FirmTech 2 firmness measurements of blueberries. Trans. ASABE 49, 113-121. https://doi.org/10.13031/2013.20219

Pons, M., Fiszman, S.M., 1996. Instrumental Texture profile analysis with particular reference to gelled systems. J. Texture Stud. 27, 597-624. https://doi.org/10.1111/j.1745-4603.1996.tb00996.x

Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2021. Data of texture profile analysis performed by different input settings on stored 'Nui' and 'Rahi' blueberries. Data in Brief. 38, 107313. https://doi.org/10.1016/j.dib.2021.107313

Rivera, S.A., Zoffoli, J.P., Latorre, B.A., 2013. Determination of optimal sulfur dioxide time and concentration product for postharvest control of gray mold of blueberry fruit. Postharvest Biol. Technol. 83, 40-46. https://doi.org/10.1016/j.postharvbio.2013.03.007

Rodriguez, J., Zoffoli, J.P., 2016. Effect of sulfur dioxide and modified atmosphere packaging on blueberry postharvest quality. Postharvest Biol. Technol. 117, 230-238. https://doi.org/10.1016/j.postharvbio.2016.03.008

Rohrbach, R.P., Mainland, C.M., 1993. Accurate low-cost measurement of blueberry firmness for research workers. Acta Hortic. 338-353. https://doi.org/10.17660/ActaHortic.1993.346.47

Rosenthal, A.J., 2010. Texture profile analysis - How important are the parameters. J. Texture Stud. 41, 672-684. https://doi.org/10.1111/j.1745-4603.2010.00248.x

Saftner, R., Polashock, J., Ehlenfeldt, M., Vinyard, B., 2008. Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. Postharvest Biol. Technol. 49, 19-26. https://doi.org/10.1016/j.postharvbio.2008.01.008

Scheidt, T.B., Silva, F.V.M., 2018. High-pressure processing and storage of blueberries: effect on fruit hardness. High Press. Res. 38, 80-89. https://doi.org/10.1080/08957959.2017.1402895

Schotsmans, W., Molan, A., MacKay, B., 2007. Controlled atmosphere storage of rabbiteye blueberries enhances postharvest quality aspects. Postharvest Biol. Technol. 44, 277-285. https://doi.org/10.1016/j.postharvbio.2006.12.009

Segade, S.R., Giacosa, S., Torchio, F., de Palma, L., Novello, V., Gerbi, V., Rolle, L., 2013. Impact of different advanced ripening stages on berry texture properties of 'Red Globe' and 'Crimson Seedless' table grape cultivars (*Vitis vinifera* L.). Sci. Hortic. 160, 313-319. https://doi.org/10.1016/j.scienta.2013.06.017

Sirisomboon, P., Tanaka, M., Kojima, T., Panmanas, S., 2012. Evaluation of tomato textural mechanical properties. J. Food Eng. 111, 618-624. https://doi.org/10.1016/j.jfoodeng.2012.03.007

Slaughter, D.C., Rohrbach, R.P., 1985. Developing a blueberry firmness standard. Transactions of the ASAE 28(3), 986-992. https://doi.org/10.13031/2013.32374

Suzuki, A., Kikuchi, T., Aoba, K., 1997. Changes of ethylene evolution, ACC content, ethylene forming enzyme activity and respiration in fruits of Highbush blueberry. J. Jpn. Soc. Hortic. Sci. 66, 23-27. https://doi.org/10.2503/jjshs.66.23

Szczesniak, A.S., 1963. Classification of textural characteristics. J. Food Sci. 28 (4), 385-389. https://doi.org/10.1111/j.1365-2621.1963.tb00215.x

Tetteh, M.K., Prussia, S.E., NeSmith, D.S., Verma B.P., Aggarwal D., 2004. Modeling blueberry firmness and mass loss during cooling delays and storage. Trans. ASABE 47(4), 1121-1127. https://doi.org/10.13031/2013.16543

Timm, E.J., Brown, G.K., Armstrong, P.R., Beaudry, R.M., Shirazi, A., 1996. Portable instrument for measuring firmness of cherries and berries. Appl. Eng. Agric. 12, 71-77. https://doi.org/10.13031/2013.25441

Trinh, K.T., Glasgow, S., 2012. On the Texture profile analysis test, Chemeca 2012: Quality of life through chemical engineering: 23-26 September 2012, Wellington, New Zealand.

Vance, A.J., Jones, P., Strik, B.C., 2017. Foliar calcium applications do not improve quality or shelf life of strawberry, raspberry, blackberry, or blueberry fruit. Hortscience 52, 382-387. https://doi.org/10.21273/HORTSCI11612-16

Vicente, A.R., Ortugno, C., Rosli, H., Powell, A.L.T., Greve, L.C., Labavitch, J.M., 2007. Temporal sequence of cell wall disassembly events in developing fruits 2 Analysis of blueberry (Vaccinium species). J. Agric. Food Chem. 55, 4125-4130. https://doi.org/10.1021/jf063548j

Vilela, A., Goncalves, B., Ribeiro, C., Fonseca, A.T., Correia, S., Fernandes, H., Ferreira, S., Bacelar, E., Silva, A.P., 2016. Study of textural, chemical, color and sensory properties of organic blueberries harvested in two distinct years: A chemometric approach. J. Texture Stud. 47, 199-207. https://doi.org/10.1111/jtxs.12173

Wang, S.Y., Zhou, Q., Zhou, X., Wei, B.D., Ji, S.J., 2018. The effect of ethylene absorbent treatment on the softening of blueberry fruit. Food Chem. 246, 286-294. https://doi.org/10.1016/j.foodchem.2017.11.004

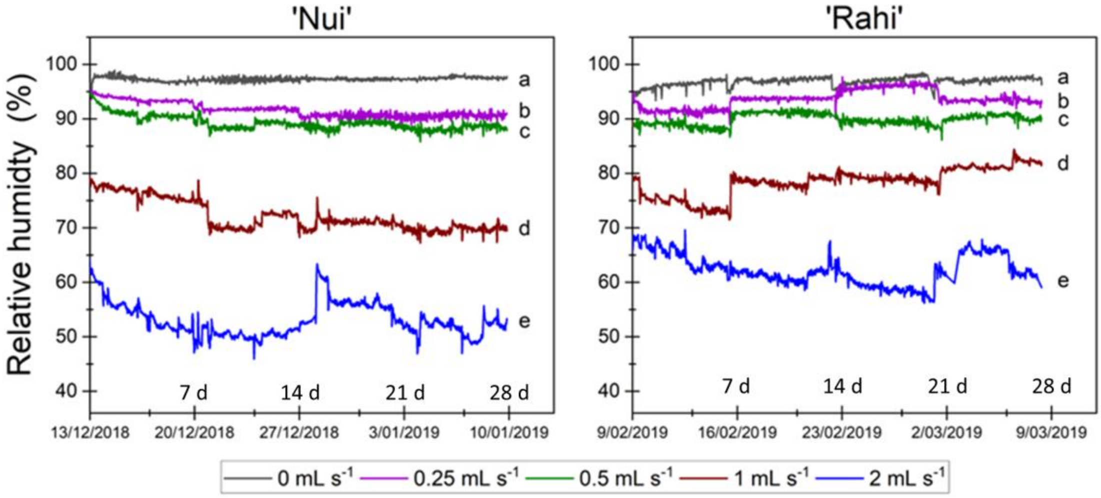
Wolfe, R.R., Abrams, M.L., Probasco, W.F., 1983. Controlling postharvest decay of blueberries using firmness separation and hot water treatments. Trans. ASABE 26(1), 312-315. https://doi.org/10.13031/2013.33927

Xie, G.F., Wang, X.H., Wei, K.H., Wang, R., Cao, S., Ji, N., Yang, X.Z., 2018. Effects of 1-methylcyclopropene on texture properties of Rabbiteye blueberry during long-term storage and simulated transportation. Food Sci. Tech. Campinas 38, 188-192. https://doi.org/10.1590/1678-457x.21816

2.9. Appendices Chapter 2.

2.9.1. Relative humidity recorded by i-Buttons loggers

The average of three replicates of the relative humidity (RH) recorded by iButton® loggers (Maxim Integrated, CA, USA) during storage at 4.6 °C is presented for each airflow used (0 mL s^{-1}, 0.25 mL s^{-1}, 0.5 mL s^{-1}, 1 mL s^{-1}, and 2 mL s^{-1}) on blueberries 'Nui' and 'Rahi' (**supplementary Fig 2.1**.).



Supplementary Fig. 2.1. Relative humidity (%) recorded by iButton® loggers (Maxim Integrated, CA, USA) throughout the storage period of blueberries 'Nui' and 'Rahi'. Data is the average of 3 replicates for each of the five airflows (0 mL s^{-1}, 0.25 mL s^{-1}, 0.5 mL s^{-1} 1 mL s^{-1} and 2 mL s^{-1}). Different letters indicate significant differences for each cultivar as obtained by the general mixed linear model (GLM), where the storage time was considered a random factor and the airflow was a fixed factor. Tukey-HSD (P<0.05) used a multiple mean comparison test.

Although the temperature and airflow used to induce the RH levels remained constant during the experiment, variability from the average RH was observed on each RH level. The coefficient of variation (CV %) of the RH obtained for stored blueberries 'Nui' were 0.43 %, 1.6 %, 1.7 %, 4.1 %, 6.2 %, for an airflow of 0 mL s^{-1}, 0.25 mL s^{-1}, 0.5 mL s^{-1}, 1 mL s^{-1}, and 2 mL s^{-1}, respectively. Similarly, for blueberries 'Rahi' the CV was 0.77 %, 1.7 %, 1.2 %, 3.3 %, 4.6 %, for 0 mL s^{-1}, 0.25 mL s^{-1}, 0.5 mL s^{-1}, 1 mL s^{-1}, and mL s^{-1}, respectively. Consequently, the higher the airflow (or lower the RH obtained), the higher the variation in RH data recorded during storage.

Although variation in the RH during the storage time was observed for a given airflow, the airflows used in the experimental design showed significant ($p < 0.05$) differences in the obtained RH. Hence, all the airflows used for 'Nui' and 'Rahi' produced different RH levels (**Supplementary Fig. 2.1**).

The author inferred that the variability in storage RH within a given airflow could be associated with the practice of opening the jars and removing berries at different evaluation moments (7 d, 14 d, 21 d). Opening the jars to remove berries can alter the steady-state humidity inside the jars. The steady-state humidity varied in function of the water vapour entering the jar, the transpiration from the total blueberry surface area and the water vapour removed from the jar as influenced by the airflow rate.

When the jar is closed, the water vapour entering the jar comes from the dry air (<20 % RH). However, opening the jar produces an abrupt (but for a short period) alteration of the water vapour entering the jar as the RH in the cold room was higher (> 80 %) than in dry air RH. The time needed for the closed jar environment to reach a new steady balance (after the humidity alteration when opening the jar) depends on the airflow and free volume in the jar. The author suggests that the RH sharp but short peaks observed after 7, 14 or 21 d can be associated with the act of opening the jars.

The transpiration from the total blueberry surface is related to the number of berries and the water vapour permeance (WVP, a measure of the ease with which the water vapour is released from the blueberry). Conseuqntekly reducing the number of berries inside the jar can decrease the balanced RH. This behaviour was visualised for airflows between 0.25 mL s^{-1} and 2 mL s^{-1} of 'Nui' blueberries (**Supplementary Fig. 2.1**). Still, only for the highest airflow (2 mL s^{-1}) of stored 'Rahi' (**Supplementary Fig. 2.1**). On the other hand, the author can not discard changes in the blueberry water vapour permeance during the storage which could explain the discrepancy in balanced RH data of 'Rahi'. Whether changes in WVP occur on stored blueberries requires further investigation.

2.9.2. Data of texture profile analysis performed by different input settings on stored 'Nui' and 'Rahi' blueberries

Abstract

Texture profile analysis is a well-established method for assessing the mechanical properties of horticultural food products and consists of two compression cycles on a repeated motion to a given strain using a flat surface probe (i.e., compression plate). Input settings of target deformation (strain %) and duration (s) between compression cycles utilized for Texture profile analysis could influence output mechanical properties. The article provides data related to the ability of different texture profile analysis operational settings to enable the separation of blueberries with variable mechanical properties. To create variable mechanical parameters of 'Nui' and 'Rahi' blueberries, fruit was stored in four relative humidity for 21 d at 4.6 °C. For each storage relative humidity, mechanical properties of hardness (BH, N), hardness slope (BHS, kN m^{-1}), apparent modulus of elasticity (*E*, MPa), and resilience (BR, -) were determined by utilizing two strain (15 % or 30 % of berry equatorial height). Meanwhile, mechanical parameters of cohesiveness (BCo, -), and springiness (BSp, -) were obtained by utilizing the combination of two strain (15 % or 30 %) and two durations between cycles (2 s and 10 s) as TPA operational settings. One-way ANOVA conducted the statistical evaluation, and the means of each storage relative humidity were separated according to the Tukey-HSD test (P = 0.05). The data presented in this article was used to select the Texture profile analysis operational settings utilized in the article entitled "Influence of water loss on mechanical properties of stored blueberries" Rivera et al. (2021).

2.9.2.1. Experimental design, materials and methods

2.9.2.1.1. Blueberry material

Highbush 'Nui' (*Vaccinium corymbosum*) and rabbiteye 'Rahi' (*Vaccinium ashei*) blueberry were hand-harvested at 100 % blue surface colour from a commercial orchard located at Flaxmere, Hawke's Bay, New Zealand on January 15th, 2018, for 'Nui' (highbush blueberry) and February 2nd, 2018 for 'Rahi' (rabbiteye blueberry). The fruit was immediately transported for 3 h in a polystyrene cooler box at 19.1 ±1.0 °C and 59.4 ±15.9 % relative humidity to the Postharvest Lab, Massey University (Palmerston North, New Zealand). Commercial maturity at harvest was determined by the ratio of total soluble solids (%) to acidity (% citric acid equivalent). The ratio of total soluble solids to acidity was 9.4 ± 1.0 for 'Nui', and 19.4 ± 2.0 for 'Rahi'.

2.9.2.1.2. Storage relative humidity treatments

Immediately after arriving at the Massey postharvest lab, 72 healthy berries of 'Nui' and 'Rahi' blueberries were randomly distributed in twelve 0.578 L glass jars. In order to create different storage relative humidity treatments, glass jars were randomly assigned to airflow through the jar of either 0 mL s^{-1}, 0.25 mL s^{-1}, 0.5 mL s^{-1}, or 1 mL s^{-1} in triplicate, for 21 d of storage in a temperature-controlled room at 4.5 ± 0.1°C. Airflow through each glass jar was generated following the methodology presented by Paniagua et al. (2013). Glass jars were closed with a lid containing two rubber septa. One rubber septa was used for dry air input (inlet) and another for air output (outlet). Airflows through the jars were regulated using needle valves, and the airflows were checked at the outlet every two days using a gas flow meter (ADM 1000, Agilent Technologies, CA, USA). For the airflow of 0 mL s^{-1}, two holes of 4 mm located on the jar lid were left open to allow air refresh by diffusion. Each airflow results in a relative humidity range, achieved as a result of the steady-state relative humidity inside the glass jar (Paniagua et al., 2006). The resulting storage relative humidity was recorded by a logger (iButton®, Maxim Integrated, CA, USA). Storage relative humidity (average ± standard deviation) of 73.3 ± 10.3 %, 88.2 ± 1.2 %, 92.3 ± 1.8 %, and 95.0 ± 0.25 % for 'Nui', and 73.9 ± 1.6 %, 81.2 ± 0.5 %, 88.5 ± 0.3 %, and 93.3 ± 0.42 % for 'Rahi' were created by the controlled supply of the airflow of 1 mL s^{-1}, 0.5 mL s^{-1}, 0.25 mL s^{-1}, and 0 mL s^{-1}, respectively.

2.9.1.4.3. Operational settings for Texture Profile Analysis

After 21 d of storage, healthy berries without any sign of mould growth or decay lesion were selected from each glass jar to assess texture profile analysis (TPA) mechanical parameters.

A texture analyser (TA.XT plus, Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell and a 25 mm cylindrical flat-ended probe (P/25, Stable Micro Systems) was operated using different settings to evaluate texture profile analysis. Mechanical properties of hardness (N), hardness slope (kN m^{-1}), apparent modulus of elasticity (MPa) and resilience (-) were assessed on 26 berries in triplicate (n= 78 berries) for each storage relative humidity, by utilising two strain targets (15 % and 30 % of berry equatorial height) as operational settings. Meanwhile, mechanical properties of cohesiveness (-) and springiness (-), were assessed on 13 berries in triplicate (n= 39 berries) for each storage relative humidity by utilizing four operational settings obtained by the combination of two strain (15 % and 30 %), and two duration (2 s and 10 s) between compression cycles.

The operation process for the texture profile analysis was performed considering a starting position of the probe of 21 mm height from the platform surface. The probe started moving at a pre-test speed of 1.6 mm s^{-1} until touching the berry as achieved by recognising the trigger force of 0.06 N. At that point, the probe starts the first compression (downstroke) at a test speed of 0.8 mm s^{-1} until the target strain of 15 % or 30 % of the berry equatorial height is achieved. After the selected deformation distance is achieved, the probe ascends (upstroke) at the test speed of 0.8 mm s^{-1} to the position where the probe recognised the trigger force for the first compression. The first compression cycle is completed, and the probe waits for the selected duration between cycles of 2 s or 10 s. After the selected waiting time is complete, the probe starts the second compression, descending at the same test speed to the same target distance as the first compression. Finally, the probe ascends at post-test speed of 0.8 mm s^{-1} to the starting position. All acquisitions were made with a resolution of 250 points per second.

During the texture profile analysis, the blueberries were placed with the stem-calyx axis oriented parallel to the surface over a flat metal ring of 10 mm internal diameter and 1 mm height. Although the compression should be performed by paralleled flat surfaces using flat surface samples (Pons et al., 1996), for small fruit such as blueberries, the preparation of cylindrical samples of flat surface by using cork borer and blade is not possible. Therefore, Texture profile analysis evaluations of intact fruit are preferred. Hence, the flat metal ring of 10 mm allows steady support of the berry to the texture Analyser platform surface, avoiding any balancing movements during and between compression cycles.

Mechanical properties of berry hardness, hardness slope, resilience, cohesiveness, and springiness were estimated from the obtained compression graphs using Exponent software (Version 6.1.14.0, Stable Micro Systems). Hardness, hardness slope, and resilience were obtained using the first compression cycle immediately before the waiting time has started, and cohesiveness and springiness, using both compression cycles. Hardness was calculated as the maximum force (N) to achieve 15 % or 30 % strain (Chiabrando et al., 2009). Hardness slope or chord stiffness as the slope of a straight line drawn between the trigger force of 0.06 N and the maximum force at 15 % or 30 % strain (Slaughter and Rohrbach, 1985). Resilience was calculated as the ratio of the area of work (force X displacement) during withdrawal (upstroke) of first compression to the area of work during downstroke of first compression (Chiabrando et al., 2009). Cohesiveness was calculated as the ratio of the area of work during the second compression cycle to the area of work during the first compression cycle (Chiabrando et al., 2009). Springiness was calculated as the ratio of displacement (mm) during downstroke compression of the second cycle to the displacement (mm) during downstroke compression of the first cycle (Hu et al., 2015).

The apparent modulus of elasticity (MPa) was calculated following the equation for parallel plate contact of spherical samples of viscoelastic behaviour (**supplementary Eq. 2.1**) (Prussia et al., 2006).

$$E = \frac{0.338\,F\,(1-\mu^2)}{D^{3/2}} \times \left[\mathrm{K_U} \times \left(\frac{1}{\mathrm{R_U}} + \frac{1}{\mathrm{R'_U}}\right)^{1/3} + \mathrm{K_L} \times \left(\frac{1}{\mathrm{R_L}} + \frac{1}{\mathrm{R'_L}}\right)^{1/3}\right]^{3/2} \quad \text{(Supplementary Eq. 2.1)}$$

Where, *E* is the apparent modulus of elasticity (Pa); F is the force (N) at selected deformation distance (15 % or 30 % strain); D is the probe displacement (m) at selected deformation distance; μ is the blueberry Poisson's ratio (dimensionless); R_U and R'_U are the minimum and maximum, respectively, radii of curvature (m) of convex shape object at the upper point of contact (plate probe); R_L and R'_L are the minimum and maximum, respectively, radii of curvature (m) of convex shape object at the lower point of contact (platform support). K_U and K_L are constants calculated from the geometry of the upper and lower point of contact, respectively. A Poisson's ratio (μ) of 0.4 was assumed for blueberries (Prussia et al., 2006). Assumptions for spherical objects were considered, thus R_U, R'_U, R_L, R'_L were equals to the radii of curvature (R) and K_U=K_L=1.351 (Prussia et al., 2006). Radii of curvature were calculated for each berry by the equatorial diameter obtained from Texture Analysis readings.

2.9.2.1.3. Statistical analysis

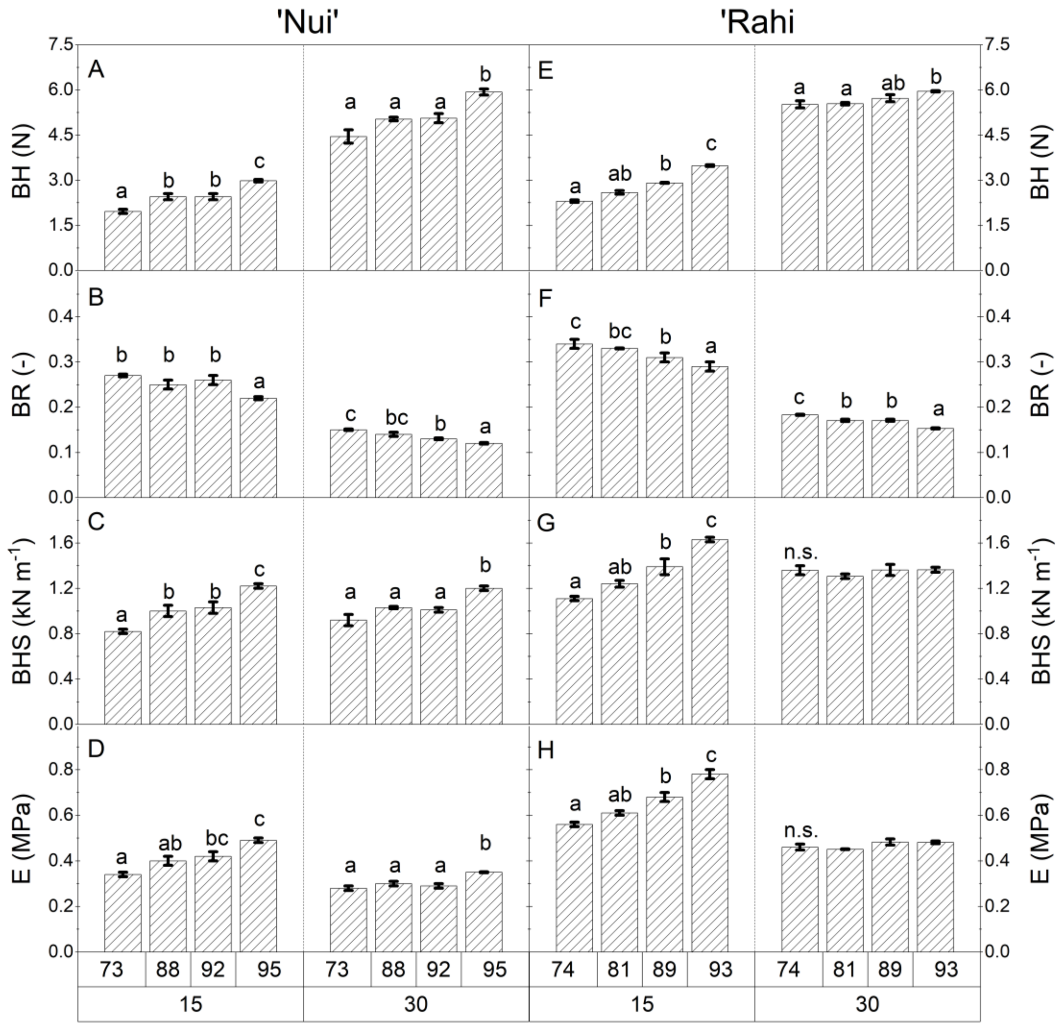
Twenty-six readings (26 berries) of each texture profile analysis response variables of hardness (N), hardness slope (kN m^{-1}), resilience (-), and apparent modulus of elasticity (MPa) obtained by the same compression displacement were averaged for each storage relative humidity treatment, in triplicate. Consequently, 78 berries were utilised for each storage relative humidity treatment to evaluate hardness, hardness slope, resilience and apparent modulus of elasticity. For each blueberry cultivar ('Nui' and 'Rahi'), the effect of the storage relative humidity treatments on hardness, hardness slope, resilience, and apparent modulus of elasticity for each of the two strains (15 % or 30 %), was estimated by one-way ANOVA and the means between relative humidity treatments were separated according to Tukey-HSD test (P-value < 0.05). Assumptions of normality and equal variance were examined before ANOVA was performed.

Thirteen readings (13 berries) of each texture profile analysis response variables of cohesiveness (-) and springiness (-) obtained by the same operational setting were averaged for each storage relative humidity level in triplicate. Consequently, 39 berries were utilised for each storage relative humidity treatment to evaluate cohesiveness and springiness. For each blueberry cultivar ('Nui' and 'Rahi'), the effect of storage relative humidity treatments on cohesiveness and springiness for each of the four operational settings (matrix of two target strain % and two duration between cycles) was estimated by one-way ANOVA, and the means were separated according to Tukey-HSD test (P-value < 0.05). Assumptions of normality and equal variance were examined before ANOVA was performed.

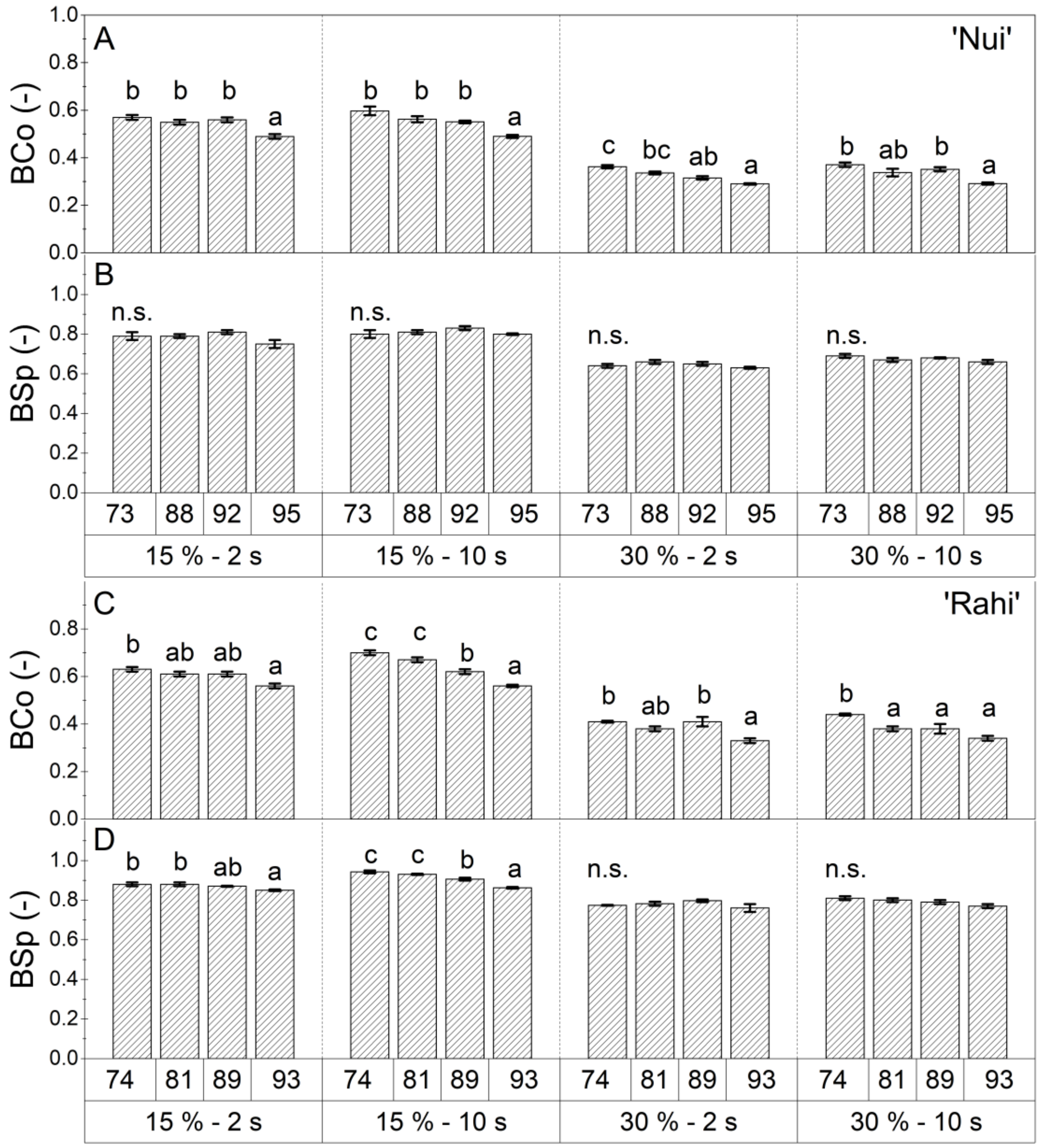
Statistical analysis was performed using statistical software InfoStat 2019 (Universidad Nacional de Córdoba, Argentina), and Figures were plotted using Origin 2019b (OriginLab Corporation, MA, USA).

2.9.2.2. Data description

Data reported in this article describe the results obtained by evaluating the influence of four storage relative humidity on mechanical parameters of blueberry stored for 21 d at 4.5 °C. After storage, the parameters of hardness (BH, N), hardness slope (BHS, kN m^{-1}), resilience (BR, -), and apparent modulus of elasticity (E, MPa) were obtained by using two strains (15 % or 30 % of berry equatorial height) for operating texture analyser for two blueberries cultivars, ‘Nui’ and ‘Rahi’ (**Supplementary Fig. 2.1**). The article also presents the data related to evaluating the influence of four storage relative humidity for 21 d at 4.5 °C on blueberry mechanical parameters of cohesiveness (BCo, -) and springiness (BSp, -). Cohesiveness and springiness were measured by using four operational settings obtained by the matrix combination of two strains (15 % and 30 %) and two durations between compression cycles (2 s and 10 s) for two blueberries cultivars, ‘Nui’ and ‘Rahi’ (**Supplementary Fig. 2.2**).



Supplementary Fig. 2.2. Influence of storage relative humidity (73 %, 88 %, 92 %, and 95 % for ‘Nui’, and 74 %, 81 %, 88 % and 93 % for ‘Rahi’) for 21 d at 4.5 °C on the average blueberry hardness (BH, A and E), resilience (BR, B and F), hardness slope (BHS, C and G) and the apparent modulus of elasticity (*E,* D and H) determined using strain of 15 % and 30 % of berry equatorial height in blueberries ‘Nui’ (A-D) and ‘Rahi’ (E- H). Columns (i.e., storage relative humidity) with the same letters for each target strain and cultivar were not statistically significant, according to Tukey´s HSD test (*P*-value < 0.05). n.s. = ANOVA reports a *P*-value of > 0.05. Columns = mean of three replications of 26 berries each (n = 78 berries). Bars = standard error of the mean of three replications.



Supplementary Fig. 2.3. Influence of storage relative humidity (73 %, 88 %, 92 %, and 95 % for 'Nui', and 74 %, 81 %, 89 % and 93 % for 'Rahi') for 21 d at 4.5 °C on the average cohesiveness (BCo, A and C) and springiness (BSp, B and D), determined using four operational settings of the combination of two strain (15 % or 30 % of berry equatorial height) and two duration between cycles (2 s or 10 s), in 'Nui' (A-B) and 'Rahi' (C-D) blueberries. Columns (i.e., storage relative humidity) with the same letters for each operational setting and cultivar were not statistically significant, according to Tukey´s HSD test (*P*-value < 0.05). n.s. = ANOVA reports a *P*-value of > 0.05. Columns = mean of three replications of 13 berries each (n = 39 berries) . Bars = standard error of the mean of three replications.

2.9.2.3. References

Chiabrando, V., Giacalone, G., Rolle, L., 2009. Mechanical behaviour and quality traits of Highbush blueberry during postharvest storage. J. Sci. Food Agric. 89, 989-992. https://doi.org/10.1002/jsfa.3544

Hu, M.H., Dong, Q.L., Liu, B.L., Opara, U.L., Chen, L., 2015. Estimating blueberry mechanical properties based on random frog-selected hyperspectral data. Postharvest Biol. Tec. 106, 1-10. https://doi.org/10.1016/j.postharvbio.2015.03.014

Paniagua, A.C., East, A.R., Hindmarsh, J.P., Heyes, J.A., 2013. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. Postharvest Biol. Tec. 79, 13-19. https://doi.org/10.1016/j.postharvbio.2012.12.016

Pons, M., Fiszman, S.M., 1996. Instrumental Texture profile analysis with particular reference to gelled systems. J. Texture Stud. 27, 597-624. https://doi.org/10.1111/j.1745-4603.1996.tb00996.x

Prussia, S.E., Tetteh, M.K., Verma, B.P., NeSmith, D.S., 2006. Apparent modulus of elasticity from FirmTech 2 firmness measurements of blueberries. Transactions of the ASABE 49, 113-121. https://doi.org/10.13031/2013.20219

Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2021a. Influence of water loss on mechanical properties of stored blueberries. Postharvest Biology and Technology 176. https://doi.org/10.1016/j.postharvbio.2021.111498.

Slaughter, D.C., Rohrbach, R.P., 1985. Developing a blueberry firmness standard. Transactions of the ASAE 28(3), 986-992. https://doi.org/10.13031/2013.32374

2.9.3. Statements of contribution Chapter *2*

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Sebastian Anibal Rivera Smith
Name/title of Primary Supervisor:	Dr. Svetla Sofkova-Bobcheva
In which chapter is the manuscript /published work: Chapter 2	

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2021. Influence of water loss on mechanical properties of stored blueberries. Postharvest Biology and Technology 176. https://doi.org/10.1016/j.postharvbio.2021.111498.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Sebastian Rivera Smith (Digitally signed by Sebastian Rivera Smith DN: cn=Sebastian Rivera Smith, c=NZ, o=School of Food and Advanced Technology, Massey University, ou= Massey AgriFood Digital Lab, email=s.riverasmith@massey.ac.nz Date: 2022.02.19 12:15:44 +13'00')
Date:	19-Feb-2022
Primary Supervisor's Signature:	Svetla Sofkova-Bobcheva (Digitally signed by Svetla Sofkova-Bobcheva DN: cn=Svetla Sofkova-Bobcheva, c=NZ, o=Massey university, ou=School of Agriculture and Environment, email=s.sofkova-bobcheva@massey.ac.nz Date: 2022.02.24 09:04:42 +13'00')
Date:	24-Feb-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

GRS Version 5 – 13 December 2019
DRC 19/09/10

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Sebastian Anibal Rivera Smith
Name/title of Primary Supervisor:	Dr. Svetla Sofkova-Bobcheva

In which chapter is the manuscript /published work: Chapter 2

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2021. Data of texture Profile Analysis performed by different input settings on stored 'Nui' and 'Rahi' blueberries. Data in Brief. 38, 107313. https://doi.org/10.1016/j.dib.2021.107313

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Sebastian Rivera Smith — Digitally signed by Sebastian Rivera Smith DN: cn=Sebastian Rivera Smith , c=NZ, o=School of Food and Advanced Technology, Massey University, ou= Massey AgriFood Digital Lab , email=s.riverasmith@massey.ac.nz Date: 2022.02.19 12:15:44 +13'00'
Date:	19-Feb-2022
Primary Supervisor's Signature:	Svetla Sofkova-Bobcheva — Digitally signed by Svetla Sofkova-Bobcheva DN: cn=Svetla Sofkova-Bobcheva, c=NZ, o=Massey university , ou=School of Agriculture and Environment, email=s.sofkova-bobcheva@massey.ac.nz Date: 2022.02.24 09:04:07 +13'00'
Date:	24-Feb-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

GRS Version 5 – 13 December 2019
DRC 19/09/10

Chapter 3. Influence of harvest maturity and storage technology on mechanical properties of blueberries

In **Chapter 2,** the mechanical parameters strongly related to water loss changes during postharvest storage have been identified for fresh 'Nui' and 'Rahi' blueberries (**Table 2.6**). This research **Chapter 3** will address an essential question opened by **Chapter 2** about the consistency of mechanical parameters data across other factors previously reported to influence blueberry firmness. Harvest maturity and controlled atmosphere storage were selected for this purpose. Both factors are critical commercial management of fresh blueberries and have been previously identified as the source of textural variance (**Chapter 1, Fig. 1.2**). This research chapter was devised to determine 'Nui' and 'Rahi' blueberries mechanical parameters that best describe harvest maturity differences and quality changes induced by different CO_2 atmosphere compositions in controlled atmosphere storage.

The data generated for this chapter resulted in one publication submitted in Elsevier Journal, Postharvest Biology and Technology. The author of this thesis has worked on the manuscript conceptualization, research methodology, formal analysis, investigation, paper visualizations, and writing > 90 % of the original draft. This chapter includes additional information not presented in the published version.

Most of the results presented in **Chapter 3** were used to write a research article:

Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2022. Influence of harvest maturity and postharvest technologies on mechanical properties of blueberries. Postharvest Biol. Technol. 191, 111961. https://doi.org/10.1016/j.postharvbio.2022.111961.

AUTHORS AND AFFILIATIONS

Sebastian Rivera[a,d], Huub Kerckhoffs[b], Svetla Sofkova-Bobcheva[a], Dan Hutchins[c], Andrew East[d]

[a] School of Agriculture and Environment, Massey University, Palmerston North, New Zealand
[b] Ministry for Primary Industries, Wellington, New Zealand
[c] Gourmet Blueberries, NZ Gourmet Ltd, Auckland, New Zealand
[d] Massey Agrifood Digital Lab, Massey University, Palmerston North, New Zealand

KEYWORDS

Vaccinium spp; controlled atmosphere; ratio of soluble solids to acidity; water loss; texture profile analysis; penetration test.

ABSTRACT

The present study contributes to the search of methodological procedures to accurately evaluate and ascribe meaning to the mechanical parameters of blueberry. To generate populations of berries with different mechanical properties, 'Nui' and 'Rahi' blueberries were harvested at three maturity stages and stored in five storage technologies for 42 d at 4.6 °C. Storage technologies included two relative humidity levels in air (high: 99 % or low: 80 % RH) and three CAs of 5 kPa, 10 kPa, or 20 kPa of CO_2 combined with 4 kPa of O_2 at high relative humidity (94-99 % RH). Two mechanical tests, texture profile analysis (TPA) and penetration test, were used to determine ten mechanical parameters. The mechanical parameter that best separated the blueberry population varied depending on the cause of textural variation. Force at skin break provided the best differentiation of harvest maturities at harvest time and after the storage period. However, force at skin break did not differentiate the effect of storage relative humidity. Hardness slope, skin break slope, and displacement at skin break provided good differentiation of berries stored in different technologies. In addition, hardness slope and skin break slope separated berries obtained by the combined effect of harvest maturity and storage technology after 42 d of storage and were able to detect both softening and firming throughout the storage period. Consequently, the results suggest that the loading slope of the force/deformation graph obtained by any of both mechanical tests (TPA plate compression or needle penetration test) separated stored berries with variable textural characteristics, independent of the mechanisms of induction of these textural changes. Hence loading slope may be the most suitable mechanical parameter to track quality changes of stored blueberries.

3.1. Introduction

Fruit firmness is referred to the textural or mechanical attributes that can denote differences in maturity and quality (Timm et al., 1996; Abbott, 1999; Lu and Abbott, 2004). Excessive blueberry softening rate (or low firmness) can negatively impact the commercialisation and consumer acceptance of fresh blueberries. However, instrumental methodologies used to assess blueberry softening vary widely across literature references (**Chapter 1, Fig 1.2**), compromising the data longevity and the cross-validation between the results obtained by the research community and industry.

Through blueberry maturation, physical and biochemical changes occur, such as the surface colour change from green to blue, softening (Vicente et al., 2007; Forney, 2009; Moggia et al., 2018), total soluble solids (TSS) accumulation and acidity (TA) reduction (Forney, 2009; Moggia et al., 2018). These developmental changes occur at different rates for the berries attached to the same fruit cluster. Consequently, several picks are usually performed for each plant during the harvest season (Suzuki et al., 1997; Moggia et al., 2017a; Lobos et al., 2018).

Surface colour has been used as a picking criterion to facilitate commercial operations, with 100 % blue colour coverage being the most used criterion (Lobos et al., 2014; Moggia et al., 2016; Moggia et al., 2017a). However, surface colour evolution for the late stages of fruit ripening occurs rapidly. Only three days are required to change from 50 % pink to 100 % blue (Forney, 2009). After the berries reach 100 % blue, surface colour cannot be considered a reliable indicator of physiological maturity (Lobos et al., 2018). Consequently, the blueberry supply chain and research community require an accurate evaluation parameter to track berry maturity, especially in the later stages of ripening.

Biochemical changes, such as the increase in the ratio of total soluble solids to acidity (TSS:TA) occur when the berry has already reached 100 % blue (Forney, 2009; Moggia et al., 2016). Consequently, TSS:TA can be used to track the physiological age of the blueberries. However, blueberry cultivar and growing conditions can result in variable TSS:TA ranges (Ballinger et al., 1978; Saftner et al., 2008; Moggia et al., 2017a), and hence a single TSS:TA range associated with blueberry maturity cannot be used as a standardised criterion.

Alternatively, changes in mechanical properties also occur during fruit ripening and can be potentially used to track harvest maturity differences (**Table 3.1**). The influence of maturity on mechanical parameters has mainly been reported using either the maximum force at a set deformation or the loading slope of the force/deformation graph by a compression test (**Table**

3.1). The most frequently studied blueberries are highbush cultivars such as 'Brigitta', 'Bluecrop' and 'Duke', and the influence of maturity on fruit performance after cool storage was previously considered only by a few studies (**Table 3.1**). Consequently, whether these reported mechanical parameters are the most useful to determine maturity differences whilst considering other causes of textural variation such as blueberry genotype and postharvest storage technologies remains unknown.

Table 3.1. Studies reporting mechanical parameters of different maturity stages of blueberry.

Cultivar	Maturity stages	Storage conditions	Mechanical parameters		Reference
			Probe Ø (mm)	Maturity effect	
'Berkeley' 'Morrow'	Green; white; blush; half red; red; purple; blue (ripe); overripe	no	Not reported	Large change in force resistance to deformation (kg m^{-1}) as fruit maturity progress from green to red. A slight reduction of the resistance to deformation from red/purple to ripe stage	Ballinger et al., 1973
'Collins'	Stages 3 (blush); 4; 5 (red); 6 (blue-red); 7; 8 (blue)	no	10	Reduction of the maximum force to deformation (dyn) as fruit maturity progress from blush to blue-red. The most significant force reduction was observed between red (stage 5) and blue-red (stage 6)	Proctor and Miesle, 1991
'Centurion'	Green; red; blue; overripe	no	Not used	Increase the contact time, of the Impact Response Analysis, as maturity progress from green to overripe	Patel et al, 1993
'Bluecrop'	Immature (low blue); near mature (medium blue); mature (high blue)	no	Plate	Reduction of mean chord stiffness (N m^{-1}) as maturity progress from immature to mature berries	Timm et al., 1996
'Bluecrop' 'Ivanhoe'	60 % blue; 100 % blue	1 or 15 d at 0 °C + 3 d at 21 °C	Hand	Higher incidence of firm berries on 60 % blue stage by subjective evaluation of hand-touch firmness	Beaudry et al. 1998
'Duke'	Large green; 25 % blue; 75 % blue; 100 % blue; blue ripe	no	2	Reduction of the maximum force (N) to 2 mm deformation as the fruit maturity progress from green to the ripe blue stage	Vicente et al., 2007
'Elliott'	50 % to 75 % blue; 75 % to 99 % blue; 100 % blue	30 d at 5 °C	Plate	Lower maximum force (N) to 1 mm deformation on 100 % blue fruit compared to 50-75 % blue fruit after the storage period	Hancock et al., 2008
'Duke' 'Brigitta'	75 % blue; 100 % blue; 100 % blue + 5-7 d on plant	45 d at 0 °C + 1 d at 18 °C	15	Lower FirmTech firmness (N m^{-1}) of overmature berries (100 % blue + 5-7 d) at harvest and after 45 d storage	Moggia et al., 2016
'Duke' 'Brigitta'	100 % blue; 100 % blue + 6 d on plant	30 or 45 d of at 0 °C	15	Lower FirmTech firmness (N m^{-1}) on overmature berries (100 % blue + 6 d) at harvest time on two years of study. Lower firmness on overmature berries after storage period only for one year of study	Lobos et al., 2018
'Duke' 'Brigitta'	100 % green; 75 % green + 25 % pink; 50 % green + 50 % pink; 25 % pink + 75 % blue; 90–100 % blue; 100 % blue + 5 d on plant	45 d at 2 °C + 1 d at 18 °C	15	Reduction of FirmTech firmness (N m^{-1}) as maturity progress from 100 % green to 100% blue + 5 d. Lower firmness of 100 % blue + 5 d compared to 90-100 % blue on storage evaluation	Moggia et al., 2018
'Bluecrop'	Pale green; reddish-purple; dark purple; dark blue	no	2	Large decrease of the maximum force (N) to 4 mm deformation from pale green to reddish-purple stage. A slight reduction of maximum force between dark purple and dark blue stage	Chea et al., 2019
'Bluecrop' 'Northblue'	Five maturity stages (1 - 5)	no	8	Reduction of the maximum force (N) to 5 mm deformation as maturity progress from stage 1 to 5	Lin et al., 2020

[a]no = postharvest storage evaluation of maturities was not included in the study.

High relative humidity (~95 % RH), complementing cool storage, has been recommended to extend the storage life of blueberry, providing benefits such as decreased rate of water loss, shrivel, and softening (Paniagua et al., 2013). However, high relative humidity storage generates optimum conditions for *Botrytis cinerea* grey mould rot (Rivera et al., 2013a). Postharvest technologies that allow modification of the atmosphere composition, such as controlled atmosphere (CA) can be used to accomplish adequate rot control (Ceponis and Cappellini, 1983; Forney et al., 2003; Harb and Streif, 2004; Alsmairat et al., 2011; Falagan et al., 2020). The effectiveness of CA on rots is mainly dependent on the atmosphere composition (O_2 + CO_2) used (Saltveit, 2003; Falagan and Terry, 2018). CO_2 higher than 6-8 kPa can reduce or retard symptoms of blueberry rot during cold storage (Forney et al., 2003; Harb and Streif, 2004; Alsmairat et al., 2011; Cantin et al., 2012; Rodriguez and Zoffoli, 2016; Falagan et al., 2020).

However, the use of CA can also affect blueberry firmness or softening rate during storage (**Table 3.2**). Excessive softening is recognised as a common risk of CO_2 higher than 8-12 kPa (Forney et al., 2003; Harb and Streif, 2004; Hancock et al., 2008; Duarte et al., 2009; Alsmairat et al., 2011; Cantin et al., 2012; Rodriguez and Zoffoli, 2016; Catuneanu et al., 2017). On the other hand, higher firmness retention has also been observed when CO_2 of 10 kPa was used during storage (Paniagua et al., 2014; Falagan et al., 2020). In addition, the combination of O_2 utilised with high CO_2 can also influence blueberry softening response. Paniagua et al. (2014) observed lower firmness of 'Maru' when stored at 2.5 kPa O_2 + 10 kPa CO_2 compared to 20 kPa O_2 + 10 kPa CO_2. Similarly, Rodriguez and Zoffoli (2016) observed lower firmness for blueberries stored under extremely low O_2 (1 kPa) compared to 15 kPa O_2, in combination with equivalent CO_2 levels (5-7 kPa).

The influence of CA technology on blueberry firmness has been evaluated by considering different instruments and evaluation methodologies (**Table 3.2**). Like fruit maturity studies, the maximum force to a certain deformation or the loading slope of the force /deformation graph determined by using a flat compression plate is the most common parameter used to characterise blueberries (Allan-Wojtas et al., 2001; Forney et al., 2003; Harb and Streif, 2004; Schotsmans et al., 2007; Hancock et al., 2008; Cantin et al., 2012; Paniagua et al., 2014; Falagan et al., 2020). However, the effect of CA on penetration force or skin toughness has also been evaluated (Forney et al., 2003; Duarte et al., 2009; Concha-Meyer et al., 2015).

This study aims to add knowledge to identifying the appropriate mechanical method to be used to measure blueberry quality. Mechanical parameters such as displacement at berry skin break obtained by a needle penetration test and the loading slope (i.e., hardness slope or chord stiffness) obtained by texture profile analysis were previously identified for their ability to separate blueberries with different water loss levels throughout storage (**Chapter 2**). However, the recommendation of these mechanical parameters to be used as a standard of firmness on quality evaluations for research and commercial purposes requires studies with alternative causes for textural variation. The author of this thesis hypothesises that the same mechanical methodologies (texture profile analysis and penetration test) used to determine the effect of water loss can be used to assess the impact of harvest maturity and controlled atmosphere on textural variation. The specific objectives of this study are:

1) Identify the mechanical parameters that best describe harvest maturity of blueberry 'Nui' and 'Rahi' for evaluations at harvest and after storage.
2) Identify the mechanical parameters that enable the best separation of mechanical descriptors of 'Nui' and 'Rahi' blueberries stored in different postharvest technologies (RH and CA).

With these investigations, a better understanding of mechanical changes as influenced by maturity and storage technology can assist quality improvements under commercial operations. In addition, it is expected to contribute to developing instrumental standards to assess blueberry firmness on quality evaluations for research and commercial purposes.

Table 3.2. Studies reporting the influence of controlled atmosphere (CA) storage on mechanical properties of blueberries in the last 20 years.

Storage conditions				Mechanical parameters		Reference
AC[a] (% or kPa)		T[b] (°C)	RH[c] (%)	Probe Ø (mm)	Controlled atmosphere (CA) effect	
O_2	CO_2					
15	15	0	NR[d]	15	Compared to air storage, lower Firmtech firmness (N m-1) of 'Burlington' and 'Coville' berries stored in CA. No differences for 'Elliot' blueberries	Allan-Wojtas et al., 2001
15	10, 15, 20 or 25	0	NR	15 (firmness); 0.3 (skin toughness)	Lower FirmTech firmness ($N\ m^{-1}$) and skin toughness (N) of highbush blueberry 'Burlington' in CA storage when CO_2 ≥15 kPa	Forney et al., 2003
18	6, 12, 18 or 24	0-1	NR	Plate	Reduction of FirmTech firmness ($g\ m^{-1}$) of 'Duke' as the CO_2 increased from 12 kPa to 24 kPa after 7 weeks of storage. 6 kPa CO_2 provided better firmness than air control.	Harb and Streif, 2004
2.5	15	1.5	98	60	Lower maximum force (N) to 2 mm compression of rabbiteye blueberry 'Centurion' and 'Maru' in CA storage compared to air	Schotsmans et al., 2007
2	8	0	>90	Plate	Lower chord stiffness ($N\ m^{-1}$) of six highbush blueberry cultivars in CA storage, in comparison to RA storage	Hancock et al., 2008
5	5, 10 or 15	0	NR	2	Lower force (N) to penetrate (tear) the skin of highbush blueberry 'Brigitta' stored on CA of 10 % and 15 % CO_2 compared to air storage	Duarte et al., 2009
2, 3, 4.5, 6, 7.5, 9, and 15	19, 18, 16, 15, 13.5, 12, and 6	0	NR	2.4	Lower durometer firmness to 2 mm compression of 7 highbush blueberry cultivars in CA storage CO_2 of 19 kPa	Alsmairat et al., 2011
3	11	0	90-95	3	Higher durometer firmness (Durofel, Copa Technology, France) after 60 d of CA storage, compared to firmness evaluation at harvest time	Chiabrando and Giacalone, 2011
3	3, 6, 12 or 24	1	NR	25	Lower maximum force (N) to 4 mm compression of highbush blueberry 'Emerald', 'Reveille' and 'Star' in CA storage CO_2 of 24 kPa	Cantin et al., 2012
2.5 or 20	10	0	90	25	Higher force (N) to 1 mm compression of 'Brigitta' stored in CA when compared to air storage. However, a lower force of Rabbiteye blueberry 'Maru' in CA storage of 10 kPa CO_2 and 2.5 kPa O_2	Paniagua et al., 2014
		4	67			
5	15	4 or 12	90-95	2	Compared to control, lower penetration peak force (g) on highbush 'Ozark Blue' stored in CA at 12 °C. No conclusive differences between treatments at 4 °C	Concha-Meyer et al., 2015
1, 5, and 15	5, 15, and 7	0	85	3.6	Lower Durofel firmness of 'Brigitta' when using CA of 15 kPa CO_2 + 5 kPa O_2 and 5 kPa CO_2 + 1 kPa O_2 compared to air or 7 kPa CO_2 + 15 kPa O_2	Rodriguez and Zoffoli, 2016
3	5 or 10	1	NR	No reported	Lower stiffness ($kg\ m^{-2}$) of highbush blueberry 'Coville' in CA storage of 10 kPa CO_2. However, not a conclusive effect of CA on blueberry 'Chandler' and 'Blueray'	Catuneanu et al., 2017
5	10	0	90-95	38	Higher maximum force (N) to 2 mm compression of 'Duke' in controlled atmosphere (CA) in comparison with regular air (RA) storage	Falagan et al., 2020

[a]AC = Atmosphere composition
[b]T = Storage temperature
[c]RH = Storage relative humidity
[d]NR = Not reported

3.2. Material and methods

3.2.1. Harvest maturity

Highbush 'Nui' (*Vaccinium corymbosum*) and rabbiteye 'Rahi' (*V. ashei*) were selected on three harvest maturity stages (immature, mature, and overmature) from a commercial orchard (Gourmet Blueberries) located in Hawke's Bay, New Zealand. All maturities stages were picked on the same day for 'Nui' (December 17th, 2019) and 'Rahi' (February 6th, 2020). During harvest, immature and mature berries were obtained by differentiation of blue colour coverage percentage on each berry surface area, 60-90 % for immature and 100 % for mature berries. To harvest overmature berries at the same time as immature and mature, approximately 1,600 berries of 90-100 % blue surface colour were labelled 6-9 d before harvesting and kept on the plant. Consequently, overmature berries are also denoted as 100 % blue + time on the plant.

The fruit maturities were sampled from 450 fruit clusters distributed in a total of 40 healthy plants. The clusters were selected 6 d before harvest for 'Nui' (December 11^{th,} 2019) and 9 d for 'Rahi' (January 29th 2020). The cluster selection was based on clusters with a similar number of berries (i.e., 6-10 berries), maturity distribution, and located on the same plant side.

On the same day of cluster selection, fruit thinning procedures were performed on each cluster to obtain different maturities on the picking day. On 250 random clusters, all the berries showing more than 20 % blue surface colour were removed, and this cluster was used to sample immature and mature berries at harvest. On the remaining 200 clusters, all berries showing <90 % blue surface colour were removed to ensure only overmature berries when harvested 6-9 days later. Very immature green-pink berries were removed by cutting at the midpoint of the pedicel of each berry using a fruit thinning scissor. Berries in the pink and blue stage were easily removed by hand. Cluster selection and berry thinning procedures were conducted on a commercial picking day. The time intervals between thinning operations and harvest of 6 d for

‘Nui’ and 9 d for ‘Rahi’, were based on the picking frequency utilised commercially for each cultivar.

Blueberry harvest was performed by hand between 10:30 am and 15:00 pm with a fruit pulp temperature of 26.7 ± 1.5 °C for ‘Nui’ and 25.1 ± 4.4 °C for ‘Rahi’ as recorded using a digital thermometer (Q1437, Dick Smith Electronics Pty Ltd, Australia). Harvest criterion for each fruit maturity was based on berries of representative and homogenous size and free of visual defects such as rots, splits, shrivelling, lack of bloom (wax), and bruises. Approximately 1.5 kg of berries for each of the three maturity stages were picked into 125 g clamshells. In addition, 7 kg of berries at the mature stage (100 % blue) were picked from same plants. These additional mature berries were used at an extra evaluation time of mechanical parameters (i.e., 21 d of storage), and as buffer fruit during the storage technology treatments.

Immediately after harvest, berries were transported for 3 h at approximately 22 °C to the postharvest lab, Palmerston North, New Zealand. One hour after arrival, three random samples of approximately 200 g of each maturity were used for blueberry maturity characterisation. The remaining berries were kept overnight (12 h) in a temperature control cabinet set at 10 °C before performing the experimental setting-up for storage technology treatments on the following day.

3.2.2. Blueberry maturity characterisation

At harvest, berry weight, mechanical parameters, total soluble solids, and acidity were evaluated at 20 °C, between 1 and 5 h after fruit arrival.

Individual berry weight was measured with a digital balance (TW423L 0.001 g, Shimadzu, Japan) on 60 berries for each of the three maturities within each cultivar.

For each maturity, mechanical parameters were evaluated by texture profile analysis and needle penetration test using a texture analyser (TA.XT plus, Stable Micro Systems, Surrey, UK). For further detail about mechanical parameters procedures, please see section 3*.2.4.3. Mechanical parameters*: *Texture profile analysis and penetration test.* The average of ten berries was calculated for each of three replicates to be consistent with author's previous study (**Chapter 2**).

Total soluble solids (TSS) and acidity (TA) for each maturity were evaluated at harvest time using six juice samples obtained from ten berries each. The concentration of TSS was determined in a ~0.3 mL juice and acidity in 1 g juice diluted in 50 mL reverse osmosis water using a digital refractometer/acid meter (PAL-BX I ACID F5, Atago, Tokyo, Japan). The validity of the digital acidity measurement was conducted in comparison to a standard titration method, details of which are provided as supplementary material (**Supplementary Fig. 1**).

3.2.3. Storage technology treatments

For each cultivar and maturity (immature, mature and overmature), groups of 25 healthy berries (absent of mechanical damage, shrivelling or rots) were created by randomisation. Each group of berries was placed inside individual fabric mesh bags of 90 cm^3, and the mesh bags were individually weighed on a digital balance to 0.001 g precision (TW423L, Shimadzu, Japan) at a flesh temperature of 20 °C. Two mesh bags at the mature stage and one mesh bag for the immature and overmature stage (4 mesh bags in total) were placed inside a 1.83 L glass jar containing buffer berries on the jar bottom. The quantity of the buffer berries varied between 275 g and 310 g and was calculated to accomplish a total sum of 450 g in each jar. Twenty jars containing the berries were prepared.

Each jar was randomly assigned to one of four replicates of five storage technologies for 42 d at 4.6 ± 0.19 °C. The storage technologies were high relative humidity air (99 % RH), low relative humidity air (80-82 % RH), and three controlled atmospheres of 5 kPa, 10 kPa, and 20 kPa of CO_2 in combination with 4 kPa of O_2 at high relative humidity (94-99 % RH). Although the selected storage temperature (4.6 °C) is higher than the commercially relevant temperature of 0 °C, this temperature was selected to be consistent (and allow comparison) with previously reported results of the same research group (Paniagua et al., 2013, 2014; Li et al., 2021). The use of a low

O_2 (4 kPa) to be used for all the CAs was selected to increase the chances of inducing a higher softening rate by high CO_2 (≥10 kPa), as reported by Paniagua et al. (2013).

Air treatments were created by continuous flow through each glass jar of environmental air supplied by an electric compressor (OM200/6, NUAIR, Italy). An airflow rate through each jar of 0.4 mL s^{-1} was used to create high relative humidity air (99 % RH), and 2 mL s^{-1} airflow was used to create low relative humidity (80- 82 % RH) air storage. Airflow through each jar was regulated by needle valves connected to a manifold to split the desired airflow into the four replicates for each air treatment. Each needle valve's flow rate was tuned through direct measurement using a portable gas flowmeter (ADM 2000, Alignet Technologies, Delwar, USA).

Controlled atmosphere (CA) was created using a continuous gas flow of three gas mixtures. Each CA mixture was achieved by a combination of different flow rates of each of three gases: environmental air from the compressor (O_2 source), nitrogen (O_2-free, BOC, Auckland, New Zealand), and food-grade CO_2 (BOC, Auckland, New Zealand). One needle valve for each gas supply was used to regulate the flow rate for each gas (air, nitrogen, and CO_2). Each CA mixture was connected to a manifold to split the gas mixture into four replicates (jars) with a continuous flow of 0.4 mL s^{-1}.

The high relative humidity for air (99 % RH) and CA (94-99 % RH) storage were assured by humidifying the gas mixture before delivering it to each jar containing the berries. Gas flow was passed through a 30 % glycerol solution (550 mL) in a 1 L sealed container. A dual temperature and RH logger (iButton®, Maxim Integrated, CA, USA) was attached inside each jar containing the berries to record the temperature and RH every 900 s.

The O_2 and CO_2 inside each jar were evaluated in the jar headspace every two or three days during 42 d of storage period. A gas sample was removed from each jar using a 100 µL glass syringe (Hamilton Company, NV, USA). The gas sample was injected into an O_2/CO_2 gas analyser equipped with an O_2 electrode (Citrell C/S type, City Technology Ltd., London, UK) in series with a miniature infrared CO_2 transducer (Analytical Development Company, Hoddesdon, UK), using

O_2-free N as a carrier. To measure CO_2 of 5.0 kPa, the gas analyser was calibrated using a certified gas standard of 21.03 ± 0.1 % O_2 and 4.98 ± 0.1 % CO_2 (BOC, Auckland, New Zealand). For the treatments where CO_2 was higher than 5 kPa, the gas analyser calibration was performed using 20 % CO_2 standards prepared weekly by mixing dry air and food-grade CO_2 into 5 L Tedlar® sample bags (CEL Scientific Corporation, CA, USA).

After 42 d of storage, three mesh bags (one mesh bag of each maturity) were removed from each jar to determine water loss, rots prevalence and mechanical parameters.

Previously reported studies of the same research group (Paniagua et al., 2013; **Chapter 2** and **Chapter 3**) have described that high relative humidity storage (or low water loss) can induce the storage firming of mature blueberries. Consequently, an additional evaluation at 21 d was set (only on blueberries harvested at the mature stage or 100 % blue) to evaluate the ability of mechanical parameters to detect softening and firming throughout the storage period, as induced by storage technologies.

3.2.4. Storage evaluations

After storage, all evaluations were conducted at 20 °C, between 2 and 4 h after the berries were removed from the cold room (4 °C). Postharvest response variables were measured in the following sequence: water loss, rot prevalence, and mechanical parameters.

3.2.4.1. Storage water loss

After 42 d of storage, three mesh bags (one mesh bag of each maturity) for each cultivar were weighed on the digital balance to 0.001 g precision (TW423L, Shimadzu, Japan), and the water loss (WL) was calculated (Eq. 3.1).

$$WL\ (\%) = \frac{Wi - Wf}{Wi} \times\ 100 \qquad \text{(Eq. 3.1)}$$

Where *Wi* is the initial weight, and *Wf* is the weight after each storage time. Water loss data was used to explain changes in mechanical parameters as influenced by air storage relative humidity.

3.2.4.2. Storage rot prevalence

Rotten berries were evaluated by naked eye on each mesh bag after 42 days of storage. The number of berries with grey mould symptoms (*Botrytis cinerea*) plus the number of berries showing signs of mould growing on the fruit surface (mainly located at the stem scar) were recorded (Rivera et al., 2013b). Prevalence was calculated as the percentage of rotten berries out of the 25 berries contained in each mesh bag. Any identified rotten berry was discarded and not included in mechanical parameter measurements.

3.2.4.3. Mechanical properties: texture profile analysis and penetration test

Mechanical tests of texture profile analysis (TPA) and penetration test were performed with a texture analyser (TA.XT plus, Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell. A 25 mm cylindrical flat-ended probe (P/25, Stable Micro Systems) was used for TPA, and a rounded tip needle probe (P/2N, Stable Micro Systems) was used for the penetration test (Giongo et al., 2022, **Chapter 2**). Each berry was placed with the stem-calyx axis oriented parallel to the texture analyser platform over a flat metal ring of 10 mm internal diameter and 1 mm height for both mechanical tests.

For TPA, the pre-test speed was set at 1.6 mm s^{-1} and the test and post-test speed at 1.0 mm s^{-1}. Each berry was compressed twice using a trigger force of 0.06 N, a target deformation of 15 % strain, and a waiting time between cycles of 10 s. All acquisitions were made with a resolution of 250 points per second. The obtained double compression graph using Exponent software was used to estimate mechanical parameters of berry hardness, hardness slope, resilience, cohesiveness, and springiness (Version 6.1.14.0, Stable Micro Systems). Hardness was calculated as the maximum force (N) to achieve 15 % strain. Hardness slope (or chord stiffness) was determined as the straight-line slope drawn during the first compression downstroke, obtained between the trigger force of 0.06 N and the maximum force at 15 % strain deformation. Resilience was calculated as the ratio of the work area (force X displacement) during withdrawal (upstroke) of the first compression to area of work during the downstroke of the first compression. Cohesiveness was calculated as the ratio of work area during the second compression cycle to area of work during the first compression cycle. Springiness was calculated as the displacement ratio during downstroke compression of the second cycle to the displacement during downstroke compression of the first cycle. Please refer to **Chapter 2** for further descriptions of TPA mechanical parameters.

The penetration test was performed to a target distance of 30 % of each berry equatorial height using a trigger force of 0.02 N. Pre-test speed was set at 5 mm s^{-1} and the test and post-test speed at 1 mm s^{-1}. Mechanical parameters of the penetration test were estimated using the Exponent software. Being, force at skin break (N), the force achieved just before irreversible rupture of the berry skin; displacement at berry skin break (mm), the distance travelled by the probe just before skin rupture; and skin break work energy (mJ), the area of work of the force/deformation curve between trigger force and the force at the berry skin break. An additional penetration test parameter was considered in the present study, the skin break slope (kN m^{-1}), determined as a straight line drawn between trigger force (0.01 N) and force at a skin break.

Data for each maturity and storage technology replication was recorded as an average of 10 berries and four replicates (n = 40 berries).

3.2.5. Statistical analysis

At harvest, maturity was distributed in a completely random design of 450 g for each of three maturities. A general linear model in ANOVA was performed to evaluate the effect of harvest maturity on the response variables of berry weight, soluble solids, acidity, and mechanical parameters. The sample size for each mechanical parameter was 10 berries for each of three replicates, and for berry weight, soluble solids, and acidity, 10 berries for each of six samples. Tukey-HSD (P-value < 0.05) was used as a multiple comparison test. Assumptions of normality and equal variance were tested before the ANOVA was performed.

After postharvest storage, treatments were distributed in a completely random design of four replicates of 25 berries contained in a mesh bag as an experimental unit. All 25 berries were used to estimate water loss and rot prevalence, and 10 healthy berries (i.e., absence of rots) were used to estimate the mechanical parameters of texture profile analysis, and the other 10 healthy berries to measure penetration test. The remaining five berries for each experimental unit were discarded.

A general linear model (GLM) in ANOVA was used to study the influence of harvest maturity, storage technologies and their interaction on the response variables of water loss and ten mechanical parameters. If the data did not meet normality assumptions, data were subjected to Box-Cox transformation considering the λ rounded estimated value obtained by the 95 % confidence interval of λ. This transformation was applied to data of water loss, displacement at skin break and skin break energy of 'Nui' and 'Rahi'; cohesiveness and springiness of 'Nui', and resilience of 'Rahi'. Tukey-HSD (P-value < 0.05) was used as a multiple comparison test.

To visualise the distribution of berry mechanical parameters within each treatment level, box-and-whisker plots were constructed considering 40 berries (10 berries X 4 replicates) for each storage technology in 'Nui' and 'Rahi'. Interquartile box range (IQR) enclosing the central 50 % of the observations starts at the 1st quartile (25th percentile) and ends at the 3rd quartile (75th

percentile). The whisker range is 1.5 IQR, and any value represents outliers out of the whisker range.

Mechanical parameters showing the most differentiation of storage technology treatments were used to study the ability to describe berry softening and firming evolution throughout the storage period of mature blueberries. Mechanical parameters of mature (100 % blue) blueberries stored on five storage technologies were evaluated at 0 d (harvest day), 21 d, and 42 d of storage. Data were subjected to a general linear model (GLM) in ANOVA. Dunnet's test (P-value < 0.05) was used as a mean comparison method, using the harvest day data as the control treatment.

Independent statistical analyses were performed for each cultivar using the statistical software Minitab (Minitab®19.1.1, USA). Figures were plotted using Origin 2019b (OriginLab Corporation, MA, USA).

3.3. Results

3.3.1. Harvest maturity characterisation

Fruit maturity influenced the berry weight, soluble solids (TSS), and acidity (TA) evaluated at harvest time (**Table 3.3**). Immature berries showed the lowest berry weight and total soluble solids (SS), and highest acidity (**Table 3.3**). Consequently, the TSS:TA increased as berries matured (**Table 3.3**). In agreement with these results, a higher ratio TSS:TA was reported for the advanced maturity stages of 'Duke' and 'Brigitta' (Moggia et al., 2016; 2018).

The TSS:TA can be considered a good indicator of the fruit maturity stage (Ballinger et al., 1978; Saftner et al., 2008; Gilbert et al., 2013; Moggia et al., 2018). However, not all blueberry cultivars generate the same range of ratio of TSS:TA as influenced by the maturity (Ballinger et al., 1978; Gilbert et al., 2013; Moggia et al., 2018). For this study, 'Rahi' showed a higher ratio range when compared to 'Nui' (**Table 3.3**). In addition, TSS:TA can be affected by the growing conditions, including season (Moggia et al., 2017a; Lobos et al., 2018). The TSS:TA of 'Rahi' at the mature stage (obtained by calculating the acidity using **supplementary Fig. 1**) was 70 % higher when compared to a previous season evaluation (**Chapter 2**).

Table 3.3. Effects of harvest maturity (immature = 60-90 % blue, mature = 100 % blue, and overmature = 100 % blue + time on the plant), berry weight (BW), total soluble solids (SS), acidity and ratio of soluble solids to acidity (TSS:TA), of blueberries 'Nui' and 'Rahi'.

Cultivar	**Harvest Maturity**	**BW[a] (g)**	**SS[b] (%)**	**Acidity[b] (%)**	**SS:A[b] (-)**
'Nui'	Immature	1.4 b[c]	9.7 c	1.6 a	6.0 c
	Mature	1.7 a	11.1 b	1.1 b	10.3 b
	Overmature	1.7 a	12.5 a	0.68 c	18.5 a
	P-value	*<0.001*	*<0.001*	*<0.001*	*<0.001*
'Rahi'	Immature	1.5 c	10.5 c	0.88 a	12.5 c
	Mature	2.0 b	12.9 b	0.49 b	26.4 b
	Overmature	2.3 a	14.3 a	0.27 c	52.7 a
	P-value	*<0.001*	*<0.001*	*<0.001*	*<0.001*

[a]Berry weight was evaluated on 60 berries.
[b]Total soluble solids and acidity were evaluated on 6 samples of 10 berries
[c]Data followed by the same letter within each response variable and cultivar were not statistically significant according to Tukey-HSD (P-value < 0.05).

Texture profile analysis (TPA) mechanical parameters of hardness, hardness slope and springiness on 'Nui', and hardness slope, resilience, cohesiveness, and springiness on 'Rahi' were influenced by the harvest maturity (**Table 3.4**). However, TPA did not provide a reliable differentiation of fruit maturity and was mainly able to separate overmature from immature berries, but not the three maturity stages simultaneously (**Table 3.4**). In addition, hardness and hardness slope of 'Nui' were found to be higher for overmature berries, which is contrary to previously reported results obtained by evaluating the loading slope of the force/deformation curve (e.g., Firmtech firmness), or the maximum force to a given fruit deformation (**Table 3.1**).

The penetration test provided a better differentiation of harvest maturity (**Table 3.4**). A decrease in the force at skin break and the skin break energy was observed as the maturity of 'Nui' and 'Rahi' progressed. For 'Rahi', force at skin break provided differentiation of the three harvest maturities, but only the immature and overmature berries were separated for 'Nui'.

Table 3.4. Mechanical parameters from texture profile analysis and penetration test as influenced by harvest maturity (immature = 60-90 % blue, mature = 100 % blue, and overmature = 100 % blue + time on the plant). Average of 3 replicates of 10 berries.

Cultivar	Maturity	Texture profile analysis[a]					Penetration test[b]			
		BH (N)	BHS ($kN\ m^{-1}$)	BR (-)	BCo (-)	BSP (-)	FSk (N)	SSk ($kN\ m^{-1}$)	DSk (mm)	WSk (mJ)
'Nui'	Immature	2.5 b[c]	1.0 b	0.22[d]	0.47	0.80 a	0.27 a	0.31	0.82 a	0.13 a
	Mature	2.6 ab	1.1 ab	0.22	0.48	0.78 ab	0.25 ab	0.30	0.75 ab	0.11 ab
	Overmature	2.9 a	1.2 a	0.23	0.48	0.77 b	0.22 b	0.29	0.69 b	0.09 b
	P-value	*0.02*	*0.04*	*0.57*	*0.71*	*0.03*	*0.04*	*0.63*	*0.02*	*0.04*
'Rahi'	Immature	3.7	1.7	0.27 b	0.52 b	0.83 b	0.22 a	0.46 a	0.44	0.06 a
	Mature	3.8	1.6	0.28 ab	0.54 b	0.84 b	0.18 b	0.42 a	0.40	0.05 ab
	Overmature	4.0	1.5	0.29 a	0.58 a	0.86 a	0.16 c	0.36 b	0.41	0.04 b
	P-value	*0.33*	*0.15*	*0.03*	*0.003*	*0.02*	*0.001*	*0.001*	*0.17*	*0.007*

[a]Texture profile analysis measured berry hardness (BH), hardness slope (BHS), resilience (BR), cohesiveness (BCo), and springiness (BSp).

[b]Penetration test measured force at skin break (FSk), skin break slope (SSk), displacement at skin break (DSk), and skin break work energy (WSk)

[c]Data followed by the same letter within each response variable and cultivar, were not statistically significant according to Tukey HSD (P-value < 0.05).

[d]Data with no letters were not significantly different across all three maturities.

3.3.2. Storage atmosphere

The resulted average O_2 + CO_2 equilibrated through the 42 days of storage at 4.6 °C, were 4.0 kPa + 5.3 kPa; 4.2 kPa + 11.4 kPa; and 4.0 kPa + 20.2 kPa in 'Nui', and 4.2 kPa + 5.1 kPa; 4.2 kPa + 10.3 kPa; and 4.1 kPa + 19.9 kPa in 'Rahi' (**Supplementary Fig. 3.2**).

Airflows of 0.4 mL s^{-1} of high relative humidity air satisfactorily allowed airflow through the jar headspace to hinder CO_2 accumulation. Average O_2 + CO_2 were 21.2 kPa + 0.18 kPa for 'Nui', and 20.9 kPa + 0.18 kPa for 'Rahi'. The higher airflow of 2 mL s^{-1} to create the low relative humidity air resulted in an average O_2 + CO_2 conditions of 21.3 kPa + 0.09 kPa for 'Nui' and 20.7 kPa + 0.08 kPa for 'Rahi'.

3.3.3. Storage relative humidity and water loss

The humidified gas at a flow rate of 0.4 mL s^{-1} used for high relative humidity (RH) air and CAs, kept RH close to the recommended RH of 95 % (Forney, 2009). For both cultivars, the measured RH was 99 % for the high RH air, 99 % for 5 kPa CO_2, 96 % for 10 kPa CO_2, and 94 % for 20 kPa

CO_2. Conversely, the dry airflow rate of 2.0 mL s^{-1} caused a low RH of 80.2 % for 'Nui' and 82.4 % for 'Rahi'.

The rate of water loss (WL) is affected by blueberry characteristics regulating the water vapour permeance (Moggia et al., 2016; Moggia et al., 2017b), and the water vapour pressure deficit as a result of steady-state relative humidity at storage temperature (Paniagua et al., 2013). This study included variability in both factors regulating the rate of WL, the fruit characteristics, and the environmental conditions (**Fig. 3.1**). Consequently, water loss (WL) in storage was influenced (P-value < 0.001) by the maturity stage and storage technology without interaction (P-value ≥ 0.05). However, no differences were observed between maturities of immature and mature 'Nui' (**Fig. 3.1 A**), and mature and overmature for **'**Rahi' (**Fig. 3.1 C**). Hence, only immature berries showed greater cumulated WL in comparison to overmature berries. Blueberries stored in high RH air and the CAs had a WL lower than 1.0 %, while WL of 12.2 % for 'Nui' (**Fig. 3.1 C**) and 9.3 % for 'Rahi' (**Fig. 3.1 D**) was induced for the low relative humidity air.

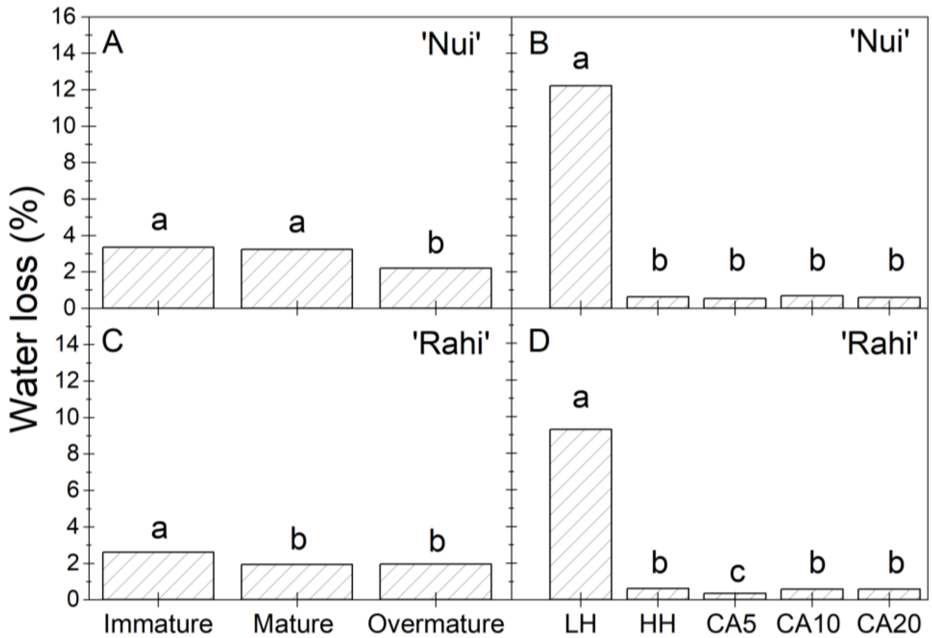


Fig. 3.1. Average cumulated water loss after 42 d of storage at 4.6 °C in 'Nui' (A-B) and 'Rahi' (C-D), as influenced by harvest maturity (A-C; immature = 60-90 % blue, mature = 100 % blue, and overmature = 100 % blue + time on the plant), and storage technology (B-D; LH = low relative humidity air; HH = high relative humidity air, CA5 = 4 kPa O_2 + 5 kPa CO_2; CA10 = 4 kPa O_2 + 10 kPa CO_2; CA20 = 4 kPa O_2 + 20 kPa CO_2). Maturity or storage technology data followed by the same letter within each cultivar were not statistically significant according to Tukey-HSD (P-value < 0.05). The water loss was calculated using 25 berries for each mesh bag.

The higher WL observed for immature blueberries agrees with previous reports for 'Duke' (Moggia et al., 2016). The differences in WL between maturity may be partially explained by a combination of the differences attributed to fruit size and wax composition. Immature berries were smaller in comparison to overmature berries (**Table 3.3**). Therefore, a higher surface-area-to-volume ratio increases the proportion of each berry available for water loss, which occurs for immature berries when compared to overmature fruit. The reduction in water loss with harvest maturity may also be attributed to cuticular wax composition. Moggia et al. (2016) reported that the proportion of the triterpenoid ursolic acid on total epicuticular wax content decreases as blueberry 'Duke' matures, and ursolic acid is positively correlated with water loss.

The RH created in the experiment is the result of the balance between water added by inflowing air, the rate of WL from the blueberry and the water removed by the outgoing airflow (Paniagua et al., 2013). Differences in RH inside the jar for high (99 % RH) and low (80-82 %) RH air are mainly attributed to the differences in the airflow rates (or water removed by the outgoing airflow) in the experimental design. However, for CA, even when setting the same airflow as high RH air, a lower RH was recorded as CO_2 increased. The authors believe that the lower RHs recorded by I-buttons sensors when CO_2 is high may be attributable to a measurement error of the sensor. Consequently, irrespective of the relative humidity reported by the sensors, the water loss observed in high RH and all CA conditions was not different (**Fig. 3.1 B, D**).

3.3.4. Postharvest rot prevalence

Reduction of rot prevalence can be considered an indicator of CA effectiveness in blueberries. Rots evaluated immediately after CA storage was expected to be lower when CO_2 > 6-10 kPa was used, following previously reported results (Harb and Streif, 2004; Cantin et al., 2012; Falagan et al., 2020). Consequently, CA of 20 kPa CO_2 did not show any decay symptom or sign of mould for all the studied maturities and cultivars (**Supplementary Fig. 3.3**). In addition, and as expected, lower rot prevalence was in low relative humidity air when compared to high relative humidity air. However, irrespective of the storage technology, average rot prevalence was lower than 7.5 %, and the coefficients of variation (CV) varied considerably, between 0 and 200 % (**Supplementary Fig. 3.3)**. Thus, statistical analysis was not performed as no differences between treatments were expected (**Supplementary Fig. 3.3**). Rotten berries were discarded and not used on mechanical parameters evaluation.

3.3.5. Postharvest mechanical parameters

Examples of graphical representations of raw mechanical data obtained by texture profile analysis (TPA) and penetration test for 'Nui' blueberry are presented for all three maturities stored in high relative humidity air (**Fig. 3.2 A, C**) and on mature berries stored in the different storage technologies (**Fig. 3.2 B, D**). The downstroke compressions of TPA (**Fig. 3.2 A, B**) and the compression portion (before the needle breaks the blueberry skin) of the penetration test (**Fig. 3.2 C, D**) are described as an approximately linear increase of force as the displacement increases. The compression/deformation loading followed a more curved pattern on low relative humidity air for both mechanical tests, as previously described in **Chapter 2** (**Fig 2.3**).

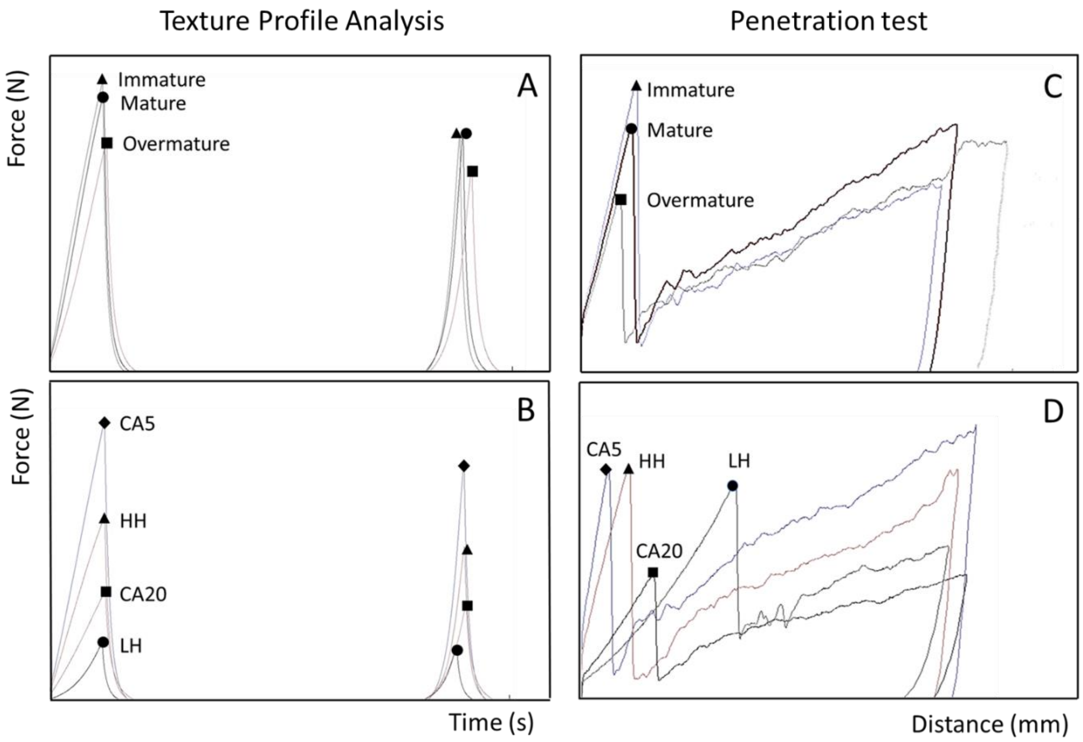


Fig. 3.2. Examples of graphic representation of the raw data obtained by texture profile analysis (A, B) and penetration test (C, D) conducted after 42 d at 4.6 °C on 'Nui' of three maturities stored in high relative humidity air (A, C; immature = 60-90 % blue, mature = 100 % blue, and overmature = 100 % blue + time on plant); and on mature berries stored in four technologies (B, D; LH = low relative humidity air; HH = high relative humidity air; CA5 = 4 kPa O_2 + 5 kPa CO_2; CA20 = 4 kPa O_2 + 20 kPa CO_2). Each plot is the resulting mechanical graph of a single berry that better represents the mean for each maturity in HH (A, C) or a mature berry for each storage technology (B, D). Differences in maximum displacement (y-axis distance or time) of plots A and C have resulted from the differences in berry size.

Mechanical parameters obtained from texture profile analysis (TPA) and penetration test on 'Nui' and 'Rahi' after 42 d of storage were influenced by harvest maturity, storage technology, and the interaction between both factors (**Table 3.5**).

All TPA mechanical parameters were affected by the harvest maturity (**Table 3.5**). Immature berries showed higher hardness and hardness slope and lower resilience, cohesiveness, and springiness when compared to overmature berries. However, differentiation between the three maturities provided by each TPA mechanical parameter was not consistent in both cultivars. Resilience, cohesiveness, and springiness differentiated all three maturities of 'Nui', while only hardness slope provided differentiation of all three maturities of 'Rahi'. The penetration test provided a better separation of harvest maturities when compared to TPA. Force at skin break and skin break slope separated the three maturities of 'Nui' and 'Rahi' (**Table 3.5**).

All mechanical parameters were influenced by the main effect of storage technology (**Table 3.5**). The TPA parameters of hardness, hardness slope, cohesiveness, and resilience provided separation of blueberries stored in high relative humidity air from the blueberries stored in either low relative humidity air or controlled atmosphere (CA). However, only the hardness and hardness slope showed separation of all the CO_2 levels of 'Nui' (**Table 3.5**). The low relative humidity air, followed by the CA of 20 kPa CO_2 showed the lowest hardness and hardness slope, while CA of 5 kPa CO_2 showed the highest values. For 'Rahi', these results were similar, except that blueberry stored in CA of 5 kPa CO_2 did not show higher results than the high relative humidity air and low relative humidity air was not different than 20 kPa CO_2. However, an interaction between maturity and storage technology was observed on hardness slope, and hence the effect of storage treatments for each harvest maturity required further analysis.

The penetration test also provided good differentiation of the blueberries stored on different technologies (**Table 3.5**). The skin break slope obtained a similar separation of treatments as the hardness slope in 'Nui' and 'Rahi'. However, the interaction between maturity and storage technology for skin break slope also requires further analysis.

The displacement at skin break provided good separation of storage treatments. Independent of harvest maturity, low relative humidity air, followed by the CA of 20 kPa CO_2 showed the highest displacement to skin break, while CA of 5 kPa CO_2 showed the lowest values.

On the other hand, force at skin break only distinguished the effect of high CO_2 on CA, being the lowest force for CA at 20 kPa CO_2. While, skin break energy only determined the impact of the low storage relative humidity air, showing higher skin break energy for this treatment (**Table 3.5**).

Table 3.5. Mechanical parameters of texture profile analysis and penetration test of 'Nui' and 'Rahi' blueberries stored for 42 d of at 4.6 °C, as influenced by harvest maturity (immature = 60-90 % blue, mature = 100 % blue, and overmature = 100 % blue + time on plant), storage technologies (LH = low relative humidity air; HH = high relative humidity air; CA5 = 4 kPa O_2 + 5 kPa CO_2; CA10 = 4 kPa O_2 + 10 kPa CO_2; CA20 = 4 kPa O_2 + 20 kPa CO_2), and the interaction between both factors.

Cultivar	Source of variation	Texture profile analysis[a]					Penetration test[b]			
		BH (N)	BHS (kN m^{-1})	BR (-)	BCo (-)	BSp (-)	FSk (N)	SSk (kN m^{-1})	DSk (mm)	WSk (mJ)
'Nui'	Maturity (M)									
	Immature	3.2 a[c]	1.5 a	0.21 c	0.45 c	0.71 c	0.27 a	0.36 a	0.86	0.13 a
	Mature	3.2 a	1.4 a	0.23 b	0.50 b	0.75 b	0.24 b	0.32 b	0.91	0.12 ab
	Overmature	2.8 b	1.2 b	0.26 a	0.56 a	0.79 a	0.21 c	0.26 c	0.88	0.10 b
	n[d]	20	20	20	20	20	20	20	20	20
	Storage tech. (St)									
	LH	1.6 e[c]	0.70 e	0.27 a	0.61 a	0.73 b	0.23 a	0.15 d	1.6 a	0.19 a
	HH	3.2 c	1.5 c	0.22 b	0.47 b	0.74 b	0.25 a	0.34 b	0.70 c	0.10 b
	CA5	4.5 a	2.0 a	0.20 c	0.41 c	0.70 c	0.27 a	0.47 a	0.56 d	0.10 b
	CA10	3.8 b	1.7 b	0.21 b	0.45 b	0.73 b	0.25 a	0.39 b	0.63 cd	0.09 b
	CA20	2.2 d	0.97 d	0.27 a	0.57 a	0.83 a	0.19 b	0.22 c	0.90 b	0.09 b
	n	12	12	12	12	12	12	12	12	12
	P-value									
	M	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.225	0.012
	St	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	M X St	<0.001	<0.001	0.002	<0.001	0.06	0.03	<0.001	0.273	0.930
'Rahi'	Maturity (M)									
	Immature	2.9 a[c]	1.4 a	0.31 b	0.62 b	0.90 b	0.26 a	0.32 a	0.80	0.11 a
	Mature	2.8 b	1.2 b	0.34 a	0.66 a	0.91 ab	0.21 b	0.26 b	0.77	0.09 b
	Overmature	2.6 b	1.1 c	0.34 a	0.68 a	0.92 a	0.19 c	0.22 c	0.80	0.09 b
	n	20	20	20	20	20	20	20	20	20
	Storage tech. (St)									
	LH	1.9 d[c]	0.84 c	0.34 ab	0.69 ab	0.87 c	0.24 a	0.19 c	1.5 a	0.16 a
	HH	3.3 a	1.5 a	0.29 c	0.61 c	0.89 bc	0.22 ab	0.32 a	0.69 c	0.08 b
	CA5	3.4 a	1.5 a	0.31 c	0.62 c	0.90 b	0.22 ab	0.31 a	0.55 c	0.08 b
	CA10	2.9 b	1.3 b	0.33 b	0.66 b	0.94 a	0.21 b	0.29 a	0.61 bc	0.08 b
	CA20	2.3 c	1.0 c	0.37 a	0.70 a	0.94 a	0.19 c	0.22 b	0.87 b	0.08 b
	n	12	12	12	12	12	12	12	12	12
	P-value									
	M	<0.001	<0.001	<0.001	<0.001	0.03	<0.001	<0.001	0.314	<0.001
	St	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	M X St	0.01	<0.001	0.001	0.02	0.193	0.04	0.003	0.07	0.015

[a]Texture Profile Analysis measured hardness (BH), hardness slope (BHS), resilience (BR), cohesiveness (BCo), and springiness (BSp), on ten berries for each replicate.
[b]Penetration test measured force at skin break (FSk), skin break slope (SSk), displacement at skin break (DSk), and work-energy at skin break (WSk), on ten berries for each replicate.
[c]Maturity or storage technology data followed by the same letter within each cultivar were not statistically significant according to Tukey-HSD (P-value < 0.05).
[d] n = number of replicates for each treatment factor.

Hardness slope and skin break slope provided good differentiation of the treatment levels on each factor (harvest maturity and storage technology), while the interaction between storage maturity and storage technologies was also significant, justifying further investigation. Box-and-whisker plots were created to visualise populations of forty berries (10 berries X 4 replicates) of each harvest maturity by storage technology combination for both mechanical parameters (**Fig. 3.3**).

The hardness slope and skin break slope provided similar inferences for the influence of the storage treatments on 'Nui' **(Fig. 3.3 A, B)**. The lowest hardness slope and skin break slope were observed for berries stored in low relative humidity air and CA of 20 kPa CO_2. These results were consistent for all the harvest maturities, except immature berries, that were less susceptible to being affected by the negative effect of high CO_2 because no differences were observed between high relative humidity air and 20 kPa CO_2 storage. On the other hand, overmature berries were less prone to show an increase in hardness slope or skin break slope due to 5 kPa CO_2, as the immature and mature berries do. In addition, overmature berries stored in 5 kPa CO_2 showed the highest data dispersion, while immature and mature berries stored in high relative humidity air and 5 kPa CO_2 showed the lowest dispersion. Remarkably, overmature berries showed lower results than immature berries when stored in 5 kPa CO_2, but not when stored in high or low relative humidity air.

For 'Rahi', hardness slope and skin break slope results were similar to 'Nui'. However, both methods provided slight differences in treatments effect. The positive effect of CA of 5 kPa CO_2 was shown by immature berries, but only the hardness slope provides differences between 5 kPa CO_2 and high relative humidity air (**Fig. 3 C, D**). The highest dispersion was observed in low relative humidity air treatment for hardness slope data.

In summary, all the harvest maturities stored in low relative humidity air or CA of 20 kPa CO_2 resulted in a lower hardness slope and skin break slope compared to high relative humidity air. These results exclude the immature 'Nui' where no differences were observed between high relative humidity air and 20 kPa CO_2. In addition, overmature berries of 'Nui' and 'Rahi' did not show the positive effect of 5 kPa CO_2 on hardness slope results, which was observed for less mature berries.

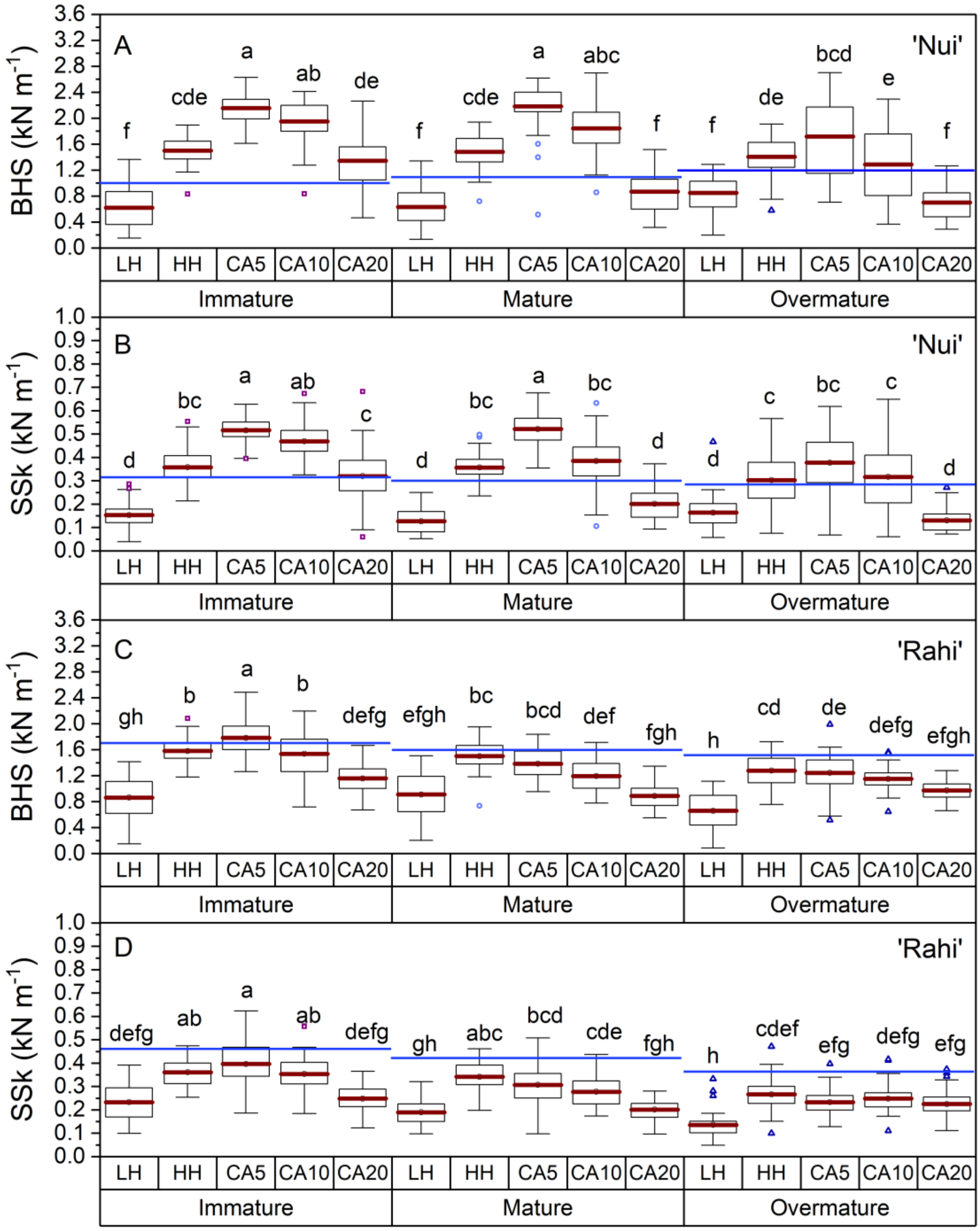


Fig. 3.3. Hardness slope (A,C, BHS) and skin break slope (B,D, SSk) of 'Nui' (A-B) and 'Rahi' (C,D) blueberries stored for 42 d at 4.6 °C, as influenced the interaction of harvest maturity (immature = 60-90 % blue, mature = 100 % blue, and overmature = 100 % blue + extra time period on plant) and storage technology (LH = low relative humidity air; HH = high relative humidity air; CA5 = 4 kPa O_2 + 5 kPa CO_2; CA10 = 4 kPa O_2 + 10 kPa CO_2; CA20 = 4 kPa O_2 + 20 kPa CO_2). Data of 40 berries for each treatment combination is presented as a boxplot where the IQR box contains the observation between the 1st (25th percentile) and 3rd quartile (75th percentile). The horizontal line inside each box represents the mean. Whiskers are calculated as the 1.5 of IQR, and outliers are represented by any value (dot) shown outside of the whisker range. Statistical analysis was performed using the average of the ten berries for each of the four replicates. Means with the

same letters were not statistically significant according to Tukey´s HSD test (P-value < 0.05). Solid blue line indicates the mechanical parameter value at harvest.

3.3.6. Berry softening and firming effect during the storage period

The ability to differentiate softening and firming throughout the storage period was evaluated by mechanical parameters of hardness slope, skin break slope, and displacement at skin break on 'Nui' and 'Rahi' harvested at the mature stage (**Fig. 3.4**). Only these three mechanical parameters were used due to the good ability to separate the storage technology effects (**Table 3.5**).

Berry firming was detected when an increase of hardness slope or skin break slope was recorded during the storage period or when a reduction of the displacement to skin break occurred. For 'Nui', hardness slope and skin break slope showed a firming effect in the berries stored in high relative humidity air and CAs of 5 kPa and 10 kPa CO_2 (**Fig. 3.4 A,B**). While displacement at skin break described berry firming in 5 kPa CO_2 storage, but not due to high relative humidity (**Fig. 3.4 C**). Firming was not observed to occur in 'Rahi' (**Fig. 3.4 D-F**)

Conversely to the firming effect, softening was detected when the berry hardness slope or skin break slope was reduced or when displacement to skin break increased during storage. For 'Nui', softening was observed on the three mechanical parameters when berries were stored in the low relative humidity air (**Fig. 3.4 A-C**). In addition, skin break slope evaluation determined the softening effect in 20 kPa CO_2 storage (**Fig. 3.4 B**). For 'Rahi', the hardness slope showed softening as induced by low relative humidity air and CA of 10 kPa and 20 kPa CO_2 (**Fig. 3.4 D**). Skin break slope and displacement at skin break indicated berry softening for all storage technologies (**Fig. 3.4 E-F**).

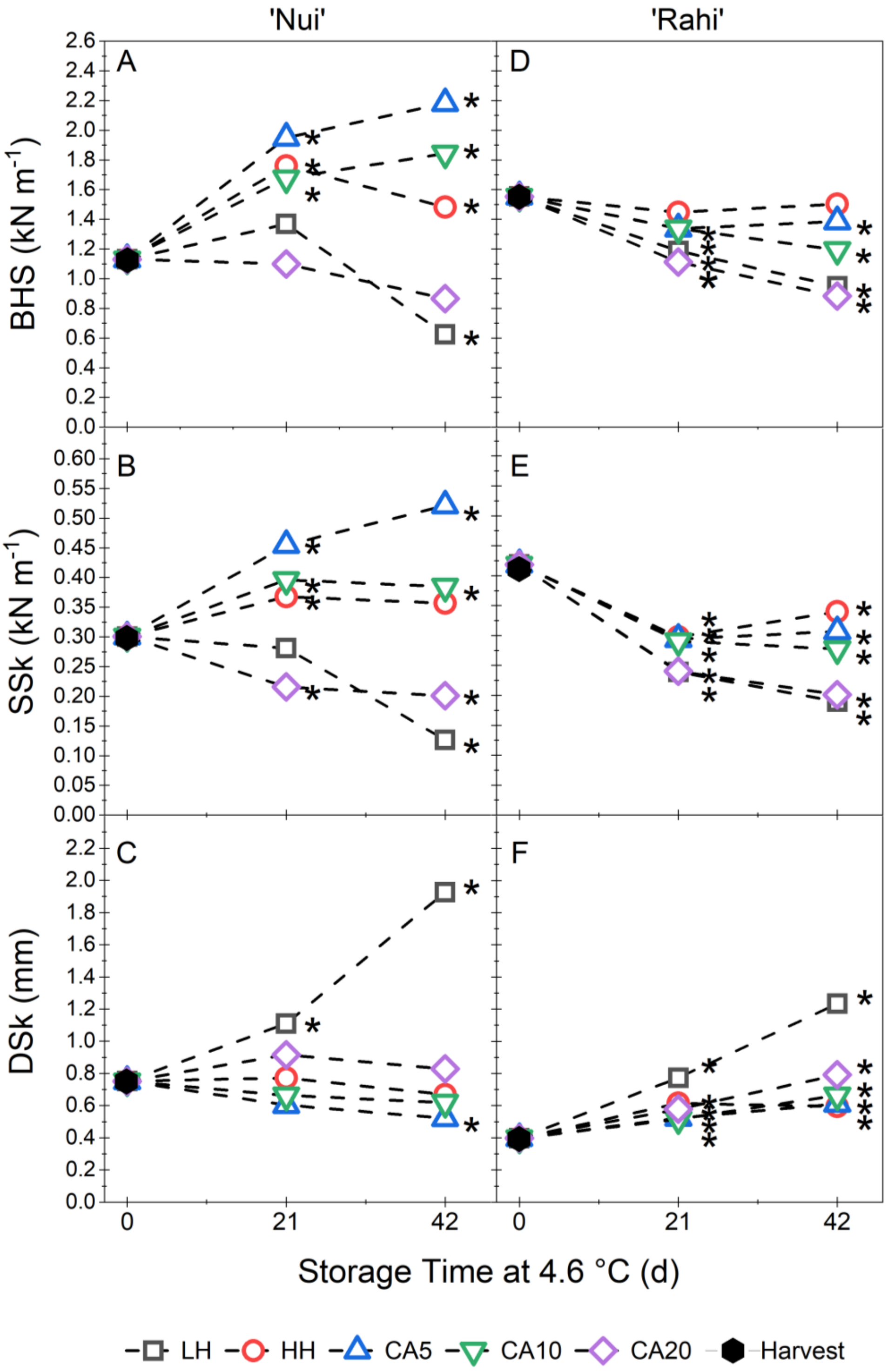


Fig. 3.4. Variation of the average hardness slope (BHS, A and D), skin break slope (SSK, B, and E), and displacement at skin break (DSk, C and F) as influenced by the storage technologies of low relative humidity air (LH); high relative humidity air (HH), CA of 4 kPa O_2 + 5 kPa CO_2 (CA5); CA of 4 kPa O_2 + 10 kPa CO_2; (CA10); CA of 4 kPa O_2 + 20 kPa CO_2 (CA20) on 'Nui' (A-C) and 'Rahi' (D-F) harvested at the mature stage (100 % blue). Mechanical parameters were evaluated at harvest day and after 21 d and 42 d of storage at 4.6 °C. Dots accompanied by an asterisk symbol (*), indicate a difference to harvest day (0 d) evaluation, according to Dunnet's test (P-value <0.05).

3.4. Discussion

This research chapter identifies those mechanical methodologies of texture profile analysis and penetration tests that can be used to separate blueberries with different textural properties after postharvest storage. However, mechanical parameters identified varied depending on the source of variation impacting the blueberry texture, including the harvest maturity and storage technology. In addition, this study supports the results of **Chapter 2**, where the displacement at skin break and hardness slope were strong descriptors of textural changes related to water loss.

The mechanical parameters that better separate the influence of harvest maturity and controlled atmosphere technology are discussed to identify a mechanical test that can measure firmness on blueberry quality evaluations.

3.4.1. Harvest maturity

As the harvest maturity progressed from immature (60-90 % blue) to overmature (100 % blue + time on the plant) blueberries, a decrease in force at berry skin break was observed at harvest (**Table 3.4)** and after 42 d of storage (**Table 3.5**). The force at skin break or skin toughness is the force (N) required to penetrate or rupture the berry skin. Previously, only the studies of Vicente et al. (2007) and Chea et al. (2019) differentiated blueberry maturities by evaluating the maximum force obtained when using a small diameter (2 mm) probe of a flat surface tip. However, neither of these earlier studies indicated if the maximum force was achieved before or after the berry skin was ruptured. In addition, no studies differentiating blueberry maturities have previously been conducted using a needle probe (**Table 3.1**). Only Vance et al. (2017) have suggested that lower skin toughness can potentially be observed on blueberries picked at a more advanced stage of maturity. Consequently, to the author's knowledge, this is the first study formally showing that force at skin break (or skin toughness) by needle penetration can differentiate blueberry maturities.

Penetration tests using a needle probe applied on tomato (Sirisomboon et al., 2012), and Japanese pears (Sirisomboon et al. 2000a), have demonstrated a similar force reduction at skin break with advancing maturity. However, for other small berries such as grapes, the force at skin break was found not to provide reliable differentiation between harvest maturities for 'Red Globe' and 'Crimson seedless' (Segade et al., 2013), or 'Cabernet Franc' (Maury et al., 2009). Conversely, Maury et al. (2009) postulated that growing conditions for grapes could be more influential on skin penetration test results in comparison to maturity. To the author's knowledge, only Vance et al. (2017) have determined skin toughness using a needle penetration as influenced by preharvest conditions (i.e., growing location and calcium management) on blueberries. However, whether and what preharvest growing conditions influence the force at skin break of blueberries required further investigation.

Changes leading to softening during maturation are likely to be related to modifications of the cell wall components (Proctor and Miesle, 1991; Vicente et al., 2007). Vicente et al. (2007) described depolymerisation of pectin and hemicellulose and solubilisation of arabinose leading to cell wall changes occurring on 'Duke' blueberry between 75 % blue and blue ripe fruit stages. In addition, Proctor and Miesle (1991) observed that the polygalacturonase enzyme activity, which catalyses the hydrolysis of de-esterified pectins, was higher at the blue-red stage for highbush blueberry 'Collins'. Chea et al. (2019), studied the ripening changes occurring between ripening stages of pale green and dark blue fruit of 'Bluecrop', and observed a strong correlation between maximum force by a 2 mm flat probe and water-soluble pectins ($r = -0.94$) or HCl-soluble pectins ($r = 0.99$).

This work demonstrated that the use of force at skin break might detect maturity differences at advanced maturities commonly found in commercial harvest and hence has the potential to destructively assist the assessment of maturity within commercial operations. However, force at skin break was in the low range of measurable forces (i.e., 0.08 - 0.45 N), and the average differences between maturities ranged between 0.027 – 0.058 N. Consequently, an instrumental device with a high resolution (≤ 0.01 N) and sensitivity such as the texture analyser, is required to measure this mechanical parameter accurately. Texture analysers are expensive devices and commonly used in scientific contexts and may not represent a realistic investment for commercial evaluations due to the high cost and low operational speed. Alternatively, using a small cylindrical flat tip probe (e.g., 2-4 mm diameter) that can potentially pierce the skin to detect maturity differences on a higher force range (when compared to a needle probe) remains to be studied using low-cost and rapid equipment.

On the other hand, hardness slope or chord stiffness, defined as the slope of the chord drawn between two specific points on the force-deformation curve (Ballinger, 1973; Slaughter and Rohrbach, 1985), may correspond to a more suitable parameter for commercial operations. Chord stiffness has the potential to be adapted on texture instrumental devices with less resolution and sensitivity, such as the FirmTech device.

FirmTech firmness is calculated as the slope of the straight line between a minimum (e.g., 0.15 N) and maximum (e.g., 1.96 N) force predefined by the operator (Prussia et al., 2006; Moggia et al., 2016; 2018). While hardness slope calculated in our study considers the force between the trigger force (0.06 N) and force at compression deformation (i.e., 15 % strain). Prussia et al. (2006), using rubber balls, have reported that the minimum and maximum force selected to calculate the slope of force-deformation can significantly influence the output result.

On the other hand, the chord stiffness calculation and interpretation may be highly dependent on the shape (straight or curved upward) of the force/deformation loading. Loading of first downstroke compression obtained using a slow test speed of 1 mm s^{-1} in TPA mainly showed a linear increase of force as the berry is deformed up to 15 % of the equatorial diameter, except when blueberries were stored in low relative humidity air (**Fig. 2 A, B**).

The authors suggest that it is worth studying the use of FirmTech to detect maturity differences as a low-cost device. Moggia et al. (2016, 2018) observed differences between maturity stages using the FirmTech firmness. However, the hardness slope results were inconsistent between the evaluations performed at harvest and after storage (**Tables 3.4** and **3.5**). Hence, based on these results and the previously reported data (**Table 3.1**), the loading slope (or chord stiffness) can only be recommended to analyse the maturity differences after postharvest storage. In addition, if the FirmTech device can detect maturities, differences remain to be studied when stored under contrasting humidities air.

3.4.2. Storage technologies

Controlled atmosphere storage induced differences in mechanical parameters, being hardness slope, skin break slope and displacement at skin break the most remarkable (**Table 3.5, Fig. 3.4**).

The hardness slope was reduced as CO_2 increased from 5 kPa to 20 kPa (**Fig. 3.3 A, C**), with 20 kPa CO_2 always having less hardness slope than high relative humidity air storage. These results agree with previous findings (**Table 3.2**). Harb and Streif (2004) observed a reduction of FirmTech firmness as CO_2 increased from 6 kPa to 18 kPa. In addition, the use of higher CO_2 (between 15 kPa and 25 kPa), compared to in air storage, commonly induced a lower maximum force (N) to a given compression distance (Schotsman et al., 2007; Cantin et al., 2012), lower FirmTech firmness (Allan-Wojtaset al., 2001; Forney et al., 2003; Harb and Streif, 2004), or lower durometer firmness (Alsmairat et al., 2011; Rodriguez and Zoffoli, 2016). In addition, Rodriguez and Zoffoli (2016) observed an increase in the percentages of soft blueberries (evaluated by hand-touch firmness) when storage CO_2 is 15 kPa, compared to air control.

The controlled atmosphere (CA) can also induce a beneficial effect on mechanical parameters. In this chapter, 5 kPa CO_2 induced better mechanical parameter results than 10 kPa CO_2 and high relative humidity air (**Table 3.5, Fig. 3, 4**). In agreement, benefits on mechanical properties caused by CA storage have previously been reported for highbush 'Brigitta' (Paniagua et al., 2014) and 'Duke' (Harb and Streif., 2014; Falagan et al., 2020) when CO_2 was 6-10 kPa.

A penetration test can also be used to study the effect of CA on blueberries (**Table 3.5**). Skin break slope and displacement at skin break provided the best differentiation of the berries stored in different CO_2 levels (**Table 3.5**). Previous research that used a penetration test to evaluate the CA effect has reported only the maximum force to penetrate the skin (**Table 3.2**), previously named skin toughness (Forney et al., 2003), or force required to tear or penetrate the blueberry skin (Duarte et al., 2009; Concha-Meyer et al., 2015). The force at skin (FSk) break using a needle probe differentiates the detrimental effect of very high CO_2 (20 kPa) storage but does not differentiate the improvement induced by 5 kPa CO_2 (**Table 3.5**). In agreement, blueberries stored at 0 °C (Duarte et al., 2009) and 12 °C (Concha-Meyer et al., 2015) showed lower maximum force to penetration when stored in CA of 15 kPa CO_2. In addition, Forney et al. (2003) observed that mechanical parameters of Firmtech firmness and skin toughness were lower for elevated CO_2 (15 kPa) storage when compared to regular air storage. However, the lowest skin toughness was observed when CO_2 was set at 15 kPa and FirmTech firmness when CO_2 was ≥ 20 kPa.

Mechanical parameters of hardness, hardness slope, resilience, and cohesiveness obtained by TPA; and displacement at skin break, skin break slope, and skin break energy obtained by penetration test differentiated the berries stored in low relative humidity air from the berries held in high relative humidity air (**Table 3.5**). In concordance with **Chapter 2** results, low relative humidity storage induced lower hardness slope values and higher skin break displacement. In addition, the highest Pearson correlation coefficient with water loss (r = 0.92) was observed for displacement at skin break (**Supplementary Table 1** and **Supplementary Table 2**). Consequently, displacement at skin break might be considered a good physical measure of how water loss during storage has influenced mechanical changes. However, displacement at skin break was not good at describing berry firming effects that may be caused in high relative humidity storage conditions or when a minimal water loss occurs (**Fig. 3.4**).

The force at skin break (N) obtained using the penetration test is achieved by the combined force responses of the blueberry flesh and skin (Silva et al., 2005). In this chapter, to remove the effect of the skin on the maximum force to penetration, the penetration test was also conducted using peeled blueberries. The flesh firmness (N) was measured on 'Nui' blueberries of the mature stage stored in each of the five storage technologies. Mechanical methods and data analysis are described in the appendices of this chapter (see *3.9.4. Penetration test on unpeeled 'Nui' blueberries*).

When the penetration test was conducted on unpeeled berries, the maximum force or force at skin break could only differentiate the detrimental effect of high CO_2 (20 kPa) CA storage, but not the textural benefits displayed when blueberries are stored in CA of 5 kPa CO_2 (**Table 3.5**). However, when the penetration test was conducted on a peeled portion of 'Nui' blueberries, the flesh firmness (N) showed a higher force of the blueberries stored in CA of 4 kPa O_2 + 5 kPa CO_2 compared to blueberries stored in HH (**Fig. 3.5**). Similarly, when the penetration test was conducted on peeled blueberries, the maximum flesh force was lower on blueberries stored in LH than in HH, but no differences were observed when the penetration test was conducted on unpeeled blueberries (**Fig. 3.5**). In addition, a negligible relationship between force at skin break and water loss was observed in **Chapter 2** results (**Fig 2.4**).

Based on these results, the author postulates that textural changes induced by water loss and CA storage mainly occur in the blueberry flesh tissue (**Fig 3.5**). Except for the detrimental effect of CA in higher CO_2 (20 kPa), the results suggested that textural changes induced by very high CO_2 also occur in the blueberry skin (**Fig 3.5**). Whether these hypotheses can be demonstrated by measuring the mechanical properties of an isolated skin of blueberries stored on different technologies requires further investigation.

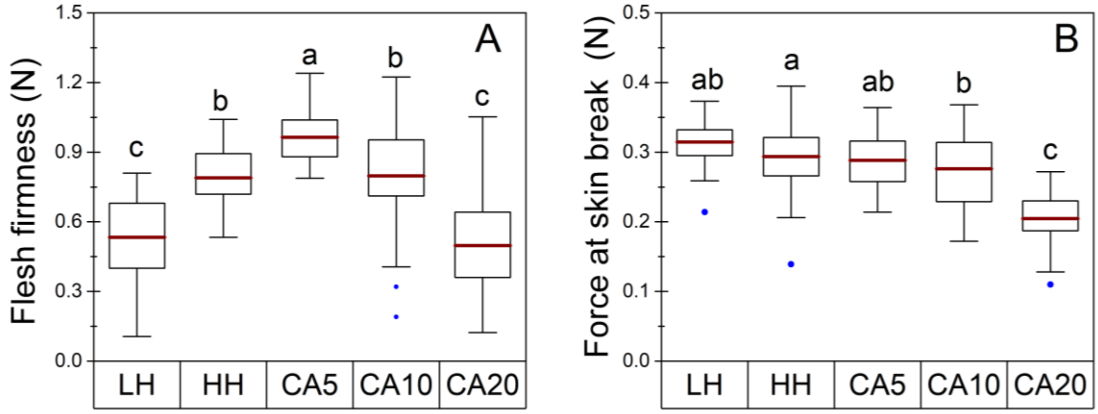


Fig. 3.5. Flesh firmness measured on unpeeled 'Nui' blueberries (A) and force at skin break of unpeeled 'Nui' blueberries (B) stored for 42 d in four storage technology (LH = low relative humidity air; HH = high relative humidity air; CA5 = 4 kPa O_2 + 5 kPa CO_2; CA10 = 4 kPa O_2 + 10 kPa CO_2; CA20 = 4 kPa O_2 + 20 kPa CO_2). Data of 40 berries for each treatment is presented as a boxplot where the IQR box contains the observation between the 1st (25th percentile) and 3rd quartile (75th percentile). The horizontal line inside each box represents the mean. Whiskers are calculated as the 1.5 of IQR, and outliers are represented by any value (dot) shown outside of the whisker range. Statistical analysis was performed using the average of the ten berries for each of the four replicates. Means with the same letters were not statistically significant according to Tukey´s HSD test (P-value < 0.05).

The present study results suggest that a CA of 5 kPa CO_2 + 4 kPa O_2 combined with high relative humidity can benefit the retention of mechanical parameters of blueberries (**Fig. 3.3**), with even a significant firming effect observed for mature 'Nui' blueberries (**Fig. 3.4**). Modified atmosphere packaging (MAP) is a commercially available technology that can provide these optimum storage conditions when CO_2 inside commercial blueberry MAPs stabilise between 4 % and 8 % (Rodriguez et al., 2016; Koort et al., 2018; Saito et al., 2020). In addition, water loss lower than 1.0 % has previously been reported for blueberries stored in MAP for up to 3 weeks at 1 °C (Saito et al., 2020) or 45 d at 0 °C (Rodriguez and Zoffoli, 2016). However, due to the high RH induced by MAP and the risk of temperature fluctuations during commercial storage conditions, favourable environmental conditions for disease expression may be a risk in MAP. Decay risk is specifically a threat when maximum CO_2 inside MAP is not high enough to produce rot control. For example, Moggia et al. (2014) reported a maximum CO_2 of 2 % using low-density polyethylene MAP on 'Brigitta' blueberries, while adequate rot control was observed when atmospheric CO_2 is higher than 10-12 kPa (Harb and Streif, 2004; Alsmairat et al., 2011; Cantin et al., 2012).

3.4.3. Interaction effect of maturity and storage technology

The combined effects of harvest maturity and storage technology indicated that immature berries could retain a higher hardness slope and skin break slope than overmature berries after storage (**Table 3.5, Fig. 3.3**). In addition, immature berries result in more textural benefits when using CA of 4 kPa O_2 + 5 kPa CO_2 than overmature berries (**Fig. 3.3**). However, immature berries can present higher water loss through storage (**Fig. 3.1**).

Many authors have shown the influence of harvest maturity on the postharvest firmness performance of blueberries (Ballinger et al., 1978; Beaudry et al., 1998; Hancock et al., 2008; Moggia et al., 2016; Lobos et al., 2018). For example, Moggia et al. (2016) determined an interaction between the maturity stage and the use of a bag for moisture control on firmness retention after storage of 'Brigitta' blueberries. Higher Firmtech firmness was obtained in immature (75 % blue) and mature blueberries (100 % blue) under bag conditions (high RH), while overmature 'Brigitta' did not benefit from high relative humidity storage. In the present study, high relative humidity air produced a higher hardness slope and skin break slope for all the maturities when compared to low relative humidity air (**Fig. 3.3**).

Previously, only Hancock et al. (2008) stated the role of harvest maturity in influencing the storage life enhancement of CA technology (**Table 2**). The authors postulated that an early stage of ripeness could be less responsive to CA. The results obtained in this study suggest the opposite. Immature blueberries showed more responsivity for CA benefits when maturity influenced response (**Fig. 3.3**). In addition, immature 'Nui' stored in CA of 20 kPa did not show lower hardness slope and skin break slope than berries stored in high relative humidity air, while mature and overmature berries did (**Fig. 3.3 A, B**). The authors believe that the present study is the first demonstration of interaction effects between harvest maturity and controlled atmosphere conditions on the resulting mechanical parameters of blueberry.

The present study considers sources of textural variation with different potential mechanisms of induction of mechanical changes. Mechanical changes induced by low storage relative humidity (or high WL) can be explained by a reduction of internal turgor pressure (Giongo et al., 2013; Panigua et al., 2013). In addition, Li et al. (2021) described a positive relationship (r = 0.69) between water loss (%) and the first cellular layer thickness (μm) immediately beneath the blueberry skin. On the other hand, the mechanical changes induced by harvest maturity can be explained by cell wall degradation (Vicente et al., 2007). Similarly, the enhancement of mechanical parameters caused by CA storage may be associated with microstructural modifications regulated by cell-to-cell adhesion and cell wall strength, as described for other fruit species such as strawberries (Harker et al., 2000). Allan-Wojtas et al. (2001) reported that storage in 15 % CO_2 inhibited the blueberry firming effect, and this CA effect is probably related to a thickening of the parenchyma cell wall in the flesh tissue. Consequently, the influence of CA on the blueberry primary cell wall and middle lamella components requires further investigation. In addition, the mechanism explaining the effect of CA on mechanical parameters is a challenge when considering that CA composition and blueberry maturity at harvest can both induce effects on the results (**Fig. 3.3**).

The present study results suggest that the loading slope of the force/deformation graph obtained by a plate compression or by penetration compression test can enable the separation of stored blueberries of different populations independent of the mechanism of induction of the textural change (**Fig. 3.3**). Hardness slope and skin break slope were identified as the parameters that allowed the best differentiation of the combined effect of harvest maturity and storage technology.

Hardness slope and skin break slope are calculated as the slope of a force/deformation graph. Due to dramatic probe shape differences (plate vs. needle) and how the maximum forces are achieved (strain % vs. force at irreversible rupture point), these methods remain different. However, whether the maximum force is achieved by a target strain (TPA) or due to maximum force to skin penetration may not be relevant in the loading slope outcome irrespective of the probe shape. On the other hand, when blueberries are stored in low relative humidity air, a curved force/deformation loading was described (**Fig. 3.2 B, D**). Consequently, a controlled target (i.e., fixed strain) may better measure the loading slope changes when a high water loss occurs. The authors recommend further study of hardness slope and skin break slope as influenced by CA storage when combined with different storage relative humidity.

3.4.4. Cultivar differences

Highbush 'Nui' and Rabbiteye 'Rahi' are blueberries cultivars obtained by breeding different parental genotypes for each cultivar in New Zealand (Patel and George, 1997; Buck et al., 2012), and hence the two cultivars can be considered genetically distant. Various previous research studies have assessed the mechanical parameters of blueberries using different genotypes as a source of textural variation (Ehlenfeldt and Martin, 2002; Silva et al., 2005; Saftner et al., 2008; Giongo et al., 2013, 2022; Blaker and Olmstead, 2015). In the present study, a formal statistical analysis of the differences between both cultivars was not performed. However, data tendencies are described for each cultivar.

At harvest, a higher hardness slope was observed in rabbiteye 'Rahi' compared to 'Nui'. However, a firming effect during storage was observed to occur for 'Nui' blueberries stored in high relative humidity air, but not in 'Rahi'. Consequently, the hardness slopes after 42 d were similar (**Fig. 3.4 A, D**). These results agree with the observed findings in a previous season using the same cultivars (**Chapter 2**).

Blueberry 'Rahi' also demonstrated less benefit of CA of 5 kPa CO_2, which was only observed in the hardness slope of immature berries (**Fig. 3.3 C**). While for 'Nui', the positive effect of CA 5 kPa was found by higher hardness and skin break slopes on mature and immature berries (**Fig. 3.3 A, B**).

A higher force at skin break for each harvest maturity was observed for 'Nui' compared to 'Rahi' (**Table 3.4**). These differences were maintained after storage evaluation (**Table 3.5**). Hence, force at skin break can be potentially used to evaluate different cultivars. Silva et al. (2005) differentiate blueberry cultivars using skin toughness. Similarly, Blaker and Olmstead (2015) found that maximum force obtained by a penetration test using a 4 mm probe accurately separated blueberry genotypes of crisp from standard texture. Nevertheless, the maturity stage can influence cultivar comparison. For example, an overmature 'Nui' can show the same force at skin break as an immature 'Rahi' (**Table 3.4**).

3.4.5. Mechanical parameters to be used as a firmness standard

An essential step when developing an instrumental method to measure blueberry firmness of fresh fruit is to identify the mechanical parameters that provide information on quality irrespectively of the source of variation (e.g., harvest maturity, storage water loss, controlled atmosphere). This chapter demonstrates that chord stiffness, as measured by hardness slope and skin break slope, provides a confident indication of postharvest blueberry quality (**Fig. 3.3**) and the changes (i.e., softening and firming) throughout the storage period (**Fig. 3.4**). Consequently, these mechanical parameters can assist the blueberry research community and facilitate the development and evaluation of novel postharvest technologies and the understanding of quality changes of stored blueberries. However, these mechanical parameters may also be studied across other sources of variation, such as blueberry genotypes (Cappai et al., 2018) or commercial postharvest treatments such as sulphur dioxide (Cantin et al., 2012; Rivera et al., 2013b; Saito et al., 2020) and ozone (Zhou et al., 2019).

Operational settings (e.g., test speed, trigger force, target mode) to measure chord stiffness may also be standardised to compare the data collected across research and commercial scenarios. Further investigation should focus on standardising instrumental settings that can be used on low-cost and rapid equipment for the blueberry supply chain.

On the other hand, for the commercial blueberry industry, a standard method to evaluate firmness may also be representative of consumer acceptability. Hence, to determine the instrumental threshold associated with consumer acceptance, further investigation should focus on the relationship between these instrumental methods and sensory evaluation of texture (hand-touch and oral mastication parameters) using a formal sensory panel setting.

3.5. Conclusions and recommendations

The combined effects of harvest maturity and storage technology indicated that immature berries could retain higher mechanical properties than overmature berries after storage. In addition, immature berries respond more to a controlled atmosphere, resulting in more textural benefits when using CA of 4 kPa O2 + 5 kPa CO2 compared to overmature berries. However, immature berries can present higher water loss through storage and a lower soluble solid to acidity ratio, increasing risk of water loss driven textural change and potentially impacting the taste profile at consumption and hence consumer acceptability.

This chapter contributes to ongoing research on the identification of suitable mechanical parameters to be used as reliable quality descriptors for blueberries. Mechanical parameters that separated blueberry quality varied by the source of textural variation. The maximum force at berry skin break provided consistent differentiation of the harvest maturity at evaluations performed at harvest and after 42 d of storage. Meanwhile, hardness slope and skin break slope, facilitated a good description of the mechanical changes induced by a controlled atmosphere, while displacement at a skin break best described the textural changes caused by relative humidity differences in air storage. Our recommendations are that the differentiation of stored berries with different textural characteristics should be described by using hardness slope obtained by a plate compression test or by the skin break slope obtained by a needle penetration test. Hence, hardness slope and skin break slope can be used as reliable indicators of the softening/firming occurring throughout the storage period.

The above-mentioned mechanical parameters could be used for commercial or research purposes such as quality checkpoint operations or assessment of postharvest technology treatments. However, the present study does not attempt to relate the measured mechanical properties to consumer perception or suggest quantitative indicators of ranges associated with consumers' sensory acceptance of quality. Consequently, future research should identify the data ranges of these instrumental measures related to consumer perceived a sensory 'soft' and 'firm' berry, in order to determine the potential thresholds of quality acceptance.

3.6. Acknowledgment

The authors acknowledge Gourmet Blueberries Ltd. for providing 'Nui' and 'Rahi' blueberries; Francisca Ducommun for the help provide in organising the plant selection and fruit sampling at the commercial plantation; Talon Sneddon, Peter Jeffery, and Sue Nicholson for the help provided at the experimental setup and during the evaluations in Postharvest Laboratory, Massey University. Sebastian Rivera acknowledges the School of Agriculture and Environment, Massey University (NZ), and Agencia Nacional de Investigacion y Desarollo (ANID), Chile for the financial support to pursuing Ph.D. studies at Massey University.

3.7. Author Statement

Sebastian Rivera: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing - Original draft; **Huub Kerckhoffs**: Conceptualization, Supervision, Writing - Review and Editing; **Svetla Sofkova-Bobcheva**: Supervision, Writing - Review and Editing; **Dan Hutchins**: Resources, Supervision, Writing - Review and Editing; **Andrew East**: Conceptualization, Methodology, Resources, Supervision, Writing - Review and Editing.

3.8. References

Abbott, H.A., 1999. Quality measurements of fruits and vegetables. Postharvest Biol. Technol. 15, 207-225. https://doi.org/10.1016/S0925-5214(98)00086-6.

Allan-Wojtas, P.M., Forney, C.F., Carbyn, S.E., Nicholas, K.U.K.G., 2001. Microstructural indicators of quality-related characteristics of blueberries-an integrated approach. Lebensm.-Wiss. U.-Technol. 34, 23-32. https://doi.org/10.1006/fstl.2000.0738

Alsmairat, N., Contreras, C., Hancock, J., Callow, P., Beaudry, R., 2011. Use of combinations of commercially relevant O2 and CO2 partial pressures to evaluate the sensitivity of nine highbush blueberry fruit cultivars to controlled atmospheres. HortScience 46(1), 74-79. https://doi.org/10.21273/HORTSCI.46.1.74

Ballinger, W.E., Kushman, L.J., Hamann, D.D., 1973. Factors affecting the firmness of highbush blueberries. J. Amer. Soc. Hort. Sci. 98(6), 583-587.

Ballinger, W.E., Maness, E.P., McClure, W.F., 1978. Relationship of stage of ripeness and holding temperature to decay development of blueberries. J. Amer. Soc. Hort. Sci. 103(1), 130-134.

Beaudry, R.M., Moggia, C.E., Retamales, J.B., Hancock, J.F., 1998. Quality of 'Ivanhoe' and 'Bluecrop' blueberry fruit transported by air and sea from Chile to North America. HortScience, 33(2), 313-317.

Blaker, K.M., Olmstead, J.W., 2015. Cell wall composition of the skin and flesh tissue of crisp and standard texture southern highbush blueberry genotypes. J. Berry Res. 5(1), 9-15. http://dx.doi.org./10.3233/JBR-140085

Buck, E.J., Scalzo, J., Wiedow, C., Hurst, R., Allan, A. C., McGhie, T.K., Bassil, N.V., Rowland, L.J., 2012. Progress in blueberry research in New Zealand. Int. J. Fruit Sci. 12(1-3), 304-315. https://doi.org/10.1080/15538362.2011.619444

Cantin, C.M., Minas, I.S., Goulas, V., Jimenez, M., Manganaris, G.A., Michailides, T.J., Crisosto, C.H., 2012. Sulfur dioxide fumigation alone or in combination with CO_2-enriched atmosphere extends the market life of highbush blueberry fruit. Postharvest Biol. Technol. 67, 84-91. https://doi.org/10.1016/j.postharvbio.2011.12.006

Cappai, F., Benevenuto, J., Ferrao, L.F.V., Munoz, P., 2018. Molecular and genetic bases of fruit firmness variation in blueberry - A review. Agronomy 8, 174. https://doi.org/10.3390/agronomy8090174

Catuneanu, I.B., Badulescu, L., Dobrin, A., Stan, A., Hoza, D., 2017. The influence of storage in controlled atmosphere on quality indicators of three blueberries varieties. Horticulture (61), 91-100.

Ceponis, M.J., Cappellini, R.A.. 1983. Control of Post-Harvest Decays of Blueberries by Carbon-Dioxide Enriched Atmospheres. Plant Disease, 67(2), 169-171.

Chea, S., Yu, D., Park, J., Oh, H.D., Chung, S.W., Lee, H.J., 2019. Fruit softening correlates with enzymatic and compositional changes in fruit cell wall during ripening in 'Bluecrop' highbush blueberries. Sci. Hortic. 245, 163-170. https://doi.org/10.1016/j.scienta.2018.10.019

Chiabrando, V., Giacalone, G., 2011. Shelf-life extension of highbush blueberry using 1-methylcyclopropene stored under air and controlled atmosphere. Food Chem. 126(4), 1812-1816. https://doi.org/10.1016/j.foodchem.2010.12.032

Concha-Meyer, A., Eifert, J.D., Williams, R.C., Marcy, J.E., Welbaum, G.E., 2015. Shelf life determination of fresh blueberries (Vaccinium corymbosum) stored under controlled atmosphere and ozone. Inter. J. Food Sci. 2015, 164143. https://doi.org/10.1155/2015/164143

Duarte, C., Guerra, M., Daniel, P., Lopez Camelo, A., Yommi, A., 2009. Quality changes of highbush blueberries fruit stored in CA with different CO_2 levels. J. Food Sci. 74(4), 154-159. https://doi.org/10.1111/j.1750-3841.2009.01118.x

Ehlenfeldt, M.K., Martin, R.B., 2002. A survey of fruit firmness in Highbush blueberry and species-introgressed blueberry cultivars. Hortscience 37, 386-389. https://doi.org/10.21273/HORTSCI.37.2.386

Falagan, N., Miclo, T., Terry, L.A., 2020. Graduated controlled atmosphere: a novel approach to increase 'Duke' blueberry storage life. Front. Plant Sci. 11. https://doi.org/10.3389/fpls.2020.00221

Falagan, N., Terry, L.A., 2018. Recent advances in controlled and modified atmosphere of fresh produce postharvest technologies to reduce food waste and maintain fresh produce quality. Johnson Matthey Technol. Rev. 62, 107-117. https://doi.org/10.1595/205651318X696684

Forney, C.F., Jordan, M.A., Nicholas, KUKG, 2003. Effect of CO_2 on physical, chemical, and quality changes in 'Burlington' blueberries. Acta Horticul. 600, 587-593.

Forney, C.F., 2009. Postharvest issues in blueberry and cranberry and methods to improve market-life. Acta Hortic. 810 (2), 785-798. https://doi.org/10.17660/ActaHortic.2009.810.105

Gilbert, J.L., Schwieterman, M.L., Coloqhoun, T.A., Clark, D.G., Olmstead, J.W., 2013. Potential for increasing southern highbush blueberry flavor acceptance by breeding for major volatile components. HortScience 48(7), 835-843. https://doi.org/10.21273/HORTSCI.48.7.835

Giongo, L., Poncetta, P., Loretti, P., Costa, F., 2013. Texture profiling of blueberries (*Vaccinium* spp.) during fruit development, ripening and storage. Postharvest Biol. Technol. 76, 34-39. https://doi.org/10.1016/j.postharvbio.2012.09.004

Giongo, L., Ajelli, M., Pottorff, M., Perkins-Veazie, Iorizzo, M., 2022. Comparative multi-parameters approach to dissect texture subcomponents of highbush blueberry cultivars at harvest and postharvest. Postharvest Biol. Technol. 183, 111696. https://doi.org/10.1016/j.postharvbio.2021.11696

Hancock, J., Callow, P., Serce, S., Hanson, E., Beaudry, R., 2008. Effect of cultivar, controlled atmosphere storage, and fruit ripeness on the long-term storage of highbush blueberries. HortTechnology 18(2), 199-205. https://doi.org/10.21273/HORTTECH.18.2.199

Harb, J.Y., Streif, J., 2004. Controlled atmosphere storage of highbush blueberries cv 'Duke'. Eur. J. Hort. Sci, 69(2), 66-72.

Harker, F.R., Elgar, H.J., Watkins, C.B., Jackson, P.J., Hallett, I.C. 2000. Physical and mechanical changes in strawberry fruit after high carbon dioxide treatments. Postharvest Biol. Technol. 19(2), 139-146. https://doi.org/10.1016/S0925-5214(00)00090-9

Koort, A., Moor, U., Poldma, P., Kaiser, C., Starast, M., 2018. Comparison of regular atmospheric storage versus modified atmospheric packaging on postharvest quality of organically grown lowbush and half-highbush blueberries. Sustainability 10(11), 3916. https://doi.org/10.3390/su10113916

Li, M., Rivera, S., Franklin, D., Nowak, E., Hallett, I., Kolenderska, S., Urbanska, M., Vanholsbeeck, East, A., 2021. Use of optical coherence tomography and light microscopy for characterisation of mechanical properties and cellular level responses of 'Centurion' blueberries during weight loss. J. Food Eng. 303, 1110596. https://doi.org/10.1016/j.jfoodeng.2021.110596

Lin, Y., Huang, G., Zhang, Q., Wang, Y., Dia, V.P., Meng, X., 2020. Ripening affects the physicochemical properties, phytochemicals, and antioxidant capacities of two blueberry cultivars. Postharvest Biol. Technol. 162, 111097. https://doi.org/10.1016/j.postharvbio.2019.111097

Lobos, G.A., Bravo, C., Valdes, M., Graell, J., Ayala, I. L., Beaudry, R.M., Moggia, C., 2018. Within-plant variability in blueberry (*Vaccinium corymbosum* L.): maturity at harvest and position within the canopy influence fruit firmness at harvest and postharvest. Postharvest Biol. Technol. 146, 26-35. https://doi.org/10.1016/j.postharvbio.2018.08.004

Lobos, G.A., Callow, P., Hancock, J.F., 2014. The effect of delaying harvest date on fruit quality and storage of late highbush blueberry cultivars (*Vaccinium corymbosum* L.). Postharvest Biol. Technol. 87, 133-139. https://doi.org/10.1016/j.postharvbio.2013.08.001

Lu, R., Abbot, J.A., 2004. Force/Deformation techniques for measuring texture, In: Kilcast, D. (Ed.), Texture in food Volume 2, Solid foods. Woodhead Publishing Limited, England.

Maury, C., Madieta, E., Moigne, M.l., Mehinagic, E., Siret, R., Jourjon, F., Le Moigne, M., 2009. Development of a mechanical texture test to evaluate the ripening process of 'Cabernet Franc' grapes. J. Text. Stud. 40(5), 511-535. https://doi.org/10.1111/j.1745-4603.2009.00195.x

Moggia, C., Lobos, G.A., Retamales, J.B., 2014. Modified atmosphere packaging in blueberries: effect of harvest time and moment of bag sealing. Acta Horticul. 1017, 153-158. https://doi.org/10.17660/ActaHortic.2014.1017.16

Moggia, C., Graell, J., Lara, I., Schmeda-Hirschmann, G., Thomas-Valdes, S., Lobos, G.A. 2016. Fruit characteristics and cuticle triterpenes as related to postharvest quality of highbush blueberries. Sci. Hortic. 211, 449-457. http://dx.doi.org/10.1016/j.scienta.2016.09.018

Moggia, C., Graell, J., Lara, I., Gonzalez, G., Lobos, G.A. 2017a. Firmness at harvest impacts postharvest fruit softening and internal browning development in mechanically damaged and non-damaged highbush blueberries (*Vaccinium corymbosum* L.). Front. Plant Sci. 8. https://doi.org/ 10.3389/fpls.2017.00535

Moggia, C., Beaudry, R.M., Retamales, J.B., Lobos, G.A. 2017b. Variation in the impact of stem scar and cuticle on water loss in highbush blueberry fruit argue for the use of water permeance as a selection criterion in breeding. Postharvest Biol. Technol. 132, 88-96. https://doi.org/10.1016/j.postharvbio.2017.05.019

Moggia, C., Gonzalez, C., Lobos, G. A., Bravo, C., Valdes, M., Lara, I., Graell, J., 2018. Changes in quality and maturity of 'Duke' and 'Brigitta' blueberries during fruit development: postharvest implications. Acta Horticul. 1194, 1495-1501. https://doi.org/10.17660/ActaHortic.2018.1194.209.

Paniagua, A.C., East, A.R., Heyes, J.A., 2014. Interaction of temperature control deficiencies and atmosphere conditions during blueberry storage on quality outcomes. Postharvest Biol. Technol. 95, 50-59. https://doi.org/10.1016/j.postharvbio.2014.04.006

Paniagua, A.C., East, A.R., Hindmarsh, J.P., Heyes, J.A., 2013. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. Postharvest Bio. Technol. 79, 13-19. https://doi.org/10.1016/j.postharvbio.2012.12.016

Patel, N., McGlone, A., Schaare, P., Hall, H., 1993. "BerryBounce": a technique for the rapid and non-destructive measurement of firmness in small fruit. Acta Hortic., 352, 189-198. https://doi.org/10.17660/ActaHortic.1993.352.26

Patel, N., George, J.P., 1997. Progress with breeding blueberries in New Zealand. Acta Hortic. 446, 149-154. https://doi.org/10.1080/15538362.2011.619444

Proctor, A., Miesle, T.J., 1991. Polygalacturonase and pectinmethylesterase activities in developing highbush blueberries. HortScience, 26(5), 579-581. https://doi.org/10.21273/HORTSCI.26.5.579

Prussia, S.E., Tetteh, M.K., Verma, B.P., NeSmith, D.S., 2006. Apparent modulus of elasticity from FirmTech 2 firmness measurements of blueberries. Trans. ASABE 49, 113-121. https://doi.org/10.13031/2013.20219

Rivera, S.A., Zoffoli, J.P., Latorre, B.A., 2013a. Infection risk and critical period for the postharvest control of gray mold (*Botrytis cinerea*) on blueberry in Chile. Plant Disease 97(8), 1069-1074. https://doi.org/10.1094/PDIS-12-12-1112-RE

Rivera, S.A., Zoffoli, J.P., Latorre, B.A. 2013b. Determination of optimal sulphur dioxide time and concentration product for postharvest control of gray mold of blueberry fruit. Postharvest Biol. Technol. 83, 40-46. https://doi.org/10.1016/j.postharvbio.2013.03.007

Rodriguez, J., Zoffoli, J.P., 2016. Effect of sulphur dioxide and modified atmosphere packaging on blueberry postharvest quality. Postharvest Biol. Technol. 117, 230-238. https://doi.org/10.1016/j.postharvbio.2016.03.008

Saftner, R., Polashock, J., Ehlenfeldt, M., Vinyard, B. 2008. Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. Postharvest Biol. Technol. 49, 19-26. https://doi.org/10.1016/j.postharvbio.2008.01.008

Saito, S., Obenland, D., Xiao, C., Xiao, C.L., 2020. Influence of sulphur dioxide-emitting polyethylene packaging on blueberry decay and quality during extended storage. Postharvest Biol. Technol. 160, 111045. https://doi.org/10.1016/j.postharvbio.2019.111045

Saltveit, M.E., 2003. Is it possible to find an optimal controlled atmosphere? Postharvest Biol. Technol. 27, 3-13. https://doi.org/10.1016/S0925-5214(02)00184-9

Schotsmans, W., Molan, A., MacKay, B., 2007. Controlled atmosphere storage of rabbiteye blueberries enhances postharvest quality aspects. Postharvest Biol. Technol. 44(3), 277-285. https://doi.org/10.1016/j.postharvbio.2006.12.009

Segade, S.R., Giacosa, S., Torchio, F., Palma, L., Novello, V., Gerbi, V., Rolle, L., 2013. Impact of different advanced ripening stages on berry texture properties of 'Red Globe' and 'Crimson Seedless' table grape cultivars (*Vitis vinifera* L.). Sci. Hortic. 160, 313-319. https://doi.org/10.1016/j.scienta.2013.06.017

Silva, J.L., Marroquin, E., Matta, F.B., Garner, J.O., Stojanovic, J., 2005. Physicochemical, carbohydrate and sensory characteristics of highbush and rabbiteye blueberry cultivars. J. Sci. Food Agr. 85(11), 1815-1821. https://doi.org/10.1002/jsfa.2083

Sirisomboon, P., Tanaka, M., Akinaga, T., Kojima, T., Panmanas, S., 2000a. Evaluation of the textural properties of Japanese pear. J. Text. Stud. 31(6), 665-677. https://doi.org/10.1111/j.1745-4603.2000.tb01027.x

Sirisomboon, P., Tanaka, M., Kojima, T., Panmanas, S., 2012. Evaluation of tomato textural mechanical properties. J. Food Eng., 111(4), 618-624. https://doi.org/10.1016/j.jfoodeng.2012.03.007

Slaughter, D.C., Rohrbach, R.P., 1985. Developing a blueberry firmness standard. Transactions of the ASAE 28(3), 986-992. https://doi.org/10.13031/2013.32374

Suzuki, A., Kikuchi, T., & Aoba, K. (1997). Changes of ethylene evolution, ACC content, ethylene forming enzyme activity and respiration in fruits of highbush blueberry. J. Jpn. Soc. Hortic. Sci. 66, 23-27. https://doi.org/10.2503/jjshs.66.23

Timm, E.J., Brown, G.K., Armstrong, P.R., Beaudry, R.M., Shirazi, A., 1996. Portable instrument for measuring firmness of cherries and berries. Appl. Eng. Agric. 12, 71-77. https://doi.org/10.13031/2013.25441

Vance, A.J., Jones, P., Strik, B.C., 2017. Foliar calcium applications do not improve quality or shelf life of strawberry, raspberry, blackberry, or blueberry fruit. Hortscience 52, 382-387. https://doi.org/10.21273/HORTSCI11612-16.

Vaughan, G.L., Carrington, C.G., 1998. Psychrometric properties of a moist carbon dioxide atmosphere. Int. J. Food Prop. 1 (1), 77-87. https://doi.org/10.1080/10942919809524567

Vicente, A.R., Ortugno, C., Rosli, H., Powell, A.L.T., Greve, L.C., Labavitch, J.M., 2007. Temporal sequence of cell wall disassembly events in developing fruits. 2. Analysis of blueberry (Vaccinium Species). J. Agr. Food Chem. 55(10), 4125-4130. https://doi.org/10.1021/jf063548j

Zhou, D., Wang, Z., Tu, S., Chen, S., Peng, J. Tu, K. 2019. Effects of cold plasma, UV-C or aqueous ozone treatment on *Botrytis cinerea* and their potential application in preserving blueberry. J. Applied Microbiol. 127, 175-185. https://doi.org/10.1111/jam.14280

3.9. Appendices Chapter 3

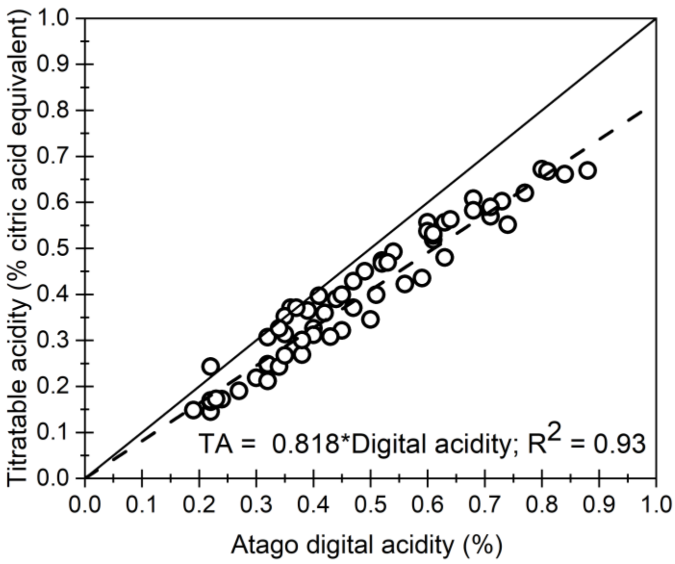
3.9.1. Relationship between Atago acid meter and titratable acidity on 'Rahi' blueberries

The Atago acid meter (PAL-BX I ACID F5, Japan) utilised in the present study is a novel method that requires comparison to standard acidity determination on blueberries. Sixty juice samples (n = 60), obtained by combining different maturities and postharvest technologies of 'Rahi', were analysed using two acidity evaluation methods, the novel Atago acid meter and standard titration method. The same juice sample and dilution ratio (1 g of juice on 50 mL reverse osmosis water) were used on both acidity methods. The standard acidity was performed using titration with NaOH (0.1 N) until pH 8.2 on a digital pH-meter (TitroLine easy, SI Analytics, Mainz, Germany), and results were expressed as grams of citric acid equivalent for 0.1 kg of fresh weight, using a milliequivalent factor for citric acid of 0.064.

A simple linear regression between digital acidity and standard titration acidity is displayed in **supplementary Eq. 3.1**. The digital acid meter displayed higher values than standard titration. However, a strong coefficient of determination (R^2) of 0.93 was estimated (**Supplementary Fig. 3.1**). Consequently, the new method (Digital acidity) can be considered valid for the objectives of the present study.

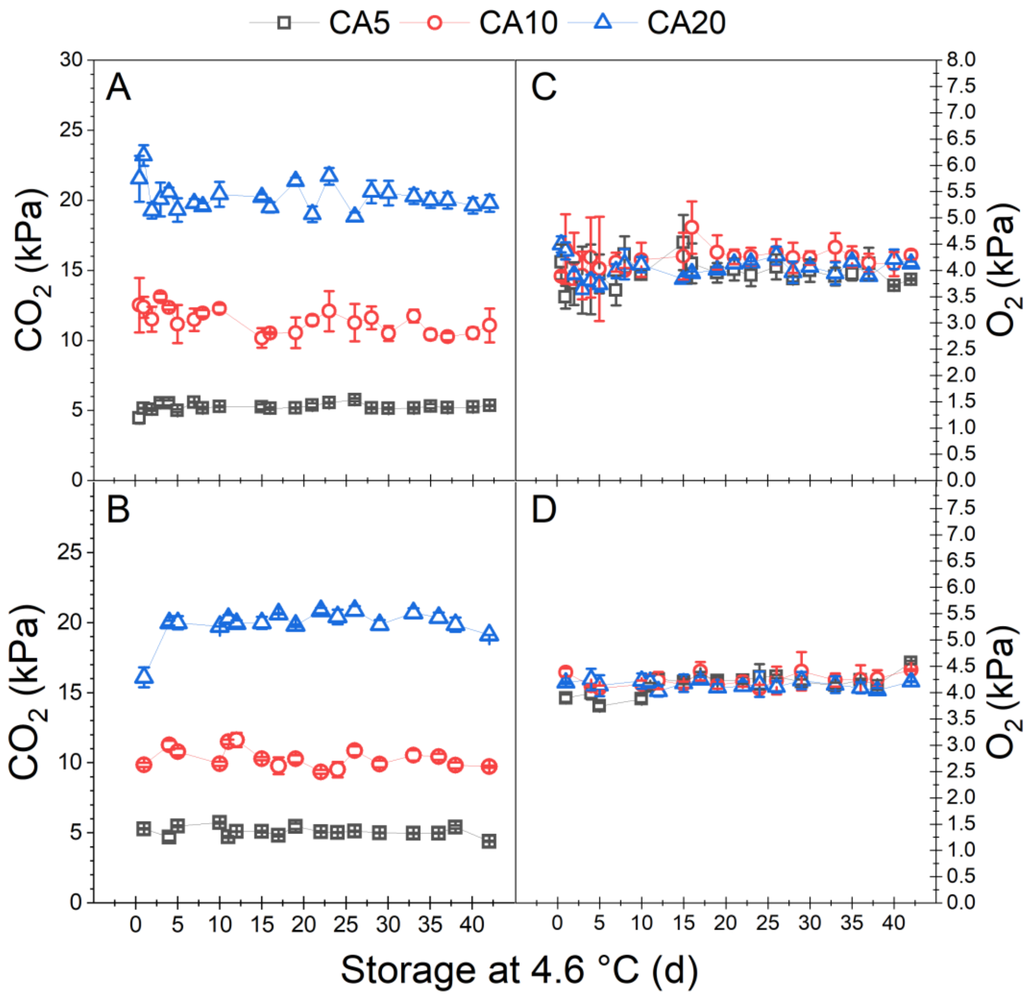
$$Titratable\ acidity\ (\%\ citric\ acid\ eq.) = 0.818 * Atago\ acid\ meter\ (\%)$$

(**Supplementary Eq. 3.1**)

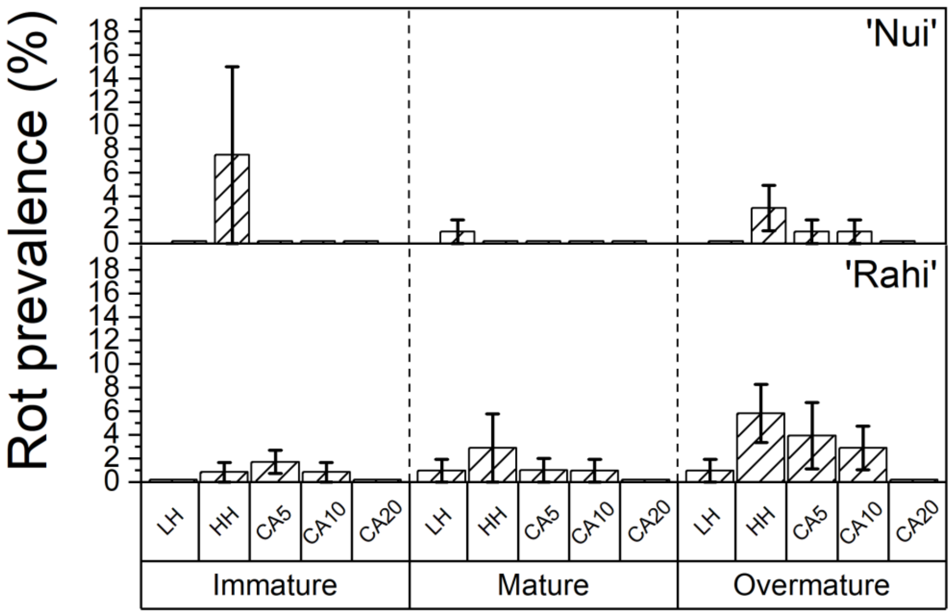


Supplementary Fig. 3.1. Regression analysis for titratable acidity as predicted by the Atago digital acidity of 'Rahi' blueberries across three harvest maturities stored in five different atmospheres for 42 d. Each of the 60 dots represents a pooled juice sample of 20 berries. Dashed line is the regression equation, and continues line represent Digital Acid meter (x) = Titratable acidity (y) plot.

3.9.2. Supplementary figures



Supplementary Fig. 3.2. Variation of gas composition of three controlled atmospheres (CA) designed to reach CO_2 + O_2 combination of 5 kPa CO_2 + 4 kPa O_2 (CA5), 10 kPa CO_2 + 4 kPa O_2 (CA10), or 20 kPa CO_2 + 4 kPa O_2 (CA20). The headspace CO_2 (A, B) and O_2 (C, D) of each jar were measured 3-4 times for each week of storage at 4.6 °C in blueberries 'Nui' (A, C) and 'Rahi' (B, D). Dots = average of four replicates. Bars = standard deviation of four replicates.



Supplementary Fig. 3.3. Rot prevalence of 'Nui' and 'Rahi' blueberries harvested at immature (60-90 % blue), mature (100 % blue), and overmature (100 % blue + time on plant) stage and stored in five technologies (LH = low relative humidity air; HH = high relative humidity air; CA5 = 4 kPa O_2 + 5 kPa CO_2; CA10 = 4 kPa O_2 + 10 kPa CO_2; CA20 = 4 kPa O_2 + 20 kPa CO_2) for 42 d at 4.6 °C. Data represent the mean ± standard error of four mesh bags. The rot prevalence was calculated using 25 berries for each mesh bag.

3.9.3. Supplementary tables

Supplementary Table 3.1. Pearson correlation analysis between quality and mechanical properties of 'Nui' blueberries harvested in three maturities and stored for 42 d at 4.6 °C in five storage treatments.

Postharvest parameter	BH	BR	BCo	BSp	BHS	FSk	SSk	DSk	WSk	BW	WL	TSS:TA
Hardness (BH)		<0.001[b]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.47	<0.001	0.06
Resilience (BR)	-0.87[a]		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.06	0.08	<0.001	<0.001
Cohesiveness (BCo)	-0.91	0.98		<0.001	<0.001	<0.001	<0.001	<0.001	0.01	<0.001	<0.001	<0.001
Springiness (BSp)	-0.59	0.82	0.74		<0.001	<0.001	<0.001	0.25	0.07	0.01	0.47	<0.001
Hardness slope (BHS)	0.99	-0.89	-0.93	-0.61		<0.001	<0.001	<0.001	<0.001	0.92	<0.001	0.01
Force at skin break (FSk)	0.55	-0.75	-0.70	-0.80	0.57		<0.001	0.15	0.02	<0.001	0.64	<0.001
Skin break slope (SSk)	0.92	-0.89	0.92	-0.62	0.95	0.66		<0.001	<0.001	0.49	<0.001	0.003
Displacement skin (DSk)	-0.81	0.62	0.72	0.15	-0.80	-0.19	-0.80		<0.001	0.07	<0.001	0.18
Skin energy (WSk)	-0.54	0.24	0.36	-0.23	-0.52	0.31	-0.47	0.86		<0.001	<0.001	0.73
Weight (BW)	0.09	0.23	0.17	0.35	-0.01	-0.38	-0.09	-0.24	-0.41		<0.001	<0.001
Water loss (WL)	-0.66	0.44	0.57	-0.10	-0.64	-0.06	-0.61	0.92	0.84	-0.36		0.45
SS to acidity (TSS:TA)	-0.24	0.51	0.50	0.39	-0.32	-0.42	-0.38	0.18	-0.05	0.71	0.10	

[a]Pearson correlation coefficients (r). Values in red represent the negligible correlations (*P* < 0.05), in black the weak to moderate correlations (r = 0.3 to 0.79), in green the strong correlations (r = 0.8 to 0.95), and in blue the very strong correlations (r > 0.95).
[b] *P*-value

Supplementary Table 3.2. Pearson correlation analysis between quality and mechanical properties of 'Rahi' blueberries harvested in three maturities and stored for 42 d at 4.6 °C in five storage treatments.

Postharvest parameter	BH	BR	BCo	BSp	BHS	FSk	SSk	DSk	WSk	BW	WL	TSS:TA
Hardness (BH)		<0.001[a]	<0.001	0.58	<0.001	0.20	<0.001	<0.001	<0.001	0.94	<0.001	0.18
Resilience (BR)	-0.63[a]		<0.001	<0.001	<0.001	<0.001	<0.001	0.10	0.73	0.01	0.59	0.02
Cohesiveness (BCo)	-0.76	**0.96**		<0.001	<0.001	<0.001	<0.001	<0.001	0.18	0.02	0.01	0.01
Springiness (BSp)	**0.07**	0.62	0.45		0.86	<0.001	0.687	<0.001	<0.001	0.11	<0.001	0.83
Hardness slope (BHS)	**0.96**	-0.69	**-0.81**	**0.02**		<0.001	<0.001	<0.001	<0.001	0.04	<0.001	<0.001
Force at skin break (FSk)	**0.17**	-0.58	-0.46	-0.52	0.36		<0.001	0.06	<0.001	<0.001	0.01	<0.001
Skin break slope (SSk)	**0.84**	-0.70	**-0.80**	**-0.05**	**0.91**	0.48		<0.001	0.003	0.011	<0.001	<0.001
Displacement skin (DSk)	-0.75	**0.21**	0.42	-0.50	-0.69	**0.24**	-0.68		<0.001	0.63	<0.001	0.21
Skin energy (WSk)	-0.57	**-0.05**	**0.17**	-0.62	-0.45	0.60	-0.38	**0.92**		0.01	<0.001	0.40
Weight (BW)	**-0.01**	0.35	0.30	**0.21**	-0.26	-0.75	-0.33	**-0.06**	-0.35		0.22	<0.001
Water loss (WL)	-0.72	**0.07**	0.31	-0.64	-0.65	0.32	-0.56	**0.92**	**0.90**	**-0.16**		0.47
SS to acidity (TSS:TA)	**-0.18**	**0.30**	0.34	**0.03**	-0.39	-0.64	-0.52	**0.17**	**-0.11**	**0.84**	**0.10**	

[a]Pearson correlation coefficients (r). Values in red represent the negligible correlations (*P* < 0.05), in black the weak to moderate correlations (r = 0.3 to 0.79), in green the strong correlations (r = 0.8 to 0.95), and in blue the very strong correlations (r > 0.95).
[b] *P*-value

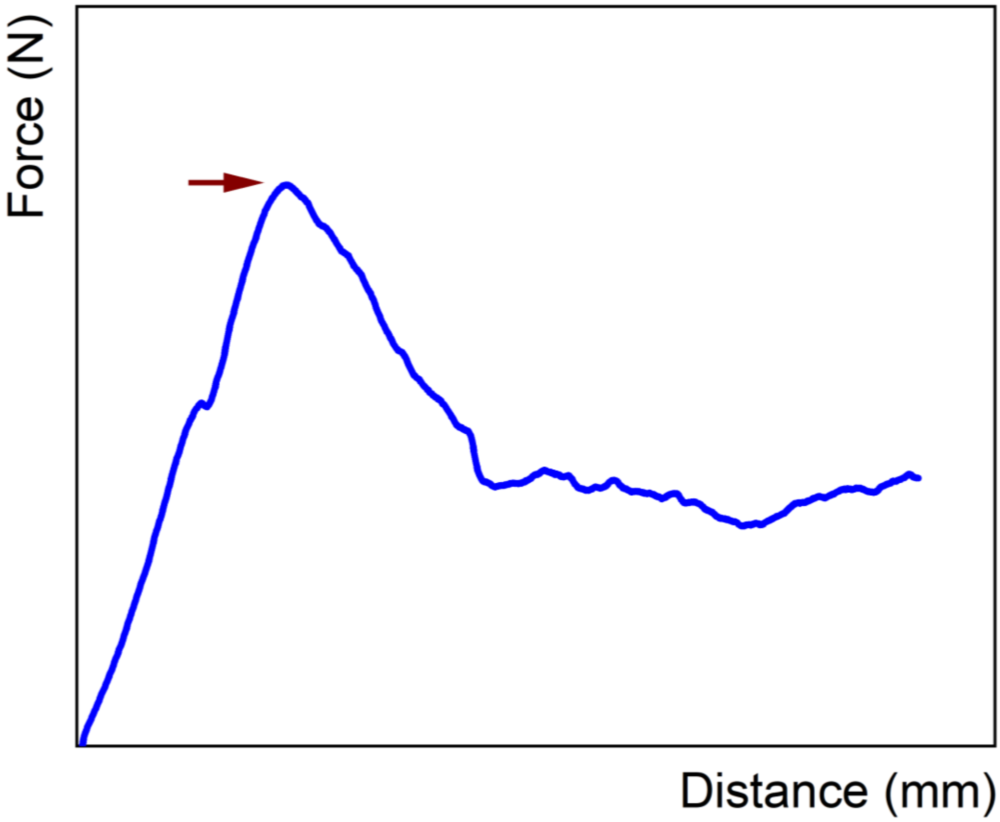
3.9.4. Penetration test of unpeeled 'Nui' blueberries

A penetration test was conducted on a peeled portion of each 40 'Nui' blueberries stored for 42 d at 4.7 °C for each of the five storage treatments (low RH air; high RH air; CA of 4 kPa O_2 + 5 kPa CO_2; CA of 4 kPa O_2 + 10 kPa CO_2; and CA of 4 kPa O_2 + 20 kPa CO_2). The buffer berries in the mature stage (100 % surface blue colour) contained in each treatment jar were used for this purpose. A peel portion of approximately 10 mm^2 of the skin area was removed from the equator of each berry using a surgical blade (size 11, Swann-Morton, Sheffield, UK) and tweezers.

A texture analyser (TA.XT plus, Stable Micro Systems, Surrey, UK) was equipped with a 5 kg load cell and a 2 mm cylindrical flat-ended probe (P/2. Stable Micro Systems). A 2 mm cylindrical probe was preferred over the needle probe to generate a higher force (N), which secured the resolution and accuracy of the measurement. Penetration of unpled berries using a needle probe generates data ranges below 0.2 N, whilst with a 2-mm cylindrical probe, the force ranges between 0.2 and 1.5 N.

The test was conducted to a penetration distance of 30 % strain of the berry flesh using a trigger force of 0.02 N, a pre-test speed of 5 mm s^{-1} and a test speed of 1 mm s^{-1}. The maximum flesh force to penetration or flesh firmness (N) was measured from the force-distance plot using the Exponent software (Version 6.1.14.0, Stable Micro Systems). An example of the force-deformation plot of a penetration test conducted on a peeled portion of blueberries is presented (**Supplementary Fig 3.4**)

Differences between storage technologies were analysed using a general linear model (GLM) in ANOVA, and the Tukey HSD test (P-value <0.05) was used as a multiple mean comparison procedure.



Supplementary Fig. 3.4. Graphic representation of penetration test conducted on a peeled portion of 'Nui' blueberries. The red arrow indicates the maximum force to penetration to measure flesh firmness (N).

3.9.5. Statement of contribution Chapter 3

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Sebastian Anibal Rivera Smith
Name/title of Primary Supervisor:	Dr. Svetla Sofkova-Bobcheva
In which chapter is the manuscript /published work:	Chapter 3

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Rivera, S., Kerckhoffs, H., Sofkova-Bobcheva, S., Hutchins, D., East, A., 2022. Influence of harvest maturity and postharvest technologies on mechanical properties of blueberries. Postharvest Biol. Technol. 191, 111961. https://doi.org/10.1016/j.postharvbio.2022.111961.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Sebastian Rivera Smith (digitally signed)
Date:	04-Aug-2022
Primary Supervisor's Signature:	Svetla Sofkova-Bobcheva (digitally signed)
Date:	8-Aug-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

GRS Version 5 – 13 December 2019
DRC 19/09/10

Chapter 4. Instrumental mechanical parameters related to hand-touch firmness measures of blueberries

In **Chapter 3,** mechanical parameters that can be used to track quality changes as induced by harvest maturity, storage water loss, and controlled atmosphere storage of blueberries 'Nui' and 'Rahi' blueberries have been identified (**Table 3.6**). However, whether the instrumental methods that can detect quality differences can also be related to consumer sensory judgment of 'soft' and 'firm' blueberries remain to be studied. This research chapter has been devised to examine the relationship between instrumental mechanical parameters and sensory evaluation of hand-touch firmness using a sensory panel setting. Blueberries' Centurion' were stored in contrasted storage humidity to induce sensory textural and instrumental mechanical changes, as previously described in **Chapter 2**.

Experimental design for the sensory evaluations used in **Chapter 4** was devised following the recommendations provided by Prof Joanne Hort (Feast, Massey University, New Zealand). Prof Joanne Hort is co-author of this chapter but not officially part of this thesis committee.

Chapter 4 has been written following the guide for authors of the Elsevier journal Postharvest Biology and Technology. The author of this thesis has worked on the conceptualization, research methodology, formal analysis and investigation, paper visualizations, and writing > 90 % of the original draft.

AUTHORS AND AFFILIATIONS

Sebastian Rivera[a,b], Joanne Hort[c], Svetla Sofkova-Bobcheva[b], Huub Kerckhoffs[b,d], Dan Hutchins[e], Andrew East[a]

[a] Massey AgriFood (MAF) Digital Lab, School of Food and Advanced Technology, Massey University, Palmerston North, New Zealand
[b] School of Agriculture and Environment, Massey University, Palmerston North, New Zealand
[c] Food Experience and Sensory Testing Laboratory (Feast), School of Food and Advanced Technology, Massey University, Palmerston North, New Zealand
[d] Start Afresh, Mt. Maunganui, New Zealand
[e]Gourmet Blueberries, NZ Gourmet Ltd, Auckland, New Zealand

KEYWORDS
Vaccinium spp; water loss; texture profile analysis; penetration test.

ABSTRACT
Hand-touch firmness is a sensory method reported to be used by the blueberry industry to characterise firmness quality. A hand-touch 'soft' blueberry may be considered unmarketable. However, the method of hand-touch to characterise firmness is questionable due to being highly influenced by the assessor's subjective judgment. Objective instrumental methods are preferred to measure firmness and create a reliable and consistent dataset. This chapter aimed to study the relationship between sensory hand-touch firmness measured by a trained sensory panel and instrumental mechanical parameters of blueberry 'Centurion'. To the author's knowledge, this is the first attempt to relate instrumental mechanical parameters to hand-touch firmness using a sensory panel setting and reference samples to exemplify hand-touch intensity differences. Seven assessors were trained to evaluate hand-touch firmness in a sensory panel setting. References samples resembling a blueberry were made of silicone and used to calibrate assessors by exemplifying four blueberry firmness intensities ('very soft', 'soft', 'firm' and 'very firm'). Six sensory sessions were conducted, where each session represented a harvest date and storage time combination (3 harvest dates X 2 storage times). Each sensory session considered 'Centurion' blueberries exposed to five storage relative humidity (71 %, 88 %, 95 %, 97 % and 100 % RH). Assessors were able to discriminate differences in hand-touch firmness as influenced by storage relative humidity (i.e., water loss). Mechanical parameters associated with the slope of force/deformation graph using a plate probe on a compression test (i.e., hardness slope) or a needle probe on a penetration test (i.e., skin break slope) were best related to sensory hand-touch firmness. Values of hardness slope and skin break slope higher than 1.7 kN m^{-1} and 0.42 kN m^{-1}, respectively, were associated with a very low (≤ 5 %) likelihood of observing unmarketable ('soft' or 'very soft') berries in a batch. This work provides a basis for the standardisation of mechanical methods that can be used to assess blueberry quality based on its ability to represent consumer sensation preferences of texture.

4.1. Introduction

Blueberry consumers prefer firmer berries (Ehlenfeldt and Martin 2002). However, firmness evaluation methods have not yet been standardised across the blueberry supply chain (**Chapter 1**). Consequently, research studies previously conducted to characterise blueberry quality (and consumer acceptance) have been evaluated using different instrumental and sensory methods (**Chapter 1, Table 1.1,** and **Table 1.2).**

Sensory methods have previously been reported to assess blueberry firmness as a consumer acceptance indicator. Sensory methods can be conducted as mouthfeel or hand-feel evaluation. Mouthfeel evaluations have previously considered firmness as the force required to fracture a blueberry using molars when chewing (Ballinger et al., 1973; Saftner et al., 2008; Blaker et al., 2014; Lobos et al., 2014; Vilela et al., 2016). While hand-feel evaluation measured firmness as slight hand-touch, including sensory procedures as squeezing or roll between the index and thumb fingers (Sanford, 1991; Nunes, 2015; Rodriguez and Zoffoli, 2016).

For quality characterisation purposes, hand-touch firmness is claimed to be a more relevant sensory assessment of blueberry than mouthfeel because:

(1) Blueberries are often manipulated using hands when consumed in many cultures. For example, when the fruit is removed from its packaging. Consequently, berry manipulation between fingers is often the first point of sensory engagement for consumers to judge the (textural) quality of the product before consumption.

(2) Hand-touch firmness can be used to simulate firmness evaluation applied at the commercial industry level (Timm et al., 1996; Beaudry et al., 1998; Schotsmans et al., 2007; Cantin et al., 2012; Nunes, 2015; Rodriguez and Zoffoli, 2016). In addition, hand-touch firmness scores representing 'soft' berries are associated with unacceptable blueberries for commercial sale.

(3) Reference samples are recommended to be used to exemplify intensity points of a sensory scale and calibrate the assessor's performance when conducting sensory evaluations (Kemp et al., 2009). For blueberry mouthfeel evaluations, the reference sample used to calibrate firmness assessment required to be foods such as boiled egg white or pitted olives (Vilela et al., 2016). However, these mouth-feel samples can be prone to the natural variation of most foods due to environmental interaction and time

(ageing). As hand-touch firmness does not require human consumption, reference samples can be made of non-edible and reusable viscoelastic materials such as silicone. Silicone products may assure higher consistency and homogeneity of reference samples compared to fresh foods. Silicones can show less variability of biological materials, are less prone to ageing and can be consistently reproduced (Ahmadzzadeh and Hukins, 2014).

The commercial blueberry industry may favour objective instrumental parameters over sensory evaluation to determine firmness (Moggia et al., 2022). This requirement can be mainly attributable to the subjectiveness associated with sensory evaluation methods. In addition, the type of data generated by the instrumental techniques (continuous interval data) Is easier to statistically be analysed (parametric methods) and later modelled when compared to discrete data (2–5-point firmness scale) produced by hand-touch firmness evaluation (**Table 4.1**).

Although the blueberry industry prefers objective instrumental data, the instrumental measurement must be informative of consumer acceptance. The statistical analysis of the relationship (e.g., correlation analysis) between instrumental mechanical parameters and sensory firmness can be used as an indicator of the ability of an instrumental method to relate (provide information) to consumer acceptance (Nunes, 2015).

Several studies had previously used sensory hand-touch and instrumental parameters to assess blueberry firmness (**Table 4.1**). To the author's knowledge, only Nunes (2015) has reported a formal statistical correlation analysis between instrumental mechanical parameters and hand-touch firmness. The Pearson correlation coefficient found by Nunes (2015) was weak ($r = 0.34$). However, the study conducted by Nunes (2015) considered a non-common method to assess the instrumental firmness of blueberries, where a group of blueberries (30 g) contained in a 100 mL beaker were compressed by a cylindrical probe (39 mm diameter and 20 mm high). The author of this thesis hypothesises that this method can potentially be subjected to experimental variation attributable to the differences in the contact area (between fruit and the cylindrical probe) and differences in maximum height as dependent on the volume occupied by the berries inside the 100 mL beaker.

In addition, none of the studies listed in **Table 4.1** (including Nunes, 2015) reported the use of a formal and trained sensory panel setting to assess hand-touch firmness. The use of a trained sensory panel is relevant to hand-touch firmness evaluation because this sensory test is questioned as being highly influenced by assessor judgment (Slaughter and Rohrbach, 1985; Schotsmans et al., 2007). In addition, a formal sensory panel will allow several assessors to characterise the blueberry hand-touch firmness using the same blueberry population, which is not commonly considered in evaluations conducted using trained individuals (**Table 4.1**).

Prior studies conducted in postharvest lab at Massey University have shown that mechanical parameters can vary by manipulating storage weight loss (Paniagua et al., 2013; Li et al., 2021; **Chapter 2, Chapter 3**). In addition, Nunes and Emond (2007) have reported differences in sensory hand-touch firmness as influenced by weight loss. Consequently, the author of this thesis hypothesises that using a range of storage relative humidity will generate enough data variability to relate instrumental mechanical parameters to sensory hand-touch firmness.

This study aims to study the relationship between sensory texture (hand-touch firmness) and instrumental mechanical parameters obtained by texture profile analysis (double compression test) and penetration test. Specific objectives were:

(1) Determine values of instrumental mechanical parameters associated with a high (> 95 %) and low (< 5 %) likelihood of unmarketable ('soft' or 'very soft') berries in a blueberry batch.

(2) Development and characterisation of reference samples made of silicone (that imitates real blueberries) to be used on hand-touch firmness evaluations.

The investigation on the identification of instrumental methods that may represent the human sensation of blueberry preferences has previously been reported on blueberries (**Table 4.1**). However, compared to studies reported in **Table 4.1**, the novelty of this chapter is the use of a trained sensory panel to assess hand-touch firmness. In addition, a more extensive range of mechanical parameters, including compression and penetration test, was considered in this chapter. The authors hope that this chapter contributes to the standardisation of instrumental firmness evaluation that can be used to characterise blueberry quality and relate to consumer preference.

Table 4.1. Postharvest blueberry research studies that have previously used hand-touch firmness methods. This table also includes Instrumental parameters and relation to hand-touch firmness.

Sensory hand-touch firmness evaluation				Instrumental evaluation		Reference
Purpose	**Method**	**Rating scores**	**Assessors profile**	**Mechanical method**	**Relationship to hand-touch firmness**	
Effect of temperature and mechanical damage	Roll between the thumb and index finger	0 (‘soft’) 5 (‘firm’)	Not reported	Compression Force to 30 mm of a group of blueberries (30 g) using a Force Gauge (Accuforce II, Ametek, Hatfield, USA)	NCAR[a], but instrumental force followed a similar trend than hand-touch firmness	Sanford, 1991
Characterise quality of blueberries shipped from Chile to North America	Touch compression	1 (‘firm’) to 3 (‘soft’)	Not reported	Chord stiffness (kN m^{-1}) using a portable compression instrument equipped with a flat plate probe (Timm et al., 1993)	NCAR, but instrumental chord stiffness followed a similar trend to percentage of soft berries by touch compression	Beaudry et al., 1998
Effect of cultivar and packaging technology	Finger pressure	0 (‘soft’) or 1 (‘firm’)	Not reported	Not applicable	Not applicable	Miller et al., 1993
Influence of storage temperature and time	Roll between thumb and index finger	1 (‘soft’) to 5 (‘firm’)	Single trained individual	Not applicable	Not applicable	Nunes et al., 2004
Effect of controlled atmosphere storage	Slight squeezing	1 (‘firm’) to 3 (‘soft’)	Not reported	Force to 2 mm compression using a Texture analyser (TA.XT, Stable Micro System, Godalming, UK) equipped with 60 mm flat plate probe	NCAR, but sensory results behaves differently than instrumental mechanics	Schotsmans et al., 2007
Relationship between weight loss and texture	Slight finger pressure	1 (‘very soft’) to 5 (‘firm’)	Trained individuals	Not applicable	Not applicable	Nunes and Emond, 2007
Effect of sulphur dioxide (SO_2) and controlled atmosphere	Slight squeezing	1 (‘very firm’) to 3 (not ‘firm’ enough for marketing)	Not reported	Force to 4 mm compression using a Texture analyser (FTA model GS, Guss manufacturing Ltd., Strand South Africa) equipped with a 25 mm plate probe	NCAR, but storage treatments produced differences on hand-touch firmness, as similar to instrumental force evalution	Cantin et al., 2012
Effect of SO_2 fumigation before storage	Slight squeezing	‘firm’ or ‘soft’	Not reported	Force to 3 mm compression using a Texture analyser (TA.XT, Stable Micro System, Surrey, UK) equipped with a 75 mm flat plate probe	NCAR, but instrumental force below 2.0 N were associated with sensory soft berries	Rivera et al., 2013
Correlate subjective quality with instrumental attributes	Slight finger pressure	1 (very poor, ‘soft’) to 5 (excellent, ‘firm’)	Trained individuals	Force to 30 mm compression of a group of blueberries (30 g) using a Texture Analyser (Texture Technologies Corp, NY, USA) equipped with a 38 mm diameter and 20 mm high cylinder probe	Pearson Corelation coefficient (r) of 0.34 (P-value <0.05)	Nunes, 2015
Effect of controlled and modified atmosphere storage	Slight finger pressure	‘Firm’ or ‘Soft’	Single trained individual	Force compression using a Durometer (Durofel, Copa Technology, France) equipped with a 0.1 cm^2 probe	NCAR, but average Durofel firmness of 2.5 N were measured on sensory soft berries	Rodriguez and Zoffoli, 2016
Identification of critical steps in supply chain to quality	Touch compression	1 (very poor, ‘soft’) to 5 (excellent, ‘firm’)	Trained personnel	Force to 30 mm compression of a group of blueberries (30 g) using a Texture Analyser (Texture Technologies Corp, NY, USA) equipped with a 38 mm diameter and 20 mm high cylinder probe	NCAR	Ktenioudaki et al., 2021

[a]NCAR = not correlation analysis reported

4.2. Materials and methods

4.2.1. Blueberry material

The experimental design used in this study considered different harvest dates of 'Centurion' blueberries (rabbiteye, *Vaccinium ashei*). The use of three harvest times during the season may facilitate variability in blueberry firmness and other quality responses (Strike, 2019). Hence the use of different harvests will allow associating the study conclusions with a range of commercial scenarios.

Blueberries 'Centurion' was collected from a commercial orchard in Flaxmere, New Zealand. Approximately 3.4 kg of berries at commercial maturity (100 % blue surface colour) were hand-harvested directly into 125 g plastic clamshells on each of three consecutive commercial harvest dates (H1 = 28th January; H2 = 7th February; H3 = 17th February 2020). Three clamshells were collected from each of the same nine mature and healthy plants used on each harvest date. Harvests were conducted between 10:00 to 13:00 on each harvest date, with fruit pulp temperature recorded with a digital thermometer (Q1437, Dick Smith Electronics Pty Ltd, Australia), of 30.7 ±1.9, 24.3 ±2.8, and 27.6 ±1.00 °C for H1, H2, and H3, respectively.

Immediately after each harvest, blueberries were transported (approximately 3 h at 22 °C) to the postharvest lab at Massey University, Palmerston North, New Zealand. Around 1 h after arriving at the lab, a sample of 90 berries was used to characterise fruit maturity and quality by measuring, in the following order, mechanical parameters by texture profile analysis, mechanical parameters by a penetration test, berry size, soluble solids, and acidity. The remaining berries were kept for 12-14 h in a temperature control cabinet set at 10 °C before setting up storage treatments.

4.2.2. Quality and maturity at harvest

Berry maturity was characterised by measuring total soluble solids (%) and acidity (%) on six juice samples of 15 berries each using a digital refractometer/acid meter (PAL-BX I ACID F5, Atago, Tokyo, Japan). For total soluble solids, an aliquot of 0.5 mL juice was used on the refractometer. In contrast, for acidity, a dilution of 1 g of juice sample in 50 mL of reverse osmosis water was conducted before taking a reading on the refractometer/acid meter.

Berry size was characterised by measuring equatorial diameter (mm) and polar length (mm, stem to calyx end) on each of 90 berries using a digital calliper (IP54, Shahe, China). Blueberry volume (cm^3) was calculated as an oblate spheroid (Moggia et al., 2017a), using the diameter (DIA) and length (LEN) (**Eq. 4.1**).

$$V = \frac{4}{3} \times \pi \times \frac{LEN}{2} \times (\frac{DIA}{2})^2 \quad \text{(Eq. 4.1)}$$

Mechanical parameters of hardness slope (kN m^{-1}), cohesiveness (-), resilience (-), and springiness (-) were obtained from texture profile analysis (TPA) on 45 berries for each harvest date. While force at skin break (N), skin break slope (kN m^{-1}), displacement at skin break (mm), and skin break energy (mJ) was obtained by penetration test on a second group of 45 berries for each harvest date. Both mechanical tests were conducted on a texture analyser (TA.XT plus, Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell. See **Chapter 2**, *2.6. Instrumental mechanical parameters* for a detailed description of TPA and penetration test methods.

4.2.3. Storage relative humidity treatments

The experiment was devised to manipulate blueberry cumulated water loss and, consequently, textural quality as detailed by previously reported works of the same research group (Paniagua et al., 2013; Li et al., 2021; **Chapter 2; Chapter 3**). Five relative humidity levels (71 %, 88 %, 95 %, 97% and 99 %) for two storage times (14 and 28 d) at 4.7 ± 0.1 °C were used on each harvest date.

Different storage relative humidity was created by manipulating airflows through a jar system (containing blueberries) based on theory and practical implications previously reported (Paniagua et al., 2013; Li et al. 2021; **chapter 2**). Approximately 20 h from each harvest, 60 groups of 24 berries of homogenous size and free of external defects (e.g., shrivel, bruises, or rotten berries) were randomised. Each group of berries was placed in ~90 cm^3 mesh bags and individually weighed on a digital balance (TW423L, Shimadzu, Japan) at 20 °C. Groups of four mesh bags were randomly assigned to each of 15 glass jars of 578 mL. Each jar contains between 45 and 55 g of buffer berries in the bottom, which were not used on any evaluation. Consequently, approximately 200 g of berries (4 mesh bags + buffer) were placed in each jar.

For each harvest date, jars were randomly assigned to one of five airflows (i.e., 2 mL s^{-1}, 1 mL s^{-1}, 0.5 mL s^{-1}, 0.25 mL s^{-1}, and 0 mL s^{-1}) in triplicate. Temperature and relative humidity inside the jar (as induced by the airflows) were recorded every 800 s by a dual data logger, which measures temperature and RH (iButton®, Maxim Integrated, CA, USA). The iButton® was attached to the inner wall of one jar for each of five airflows. Storage relative humidity (average ±standard deviation) of 71.4 ±7.3, 88.2 ±3.5, 94.7 ±0.1, 97.0 ±0.1, 99.1 ±0.05 were recorded for airflows of 2 mL s^{-1}, 1 mL s^{-1}, 0.5 mL s^{-1}, 0.25 mL s^{-1}, and 0 mL s^{-1}, respectively.

4.2.4. Storage water loss and shrivel evaluation

After completing a storage time of 14 d and 28 d at 4.7 °C, two mesh bags for each glass jar were removed and weighed separately to calculate weight loss (WL) using **Eq. 4.2.**

$$WL\ (\%) = \frac{Wi - Wf}{Wi} \times 100 \qquad \textbf{(Eq. 4.2)}$$

Wi is the initial weight of berries of each mesh bag, and *Wf* is the weight of berries of each mesh bag after each storage time. Average WL of the two mesh bags for each jar was used as one of three replicates.

Shrivel symptoms were visually assessed by naked eye evaluated for each individual berry using a four-point scale based on Rodriguez and Zoffoli (2016) with modifications. Where 1 = no symptoms, 2 = slight shrivel (fine lines of 3-5 mm length located at the stem scar surrounding area), 3 = moderate shrivel (fine lines of 6-9 mm length mainly located at the stem scar surrounding area), and 4 = severe shrivel (wide lines of > 6 mm length located on any fruit surface area). Percentage of berries with a shrivel score of 3 or 4, out of the total number of berries for each storage relative humidity, was calculated and recorded as dehydration (Rodriguez and Zoffoli, 2016).

4.2.5. Fruit randomisation for textural and mechanical parameters

After WL evaluation, rotten or external bruised berries were discarded from each mesh bag. The remaining berries of both mesh bags were combined to make a single replication. For each replication, 14 healthy berries were randomly assigned to any of the three groups, with the remaining berries omitted. Each group of berries was then randomly assigned to one textural quality evaluation

(1) Sensory analysis (hand-touch firmness).
(2) Instrumental mechanical parameters of texture profile analysis (double compression test).
(3) Instrumental mechanical parameters of the penetration test.

The above-mentioned process was repeated ninety-fold (three harvest dates x two storage times x five storage relative humidity x three replication). Six independent textural quality evaluations (three harvest dates x two storage times) were performed.

4.2.6. Sensory analysis

4.2.6.1. Hand-touch firmness

Sensory firmness was non-destructively evaluated by tactile hand-feel, squeezing individual berries between thumb and index finger. Hand-touch firmness was scored using a 4-point categorical scale, an adaptation from firmness categories and descriptions reported by Nunes et al. (2004). However, the present study differentiated firmness categories by applying two consecutive squeezes of 1-2 s duration each. A first squeeze was applied with gentle hand pressure at the blueberry equator (stem scar is facing upward), and if the blueberry was easily depressed, it was scored as 'very soft'. If the berry was not easily depressed, the berry with stem scar facing upward was rotated 90° over its axis, and a second squeeze of moderate pressure was applied on the blueberry equator to differentiate 'soft', 'firm' and 'very firm' blueberries (**Table 4.2**). The decision of using two squeezes to discriminate hand-touch firmness categories was a consensus between assessors and moderator (first author) during panel training sessions.

Table 4.2. Evaluation scale of hand-touch firmness. Score and description are provided for each firmness category. This scale is an adaption of firmness categories reported by Nunes et al., (2004).

Category	Score	Description
'Very soft'	1	Berry surface easily depressed upon gentle squeeze
'Soft'	2	Berry surface is slightly depressed upon gentle squeeze but easily depressed when the second squeeze is moderate.
'Firm'	3	Not apparent surface yielding upon gentle squeeze, but slightly depressed when the second squeeze is moderate.
'Very firm'	4	Berry feels solid in hand. Not yielding to moderate squeeze

Sensory evaluation of blueberries using hand-touch can be inconsistent as it is influenced by assessor judgement (Slaughter and Rohrbach, 1985; Schotsmans et al., 2007). To ensure consistency on each evaluation, prerequisites included in the sensory analysis design included training activities, assistance of sensory evaluation using reference samples (or calibration standards), and sensory sessions performed by same group of assessors.

4.2.6.2. Development and characterisation of reference samples (silicone spheroids)

For each assessor and sensory session, spheroids (imitating real blueberries) made from different silicone formulations were made available as reference samples to illustrate intensity of the four points on the hand-touch firmness scale (**Table 4.2**) and calibrate the performance of assessors (Kemp et al., 2009).

The decision to use silicone to create reference samples was based on the ability of silicone to deform when subjected to similar compression forces that can deform a blueberry using human fingers. In addition, silicone materials can be used to imitate the mechanical behaviour of biological tissues such as human organs and other viscoelastic materials (Ahmadzzadeh and

Hukins, 2014). Soft fruits, such as blueberries, show viscoelastic behaviour under small compression force (Abbott, 1999).

Silicone spheroids with seven different hand-touch firmness intensities were created by mixing commercial silicone chemicals used for modelling tissues for theatrical make-up, impressions, or sculpturing. Commercial chemicals mixtures were PlatSil® Gel-00 (Part A and B, Barnes Products Pty Ltd, Australia), Transil™ (Part A and B, Barnes Products Pty Ltd, Australia), Elastosil® Vario 15 (Part A and B, Wacker Chemie AG, Munich, Germany), and Elastosil® Vario 40 (Part A and B, Drawin Vertriebs-GmbH, Munich, Germany). All chemicals were acquired from Barnes NZ Pty Ltd (Auckland, New Zealand) and are classified as not hazardous substances or mixtures. Further description of composition and hazards classification for each commercial product is provided in **Supplementary Table 4.1**. The above-mentioned chemicals were mixed at 20 °C in a Pyrex beaker of 100 mL. Mixing procedures were conducted as recommended by Barnes New Zealand or the manufacturer. In addition, a drop of a dark blue pigment suitable for silicone (Barnes Products Pty Ltd, Australia) was added to each mixture to imitate the blueberry surface colour.

The silicone mixes were placed on ABS re-utilisable moulds that imitate blueberry oblate spheroid shape. Spheroid moulds were created in two sizes, which imitate the commercial size ranges of 'Centurion' blueberries, 1.1 cm^3 (14 mm equatorial diameter and 11 mm polar length) and 1.4 cm^3 (15 mm equatorial diameter and 12 mm polar length). The moulds were printed by a 3D printer. Working time (pot life), curing and demould conditions were conducted in a laboratory at 20 °C, following the manufacturer's directions for each chemical mixture. This process was repeated to generate seven silicone spheroids for each firmness category and size combination. Examples of 3D-print moulds and silicone spheroids of two sizes are shown in **Fig. 4.1**.

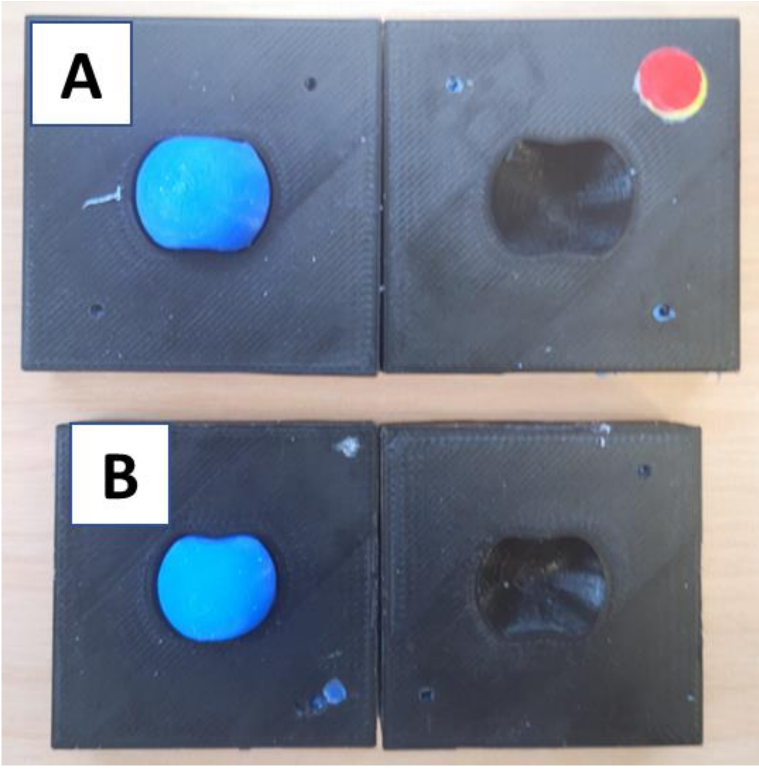


Fig. 4.1. Example of the 3D-print moulds and silicone spheroids of two sizes, 1.4 cm^3 (A) and 1.1 cm^3 (B).

The silicone spheroids illustrating the four hand-touch scores (**Table 4.2**) used in the sensory session were selected from a total of seven silicone mixtures. The agreement on the selection of the four attributes was firstly assisted by sensory criteria of personnel in charge of grading operations of a commercial blueberry packhouse. Later, during the training sessions, the trained assessors agreed the four silicone spheroids categories were selected as good sensory indicators of the attribute (hand-touch firmness).

To characterise the selected silicone spheroids for each hand-touch category, approximately one week before conducting the first sensory session, a uniaxial compression to 15 % deformation of the silicone spheroids was conducted using a texture analyser equipped with a 25 mm cylindrical flat-ended probe (P/25, Stable Micro Systems), and a 5 kg loading cell. The compression settings were 0.06 N as trigger force, pre-test speed of 1.6 mm s^{-1} and a test speed of 1.0 mm s^{-1}. The maximum force (N) to 1 mm deformation was determined from the compression loading curve. In addition, as sensory sessions were conducted over an approximately two-month timeframe and the same silicone blueberries were randomised on each sensory session, confirmation that little change in the mechanical parameters of the silicone blueberries through time was required. Maximum force to 1 mm was also measured, after the 3rd session (i.e., H2- 14 d) and 6th sensory sessions (i.e., H3 – 28 d).

4.2.6.3. Training sessions and performance check

A group of ten Massey University postgraduate students or staff (ages 21-35 years) with a food technology or horticultural background were trained over three 1 h sessions to develop a consensus of hand-touch firmness procedures and intensity scores (**Table 4.2).** In addition, assessor judgments were calibrated by familiarisation with the reference silicone blueberries. Training sessions were conducted in a food quality lab (Massey University) and included a series of activities where the assessors were asked to create firmness categories of silicone spheroids and real blueberries with predetermined and contrasting hand-touch firmness. All the training sessions were moderated by the first author.

The last training session was a performance check session. This was conducted in individual sensory booths (Food Experience and Sensory Testing Laboratory (FEAST), Massey University, New Zealand) at room temperature (approximately 20 °C). The assessors were asked to evaluate 'Centurion' blueberries (obtained from harvest 1, January 28th) with a balanced number of berries (n = 28) in two batches with contrasting firmness, 'firm' + 'very firm' (n = 14) and 'soft' + 'very soft' (n = 14). Each firmness batch was created by manipulating blueberry water loss (<1% vs 8 %) through 7 d storage at 20 °C. Blueberries were presented to each assessor on a white plastic plix tray of 1,363 cm^2 (commercial kiwifruit tray, Zespri®, New Zealand) in random order, using a 3-digit random code for each blueberry. Assessors that reached a scoring accuracy higher than 60 % of correctly evaluated berries were further selected to conduct formal sensory evaluations. Consequently, 7 out of the 10 assessors were selected (**Supplementary Table 4.2**).

4.2.6.4. Sensory sessions

Seven selected assessors (four females and three males) were asked to carry out six sensory sessions executed between February and March 2020. Each sensory session represented a harvest date (H1, H2, and H3) and storage time (14 and 28 d at 4.7 °C) combination.

To reach a blueberry flesh temperature of approximately 20 °C for each sensory session, blueberries were left in a temperature-controlled laboratory set at 20 °C for two hours after removing them from storage treatments at 4.7 °C. During blueberry warming, the berries were randomly placed on seven white plastic plix trays (commercial kiwifruit tray). Each tray contained 30 blueberries, obtained by combining two blueberries randomly selected from each of the three replications of each of five storage relative humidity treatments (71 %, 88 %, 95, 97 % and 99 %). Hence, the number of blueberries with different textural properties was balanced for each of the seven trays. Each of the 30 blueberries was individually labelled with a three-digit random code created using a free online service website (random.org/integers). All the sample preparation was led by the Panel Leader (first author), who was not involved in any of the sensory evaluations.

All sensory sessions were performed in sensory booths, at room temperature, and each session lasted 0.5 h. Each tray was randomly assigned to each of seven individual sensory booths. In addition to the 30 blueberries to be evaluated, each sensory booth contained a set (four intensities) of silicone spheroids of each size (**Supplementary Fig. 4.1**).

Assessors were asked to randomly sit in a sensory booth, and the panel leader provided instructions to conduct the sensory evaluation for each berry

1. Place the blueberry with the stem scar facing the room ceiling.
2. Select the silicone spheroid size that better matches the berry size to be assessed and perform a gentle squeeze on each of four silicone spheroids.

3. Perform a first gentle squeeze of 1-2 seconds duration in the equatorial plane of the blueberry to be assessed. If the berry feels 'very soft' (**Table 4.2**, score 1), record your result on the sheet provided. If not, continue the assessment.
4. Perform a moderate squeeze on silicones spheroids referencing a 'soft', 'firm' and 'very firm' blueberry.
5. Rotate the real blueberry 90 ° over its axis (stem scar facing upward) and perform a second squeeze of moderate intensity on the blueberry equator.
6. Manually record your hand-touch firmness result using the pencil and sheet provided.

The responses for each of 30 berries evaluated by each of the seven assessors were grouped considering the jar (storage relative humidity and replicate) where berries were originated. Data obtained from each sensory session considered fourteen scores for each replication and consequently 42 scores (14 berries X 3 replicate) for each of five storage relative humidity. Blueberry scores of each replication were counted, distinguishing each of the four hand-firmness categories.

Blueberry hand-touch firmness categories of 'very soft' or 'soft' may represent commercial rejection or unmarketable berries (Beaudry et al., 1998; Nunes et al., 2004; Schotsmans et al., 2007; Cantin et al., 2012; Rodriguez and Zoffoli, 2016). Hence, in addition to the count data for each hand-touch category, the number of 'soft' or 'very soft' berries were summed to calculate the number of unmarketable berries out of 14 berries of each replication.

4.2.7. Instrumental mechanical parameters

Immediately after each sensory session, instrumental mechanical parameters of texture profile analysis (TPA) and penetration test were assessed on new randomly assigned blueberries from the same batch (treatment replication) that were used for the sensory evaluations.

All mechanical parameters were evaluated with a texture analyser equipped with a 5 kg load cell and all acquisitions were made with a resolution of 250 points per second. Each blueberry was analysed with the stem-calyx axis oriented parallel to the texture analyser platform over a flat metal ring of 10 mm internal diameter and 1 mm height (**Chapter 2**).

4.2.7.1. Texture profile analysis

Texture profile analysis (TPA) was conducted with a 25 mm cylindrical flat-ended probe (P/25, Stable Micro Systems) travelling at a pre-test speed of 1.6 mm s^{-1} and a test and post-test speed of 1.0 mm s^{-1}. Each berry was compressed twice using a trigger force of 0.06 N, a target deformation distance of 15 % strain, and a waiting time between cycles of 10 s (**Chapter 2**). Mechanical parameters of hardness slope, resilience, cohesiveness, and springiness were estimated for each berry using the double compression in Exponent software (Version 6.1.14.0, Stable Micro Systems). Hardness slope was calculated as the slope of a straight line drawn between the trigger force and maximum force to 15 % strain on the first compression downstroke. Resilience was calculated as the ratio of the work area (force x displacement) during withdrawal (upstroke) of the first compression to the area of work during the downstroke of the first compression. Cohesiveness was calculated as the ratio of the area of work during the second compression cycle to the area of work during the first compression cycle. Springiness was calculated as the ratio of displacement (mm) during downstroke compression of the second cycle to the displacement during downstroke compression of the first cycle.

In addition, to compare the data of silicone spheroids with real 'Centurion' blueberries the maximum force to 1 mm deformation was determined on first compression loading. This data was only used to determine if ranges of silicone spheroids were in similar values than real blueberries, and not was used on any other evaluation.

For each harvest date and storage time combination (i.e., six evaluations), average mechanical parameters of 14 berries were calculated for each of five storage relative humidity in triplicate.

4.2.7.2. Penetration test

The penetration test was conducted with a needle probe (SMS P/2N, Stable Micro Systems) to a target distance of 30 % of each berry's equatorial height. Pre-test speed was set at 5 mm s^{-1}, test and post-test speed at 1 mm s^{-1} and trigger force at 0.01 N. Mechanical parameters of force at skin break (N, force achieved just before irreversible rupture of the berry skin), skin break slope (kN m^{-1}, determined as the straight-line slope drawn between trigger force and force at a skin break), displacement at berry skin break (mm, distance travelled by needle probe just before skin rupture), skin break work energy (mJ, area of work of the force/deformation curve between trigger and force at the berry skin break) were determined from the force/deformation graph using the Exponent software.

For each of the six evaluations, the average mechanical parameters of 14 berries were calculated for each storage relative humidity in triplicate.

4.2.8. Statistical analysis

4.2.8.1. Blueberry characterisation at harvest

General linear model (GLM) in ANOVA was used to determine the influence of harvest date on berry size, maturity indicators (soluble solids and acidity), and mechanical parameters at harvest. For each harvest date, maturity was assessed on 6 juice samples, berry size on 90 berries and mechanical parameters on 45 berries. Assumptions for conducting ANOVA were analysed. If normality did not exist, data were subjected to Box-Cox transformation considering the rounded λ value from the 95 % confidence interval of λ. Means were separated according to Fisher-LSD (P-value < 0.05).

For each independent harvest date (H1, H2, and H3) and storage time (14 or 28 d) combination, general linear model (GLM) in ANOVA was used to determine the influence of storage relative humidity (77 %, 88 %, 95 %, 97 % and 99 %) on average water loss. Six independent GLM were performed. Two mesh bags (24 berries each) obtained from the same jar were averaged for each replication. If normality was not passed data was subjected to Box-Cox transformation. Means were separated using LSD-Fisher at *P*-value < 0.05. In addition, the influence of storage time (14 and 28 d) on water loss for each storage relative humidity was analysed using mixed linear model (MLM). Jars were treated as random factor and storage time as a fixed factor.

4.2.8.2. Sensory analysis

Sensory data for each of six sensory sessions (3 harvest X 2 storage times) was presented by the count of berries with the same hand firmness score (1-4) from a total of 42 berries for each storage relative humidity (14 berries x 3 replication). Statistical mode and median were used to report descriptive statistics of categorical sensory data (Kemp et al., 2009).

Statistical comparison of hand-touch firmness scoring between relative humidity treatments was performed by nonparametric Kruskal Wallis H test (P-value < 0.05). Count of berries of each hand-touch firmness was obtained for each replication (n = 14 berries), and independent analyses were performed for each of the six sensory sessions.

4.2.8.3. Relationship between instrumental and sensory response variables

Principal component analysis (PCA) was used to visualise the relationship between instrumental and sensory variables in a loading plot, and between source of data variation, including harvest dates and storage relative humidity, in a score plot. PCA was performed using the correlation matrix (rescaled values) of instrumental and sensory data. For each replication, instrumental data was average water loss (WL), berry diameter (BD), mechanical parameters of TPA (hardness slope (BHS), cohesiveness (BCo), resilience (BR) and springiness (BSp)), and parameters of penetration test (force at skin break (FSk), skin break slope (SSk), displacement at skin break (DSk), and skin break energy (WSk)). Sensory data included five variables, median of hand-touch firmness category and count of berries for each hand-touch category of 'very soft', 'soft', 'firm' and 'very firm' on each replication. All these response variables were obtained from ninety (90) experimental units (3 harvest dates X 2 storage times X 5 storage relative humidity X 3 replications). Fourteen berries were used for instrumental mechanical parameters, diameter, and sensory evaluation, and 48 berries for water loss. Blueberries used to calculate sensory data were different to those used to calculate BSP, BR, BCo, and BD, which were different to blueberries used for DSk, WSk, FSk, and SSk. Water loss (WL) is calculated from all berries on each observation.

As instrumental variables were continuous data, the linear relationship between the average of each instrumental variable was quantified by Pearson's Correlation (r, $P \leq 0.05$). As sensory data was discrete data, monotonic relationships between the average of each instrumental variable and sensory variables (i.e., median hand-touch firmness and count for each hand-touch category) were calculated using Spearman's correlation (r_s, $P \leq 0.05$).

To calculate unmarketable berries (%), hand-touch firmness results of each berry were converted to binary data, where 1 (unmarketable) was used for 'very soft' or 'soft' blueberries and 0 (saleable) for 'firm' or 'very firm'. A binary logistic model was fitted to determine the likelihood of unmarketable berries (i.e., number of berries categorised as 1, out of 14 berries on each replication) as dependent variables and average of each mechanical parameter, as an explanatory or independent variable. As determined by PCA and Spearman correlation, only the most related mechanical parameters to sensory data were used for logistic modelling. Independent logistic regression was performed for each explanatory mechanical parameter of ninety (90) observations (3 harvest dates X 2 storage times X 5 storage relative humidity X 3 replications). Logit was used as a link function. Regression equation, significance level (Chi-square *P*-value), deviance R^2, and odds ratios were determined. Odds are calculated based on ratio of likelihood of event success (i.e., unmarketable berries) to the likelihood of failure (i.e., saleable berries). Consequently, odds ratio allowed determination of the likelihood of unmarketable fruit to increase (odd ratio > 1) or decrease (odd ratio <1) as influenced by an additional increase in one unit for each mechanical parameter. In addition, a logistic regression equation was used to calculate values of mechanical parameters associated with a low (≤ 5 %) or high (≥ 95 %) likelihood of unmarketable berries.

4.2.8.4. Refence samples characterisation (silicone spheroids)

Differences in average maximum force to 1 mm between four hand-firmness intensities of reference silicone spheroids were evaluated before the first sensory session, using a general linear model (GLM) in ANOVA. Independent analyses were performed on each spheroid size (1.1 cm^3 or 1.4 cm^3); hence, for each hand-touch intensity, seven replications of one silicone spheroid were used. Means were separated using LSD-Fisher at *P*-value < 0.05. In addition, the influence of silicone spheroid size was analysed by a mixed linear model (MLM), where the hand-firmness intensity was used as a random factor and spheroid size as a fixed factor.

Variation of silicone spheroids during the time was analysed using MLM in ANOVA, where each silicone blueberry (including two sizes) was used as a random effect and evaluation time (Sensory session 1st, 3rd, and 6th) as a fixed effect. Fourteen replications of one silicone spheroids were used.

Statistical analysis of the general linear model (GLM), mixed linear model (MLM) and binary logistic regression were analysed using the statistical software Minitab® 18 (Minitab Pty Ltd, Australia). Kruskal Wallis H test and PCA were performed using statistical software InfoStat version 2020 (Universidad Nacional de Córdoba, Argentina). All Figures were plotted using Origin 2019b (OriginLab Corporation, MA, USA), except for principal component analysis (PCA) and binary fitted line plot, which were plotted using Infostat and Minitab® 18, respectively.

4.3. Results

4.3.1. Harvest maturity and quality

Berry size, measured by equatorial diameter, polar length, and volume, was influenced by harvest date (**Table 4.3**). Blueberry size decreased as the harvest date progressed, and hence blueberries of H1 were larger than H3, but no differences were observed between blueberries of H1 and H2 for polar length and volume (**Table 4.3**). Consequently, average blueberry volume varied between 1.1 cm^3 and 1.3 cm^3 with a dispersion from the mean, as measured by coefficient of variation (CV), of approximately 17 % (**Table 4.3**). Hence, size differences designed for silicon spheroids (reference samples) of 1.1 cm^3 (14 mm equatorial diameter and 11 mm polar length) and 1.4 cm^3 (15 mm equatorial diameter and 12 mm polar length), were in the range of 'Centurion' blueberries used on the present study (**Table 4.3**).

Harvest dates influenced maturity indicators of soluble solids (SS), acidity (A), and the ratio of soluble solids to acidity (TSS:TA) (**Table 4.3**). However, maturity differences did not follow a consistent pattern as the harvest date progressed. Berries of H2 showed higher SS, lower acidity, and consequently higher TSS:TA than H1. While, H3 presented lower soluble solids than H1, and there were no differences in acidity and TSS:TA when compared to H1. For all harvest dates, TSS:TA was with ranges for commercial harvest maturity of 10 to 33 (Saftner et al., 2008) or 15 to 29 (Moggia et al., 2017b). Nevertheless, blueberry genotypes can require different optimal ranges in TSS:TA for mature berries destinated to storage (Gilbert et al., 2013).

Table 4.3.Fruit dimensions and maturity indicators of 'Centurion' blueberries harvested at 100 % blue surface colour on three opportunities (H1, H2, H3).

Harvest Date (2020)		**Fruit size**				**Maturity indicators**			
		Diameter (mm) [a]	**Length (mm)**	**Volume**		**SS**[c] **(%)**[d]	**TA (%)**[d]	**TSS:TA**	
				(cm^3)	**CV**[b]			**(-)**	**CV**
H1	January 28th	14.3 a[e]	11.7 a	1.3 a	14.1	15.5 b[e]	0.61 a	25.5 b	12.8
H2	February 7th	13.9 b	11.6 a	1.2 a	19.1	16.4 a	0.53 b	31.3 a	6.6
H3	February 17th	13.6 c	11.1 b	1.1 b	17.6	14.2 c	0.58 ab	24.8 b	13.3

[a]Mean of 90 berries
[b]CV= coefficient of variation (%) calculated as $(\frac{standard\ deviation}{mean}) \times 100$.
[c]SS = total soluble solids, TA = acidity
[d]Mean of 6 juice samples of 15 berries each
[e]Harvest dates mean with the same letters were not statistically significant according to LSD-Fisher (P-value > 0.05).

For all mechanical parameters from texture profile analysis (TPA), box plots described similar dispersion for each harvest date (**Fig. 4.2**). Hardness slope (BHS) was the only TPA parameter influenced by harvest date (**Fig. 4.2**), with berries of H2 having higher BHS than H3, while H1 was not different to H2 and H3. All mechanical parameters from the penetration test were influenced by harvest date. Blueberries of H1 showed higher force at skin break (FSk) and skin break energy (WSk) than H2 and H3, but only higher displacement at skin break (DSk) than H2. In addition, the same differences described for BHS were observed for skin break slope (SSk). The differences in mechanical parameters between harvest dates support that this study generated data that can be associated with a range of commercial scenarios.

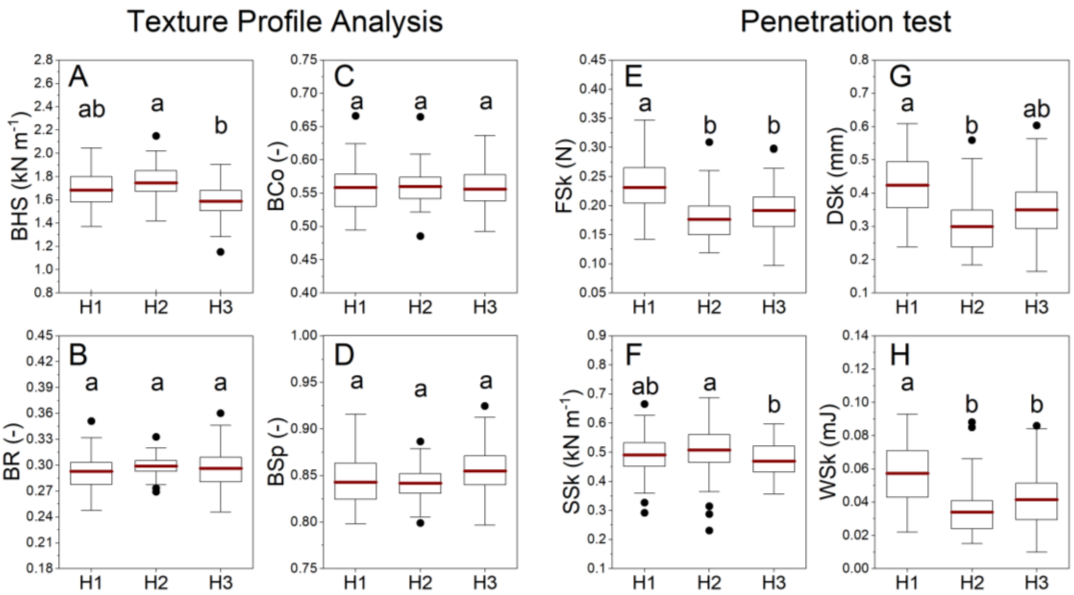


Fig. 4.2. Mechanical parameters from Texture profile analysis (TPA. A-D) and penetration test (E-H) of 'Centurion' blueberry as influenced by three harvest dates (H1 = 28th January; H2 = 7th February; H3 = 17th February 2020). TPA measured berry hardness slope (BHS, A), resilience (BR, B), cohesiveness (BCo, C), and springiness (BSp, D). Penetration test measured force at skin break (FSk, E), skin break slope (SSk, F), displacement at skin break (DSk, G), and skin break work energy (WSk, H). Data of 45 berries for each harvest is presented as a boxplot where the IQR box contains the observation between the 1st (25th percentile) and 3rd quartile (75th percentile). The horizontal line inside each box represents the mean. Whiskers are calculated as the 1.5 of IQR, and outliers are represented by any dot shown outside of the whisker range. Means with the same letters were not statistically significant according to LSD-Fisher (P-value > 0.05).

4.3.2. Water loss and shrivel

Storage in five relative humidity was utilised to generate blueberry populations with different cumulated water loss (**Fig. 4.3**). Blueberries stored at lower relative humidity (71 % or 88 %) always showed higher water loss when compared to berries stored at high relative humidity (≥ 97 %). In addition, water loss increases from 14 d to 28 d of storage for all humidities ≤ 97 % (**Fig. 4.3**, see asterisk symbols). Consequently, maximum cumulated water loss was observed on berries stored at 71 % for 28 d, being 12.7 %, 13.9 %, and 16.3 % for H1, H2, and H3, respectively. In contrast, for 99 % relative humidity, water loss was always less than 1.0 %. These WL differences were expected to generate a variety of textural quality (**Chapter 2**).

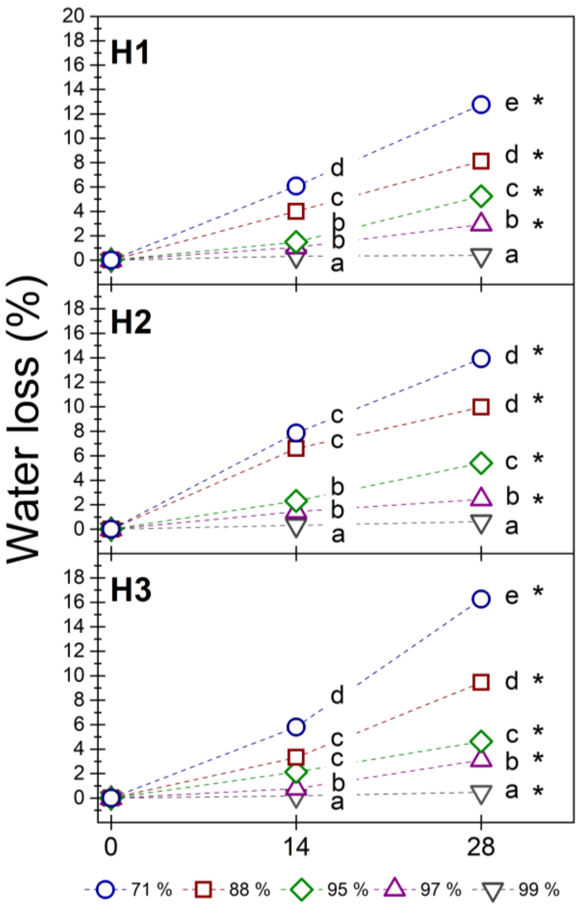


Fig. 4.3. Average of cumulated water loss (%) of 'Centurion' blueberries as influenced by storage relative humidity (71 %, 88 %, 96 %, 98 % or 99 %). Differences are presented for each storage time (14 d or 28 d) at 4.7 °C on each of the three harvest date (H1 = 28th January; H2 = 7th February; H3 = 17th February 2020). Dots = mean of three replications, where each replication is the average of two mesh bags with 24 blueberries each. Means with the same letters on each harvest date (H1, H2, H3) and evaluation time (14 or 28 d) were not statistically significant according to LSD-Fisher (P-value > 0.05). Asterisks (*) indicate significant differences (P <0.05) in WL between storage times (14 d and 28 d) for each storage relative humidity and harvest date.

The percentage of visual dehydration (berries in moderate or severe shrivel category) increased as storage relative humidity decreased. In addition, dehydration increased as storage time progressed. Dehydration was maximum on berries stored at 71 % relative humidity for 28 d being, 52.4%, 66.7 % and 54.8 % for H1, H2, and H3, respectively. In contrast, dehydration was not observed (0.0 %) for 99 % relative humidity even after 28 d (**Table 4.4)**.

Table 4.4. The percentage of berries with visual dehydration (berries with moderate or severe shrivel symptoms) as influenced by storage relative humidity conducted on 'Centurion' blueberries on three harvest dates (H1 = 28th January; H2 = 7th February; H3 = 17th February 2020) and two storage times (14 d or 28 d) at 4.7 °C. Data represent the mean percentage of three replications, and each replication considered the percentage of dehydrated berries out of 14 berries.

Relative humidity (RH)	H1		H2		H3	
	14 d	28 d	14 d	28 d	14 d	28d
99 %	0.0 a[a]	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
97 %	0.0 a	2.4 ab	0.0 a	0.0 a	0.0 a	2.4 a
95 %	0.0 a	14.3 bc	0.0 a	0.0 a	2.4 a	11.9 ab
88 %	4.7 ab	16.7 c	16.7 ab	52.4 b	2.4 a	26.2 b
71 %	11.9 b	52.4 d	11.9 b	66.7 b	2.4 a	54.8 c

[a] means dehydration for each storage relative humidity with the same letters were not statistically different according to LSD-Fisher (P-value > 0.05).

4.3.3. Sensory evaluation of hand-touch firmness

In agreement with water loss results, the experimental design was properly devised to generate variations on hand-touch firmness, which was influenced by storage relative humidity in all six sensory sessions (**Fig. 4.4**). As hand-touch firmness is categorical data, descriptive statistics are reported based on median and mode rather than mean. 'Very firm' blueberries (score 4) were the most frequent (mode) hand-touch firmness category when blueberries were stored in high relative humidity (≥ 97 %) for 14 d or 28 d at 4.7 °C (**Fig. 4.4 A-F**). Similarly, median hand-touch firmness of high relative humidity storage ranged between 'firm' (score 3) and 'very firm'. On the other hand, blueberries stored in low relative humidity (i.e., 71 % or 88 % relative humidity) for 14 d at 4.7 °C median and mode categories were 'firm' (**Fig. 4.4 A, C, E**). However, when low relative humidity storage was extended for 28 d, the median and mode of hand-touch firmness category ranged between 'very soft' and 'soft' (**Fig. 4.4 B, D, F**)

Non-parametric ANOVA Kruskal Wallis test confirmed the differences observed by the descriptive data. For all sensory sessions, blueberries stored in high relative humidity (≥ 97 %) showed higher hand-touch firmness than berries stored at low relative humidity (71 % or 88 %) (**Fig. 4.4 A-F**), except for H3 stored for 14 d, where 88 % relative humidity was not different to 97 % relative humidity (**Fig. 4.4 E**).

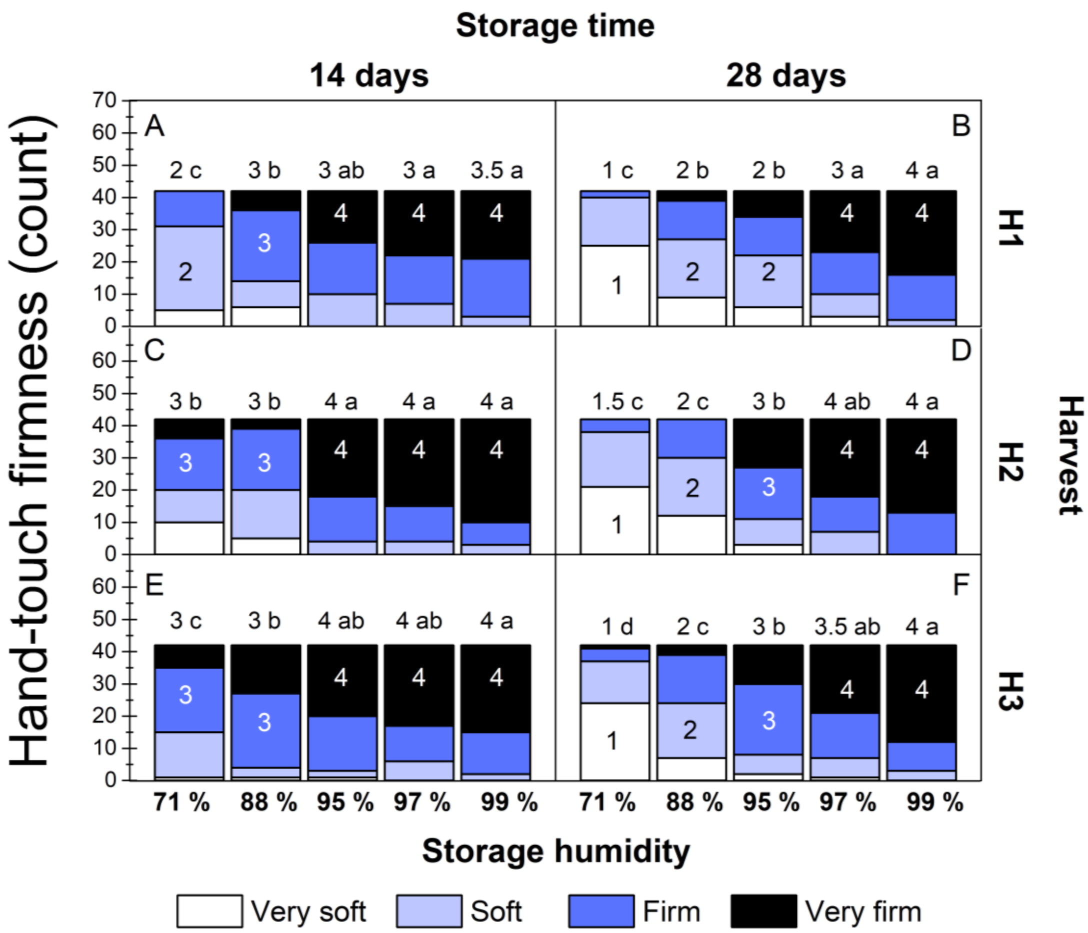


Fig. 4.4. Count of berries for each hand-touch firmness category in each storage relative humidity as evaluated by seven assessors during six sensory sessions (A-F). Each sensory session was a combination of harvest date of H1 (A, B), H2 (C, D), H3 (E, F) and storage time of 14 d (A, C, E) and 28 d (B, D, F) at 4.7 °C. Each column (i.e., storage relative humidity) is a total of 42 berries, where each of the seven assessors evaluated six berries. The number displayed inside each column is the mode hand-touch firmness category of 42 berries, while the number over the column is the median score. According to ANOVA on Ranks (Kruskal Wallis Test), columns with the same letters on each sensory session were not statistically significant (P-value > 0.05).

4.3.4. Relationship between hand-touch firmness and instrumental mechanical parameters

4.3.4.1. Principal Component Analysis and Correlation coefficients

A multivariate descriptive analysis, principal component analysis (PCA), was used to visualise relationships between instrumental and sensory variables in a loading plot (**Fig. 4.5 A**), and between observations (harvest date and storage relative humidity) in a score plot (**Fig. 4.5 B**). Only PC1 and PC 2 are shown because both PC explained around 75 % of data variation.

The first two principal components (PC1 + PC2) accounted for 74.7 % of data variation, where PC1 accounted for most of the variation (62.5 %) and PC2 for 12.1 %. From the loading plot, the most associated variables to PC1 were average hardness slope (BHS, kN m^{-1}), skin break slope (SSk, kN m^{-1}), displacement at skin break (DSk, mm), an average of water loss (WL. %), and median of hand-touch firmness category (HFM, 1-4) (**Fig. 4.5 A).** PC 2 was mainly associated with average springiness (BSp, -), and the count of berries in 'firm' category (**Fig. 4.5 A**).

The score plot shows that for all harvest dates (H1, H2, H3), blueberries stored in high relative humidity (97 % - 99 %) were positioned on the left side of PC1, while blueberries of low relative humidity (71 % - 88 %) were positioned on the right side of PC1 (**Fig. 4.5 B**). Consequently, blueberries in high relative humidity were associated with higher values of BHS, SSk, and HFM (i.e., firmer berries). In contrast, low relative humidity was associated with higher values of DSk, and WL (**Fig. 4.5 A, B**). The score plot also showed that more H1 data points were positioned on the upper quadrant of PC2 compared to H2 and H3. Hence, H1 can be associated with higher values of BSp and the count of berries in 'firm' category (**Fig. 4.5 A, B**).

A relationship between instrumental variables observed from the PCA (**Fig. 4.5 A**) and quantified by Pearson's correlation coefficients (r) confirms previous study results (**Chapter 2**). Mechanical parameters of BHS, DSk, SSk and skin work energy (WSK, mJ) were the most strongly correlated ($r \geq \pm 0.92$) to water loss (**Supplementary Table 4.3**). In addition, mechanical parameters of TPA, BHS, cohesiveness (BCo, -) and resilience (BR, -) were strongly correlated between each other (r ≥ ±0.75), and DSk, SSk and WSK of penetration test were strongly associated between each other (r ≥ ±0.75) and with BHS (r ≥ ±0.88) (**Supplementary Table 4.3)**.

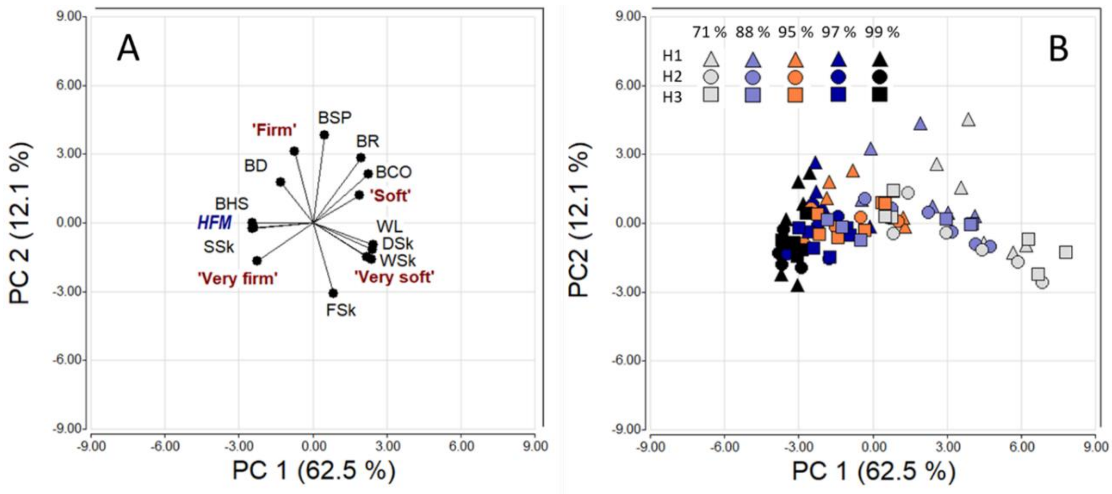


Fig. 4.5. Loading (A) and score (B) plots of principal component (PC) 1 and 2, of instrumental and sensory variables (A) of blueberries of three harvests (B, H1 (triangles), H2 (circles), and H3 (squares)) stored in five relative humidity (B, 71 % (grey), 88 % (purple), 95 % (orange), 98 % (blue), 99 % (black)) for 14 d and 21 d at 4.7 °C. Instrumental variables are in clockwise order, BSp = springiness (-), BR = resilience (-), BCo = cohesiveness (-), WL = water loss (%), DSk = displacement at berry skin break (mm), WSk = skin break work energy (mJ), FSk = force at berry skin break (N), SSk = skin break slope (kN m^{-1}), BHS = hardness slope (kN m^{-1}), BD = berry diameter (mm). Sensory data is 'very soft', 'soft', 'firm' and 'very firm', which represent the count of berries (n = 14) in each hand-touch firmness category; and HFM = median hand-touch firmness category (1-4; where 1 = 'very soft', 2 = 'soft', 3 = 'firm' and 4 = 'very firm'). For score plots, each dot (n = 90) accounts for 14 berries for instrumental and sensory data, except water loss which is calculatedd from 2 mesh bags of 24 berries each. Berries used to calculate sensory data were different from those used to calculate BSP, BR, BCo, and BD, which were different blueberries than DSk, WSk, FSk, and SSk. Water loss (WL) is calculated from all berries combined.

In addition to PCA (**Fig. 4.5 A)**, Spearman correlation coefficients (r_s) were calculated to quantify relationships between instrumental and sensory variables (**Table 4.5**). A strong negative correlation (r_s = -0.86) was calculated between HFM and water loss (**Table 4.5),** which was an expected result when considering results for water loss (**Fig. 4.3)** and sensory sessions (**Fig. 4.4**).

Mechanical parameters of BHS obtained by plate compression and SSk obtained by needle probe showed the strongest correlations (r_s ≥ ±0.84) with HFM. In addition, these mechanical parameters were the strongest correlated with count of berries in hand-touch firmness category of ‘very soft’, ‘soft’ or ‘very firm’ (**Table 4.5**). However, the count of berries in the ‘firm’ category was not correlated to any mechanical parameter, except BSp, but it was a weak (r_s = 0.34) correlation (**Table 4.5**). Graphical visualisation of the Spearman correlation between the mechanical parameters best related (i.e., BHS or SSk) to sensory responses is also presented (**Supplementary Fig. 4.2**).

Table 4.5. Spearman correlation coefficient (r_s) between sensory variables, water loss, and instrumental mechanical parameters of Texture profile analysis and Penetration test (n = 90).

Sensory variables[a]	Instrumental variables									
	Water loss	Texture Profile Analysis[b]				Penetration test[c]				Diameter
		BHS	BR	BCo	BSp	FSk	SSk	DSk	WSk	
HFM	-0.86[d]***	0.84***	-0.75***	-0.83***	-0.10	-0.15	0.88***	-0.83***	-0.77***	0.30**
Count of ‘very soft’	0.82***	-0.82***	0.72***	0.79***	-0.03	0.25*	-0.79***	0.79***	0.76***	-0.30**
Count of ‘soft’	0.72***	-0.73***	0.66***	0.72***	0.06	0.09	-0.76***	0.70***	0.64***	-0.21*
Count of ‘firm’	-0.13	0.14	-0.04	-0.10	0.34**	-0.01	0.13	-0.14	-0.16	0.01
Count of ‘very firm’	-0.87***	0.87***	-0.78***	-0.87***	-0.18	-0.18	0.89***	-0.83***	-0.77***	0.30**

[a]HFM= Median of hand-touch firmness category (1-4); where 1 = ‘very soft’, 2 = ‘soft’, 3 = ‘firm’ and 4 = ‘very firm’. ‘Very soft’, ‘soft’, ‘firm’ and ‘very firm’ = count of berries on each category.
[b]BHS = hardness slope (kN m^{-1}), BR = resilience (-), BCo = cohesiveness (-), BSp = springiness (-)
[c]FSk = force at berry skin break (N), SSk = skin break slope (kN m^{-1}), DSk = displacement at berry skin break (mm), WSk = skin break work energy (mJ)
[d]Spearman correlation coefficient (r_s) in red represent negligible correlation (*P*-value > 0.05), in black weak to moderate correlation (r = 0.25 - 0.79), in blue strong correlations (r ≥ 0.8).
Asterisk (*) represent *P*-value, where * ≤ 0.05; ** ≤ 0.01; and *** ≤ 0.001

4.3.4.2. likelihood of unmarketable berries

From the total number of berries (n = 1,260) used on all six sensory sessions, 142 were classified by hand-touch firmness as ‘very soft’ and 268 berries as ‘soft’. Accordingly, unmarketable (‘very soft’ or ‘soft’) cases occurred for 410 berries, representing 33 % of the total evaluated population.

The most related mechanical parameters to sensory variables, hardness slope (BHS) and skin break slope (SSk) (**Fig. 4.5, Table 4.5**), were selected to logistically model likelihood of unmarketable berries. Average BHS and SSk were significantly (Chi-square P-value <0.001) and strongly (deviance R^2 = 0.8) related to likelihood of unmarketable berries. In addition, odd ratios were less than one for both mechanical parameters, and consequently, as BHS or SSk increases the likelihood of unmarketable berries decreases (**Fig. 4.6**).

Considering binary logistic plot equations (**Fig. 4.6**), average BHS or SSk lower to 0.47 kN m^{-1} or 0.13 kN m^{-1}, respectively, were related to a high likelihood (≥ 95 %) of unmarketable berries. Conversely, low likelihood (≤ 5 %) of unmarketable berries (or high likelihood of 'firm' or 'very firm' berries) was estimated when average BHS or SSk were higher to 1.71 kN m^{-1} or 0.42 kN m^{-1}, respectively (**Fig. 4.6**).

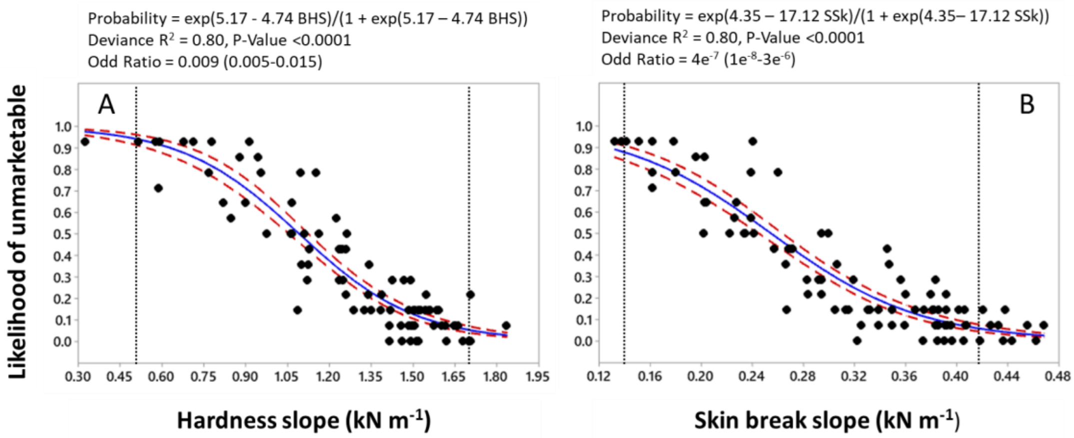


Fig. 4.6. Binary logistic plot for likelihood of unmarketable ('soft' or 'very soft' hand-touch firmness) as related to average hardness slope (A) and skin break slope (B) in 'Centurion' blueberries of three harvest dates and stored on five RH for 14, or 28 d at 4.7 °C. Dots (n= 90) represents results of groups of 14 berries. Continues line = Fitted line. Dash lines = 95 % confidence interval for likelihood of unmarketable berries fit line. Vertical dotted lines = from left to right, high (≥ 95 %) and low (≤ 5 %) likelihood of unmarketable blueberries.

4.3.5. Reference samples (silicone spheroids) characterisation

This study has developed silicone spheroids for four hand-touch firmness intensities to be used to assist and calibrate assessor performance on each sensory session. The verification that each silicone reference had different mechanical characteristics was performed by instrumentally measuring maximum force to 1 mm deformation (N). Four maximum forces to 1 mm levels were satisfactorily obtained (**Table 4.6**). No differences in average maximum force to 1 mm were observed between silicone spheroids made of two sizes (P-value = 0.06). Consequently, average maximum force to 1 mm before initiating sensory sessions were 0.56 N, 1.32 N, 1.87 N and 2.58 N, for silicone spheroids referencing a hand-touch intensity of 'very soft', 'soft', 'firm' and 'very firm', respectively (**Table 4.6**). Consequently, force to 1 mm deformation of silicone spheroids is separated from subsequent category (i.e., ‘very soft’ vs ‘soft’; ‘soft’ VS ‘firm’ and ‘firm’ VS ‘very firm’) by approximately 0.7 N.

As expected, silicone spheroids within each intensity did not vary strongly, as the coefficient of variation (CV) was below 7 % for silicones referencing a ‘very soft’, ‘firm’ and ‘very firm’ blueberry 12.3 % for ‘soft’ silicone. The authors infer that variation when creating silicones spheroids can be reduced if the same mixture is used to create all samples. Consequently, the number of ABS moulds must coincide with required number of silicone blueberries of the same intensity (i.e., 7).

Silicone spheroid of each category did not show differences in maximum force to 1 mm between evaluation times (**Table 4.6**). Consequently, silicone spheroids remained consistent across the two-month frame that lasted the sensory study.

Table 4.6. The average maximum force (N) to 1 mm deformation of silicone spheroids of four hand-touch firmness intensities of two sizes (1.1 cm^3 and 1.4 cm^3). Chord stiffness was evaluated three times (before 1st sensory session, after 3rd session, and after 6th session).

Hand-touch firmness	Spheroid dimension[a]			Evaluation time			
	1.1 cm^3	**1.4 cm^3**	**CV[b]**	**1st**	**3rd**	**6th**	**P-value[e]**
Very soft (1)	0.54 d[c]	0.58 d	5.7	0.56	0.56	0.54	0.11
Soft (2)	1.33 c	1.30 c	12.3	1.32	1.30	1.33	0.35
Firm (3)	1.83 b	1.91 b	6.7	1.87	1.84	1.84	0.53
Very firm (4)	2.55 a	2.60 a	6.8	2.58	2.52	2.49	0.16
n[d]	7	7	14	14	14	14	

[a]Average maximum force for each spheroid size and hand-touch firmness intensity was measured one week before starting first session

[b]CV= coefficient of variation (%) calculated as $(\frac{standard\ deviation}{mean}) \times 100$. The CV for each hand-touch category considered two spheroid sizes evaluated before starting 1st sensory session.

[c]Means with the same letters across hand-touch firmness categories were not statistically significant according to LSD-Fisher (P-value > 0.05).

[d]n= number of silicone spheroids used to estimate the mean and standard deviation.

[e]P-value of influence of evaluation time on chord stiffness of silicone spheroids of two sizes. Independent analyses were performed for each hand-touch firmness intensity.

A silicone spheroid recorded a less linear increase in force as the sample was deformed to 15 % strain (**Fig. 4.7 A**) when compared to a real ‘Centurion’ blueberry (**Fig. 4.7 B**). This is especially evident for ‘Centurion’ blueberries at harvest time and stored at high relative humidity (99% RH) (**Fig. 4.7 B**).

The distribution, within storage relative humidity, of force to 1 mm deformation of each individual blueberry of three harvests (n = 252 berries for each relative humidity) show that silicone spheroids are referencing a 'very soft', 'soft' and 'firm' blueberry where inside values that can display real 'Centurion' blueberries (**Fig. 4.7 A, C**). However, for 'very firm' silicones average force of 2.53 N was higher than any value (maximum = 2.19 N) observed of stored 'Centurion'.

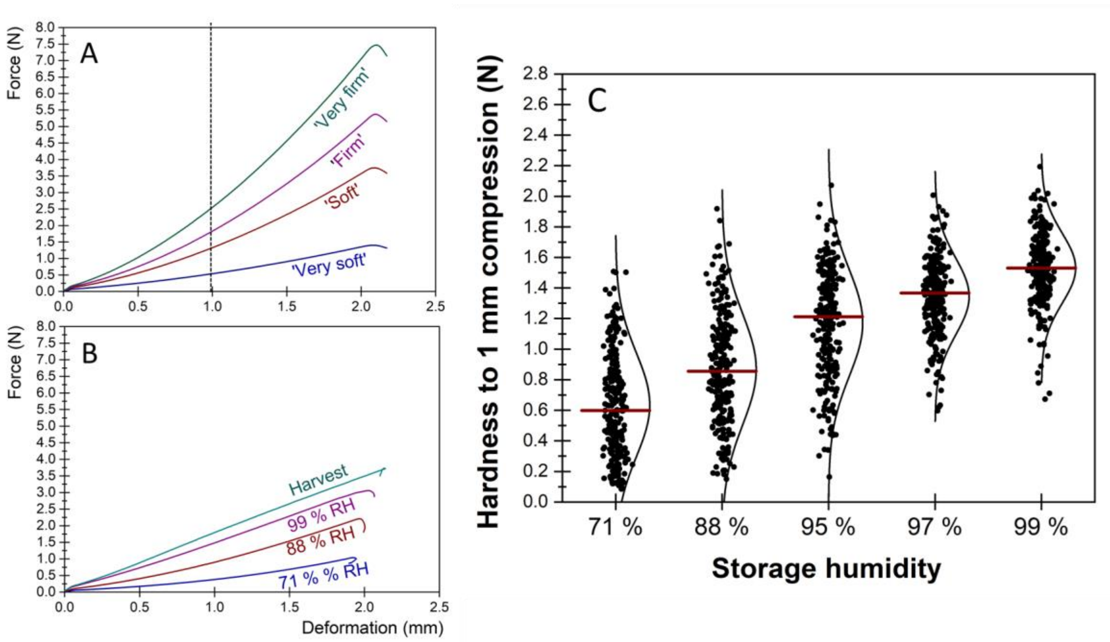


Fig. 4.7. (**A**) Graphical representation of force-deformation plot obtained by compression test to 15 % deformation of silicone spheroids (size: 1.1 cm^3) of each hand-touch category ('very soft', 'soft', 'firm' and 'very firm'). The dashed vertical line shows the force to 1 mm deformation. (**B**) Graphical representation of force-deformation of first compression loading on TPA of 'Centurion' blueberry of Harvest 1 at harvest time and after 28 d of storage at 4.6 °C on three storage relative humidity levels (71 %, 88 % and 99 %). (**C**) Distribution of the force to 1 mm deformation for each individual blueberry of H1, H2 and H3 stored on five different storage relative humidity levels for 14 d and 28 d at 4.6 (n = 252 berries for each storage relative humidity). The red line in plot C indicates the median for each storage's relative humidity. Figures A and B were plotted on Origin software (OriginLab Corporation, MA, USA) using the raw data obtained from Texture Analyser Exponent software (silicone spheroids = 7; 'Centurion' blueberries = 42 berries for each condition).

4.4. Discussion

4.4.1. Relationship between water loss and texture evaluation

As expected, storage relative humidity influenced the sensory evaluation of hand-touch firmness. As relative humidity decreased (from 97 % to 71 %) and storage time progressed (14 d or 28 d), water loss increased (**Fig. 4.3**), and blueberries were rated with a lower hand-touch firmness score (i.e., softer berries) (**Fig. 4.4)**. Consequently, a strong Spearman correlation coefficient negatively (r_s = -0.86) relates water loss to the median hand-touch firmness. In agreement with these results, Nunes and Emond (2007) reported for 'Patriot' blueberry a strong negative correlation (r = -0.9) between WL and sensory hand-touch firmness using a 5-point scale (1 = 'very soft' to 5 = 'firm'). Conversely, Cantin et al (2012) did not observe any correlation between water loss and hand-touch firmness evaluated using a 3-point scale (1 'very firm' to 3 'not firm enough') on eight blueberries genotypes. This difference can potentially be associated with differences in hand-touch evaluation scale, assessor's subjectiveness, or blueberry genotype variation.

In the present study, the relationship between water loss and each hand-touch firmness category showed a moderate to strong Spearman correlation (r_s) for counts of 'very soft' (r_s = 0.82), or 'soft' (r_s = 0.72) berry category. Likewise, Miller et al. (1993) observed that after 21 d at 1 °C storage of 'Climax' and 'Sharpblue' blueberries in polystyrene packs showed a lower percentage of 'soft' berries rated by hand-touch firmness, accompanied by less WL (0.9 %), when compared to a fibre-cellophane pack with higher WL (5.3 - 6.1 %).

Nevertheless, in the present study, the correlation to water loss was negligible to count of berries considered 'firm' (r_s = -0.13), but strong for 'very firm' (r_s = -0.87). Consequently, lower differences in the distribution of berries in the 'firm' category were observed for different storage RHs compared to 'very soft', 'soft', or 'very firm' categories (**Fig. 4.4**). For example, the average of all six sensory sessions of berry count for the 'firm' category was 17 for 88 % RH and

12 for 99 % RH, while the average count for 'very firm' was 5 for 88 % and 28 for 99 % berries. It cannot be eliminated that lower differences between RHs for berries rated as 'firm' can be attributed to central tendency error induced by assessor's reasoning psychology, which may affect the sensory evaluation performance. Central tendency error is produced when sensory assessors avoid using the extremes of an evaluation scale and decide to score samples using the categories located in the middle portion of the evaluation scale (Kemp et al., 2009). This study asked assessors to touch the blueberry twice before scoring the blueberries in one of the four-point hand-touch categories (**Table 4.2**). The first touch provided information to differentiate berries in the 'very soft' category from the other three hand-touch categories. On the second touch, assessors chose between three score categories 'soft', 'firm' and 'very firm'. Consequently, on second hand-touch assessment, assessors may have been persuaded to score the berries as 'firm' (i.e., middle category of the scale) when not sure of which category to choose. However, this error is more likely to occur with untrained assessors or assessors not familiarised with blueberry hand-touch firmness categories (Kemp et al., 2009). However, it is unlikely that this was the case in our sensory design because all assessors were trained, and reference samples (silicone spheroids) were provided to each assessor. Alternatively, central tendency error can be minimised when using a larger scale to assess hand-touch firmness (Kemp et al., 2009). Whether the similar distribution of berries in the 'firm' category between distant relative humidity (i.e., 88 % vs 99 %) is a consistent outcome on a 'Centurion' blueberry or a result of central tendency error requires further study, for example, using a larger hand-touch firmness scale (≥ 5-point).

Another bias that can be attributed to assessor psychology is a logical error (Kemp et al., 2009). Assessors were asked to evaluate texture using only their sense of touch. However, assessors may have based differences between blueberries on visual characteristics such as shrivel symptoms (as influenced by water loss), generating a stimulus and logical error. This error is driven by additional information unconsciously sought by the assessor (Kemp et al., 2009).

Blueberries that present visual shrivel symptoms (i.e., skin corrugation detectable by naked eye) are expected to be softer (Paniagua et al., 2013). Experienced assessors can be aware of this additional information. In the present study, berries with dehydration (i.e., percentage of berries with moderate or severe shrivel) were mostly observed at the 28 d evaluation and for RHs lower than 95 % (**Table 4.4**), which coincided with a hand-touch firmness median and mode varying between 'soft' or 'very soft' category (**Fig. 4.4**). A future sensory study should consider presenting berries to assessors with variation in texture properties but consistent visual characteristics. The authors hypothesise that blueberries stored in different CO_2 and O_2 atmospheric compositions (i.e., controlled atmosphere) can be used for providing berries with different textural properties but similar weight loss and visual appearance (Forney et al., 2003; Schotsmans et al., 2007; Panigua et al., 2014; Concha-Meyer et al., 2015).

In relation to instrumental mechanical properties, the results of this study confirm previous results (**Chapter 2**). Displacement at skin break (mm) and skin break work energy (mJ) by penetration test and hardness slope (KN m^{-1}) by compression show the strongest relation to water loss (**Fig. 4.5, Supplementary Table 4.3**), even though a different cultivar was used in this study, 'Centurion' compared to 'Nui' or 'Rahi' used in **Chapter 2**. In addition, the skin break slope also showed a strong relationship to water loss (**Fig. 4.5, Supplementary Table 4.3**).

4.4.2. Relationship between sensory hand-touch firmness and instrumental parameters

To the author's knowledge, this is the first attempt to relate instrumental mechanical parameters to sensory hand-touch firmness assessed in a formal sensory panel setting for blueberries. Mechanical parameters of TPA such as hardness slope, cohesiveness, and resilience, and penetration test results of skin break slope, displacement at skin break, and skin break work energy were moderate to strongly correlated (r_s = 0.75 - 0.88) to hand-touch firmness evaluation (**Fig. 4.5, Table 4.4**).

From previous studies that evaluated blueberry sensory hand-touch firmness (**Table 4.1**), only Nunes (2015) reported a statistical relationship between firmness evaluated by sensory and instrumental method results. However, even when using a similar experimental setting to the present study (i.e., storage relative humidity), Nunes (2015) observed only a weak Pearson correlation coefficient ($r = 0.342$; P-value < 0.05). The difference in results is likely caused by differences in instrumental methods or sensory evaluation methods. The instrumental methodology of Nunes (2015) considered the average compression force of a group (30 g) of berries simultaneously. The present study considered the average mechanical parameters of a group of fruit (14 berries), where each blueberry was individually analysed on a texture analyser. In addition, Nunes (2015) used trained quality evaluators, while in this chapter, a formal sensory panel including reference samples (silicone spheroids) was used.

The results of Schotsmans et al. (2007) suggest that sensory hand-touch firmness evaluated by different quality evaluators may generate differences in reproducing the storage history of 'Centurion' blueberries. For example, blueberries stored in a regular atmosphere for 21 d at 1.5 °C + shelf life (6 d at 20 °C) were scored as being softer than 28 d + shelf life using hand-touch, while instrumental compression force did not show differences between both storage times (Schotsmans et al., 2007). In the present study, storage impact caused by relative humidity < sensory panellists differentiated 95 %, sensory sessions performed at 14 d showed higher median and mode scores (i.e., firmer berries) than after 28 d at 4.7 °C (**Fig. 4.4**).

The present study attempted to determine values of instrumental mechanical parameters associated with a high (≥ 95 %) and low (≤ 5 %) likelihood of unmarketable berries due to softening. Threshold values were estimated for the hardness slope of the compression test (**Fig. 4.6**). Similarly, Moggia et al. (2017b), indicating that Firmtech firmness ranges of < 1.6 kN m^{-1}, 1.6-1.8 kN m^{-1}, and 1.81-2.0 kN m^{-1} can be related with a 'soft', 'medium', and 'firm' blueberry. In addition, Lobos et al. (2018) reported that FirmTech firmness < 1.4 kN m^{-1} can represent a 'very soft' blueberry. Firmtech firmness is calculated as a slope line between a minimum force

(0.15 N) and a maximum force threshold (1.96 N) (Moggia et al., 2017b; Prussia et al., 2006). This is the same principle of hardness slope calculation but uses different minimum (trigger force: 0.06 N) and maximum (force at 15 % strain) force thresholds. For a blueberry compression test, an approximately linear increase of force as the blueberry is deformed up to 25 % of the equatorial diameter can be assumed (Ballinger et al., 1973; Rohrbach and Mainland, 1993). Therefore, the reported thresholds for a 'soft' and 'firm' blueberry by Moggia et al. (2017b) and Lobos et al. (2018) are comparable to the threshold obtained by the present study. Low likelihood (< 5 %) of unmarketable berries (and consequently 'firm' berries) was observed when hardness slope was higher to 1.71 kN m^{-1} (**Fig. 4.6**), which is closer to reported 'firm' values by Moggia et al. (2017b). However, the threshold representing a very high likelihood (> 95 %) of 'soft' + 'very soft' berry from the present study is lower (0.47 kN m^{-1}) than the maximum FirmTech firmness threshold of < 1.6 kN m^{-1} for 'soft' (Moggia et al., 2017b) and < 1.4 kN m^{-1} for 'very soft' (Lobos et al., 2018) blueberries. These differences can be associated with equipment precision and accuracy, which is lower for FirmTech than the texture analyser used in the present study. In addition, Lobos et al. (2018) reported compression speed was considerably higher (16 mm s-1) for FirmTech than used in the present study. Higher compression speed can generate higher maximum force to compression (Rosenthal, 2010). Nevertheless, previous studies do not clarify how the FirmTech firmness threshold for firmness categories of 'very soft', 'soft', 'medium' and 'firm' berries were obtained. The present study has used a sensory analysis panel to determine instrumental thresholds (**Fig. 4.6**).

The thresholds indicated by Moggia et al. (2017b) and Lobos 2018 were reported for 'Duke' and 'Briggita' blueberries, while the present study used 'Centurion'. Whether different blueberry genotypes require different firmness thresholds associated with a 'soft' blueberry needs further investigation. In addition, whether other sources of firmness variation such as storage technologies (i.e., controlled atmosphere) generate different thresholds also requires further investigation.

Allan-Wojtas et al. (2001) observed that slight compression to 1.5 N can induce micro-crack ruptures of the cellular structure. Consequently, there is a chance the sensory analysis may alter the mechanical properties of blueberries due to being hand-touched twice. The present study design used different blueberries (obtained from the same batch treatment) to evaluate sensory hand-touch from those blueberries used to measure mechanical parameters instrumentally. Consequently, an average of the mechanical parameters was used rather than individual values for each blueberry. Considering the high biological variance of blueberries from the same harvest batch (as presented by Lobos et al., (2018)), further investigations may consider a methodology where each blueberry can be assessed sensorially and instrumentally and hence enable elucidation of further texture insights. Alternatively, the study of the influence of touching blueberries using the hand-touch protocol (**Table 4.2**) on their instrumental mechanical parameters may be warranted.

4.4.3. Reference samples (silicone spheroids)

The obtained results confirm that mixtures of commercial silicone products originally produced to create theatrical make-up can be utilised to generate silicone spheroids of different forces to 1 mm (**Table 4.5**). In addition, forced to 1 mm of silicone blueberries remained consistent across six sensory sessions carried out over a two-month window (**Table 4.5**).

The use of reference samples (e.g., silicone spheroids) of different hand-touch firmness categories represents a low-cost opportunity to reduce variability in the evaluation of firmness in commercial conditions where texture equipment is not economically affordable or practical for assisting the decision-making. In addition, silicone blueberries of different mechanical parameters can be further designed to be used as a calibration tool for instrumental equipment used on blueberries such as Durometers or FirmTech, as previously proposed by NeSmith et al. (2005) and Prussia et al. (2006) by using rubber balls on a FirmTech.

A ‘Centurion’ blueberry exhibits a more linear increase in force as it is deformed to 15 % strain when compared to silicone spheroids (**Fig. 4.7 A, B)**. In addition, the maximum force to 15 % deformation of ‘firm’ and ‘very firm’ silicones was out of the scale compared to the force to 15 % strain of real blueberries (**Fig 4.7 A, B**). Despite these differences, force values to 1 mm silicone spheroid (**Table 4.6**) were in a similar range for ‘Centurion’ blueberries (**Fig. 4.7 C**). in addition, the median of blueberries in low relative humidity (71 % RH) and consequently the high proportion of ‘very soft’ fruit (**Fig. 4.4**), was 0.59 N (**Fig 4.7 C**), which was very close to the silicone spheroids referencing a ‘very soft’ blueberry. Conversely, ‘very firm’ silicone values were out of the range of values displayed by real blueberries. Future work must re-design reference samples that resemble a ‘very firm’ blueberry to match better mechanical ranges observed on real blueberries. In addition, further investigation is required to determine whether the maximum deformation that assessors applied when hand-feel testing a ‘firm’ and ‘very firm’ real and fake blueberry is below 1 mm.

However, it cannot be precluded that the difference in mechanical profiles between silicone spheroids and real blueberries (**Fig. 4.7 A, B**) may affect assessor performance and consequently remains to be studied. Alternatively, other non-biological materials better imitating a real blueberry mechanical profile could be reviewed and studied.

The author suggests that silicone blueberries can be optimised to resemble a high and low likelihood of unmarketable blueberries that can potentially be used to assist, train, or act as consistent reference points for evaluators in commercial blueberry operations such as quality checkpoints.

4.5. Conclusion and recommendations

This work shows that water loss differences (as induced by different storage relative humidity) influence textural changes that can be perceived by sensory analysis using hand-touch firmness. Mechanical parameters of compression and penetration tests, which were previously reported to be influenced by water loss (**Chapter 2**), were related to hand-touch firmness using a formal sensory panel setting. In addition, threshold values for hardness slope and skin break slope were estimated to be associated with a high and low likelihood of unmarketable (‘soft’ or ‘very soft’) ‘Centurion’ blueberries. Future research studies should focus on validating these instrumental values on different blueberry genotypes and sources of textural variation, such as postharvest technologies (e.g., controlled atmosphere).

Silicone spheroids developed in this study can be used as reference samples to secure consistency between assessors (low variation between reference samples) and sensory sessions (low variation through time). Further study will require developing reference samples of a non-biological material that better imitates the mechanical behaviour of real blueberries and better matches with mechanical parameters (i.e., hardness slope) ranges that can be observed on blueberries of different genotypes.

4.6. Acknowledgment

The authors acknowledge Gourmet Blueberries Ltd. for providing ‘Centurion’ blueberries; Francisca Ducommun for the help provided in organising the plant selection and fruit sampling at the commercial plantation; Micaela Reyes and Talon Sneddon for the help provided at the experimental setup and during the evaluations in Postharvest Laboratory, Massey University. Sebastian Rivera acknowledges the School of Agriculture and Environment, Massey University (NZ), and Agencia Nacional de Investigacion y Desarollo (ANID), Chile, for the financial support in pursuing his PhD studies at Massey University.

4.7. Author Statement

Sebastian Rivera: Conceptualisation, Methodology, Formal analysis, Investigation, Visualization, Writing - Original draft; **Joanne Hort:** Conceptualisation, Methodology, Writing - Review and Editing; **Huub Kerckhoffs**: Conceptualisation, Supervision, Writing - Review and Editing; **Svetla**

Sofkova-Bobcheva: Supervision, Writing - Review and Editing; **Dan Hutchins**: Resources, Supervision, Writing - Review and Editing; **Andrew East**: Conceptualisation, Methodology, Resources, Supervision, Writing - Review and Editing.

4.8. References

Abbott, H.A., 1999. Quality measurements of fruits and vegetables. Postharvest Biol. Technol. 15, 207-225. https://doi.org/10.1016/S0925-5214(98)00086-6

Ahmadzadeh, S.M.H., Hukins, D.WL., 2014. Feasibility of using mixtures of silicone elastomers and silicone oils to model the mechanical behaviour of biological tissues. J. Eng. Med. 228, (7), 730-734. https://doi.org/10.1177/0954411914540138

Allan-Wojtas, P.M., Forney, C.F., Carbyn, S.E., Nicholas, K.U.K.G., 2001. Microstructural indicators of quality-related characteristics of blueberries - An integrated approach. Lebensm.-Wiss. U.-Technol. 34, 23-32. https://doi.org/10.1006/fstl.2000.0738

Ballinger, W.E., Kushman, L.J., Hamann, D.D., 1973. Factors affecting the firmness of highbush blueberries. J. Amer. Soc. Hort. Sci. 98(6), 583-587.

Beaudry, R.M., Moogia, C., Retamales, J.B., Hancock, J.F., 1998. Quality of 'Ivanhoe' and 'Bluecrop' blueberry fruit transported by air and sea from Chile to North America. HortScience 33(2), 313-317.

Blaker, K.M., Plotto, A., Baldwin, E.A., Olmstead, J.W., 2014. Correlation between sensory and instrumental measurements of standard and crisp-texture southern Highbush blueberries (*Vaccinium corymbosum* L. interspecific hybrids). J. Sci. Food Agric. 94, 2785-2793. https://doi.org/10.1002/jsfa.6626

Cantin, C.M., Minas, I.S., Goulas, V., Jimenez, M., Manganaris, G.A., Michailides, T.J., Crisosto, C.H., 2012. Sulfur dioxide fumigation alone or in combination with CO_2-enriched atmosphere extends the market life of Highbush blueberry fruit. Postharvest Biol. Tec. 67, 84-91. https://doi.org/10.1016/j.postharvbio.2011.12.006

Concha-Meyer, A., Eifert, J.D., Williams, R.C., Marcy, J.E., Welbaum, G.E., 2015. Shelf life determination of fresh blueberries (Vaccinium corymbosum) stored under controlled atmosphere and ozone. Inter. J. Food Sci. 2015, 164143. https://doi.org/10.1155/2015/164143

Kemp, S.E., Hollowood, T., Hort, J., 2009. Sensory evaluation: a practical handbook. Wiley-Blackwell, John Willey & Sons Ltd, United Kingdom.

Ktenioudaki, A., O'Donnell, C.P., Emond, J.P., Nascimento Nunes, M.C., 2021. Blueberry supply chain: critical steps impacting fruit quality and application of a boosted regression tree model to predict weight loss. Postharvest Biol. Technol. 179, 111590. https://doi.org/10.1016/j.postharvbio.2021.111590

Ehlenfeldt, M.K., Martin, R.B., 2002. A survey of fruit firmness in Highbush blueberry and species-introgressed blueberry cultivars. Hortscience 37, 386-389. https://doi.org/10.21273/HORTSCI.37.2.386.

Forney, C.F., Jordan, M.A., Nicholas, K.U.K.G., 2003. Effect of CO_2 on physical, chemical, and quality changes in 'Burlington' blueberries. Acta Horticulturae 600, 587-593.

Gilbert, J.L., Schwieterman, M.L., Coloquhoun, T.A., Clark, D.G., Olmstead, J.W., 2013. Potential for increasing southern highbush blueberry flavour acceptance by breeding for major volatile components. HortScience 48(7), 835-843. https://doi.org/10.21273/HORTSCI.48.7.835

Li, M., Rivera, S., Franklin, D., Nowak, E., Hallet, I., Kolenderska, S., Urbanska, M., Vanholsbeeck, F. East, A., 2021. Use optical coherence tomography and light microscopy to characterise

mechanical properties and cellular level responses of 'Centurion' blueberries during weight loss. Journal of Food and Engineering. 303, 110596. 10.1016/j.jfoodeng.2021.110596
Lobos, G.A., Callow, P., Hancock, J.F., 2014. The effect of delaying harvest date on fruit quality and storage of late highbush blueberry cultivars (*Vaccinium corymbosum* L.). Postharvest Biol. Technol. 87, 133-139. https://doi.org/10.1016/j.postharvbio.2013.08.001
Lobos, G.A., Bravo, C., Valdes, M., Graell, J., Ayala, I.L., Beaudry, R.M., Moggia, C., 2018. Within-plant variability in blueberry (*Vaccinium corymbosum* L): maturity at harvest and position within the canopy influence fruit firmness at harvest and postharvest. Postharvest Biol. Tec. 146, 26-35. https://doi.org/10.1016/j.postharvbio.2018.08.004
Miller, W.R., McDonald, R.E., Cracker, T.E., 1993. Quality of two Florida blueberry cultivars after packaging and storage. HortScience 28(2), 144-147. https://doi.org/10.21273/HORTSCI.28.2.144
Moggia, C., Beaudry, R.M., Retamales, J.B., Lobos, G.A. 2017a. Variation in the impact of stem scar and cuticle on water loss in highbush blueberry fruit argue for the use of water permeance as a selection criterion in breeding. Postharvest Biol. Technol. 132, 88-96. https://doi.org/10.1016/j.postharvbio.2017.05.019
Moggia, C., Graell, J., Lara, I., Gonzalez, G., Lobos, G.A., 2017b. Firmness at harvest impacts postharvest fruit softening and internal browning development in mechanically damaged and non-damaged Highbush blueberries (*Vaccinium corymbosum* L.). Frontiers in Plant Science 8. https://doi.org/ 10.3389/fpls.2017.00535
NeSmith, D.S., Nunez-Barrios, A., Prussia, S.E., Aggarwal, D., 2005. Postharvest berry quality of six rabbiteye blueberry cultivars in response to temperature. Journal of the American Pomological Society 59(1), 13-17. https://doi.org/10.21273/HORTSCI.46.9.1278
Nicolai, B., Ketelaere, B.D., Dizon, A., Wouters, N., Postelmans, A., Saeys, W., Van de Looverbosch, T., Verboven, P., Hertog, M.L.A.T.M., 2022. Chapter 14. Nondestructive evaluation: detection of external and internal attributes frequently associated with quality and damage. In: Postharvest Handling (Fourth Edition), A Systems Approach. Eds: Florkowski, W.J., Shewfelt, R., Banks, N.H., Prussia, S. Academic Press, Elsevier, The Netherlands, 399-433. https://doi.org/10.1016/B978-0-12-822845-6.00014-2
Nunes, M.C., Emond, J.P., Brecht, J.K., 2004. Quality curves for highbush blueberries as a function of the storage temperature. Proceedings of the Ninth North American Blueberry Research. Small Fruits Review, 423-438. https://doi.or/10.13000/J301v03n03_10
Nunes, M.C. Emond, J.P., 2007. Relationship between weight loss and visual quality of fruits and vegetables. Proc. Fla. State Hort. Soc. 120, 235-245.
Nunes, M.C., 2015. Correlation between subjective quality and physicochemical attributes of fresh fruits and vegetables. Postharvest Biol. Technol. 107, 43-54. http://dx.doi.org/10.1016/j.postharvbio.2015.05.001
Paniagua, A.C., East, A.R., Hindmarsh, J.P., Heyes, J.A., 2013. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. Postharvest Biol. Tec. 79, 13-19. https://doi.org/10.1016/j.postharvbio.2012.12.016
Paniagua, A.C., East, A.R., Heyes, J.A., 2014. Interaction of temperature control deficiencies and atmosphere conditions during blueberry storage on quality outcomes. Postharvest Biol. Technol. 95, 50-59. https://doi.org/10.1016/j.postharvbio.2014.04.006
Prussia, S.E., Tetteh, M.K., Verma, B.P., NeSmith, D.S., 2006. Apparent modulus of elasticity from FirmTech 2 firmness measurements of blueberries. Trans. ASABE 49, 113-121. https://doi.org/10.13031/2013.20219
Rivera, S.A., Zoffoli, J.P., Latorre, B.A., 2013. Determination of optimal sulfur dioxide time and concentration product for postharvest control of gray mold of blueberry fruit. Postharvest Biol. Tec. 83, 40-46. https://doi.org/10.1016/j.postharvbio.2013.03.007

Rodriguez, J., Zoffoli, J.P., 2016. Effect of sulphur dioxide and modified atmosphere packaging on blueberry postharvest quality. Postharvest Biol. Technol. 117, 230-238. https://doi.org/10.1016/j.postharvbio.2016.03.008

Rohrbach, R.P., Mainland, C.M., 1993. Accurate low-cost measurement of blueberry firmness for research workers. Acta Hortic., 338-353. https://doi.org/10.17660/ActaHortic.1993.346.47.

Rosenthal, A.J., 2010. Texture profile analysis - How important are the parameters. J. Texture Stud. 41, 672-684. https://doi.org/10.1111/j.1745-4603.2010.00248.x

Saftner, R., Polashock, J., Ehlenfeldt, M., Vinyard, B., 2008. Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. Postharvest Biol. Tec. 49, 19-26. https://doi.org/10.1016/j.postharvbio.2008.01.008

Sanford, K.A., Lidster, P.D., McRae, K.B., Jackson, E.D., Lawrence, R.A., Stark, R., Prange, R.K., 1991. Lowbush blueberry quality changes in response to mechanical damage and storage temperature. J. Amer. Soc. Hort. Sci. 116(1), 47-51. https://doi.org/10.21273/JASHS.116.1.47

Schotsmans, W., Molan, A., MacKay, B., 2007. Controlled atmosphere storage of rabbiteye blueberries enhances postharvest quality aspects. Postharvest Biol. Technol., 44(3), 277-285. https://doi.org/10.1016/j.postharvbio.2006.12.009

Slaughter, D.C., Rohrbach, R.P., 1985. Developing a blueberry firmness standard. Transactions of the ASAE 28(3), 986-992. https://doi.org/10.13031/2013.32374

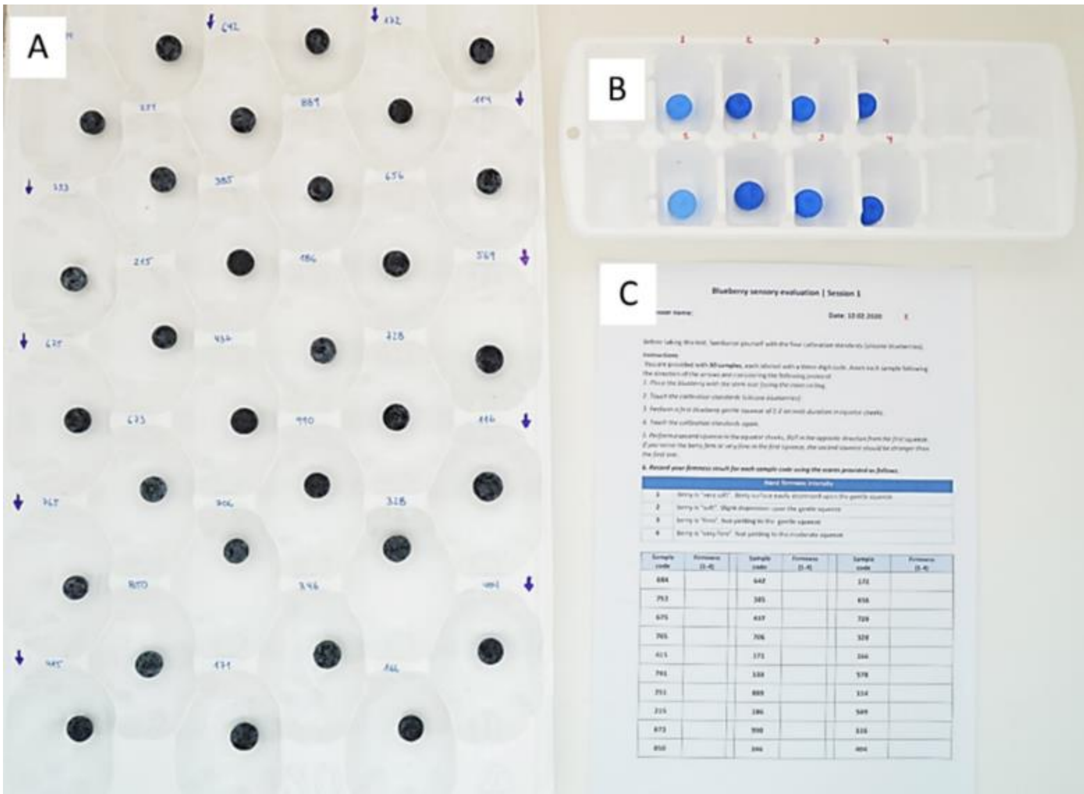
Strike, 2019. Frequency of harvest affects berry weight, firmness, titratable acidity, and percent soluble solids of highbush blueberry cultivars in Oregon. J. Amer. Pom. Soc. 73(4):254-268.

Timm, E.J., Brown, G.K., Armstrong, P.R., Beaudry, R.M., Shirazi, A., 1996. Portable instrument for measuring firmness of cherries and berries. Appl. Eng. Agric. 12, 71-77. https://doi.org/10.13031/2013.25441

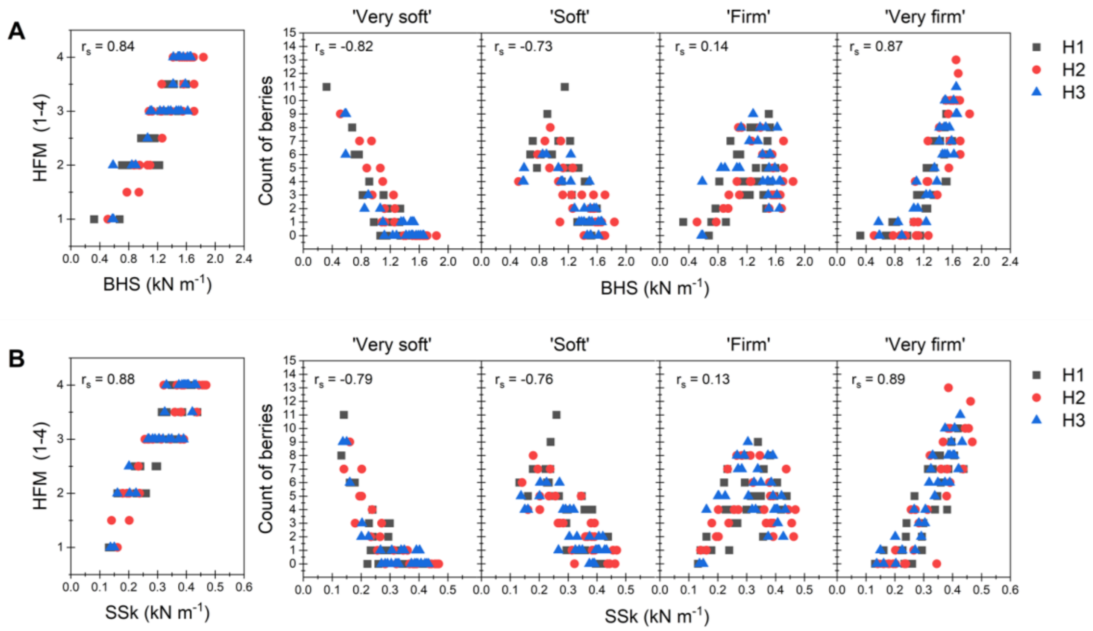
Vilela, A., Goncalves, B., Ribeiro, C., Fonseca, A.T., Correia, S., Fernandes, H., Ferreira, S., Bacelar, E., Silva, A.P., 2016. Study of textural, chemical, color and sensory properties of organic blueberries harvested in two distinct years: A chemometric approach. J. Texture Stud. 47, 199-207. https://doi.org/10.1111/jtxs.12173

4.9. Appendices Chapter 4

4.9.1. Supplementary figures



Supplementary Fig. 4.1. Materials that were available to each assessor for each of the six sensory sessions. (A) Blueberries to be assessed were presented on a plastic plix tray (commercial kiwifruit tray). Each tray contains 30 berries individually labelled with 3-digits random codes. (B) two sets of silicone spheroids (references samples) of the four-hand firmness categories. Each set mimics a blueberry size of 1.1 cm^3 or 1.4 cm^3. (C) The paper sheet includes the assessment instructions and a table to manually record sensory results for each berry.



Supplementary Fig. 4.2. Graphical representation of Spearman correlations (n=90) between average hardness slope (BHS, A) or skin break slope (SSk, B) and sensory variables of the median of hand-touch firmness category (HFM) and count of berries on each hand-touch category of 'very soft', 'soft', 'firm' and 'very firm'. Each dot represents the data of each of three replications of a harvest date (H1 = 28th January; H2 = 7th February; H3 = 17th February 2020) stored for 14 d or 28 d in either of five storage humidities (71 %, 88 %, 95 %, 97 % and 99%).

4.9.2. Supplementary tables

Supplementary Table 4.1. Composition, hazards identification, and manufacturer information of the commercial chemicals mixture used to create silicone blueberries

Commercial product name	**PlatSil® Gel-00**		**Transil™**		**Elatstosil® Vario 15**		**Elatstosil® Vario 40**	
Part	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Composition	Platinum silicones		Dimethylsiloxane, hydrogen terminated (CAS No 70900-21-9), 20-50%; dimethylsiloxane, methyl hydrogen, methyl phenol terminated, (CAS No 232261-92-6), < 10 %; dimethylsiloxane, methylhydrogen-, (CAS No 68037-59-2), < 10 %; gamma-glycidoxypropyltrimethoxysilane, (CAS No 2530-83-8), < 3 %; methanol	Modified polydimethylsiloxane, (CAS Not available), notspecified %; 1,5-cyclooctadienylbis[4-(trimethylsilyl) phenyl] ethinyl platinum, (CAS No 234112-62-0), <0.3%; methylclyclopentadienyl(trimethyl) platinum, (CAS No 94442-22-5), <0.2%	Polydimethylsiloxane with functional groups and auxiliaries for addition cross-linking			
Hazards identification	Non-hazardous chemical per 29 CFR 1910.1200.		Non-hazardous chemical, according to the WHS regulation. Non-hazardous goods the ADG Code		Non-Hazardous substance according to the criteria of NOHSC. NON-dangerous good according to the ADG code)			
Manufacturer	Barnes Products Pty Ltd, Australia		Barnes Products Pty Ltd, Australia		Wacker Chemie AG		Drawin Vertriebs-GmbH	
Source	Safety data sheet for GEL00A and GEL00B (Polytek, development corp, PA, USA)		Safety data sheet according to WHS and ADG requirements (Chemwatch, Australia: 5252-58, version 2.1.1.1)	Safety data sheet according to WHS and ADG requirements (Chemwatch, Australia: 5252-59, version 2.1.1.1)	Safety Data sheet (NOHSC:2011) Material: 60083216		Safety Data sheet (NOHSC:2011) Material: 60082729	

Supplementary Table 4.2. Performance check based on scoring accuracy to evaluate hand firmness of soft and firm 'Centurion' blueberries for each of 10 assessors.

Scoring performance	**Assessor**									
	1[a]	**2**	**3**	**4**	*5*[b]	**6**	*7*	*8*	**9**	**10**
Correct (n)	20	18	18	20	*15*	19	*13*	*16*	18	20
False (n)	8	10	10	8	*13*	9	*15*	*12*	10	8
Accuracy (%)	71	64	64	71	*57*	68	*46*	*57*	64	71

[a]bold font are the assessor that were selected to conduct sensory evaluations
[b]cursive font are the assessor that were rejected to conduct sensory evaluations

Supplementary Table 4.3. Pearson correlation between instrumental evaluations of average cumulated water loss, mechanical parameters of texture profile analysis and penetration test, and berry diameter.

Instrumental variables	Water loss (WL)	Texture Profile Analysis[a]				Penetration test [b]				Diameter (BD)
		BHS	BR	BCo	BSp	FSk	SSk	DSk	WSk	
WL		*<0.001*[d]	*<0.001*	*<0.001*	*0.41*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
BHS	-0.94[c]		<0.001	*<0.001*	*0.17*	*0.04*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
BR	0.64	-0.75		*<0.001*	*<0.001*	*0.84*	*<0.001*	*<0.001*	*<0.001*	*0.11*
BCO	0.79	-0.88	0.93		<0.001	*0.95*	*<0.001*	*<0.001*	*<0.001*	*0.01*
BSp	0.09	-0.15	0.43	0.39		*0.26*	*0.11*	*0.65*	*0.72*	*<0.001*
FSK	0.39	-0.27	0.05	0.09	-0.09		*0.14*	*<0.001*	*<0.001*	*<0.001*
SSk	-0.92	0.93	-0.72	-0.85	-0.17	-0.24		<0.001	*<0.001*	*<0.001*
DSK	0.96	-0.91	0.62	0.76	0.06	0.38	-0.92		*<0.001*	*<0.001*
WSK	0.96	-0.88	0.57	0.71	0.06	0.54	-0.89	0.98		*<0.001*
BD	-0.56	0.43	-0.17	-0.28	-0.30	-0.59	0.44	-0.55	-0.61	

[a]BHS = hardness slope (kN m^{-1}), BR = resilience (-), BCo = cohesiveness (-), BSp = springiness (-)
[b]FSk = force at berry skin break (N), SSk = skin break slope (kN m^{-1}), DSk = displacement at berry skin break (mm), WSk = skin break work energy (mJ)
[c]Pearson correlation coefficient (r_s) in red represent negligible correlation (*P*-value > 0.05), in black weak to moderate correlation (r = 0.3 - 0.79), in blue strong correlations (r ≥0.8).
[d] values in cursive font are P-value

4.9.3. Statement of contribution Chapter 4

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Sebastian Anibal Rivera Smith
Name/title of Primary Supervisor:	Dr. Svetla Sofkova-Bobcheva

In which chapter is the manuscript /published work: Chapter 4

Please select one of the following three options:

○ The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

○ The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

◉ It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Sebastian Rivera Smith — Digitally signed by Sebastian Rivera Smith DN: cn=Sebastian Rivera Smith , c=NZ, o=School of Food and Advanced Technology, Massey University, ou= Massey AgriFood Digital Lab , email=s.riverasmith@massey.ac.nz Date: 2022.02.19 12:15:44 +13'00'
Date:	19-Feb-2022
Primary Supervisor's Signature:	Svetla Sofkova-Bobcheva — Digitally signed by Svetla Sofkova-Bobcheva DN: cn=Svetla Sofkova-Bobcheva, c=NZ, o=Massey university , ou=School of Agriculture and Environment, email=s.sofkova-bobcheva@massey.ac.nz Date: 2022.02.24 09:05:48 +13'00'
Date:	24-Feb-2022

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

GRS Version 5 – 13 December 2019
DRC 19/09/10

Chapter 5. Overall discussion and future work

5.1. Main results overview

This thesis has addressed the problem of the lack of a standard instrumental method to measure firmness for postharvest quality evaluations of blueberries. An essential step in standardising an instrumental method to measure fruit firmness is identifying a mechanical parameter that provides information of the textural changes irrespectively of the factors (e.g., harvest maturity, storage water loss, controlled atmosphere) or the mechanism (e.g., turgor loss, cell wall degradation) inducing the quality changes. This thesis identified the mechanical parameters that can be used to evaluate the quality of fresh blueberries:

(1) **Storage relative humidity (water loss)**. Displacement at skin break (DSk, mm) of penetration test was strongly related (Pearson r = 0.96) to postharvest water loss increase of highbush 'Nui' (**Table 2.4**), Rabbiteye 'Rahi' (**Table 2.4**) and Rabbiteye 'Centurion' (**Supplementary Table 4.4)**. In addition, the hardness slope (BHS, kN m^{-1}) of the compression test also showed a strong relationship (r = -0.94) to water loss increase of blueberry 'Nui' (**Table 2.4**), 'Rahi' (**Table 2.5**) and 'Centurion' (**Supplementary Table 4.4**). Multiple linear regression analyses of **Chapter 2** data suggest that DSk alone or in combination with BHS can be used to track water loss changes (**Table 2.6**).

(2) **Harvest maturity**. Maximum force at skin break (FSk, N) of penetration test provides a consistent differentiation of maturity at harvest (**Table 3.4**) and after 42 d of storage at 4.7 °C of blueberries 'Nui' and 'Rahi' (**Table 3.6**). In addition, BHS, and skin break slope (SSk, kN m^{-1}) of penetration test was able to differentiate harvest maturities after 42 d of postharvest storage (**Table 3.6 and Fig. 3.4**), but not at harvest time (**Table 3.4).**

(3) **Controlled atmosphere**. BHS provided the best differentiation between different CO_2/O_2 (kPa) compositions in controlled atmosphere storage of 'Nui' and 'Rahi' (**Table 3.6, and Fig. 3.4**). In addition, DSk was also able to differentiate between controlled atmosphere storage of 0.4 kPa CO_2, 5 kPa CO_2, and 20 kPa CO_2.

(4) Firming throughout storage time. Compared to mechanical properties measured at harvest time, blueberries can also experience a positive firmness improvement (i.e., firming). BHS can be used to detect storage firming (i.e., higher BHS than harvest) as induced by storage in high relative humidity (**Fig. 2.4** and **Fig. 3.5).** In addition, BHS and SSk were able to detect firming as induced by controlled atmosphere of 5 kPa CO_2 **(Fig. 3.5).** Firming was only detected to occur in 'Nui' and not in 'Rahi' (**Fig. 2.4** and **Fig. 3.5**).

(5) **Sensory Texture.** BHS and SSk were better related to sensory texture, evaluated by hand-touch firmness, for stored blueberries with different water loss levels (**Table 4.4**). In addition, a blueberry batch (i.e., 10 berries) with an average BHS ≤0.47 kN m^{-1} and SSk ≤0.13 kN m^{-1} may represent a very high likelihood (≥ 5 %) of unmarketable blueberries (berries are 'soft' or 'very soft') (**Fig 4.6**).

To complement these findings, an overall summary of the main results for each mechanical parameter is provided (**Table 5.1).** The check (✓) symbol indicates when the mechanical parameter provides a consistent differentiation (or strong correlation) of the factor affecting texture and the cross (✗) symbol is used to denote a lack of the mechanical parameter to consistently differentiate textural changes or not strong correlation. For Texture Profile Analysis, BHS is the mechanical parameter that provides better differentiation across all sources of textural variability and with sensory hand-touch firmness. In addition, BHS was often strongly ($r > 0.9$) correlated with cohesiveness (BCo), and consequently, BCo can provide similar insights (**Table 5.1**). For penetration test, SSk is the parameter that provides better differentiation of factors. Nevertheless, it is important to denote that FSk provides consistent detection of maturity differences, and DSk provides good differentiation of softening as induced by water loss and controlled atmosphere and was also well related to sensory hand-touch firmness (**Table 5.1**).

Table 5.1. Summary of the ability of mechanical parameters to detect differences as influenced by the factors of water loss, maturity at harvest, maturity after storage, controlled atmosphere, firming effect and sensory hand-touch firmness.

Mechanical parameter		Units	Factor					
			Water loss[a]	Maturity at harvest[b]	Maturity storage[c]	Controlled atmosphere[d]	Firming[e]	Sensory firmness[f]
Texture Profile Analysis								
Hardness slope	BHS	$kN\ m^{-1}$	✓	✗	✓	✓	✓	✓
Resilience	BR	(-)	✗	✗	✓	✗	NE[g]	✗
Cohesiveness	BCo	(-)	✗	✗	✓	✓	NE	✓
Springiness	BSp	(-)	✗	✗	✓	✓	NE	✗
Penetration test								
Force at skin break	FSk	(N)	✗	✓	✓	✗	NE	✗
Displacement at skin break	DSk	(mm)	✓	✗	✗	✓	✗	✓
Skin break slope	SSk	($kN\ m^{-1}$)	✓	✗	✓	✗	✓	✓
Skin break work-energy	WSk	J	✓	✗	✗	✗	NE	✗

[a] water loss was constructed using the Pearson correlation coefficient of **Chapter 2** (**Table 2.4** and **2.5**) and **Chapter 4** (**Supplementary Table 4.4**). A ✓ was used when the correlation to water loss is consistent> 0.9.

[b]Maturity at harvest was constructed using **Chapter 3, Table 3.4**. A ✓ was used when consistent differentiation of maturity differences.

[c]Maturity after storage was constructed using **Chapter 3, Table 3.6**. A ✓ was used when consistent differentiation of maturity differences.

[d]Controlled atmosphere was constructed using **Chapter 3, Table 3.6**. A ✓ was used when consistent differentiation of controlled atmosphere CO_2 level differences.

[e]Firming was constructed using **Chapter 2, Fig 2.4** and **Chapter 3, Fig 3.5**. A ✓ was used when consistent firming detection on 'Nui'.

[f]Sensory firmness was constructed using **Chapter 4, Table 4.5**. A ✓ was used when correlation to HFM (median of hand firmness) is > 0.8.

[g]NE= not evaluated.

5.2. Development of a firmness standard

To determine the influence of a novel postharvest management practice/technology on blueberry firmness improvements, the evaluation is frequently initiated at the research level and later validated to be implemented commercially. Hence, informative firmness data must be collected on different scenarios over time (e.g., blueberry seasons) and locations. Different equipment with different resolutions, automatization and portability, are also expected to be used in these scenarios (**Fig 1.2**). Under this condition, obtaining firmness data that can be confidently comparable, independent of the collection scenario, will require the development of a universally accepted standard methodology to measure the firmness of blueberries.

Consequently, and due to the ease of measurement (and calculation procedures) using different instruments and the ability to differentiate postharvest blueberry quality (i.e., softening and firming) (**Table 5.1**.), BHS or chord stiffness may be the best candidate for measuring instrumental firmness of stored blueberries. In addition, chord stiffness showed the strongest relationship to hand-touch firmness; hence, it can be used to understand consumer acceptability, assisting blueberry marketability on commercial quality checkpoints.

Chord stiffness can assist the development of a standard firmness method for stored blueberries. However, developing a standard will require studying the data consistency across different data collection scenarios, including operational settings and calculation procedures and sources of methodological error such as blueberry size differences. All these considerations will be discussed in this chapter. Discussion of the relationship between mechanisms of textural changes (e.g., water loss, cell wall degradation) and mechanical parameters evaluated will also be provided. In addition, implications of the relationship between sensory and mechanical parameters to the commercial blueberry industry will also be provided.

5.2.1. Operational settings for hardness slope measurement

5.2.1.1. Test speed

An important operational parameter previously studied that can influence the obtained results is loading speed (mm s^{-1}). To study the general effect of loading speed on maximum compression force (hardness, N), Rosenthal (2010) utilised experimental gel cylinders made of a mixture of glycerol, starch, and water. The study showed a logarithmic hardness increase (N) as compression speed increased from 0.1 mm s^{-1} to 10 mm s^{-1}. Consequently, in this thesis, for both mechanical tests, double compression and penetration, the loading speed was fixed to 0.8 mm s^{-1} and 1.0 mm s^{-1}.

A constant predefined test speed is easier to control when sophisticated automatic equipment (i.e., texture analyser, Instron universal testing machine) is used. However, at the commercial level, equipment usually requires higher portability and lower cost. Consequently, not all the equipment available to be used in the industry will have the same resolution level to control a low loading speed. For example, Prussia et al. (2006) have indicated that the minimum compression speed of the FirmTech is 7 mm s^{-1}, which is considerably higher than the texture analyser used for this thesis. Whether an alternative loading speed than that used in this thesis can change the accuracy of BHS to detect textural changes (i.e., softening and firming) requires to be studied.

5.2.1.2. Compression strain distance

Chapter 2 has shown that changes in compression distance (strain %) for double compression test (texture profile analysis) can generate similar BHS ranges but provides a different understanding of BHS results as influenced by water loss for stored blueberries (**supplementary Fig. 2.1**). Compression to 15 % strain (approx. 2 mm deformation) provided better differentiation of blueberry BHS caused by different storage relative humidity levels than compression to 30 % (approx. 4 mm) (**supplementary Fig. 2.1)**.

Only Ferraz et al. (2000) have previously evaluated the influence of compression distance on blueberry firmness measurement. Compressions to 1 mm deformation were more sensitive to detect firmness (measured as maximum force) changes through time at 2 °C, when compared to 3 mm (Ferraz et al., 2000). Ferraz et al. (2000) result address the question of using a fixed deformation distance (i.e., 1 mm) rather than a percentage of deformation (strain %) of blueberry diameter (i.e., 15 %) as a target distance. This question is particularly important considering that breeding programs may be interested in selecting genotypes with improved berry size (Lobos and Hancock, 2015). Consequently, a constant strain (%) will generate a higher deformation distance (mm) for larger berries. Whether it is better to select a predefined deformation distance (mm) than a strain (%) as a compression target mode to evaluate quality remains to be studied, considering different genotypes of variable fruit size ranges.

5.2.1.3. Platform shape to support fruit during mechanical testing

Most studies previously conducted on blueberries have considered evaluating mechanical parameters with blueberries oriented with the stem-calyx axis perpendicular to the compression or penetration probe. Consequently, the load is applied across the fruit equator (Ballinger et al., 1973; Donahue and Work, 1998; Ferraz et al., 2001; Ehlenfeldt and Martin, 2002; Saftner et al., 2008; Ochmian, 2012; Blaker et al., 2014; Paniagua et al., 2014). In addition, Ferraz et al. (2001) have observed that applying the load to the base of the berry (stem end to blossom end) produces less smooth and less consistent force-deformation curves when compared to using the load through the blueberry equator.

The problem with compressing the blueberry through the equator is the unbalanced movement due to the prominent curvature associated with the oblate spheroid shape of blueberry. Consequently, different studies have considered assistance of the mechanical test by using a washer ring of 7-10 mm internal diameter on the texture equipment platform (Ballinger et al., 1973, Paniagua et al., 2013). Alternatively, FirmTech provides a platform with convex shallow depressions of 10 mm diameter and 2 mm depth where berries are held during the compression test (Prussia et al., 2006).

The use of a washer or a convex shape support platform instead of a flat smooth surface may generate bias in BHS measurements. Prussia et al. (2006) indicated that berries of different sizes contact the shallow depressions of the Firmtech platform differently, which can generate different measurement conditions depending on berry size. A large berry can sit on the rim of the depression or a washer, while a small berry will contact the bottom.

The bias generated by not using a flat platform is significant for mechanical parameters obtained using geometric assumptions, such as the apparent modulus of elasticity, where calculation procedures assume data was obtained using a parallel plate compression (ASAE, 2008). However, mechanical parameters of this thesis are needed for quality purposes rather than for modelling mechanical behaviour. Consequently, absolute values of mechanical parameters are not strictly needed if the method can secure data that is in comparable ranges or if the data ranges can be associated to sensory assessment. Whether different platform support shapes may affect mechanical output for quality purposes, remain to be studied.

5.2.2. Chord stiffness (hardness slope) calculation procedures

Early studies conducted on blueberries defined chord stiffness (kN m^{-1}) as the slope of the chord drawn between two specific points on the force-deformation curve (Ballinger, 1973; Slaughter and Rohrbach, 1985, **Fig. 1.1.**). This definition assumes an approximately linear increase of force for blueberries as the fruit is deformed up to 25 % strain of equatorial diameter (Ballinger, 1973; Slaughter and Rohrbach, 1985). Hence chord stiffness should provide similar results independent of the selected minimum and maximum force or deformation threshold when below 25 % strain. However, the force-deformation graphs of compression and penetration tests collected in this work demonstrate that blueberries with high water loss followed a less linear increase of force as the fruit is deformed (or distance increase) when compared to high relative humidity (low water loss) and controlled atmosphere effect (**Fig. 3.3)**.

The data of **Chapter 2** was used to investigate the shape of the increase in force as the blueberry is deformed as dependent on storage relative humidity treatment (or water loss changes). The raw data of the first compression cycle of Texture profile analysis (TPA) obtained after 28 d at 4.6 °C at the five storage relative humidity levels was used to plot the force-deformation during compression loading (**Fig. 5.1 A, C**). An approximate linear increase of force as the blueberry is compressed was observed when storage relative humidity was ≥ 92-94 % (or average water loss ≤3.4 %). When relative storage humidity ≤ 90%, a considerably less linear (or more curved) increase of force as the blueberry is deformed is observed (**Fig.5.1 A, C**).

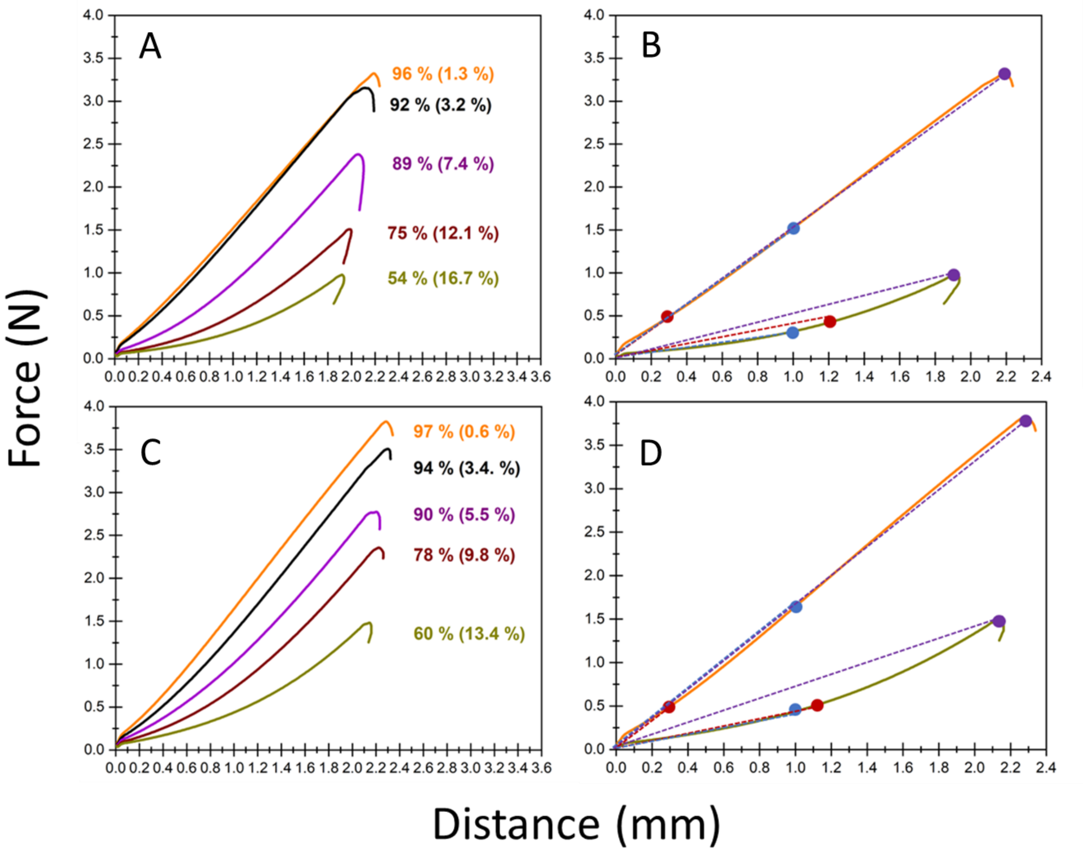


Fig. 5.1. Graphic representation of the average force-distance plot obtained by compression loading of the first cycle of texture profile analysis (TPA) of blueberry 'Nui' (**A**, **B**) and 'Rahi' (**C**, **D**) in five storage humidities for 28 d at 4.6 °C (**Chapter 2** data). Legend for each plot indicates the storage relative humidity (%) and inside the brackets is the associated average water loss (%). Examples of calculation procedures for different chord stiffness on blueberries stored in 96-97 % RH and 54-60 % RH, are shown (**B-D**). Plots and dashed lines of different colours represent the chord stiffness method. Purple for hardness slope (15 % strain of maximum threshold), blue for fixed distance (1 mm as maximum threshold) and red for fixed force (0.15 N and 0.5 N as a minimum and maximum threshold. This Figure was plotted on Origin software (OriginLab Corporation, MA, USA) using the average raw data of 30 berries for each storage relative humidity and cultivar ('Nui' or 'Rahi') obtained by texture analyser Exponent software.

To determine if differences in calculation procedures for chord stiffness can provide the same results or sensitivity to differentiate storage relative humidity treatments, the data of the first compression cycle of the TPA test of 'Nui' and 'Rahi' blueberries (**Chapter 2)** was re-analysed using different minimum and maximum thresholds to calculate the slope of the chord, as follows:

(1) **Hardness slope**. It is calculated between trigger force (0.06 N) and maximum force at 15 % deformation of the berry equator (**Fig 5.1 B, D**, purple dot and dashed lines).

(2) **Fixed distance**. The calculation was performed between trigger force (0.06 N) and force at 1 mm deformation. The compression deformation of 1 mm was based on the recommendation of Ferraz et al. (2000) (**Fig 5.1 B, D**, blue dot and dashed lines).

(3) **Fixed force**. The FirmTech calculates the slope of the force-deformation chord between a minimum and maximum force (Prussia et al., 2006). FirmTech operational settings of 0.15 N and 1.96 N have previously been reported by several studies (Ehlenfeldt and Martin, 2002; Moggia et al., 2016; 2017; 2018; Lobos et al., 2018; 2021). However, in **Chapter 2**, the maximum force to 15 % strain for low relative humidity treatment (54-60 %) was not higher than 0.6 N for some blueberries. Consequently, the chord stiffness for a fixed force was calculated between 0.15 N and 0.5 N. (**Fig 5.1 B, D**, red dot and dashed lines).

The differences between chord stiffness calculation procedure methods for the same storage relative humidity were analysed using the linear mixed model in ANOVA (P-value < 0.05). Each of the three replicates was used as a random factor and the chord stiffness method as a fixed factor. In addition, differences between storage relative humidity levels for each chord stiffness method were analysed using a general linear model in ANOVA (P-value < 0.05). The LSD-Fisher (P-value < 0.05) was used for both statistical analyses as a multiple mean comparison test. All the data were analysed in the statistical software Minitab (Minitab®19.1.1, USA)**.**

Chord stiffness calculation procedures can generate different data ranges, especially for RH ≤ 89 % for 'Nui' and ≤ 94 % for 'Rahi' (**Table 5.2**, see capital letters). These results reveal the non-linearity of force increases as the blueberry is deformed, which is more abrupt for low storage relative humidity (or higher water loss). Using a FirmTech and Instron Universal Testing Machine, Prussia et al. (2006) have reported similar results using rubber balls. The minimum and maximum force selected to calculate the slope of force-deformation can significantly influence the output result, and a higher minimum and maximum force threshold will produce a higher slope (chord stiffness). In agreement for blueberries, measurement using the force at 15 %

deformation (approximately 2 mm) as a maximum force (i.e., hardness slope) produce a higher slope than the slope obtained at smaller deformation to 1 mm (i.e., fixed distance), especially for RH ≤ 94 % (**Table 5.2**).

Although the absolute values differ, storage relative humidity level effect on chord stiffness can be similarly determined irrespectively by the calculation procedure (**Table 5.2**., see minor letters). Hence, the different calculation procedures of chord stiffness provide similar differentiation of storage relative humidity treatments.

As the results of chord stiffness calculated between trigger force and force at 15 % strain (i.e., BHS) provide a good differentiation of storage relative humidity (**Table 5.2.**, see minor letters), this calculation procedure is recommended.

To make the data confidently comparable, it is recommended that the minimum and maximum thresholds (force or distances) to calculate chord stiffness are fixed across data collection scenarios. If it is not possible to use the same settings, the calculation procedures must always be reported. In addition, it is recommended to use the same texture equipment to determine the parameters. However, whether different equipment (e.g., Firmtech and texture analyser) can provide similar results when using the same calculation procedures for chord stiffness remains to be confirmed.

Table 5.2. Influence of different calculation procedures to determine the slope of the chord between two points of the force deformation graph (i.e., chord stiffness (kN m^{-1}). Data was obtained from **Chapter 2** of blueberries stored for 28 d at 4.6 C in relative humidity of 96 %, 92 %, 89 %, 75 % and 54 % for 'Nui' and 97 %, 94 %, 90 %, 78 %, 60 % for 'Rahi'. Water loss and chord stiffness data was calculated using 3 replicates of 10 blueberries each for each relative humidity and blueberry cultivar.

RH (%)	**Water loss** (%)	**Hardness slope**[a] (kN m^{-1})	**Fixed distance**[b] (kN m^{-1})	**Fixed force**[c] (kN m^{-1})
96	1.3	1.60 A[d] a[e]	1.59 A a	1.53 A a
92	3.2	1.55 A a	1.45 AB a	1.30 B a
89	7.4	1.20 A b	0.88 B b	0.69 C b
75	12.1	0.81 A c	0.49 B c	0.45 B c
54	16.7	0.54 A d	0.30 B d	0.33 C c
RH (%)	**Water loss** (%)	**Hardness slope** (kN m^{-1})	**Fixed distance** (kN m^{-1})	**Fixed force** (kN m^{-1})
97 %	0.6	1.69 A a	1.64 A a	1.46 B a
94 %	3.4	1.57 A b	1.36 B b	1.14 C b
90 %	5.5	1.33 A c	1.01 B c	0.82 C c
78 %	9.8	1.12 A d	0.72 B d	0.58 C d
60 %	13.4	0.76 A e	0.43 B d	0.40 B e

[a]Hardness slope is calculated as the slope between the trigger (0.06 N) and maximum force at 15 % strain
[b]Fixed distance is calculated as the slope between trigger force (0.06 N) and force at 1 mm deformation.
[c]Fixed force is calculated as the slope between 0.15 N and 0.5 N force.
[d]Capital letter differences represent significant differences between chord stiffness calculation procedures for the same RH (read horizontally) by LSD-Fisher (P-value < 0.05).
[e]Minor letter differences represent significant between storage humidities for the same chord stiffness calculation procedure (read vertically) by LSD-Fisher (P-value < 0.05).

5.2.3. Chord stiffness consistency across blueberry size differences

Blueberry size has previously been indicated as a source of firmness variation when using the slope of force-deformation (or chord stiffness) by a universal testing machine (Slaughter and Rohrbach, 1985) or FirmTech (Donahue et al., 2000; Prussia et al., 2006).

Research conducted during the preparation of this thesis has evaluated the influence of fruit size on chord stiffness as measured by BHS. However, the results were out of the scope of the previous research chapters, and consequently, the results are not previously presented.

'Bluemoon' and 'Nui' blueberries (*Vaccinium corymbosum*) were hand-harvested at commercial maturity (100 % blue surface colour) from Gourmet Blueberry (Hawke's Bay, New Zealand) at commercial maturity (100% blue surface colour), on the harvest season of

2019/2020. Berries were harvested in a large range of equatorial diameters from 10.7 mm to 18.9 mm for 'Bluemoon' and from 11.4 mm to 18.8 mm for 'Nui'. Immediately after transportation to the Postharvest Lab (3 h), the blueberries were visually graded in three sizes: small, medium, and large. Ninety healthy berries were selected for each size class (n = 270 berries). Equatorial diameter and BHS (at 15 % strain) were measured on each berry on a texture analyser with a plate platform of 25 mm diameter loading speed of 1 mm s^{-1}. In addition, the chord stiffness to a fixed distance (between trigger force a force at 1 mm) was calculated. Data were analysed by Pearson correlation (P-value <0.05) using statistical software Minitab, and figures were plotted using Origin 2019b (OriginLab Corporation, MA, USA).

Correlation analysis shows a positive relationship between equatorial diameter and BHS or chord stiffness at 1 mm, and consequently, as berries are larger, a higher BHS or chord stiffness to 1 mm is observed. However, Pearson correlation coefficient (r) were weak (r= 0.29 - 0.35) for 'Nui' and moderate (r = 0.48 - 0.50) for 'Bluemoon' (**Fig. 5.2**).

Donahue et al. (2000) observed higher FirmTech firmness (N m^{-1}) on lowbush blueberries of 6-8 mm equatorial diameter when compared to berries of 11-12 mm, which is contrary to the relationship between size and BHS in our study (**Fig. 5.2**). However, Slaughter and Roahrbach (1985) results show that differences in the relationship between chord stiffness and berry size can depend on the blueberry genotype.

The results described in **Table 5.2** can partially help to understand the higher hardness slope. A higher maximum force due to a higher distance to reach 15 % deformation can generate higher BHS data. However, when analysing chord stiffness to a fixed distance (1 mm) the relationship to equatorial diameter were similar (**Fig 5.2**).

On the other hand, a blueberry with advanced maturity (i.e., overmature) can be bigger than mature berries, even when both show 100 % surface blue colour (**Table 3.3**.). Maturity indicators of average ratio of soluble solids to acidity of 'Nui' were 14.5, 18.1 and 32.5;and for 'Bluemoon', 21.9, 22.3 and 23.5, for small, medium and large berries respectively. Consequently, differences in berry size can also represent important differences in 'Nui' maturities, but not for 'Bluemoon'. In addition, Nui showed higher variability in BHS. It is expected that the slope of force-deformation at harvest time should decrease as the maturity advance (Moggia et al., 2016; Moggia et al., 2018). Consequently, considering **Table 5.2** results and maturity effect can help to explain why the correlation between hardness slope and berry size was weaker on 'Nui' compared to 'Bluemoon'. In addition, this can also help to elucidate why the hardness slope was not a good indicator of maturity differences evaluated at harvest (**Table 3.6**).

Due to the biological variability of real blueberries, it will be interesting to see how this relationship changes when using silicone spheroids of different properties (**Chapter 4**) considering a range of sizes.

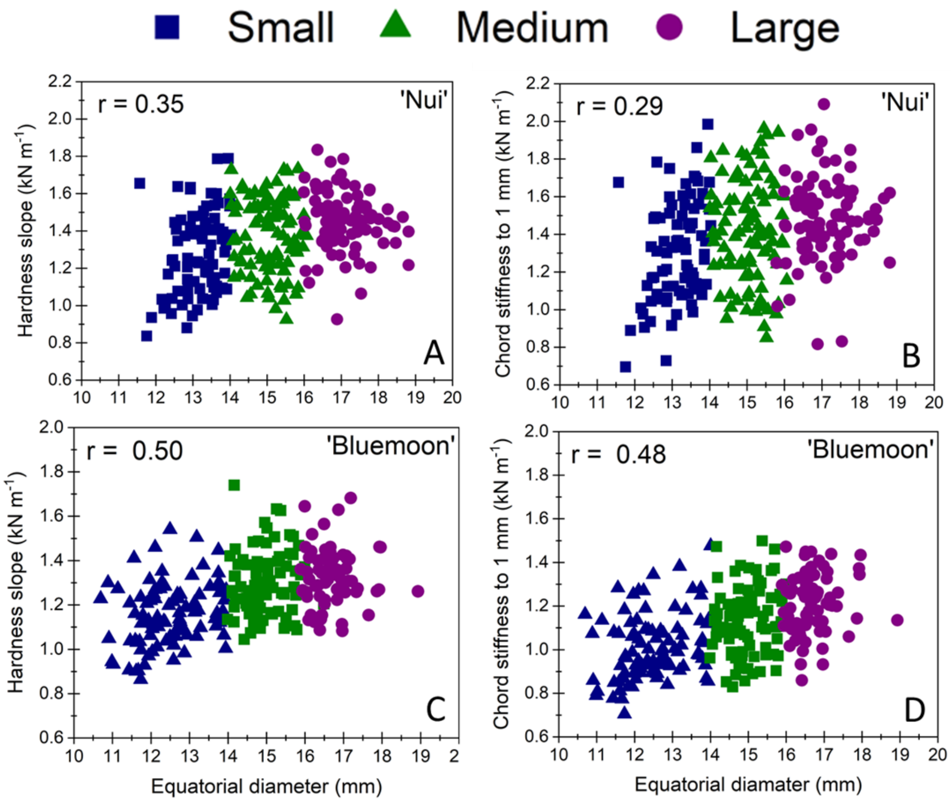


Fig. 5.2. Pearson correlation coefficient (r) between equatorial diameter and hardness slope (A-C) or chord stiffness to 1 mm (B-D) and of 'Nui' (A,B) and 'Bluemoon' (C,D) Blueberry. For each cultivar, 90 berries were used for each of three size categories (small, medium, and large). Each dot represents a single blueberry (n = 270).

5.2.4. Factors (mechanisms) of textural changes

As previously mentioned, to develop a firmness standard, the method must be informative of the quality independent of the factors or mechanisms inducing textural differences. This thesis used three factors: storage relative humidity (water loss), controlled atmosphere, and harvest maturity, to manipulate mechanical parameters. In addition, these factors were used in **Chapters 2** and **3** on two blueberry cultivars, 'Nui' (*Vaccinium corymbosum)* and 'Rahi' (V. *ashei*). In addition, 'Centurion' (V. *ashei*) was exposed to different water loss levels in the sensory study (**Chapter 4**). The author hypothesises that a mechanical parameter that better represents these factors (including genotypes) can be related to the different mechanisms that induce textural changes.

5.2.4.1. Water loss

Water loss can be associated with a decrease in cell turgor pressure (Allan-Wojtas et al., 2001; Giongo et al., 2013; Paniagua et al., 2013) and changes in the thickness of most external cellular layers (Li et al., 2021). Softening due to water loss can be detected by the increase in berry deformation before a needle probe breaks the berry skin (DSk) and by reducing the slope of force-deformation by plate compression (BHS). Giongo et al. (2013) postulated that a lower turgidity of blueberry would imply a higher ability of the tissue to deform by an applied external force before irreversible rupture of the tissues occurs, which is concordant to this thesis results when measuring DSk. In addition, for BHS a lower maximum force for reaching 15 % strain was observed for high water loss berries. Consequently, if compression is conducted to a target force (e.g., 0.5 N), a blueberry with higher water loss will exhibit a higher deformation (mm) to reach 0.5 N when compared to low water loss (**Fig 5.1**).

5.2.4.2. Controlled atmosphere

The controlled atmosphere effect on textural changes is not entirely elucidated for blueberries. It has previously been suggested to be associated with changes in the cell wall of flesh parenchyma cells (Allan-Wojtas et al., 2001). Similarly, studies on strawberries suggest that a controlled atmosphere may generate microstructural modifications regulating cell-to-cell adhesion and cell wall strength (Harker et al., 2000). The DSk and BHS were the parameters that best describe the mechanical changes induced by the controlled atmosphere. Consequently, this suggests that both parameters can provide information about blueberry microstructural modification affecting cell wall strength. Olmedo et al. (2021) have shown that lower hardness to 30 % blueberry deformation using a compression plate can be associated with lower cell wall hemicellulose quantification in stored blueberries. Whether a controlled atmosphere affects primary cell wall components requires further study for blueberries.

On the other hand, both parameters, DSk and BHS, have been shown to provide insights related to cell turgor reduction induced by water loss. However, it is also possible that water loss induces changes in the cell wall if water loss constitutes stress that results in faster cell wall degradation (components and cell wall enzymatic activity). Whether water loss can affect cell wall degradation of blueberry remain to be studied.

5.2.4.3. Harvest Maturity

Harvest maturity has previously been associated with changes in whole fruit cell wall components, such as pectin and hemicellulose (Proctor and Miesle, 1991; Vicente et al., 2007). Based on previous data, it is expected that advanced maturity (and consequently higher cell wall degradation) generates a reduction in the slope of force deformation (Moggia et al., 2016; Moggia et al., 2018) and maximum force (N) to 2 mm deformation (Vicente et al., 2007). However, when evaluating maturities at harvest, force at skin break (FSk) was the most consistent parameter to detect maturity changes in 'Nui' and 'Rahi' (**Table 3.4**), and BHS was only reasonable to detect maturity differences after postharvest storage (**Table 3.6**).

The FSk may represent the combined resistance of the blueberry skin and flesh tissue loading. Silva et al. (2005) performed a penetration test by piecing the skin from inside to outside and from outside to inside the berry; the results show that FSk (N) was approximately 45 % less when skin is pierced from inside to outside. Consequently, the contribution of skin and flesh on FSk is approximately even. In addition, Blaker and Olmstead (2015) found that differences in force to berry rupture by a 4 mm probe between different blueberry genotypes were not related to cell wall materials of the blueberry flesh or skin. As a result, whether the FSk differences as influenced by blueberry maturity are mostly contributing to the skin's degradation of cell wall materials rather than flesh or vice versa requires further studies considering mechanical parameters and cell wall degradation of isolated skin of blueberry maturities.

To obtain FSk, using a needle probe, this parameter requires texture equipment with high precision and resolution for small forces (0.15 – 0.3 N). Consequently, a penetration test using a needle probe can only be recommended to be used in research studies where a piece of sophisticated equipment (e.g., texture analyser) can be used. In addition, it is expected that differences in the needle tip geometry of the needle probe can generate different results. Whether obtaining the force at skin break using a flat type of cylinder probe of small dimension (2-4 mm diameter) that will provide higher force ranges is also consistent for detecting maturity differences remain to be studied.

5.2.4.4. Blueberry genotype

When evaluating genotype differences in firmness and genetic expression, Cappai et al. (2018; 2020) have suggested that blueberry firmness is a genetically controlled trait and is expected significant differences in firmness to the blueberry genotype. To examine the differences between genotypes used in this thesis, BHS of compression test and maximum force ta skin break (FSk) of penetration test were obtained from **Chapter 2** (‘Nui’ and ‘Rahi’) and **Chapter 4** (‘Centurion’), considering the data at harvest and for similar ranges of storage relative humidity (54-71 %, 88-90 % and 96-99 %) after 28 d of storage at 4.6 °C. No formal statistical analysis was conducted.

Results of Table 5.3. describe whether BHS of cultivars can have or not similar results as influenced by the evaluation time and the storage relative humidity. BHS was in similar ranges for all three cultivars after 28 d in very high relative humidity (96-99 %) or low relative humidity (54-71 %), but in different ranges at 88-90 % RH and at harvest time. For FSk, 'Centurion' showed different average values than 'Nui' and 'Rahi' at harvest and after storage in different humidities.

Differences in mechanical parameters between genotypes have previously been associated with microstructural changes, including cell size, degree of cell-to-cell contact, cell wall composition, the calcium content in the cell wall, thickening of the parenchyma cell wall, and the number of stone cells (Allan-Wojtas, 2001; Silva et al., 2005; Olmedo et al., 2021). Hence, several mechanisms can influence the observed textural changes due to genotype differences. In addition, part of these mechanisms (e.g., cell size, thickening of the parenchyma cell wall, cell wall composition) can be affected by the storage conditions of blueberries (Allan-Wojtas, 2001; Chen et al., 2015; Ortiz et al., 2018; Li et al., 2021). Consequently, when studying the relationship between mechanism and textural differences between cultivars is essential to standardise the evaluation time and the storage conditions, especially for cultivars harvested in different moments and stored over different time periods.

Table 5.3. Average mechanical parameters of hardness slope, force at skin break of blueberries 'Nui', 'Rahi' and 'Centurion' at harvest and after storage of 28 d at 4.6 °C in different relative humidity.

Evaluation	Water loss (%)			BHS (kN m^{-1})			FSk (N)		
	Nui[a]	Rahi	Centurion	Nui[a]	Rahi[a]	Centurion[b]	Nui	Rahi	Centurion
Harvest	0.0	0.0	0.0	1.13	1.61	1.68	0.27	0.27	0.23
54-71%	16.7	13.4	12.5	0.53	0.73	0.56	0.31	0.28	0.23
88-90 %	7.4	5.5	7.9	1.17	1.33	0.88	0.30	0.28	0.23
96-99 %	1.3	0.6	0.38	1.50	1.65	1.54	0.30	0.28	0.24

[a]Nui and Rahi blueberries data was obtained from **Chapter 2**
[a]Centurion data was obtained from **Chapter 4**, Harvest 1.

5.2.5. Consumer acceptability and sensory response

This thesis has previously indicated that identifying a mechanical parameter as a "gold standard" will require a consistent indication of quality changes across different factors. However, for the commercial blueberry industry, the "gold standard" of firmness should be the one that can be directly related to consumer acceptability. This thesis evaluated consumer acceptability using the hand-touch feeling using a scale of four-point firmness categories ('very soft', 'soft', 'firm', 'very firm'). The hand-touch firmness can represent an essential (or first) sensory judgment that consumers can have on the texture of blueberries from a blueberry batch just before consumption. In many cultures, blueberries are consumed directly from the package using the fingers or hand when taking them to the mouth. In addition, across time and world locations, the commercial supply chain has adopted the hand-touch feel to evaluate firmness (Beaudry et al., 1998; Schotsmans et al., 2007; Cantin et al., al., 2012; Rodriguez and Zoffoli, 2016). Nevertheless, the author does not reject the idea that an experienced consumer can first judge blueberry texture by its look, for example, observing the presence of shrivelling associated with a soft berry.

5.2.5.1. Relationship between hand-touch firmness and instrumental mechanical parameters

Hardness slope (BHS), cohesiveness (BCo) of double compression test and skin break slope (SSk) and displacement at skin break (DSk) of a penetration test of 'Centurion' blueberries showed a strong relationship (Spearman r_s = 0.83 - 0.88) to the median of sensory texture category as measured by hand-touch firmness using a sensory panel setting (**Table 4.5**). Hence, these parameters can provide information related to consumer acceptability and can assist commercial decisions, such as the commercialisation of fresh produce, the destination market, or other food systems.

The different sensory responses of hand-touch of 'Centurion' blueberries related to the above-mentioned mechanical parameters were obtained by manipulating the storage environmental relative humidity and, consequently, the blueberry water loss (**Fig. 4.4**). However, BHS, BCo, DSk, shown to be very strongly related to water loss changes (**Table 2.6**), which opens the questioning about the consistency of the parameters to provide information about consumer acceptability when other sources of texture variation are used.

On the other hand, the questioning can also be extended to the ability of other mechanical parameters to be related to sensory when a different source of textural variability is used. For example, force at skin break (FSk) was not associated with sensory hand-touch firmness (**Table 4.5**), but FSk was shown to be not affected by water loss (**Table 2.5**). Alternatively, FSk can provide information about maturity (**Table 3.4** and **3.6**) and genotype (**Table 5.3**). It will be worth repeating the sensory panel evaluation (**Chapter 4**), including a combination of sources of textural variation, such as maturity, controlled atmosphere, and genotypes, to generate a higher range of mechanical parameter changes. Doing this will facilitate the relationship of hand-touch firmness to whether a specific mechanical parameter is consistent or is the result of the source of textural variability used.

5.2.5.2. Relationship to texture profile analysis

The potential relationship to sensory descriptors is the main benefit of using texture profile analysis (TPA) to characterize fruit produce. However, the descriptors obtained by TPA are mechanical parameters, not sensory properties. Up to date, no studies have related TPA parameters with sensory evaluations of fresh blueberry, and the relationship with sensory parameters cannot be directly extrapolated from previous evaluations obtained for other fruit commodities such as peaches (Contador et al., 2016). In addition, a blueberry deformation of 15 % strain, used in this research to measure TPA parameters in blueberries, does not generally produce a visible fracture of the berry as chewing between molars does. Hence the relationship between TPA and sensory parameters should be considered in future blueberry studies. In addition, careful interpretation of the relationship between sensory and TPA results is required, due to the moderate to strong correlation between TPA parameters of cohesiveness, resilience, and hardness, as reported in different blueberry studies (Hu et al., 2015; Xie et al., 2018; Giongo et al., 2022).

5.2.5.3. Real-world applications of mechanical threshold associated with consumer perception

This thesis also identified average threshold values of BHS associated with the likelihood of unmarketable blueberries (berries are 'soft' or 'very soft') in a fruit batch (i.e., a group of 10 berries) using binary logistic regression. However, this relationship only associates the mechanical parameters with the proportion of 'very soft' + 'soft' berries in a batch but does not indicate the commercially relevant level of likelihood. To obtain that information is relevant to perform consumer testing or surveys imitating environments where blueberries are consumed in different cultures.

Although the uncertainty of which levels of likelihood of unmarketable berries provide relevant information to be used as a "rule of the thumb" on commercial decisions (i.e., product destination or use), is expected that values over the 50 % threshold can negatively influence consumer decisions to eat a blueberry as a fresh product. Considering the Binary Logistic model provided in **Fig 4.6**, the average BHS of 1.09, 0.86, and 0.47 kN m^{-1} is associated with likelihoods of 50 %, 75 % and 95 % of unmarketable berries on a blueberry batch. As an example, 'Centurion' blueberries of H1 with 7.9 % water loss (water loss value realistically observed in a commercial scenario of bad relative humidity management) showed an average BHS of 0.88 kN m^{-1} (**Table 5.3**), which is very close to 75 % likelihood of unmarketable berries (0.86 kN m^{-1}). Consequently, is it expected higher chance of consumer rejection for experienced or non-tolerant consumers. Hence, those blueberries may be destined to an alternative food system or marketed at a lower price for a tolerant consumer.

On the other hand, these thresholds were obtained using 'Centurion' blueberries, and BHS can change depending on the cultivar (**Table 5.3**). 'Nui' blueberries at harvest displayed a BHS of 1.13 kN m^{-1}, which is very close to 50 % likelihood (1.09 kN m^{-1}). It seems unlikely that 'Nui' used in **Chapter 2** presented a high likelihood of 'soft' or 'very soft' berries at harvest evaluation. Hence, BHS ranges associated with unmarketable sensory berries ('soft' or 'very soft') as reported in **Fig 4.6** may only be valid for the texture operational procedures, fruit material and methods reported in this thesis. Whether different cultivars or ways to manipulate textural properties (e.g., use of controlled atmosphere or maturity) can produce different ranges associated with a sensory hand touch firmness category remains to be studied.

Another important consideration of the relationship between sensory and instrumental parameters is how the mechanical data should be statistically described or presented to provide relevant information for the "real world" or commercial industry. In **Chapter 4**, berries used for the sensory analysis were different from those used to measure mechanical parameters (but obtained from the identical replication). As a result, central tendency statistics such as the mean of mechanical parameters or median hand-touch firmness category were used, rather than individual berry-to-berry data, to relate instrumental to sensory variables (**Table 3.6**). However, high biological variability on firmness distribution within a batch is expected in a commercial scenario, which can influence central tendency statistics of average. For example, Lobos et al. (2018) observed for 'Duke' blueberries at the peak of the harvest season (2014/2015), FirmTech firmness at harvest averaged 1.8 kN m^{-1}, but data ranged between 1.00 kN m^{-1} and 2.50 kN m^{-1}. Consequently, average data may not be a good representation of the variability and distribution of the firmness population. Alternatively, Lobos et al. (2018) proposed the use of cumulative frequency distribution (%) to identify the % of a batch associated with firmness categories ('soft', 'firm'). The instrumental (i.e, BHS, SSk) relationship to the likelihood of unmarketable berries ('soft' + 'very soft') obtained in this thesis (**Fig 4.6**) can be used for this purpose. This information can be used to establish an instrumental threshold to a given fractile (e.g., a BHS value that indicates a given fraction or quantile of the population distribution), which can assist commercial decisions based on consumer acceptability/tolerance of soft berries. For example, Jordan and

Loeffen (2013) indicated that the NZ kiwifruit industry had considered the use of critical values of quality measurements (e.g., Hue colour) when lye below or exceed a pre-defined fractile of the fruit distribution, to support decisions such as harvest timing.

On the other hand, considering these approaches, the use of mechanical parameters (i.e, BHS) distribution of individual berries is required. Hence it is recommended that the sensory and instrumental relationship threshold must be revisited, considering the relationship between instrumental and sensory data for individual berries rather than the average data of a batch. This will require quantifying and compensating the changes on mechanical parameters as induced by the berry compression between fingers of the assessor. In addition, it will be important to complement the information with consumer/market testing studies to establish tolerance levels for soft berries.

5.2.5.4. Crispness as a sensory descriptor

Among sensory descriptors, special attention has been given to crispness (**Table 1.1**). It is well known that the consumer satisfaction experience when eating small fruit commodities such as cherries (Hampson et al., 2014), grapes (Giacosa et al., 2015), and blueberry (Blaker et al., 2014) can be positively influenced by offering fruit with predominant crispness sensory attributes. Sensory crispness is associated with freshness and is often evaluated using touch and hearing senses when biting or suddenly fracturing a fruit sample (Saftner et al., 2008; Hampson et al., 2014; Giacosa et al., 2015; Vilela et al., 2016). Alternatively, the crispness of blueberries can be described from a sensory point of view as an audible grape-like pop in the mouth when breaking the blueberry skin during the initial mastication or the experience of eating celery stalks (Gilbert et al., 2014).

Is unknown if the mechanical parameters of BHS will provide reliable representation of crispness. Saftner et al., (2008) observed that Firmtech firmness (same parameter as BHS but different calculations procedure and equipment use) was positive, but weak ($r = 0.44$) correlated to crispiness. Conversely, Blaker et al. (2014) observed a stronger correlation ($r > 0.8$) between crispness and mechanical parameters of chord stiffness (N mm^{-1}) and skin break rupture (N). However, these strong correlations were obtained using southern highbush cultivars with predetermined crispness differences (crisp VS standard texture genotypes). In addition, Cappai et al. (2020) identified genetic markers partially associated with phenotypic variance in fruit FirmTech firmness in 237 genotypes derived from the cross between two crisp highbush cultivars ‘Sweetcrisp’ and ‘Indigocrisp’. Further study is required to determine whether the relationships between crispness and chord stiffness (hardness slope) are consistent when using diverse blueberry genotypes and growing conditions.

Alternative instrumental methods to evaluate crispness include combining mechanical and acoustic techniques reported in grapes (Giacosa et al., 2015) and apples (Piazza and Giovenzana, 2015). An acoustic envelope detector can be connected to a testing machine (i.e., texture analyzer) to record the acoustic response through instrumental rupture mechanics (Giacosa et al., 2015; Piazza and Giovenzana, 2015). For blueberries, as far as the authors found, no studies have considered the use of combined acoustic and mechanical parameters to evaluate blueberry crispness. Hence future studies for blueberries should consider the combination of BHS, acoustic sensors, and sensory panel systems in order to investigate the evaluation of crispness using objective instrumental measurements.

5.3. Conclusions and further research recommendations

This thesis demonstrates that combining different mechanical tests can provide information for postharvest quality evaluations. Displacement at skin break (DSk), alone or in combination with hardness slope (BHS), can provide a good differentiation of water loss changes occurring ob blueberries. On the other hand, a given mechanical test can generate mechanical parameters that can be related to diverse factors. For example, using the penetration test, the harvest maturity can be examined with force at skin break (FSk), and the water loss and controlled atmosphere effects can be examined using the DSk.

The studied mechanical parameters can assist the blueberry research community and facilitate the development and evaluation of novel postharvest technologies and the understanding of quality changes of stored blueberries. However, some of these parameters (i.e., FSk) require sophisticated texture equipment (i.e., texture analyser) for accurate data collection.

Alternatively, selecting a single mechanical parameter that can be used across sources of textural variance seems like a better approach to collecting firmness data in commercial scenarios. Considering that hardness slope (BHS) is easier to measure and less demanding on equipment prerequisites when compared to DSk or skin break slope (SSk), and BHS provides a confident indication of postharvest blueberry quality (i.e., softening and firming) across the different source of postharvest textural variation (i.e., water loss, controlled atmosphere, maturity). BHS may be a good candidate for developing a standard method to measure firmness in quality evaluations of fresh blueberries. In addition, BHS may provide insights about consumer acceptability (strong relationship to hand-touch firmness evaluated using a sensory panel setting), which can assist commercial quality evaluations and technology improvements.

This thesis has also identified BHS ranges associated with the likelihood of unmarketable blueberries (sensory 'soft' or 'very soft' berries) on a batch. A BHS of ≤0.47 kN m^{-1} represent a very high likelihood (≥ 95 %) of unmarketable blueberries (berries are 'soft' or 'very soft') on a

‘Centurion’ blueberry batch. Consequently, berries in a batch with average firmness close to or below the threshold should be destined to alternative commercial systems such as frozen fruit or other food ingredients. However, the author suggests that this BHS threshold can change depending on the data collection conditions (e.g., operational settings, calculation procedures) and the blueberry genotype. The standardisation of the BHS thresholds associated with consumer acceptance of different genotypes requires further study.

Later, it will be essential to study how individual blueberry firmness data will be used. In this thesis, average mechanical parameters of a fruit batch (e.g., 30 berries) were used. However, the author recommends further study of post-data collection management, such as berry dispersion statistics or interval ranges (i.e., percentile k^{th}) use, that better represent the commercial quality and consumer acceptability of a package of fruit rather than individuals.

Finally, questions remain to be answered, whether these mechanical parameters can be used to predict a blueberry batch's maximum storage life potential and whether these mechanical parameters can be associated with other critical sensory attributes such as crispness in the consumer mouthfeel.

5.4. References

Allan-Wojtas, P.M., Forney, C.F., Carbyn, S.E., Nicholas, K.U.K.G., 2001. Microstructural indicators of quality related characteristics of blueberries - An integrated approach. Lebensm.-Wiss. U.-Technol. 34, 23-32. https://doi.org/10.1006/fstl.2000.0738

ASAE, 2008. Compression test of food material of convex shape. ASABE Standards S368.4., 1-8.

Ballinger, W.E., Kushman, L.J., Hamann, D.D., 1973. Factors affecting the firmness of highbush blueberries. J. Amer. Soc. Hort. Sci. 98(6), 583-587

Blaker, K.M., Olmstead, W., 2015. Cell wall composition of the skin and flesh tissue of crisp and standard texture southern highbush blueberry genotypes. J. Berry Res. 5, 9-15.

Blaker, K.M., Plotto, A., Baldwin, E.A., Olmstead, J.W., 2014. Correlation between sensory and instrumental measurements of standard and crisp-texture southern Highbush blueberries (*Vaccinium corymbosum* L. interspecific hybrids). J. Sci. Food Agric. 94, 2785-2793. https://doi.org/10.1002/jsfa.6626

Cappai, F., Benevenuto, J., Ferrao, L.F.V., Munoz, P., 2018. Molecular and genetic bases of fruit firmness variation in blueberry - A review. Agronomy 8, 174. https://doi.org/10.3390/agronomy8090174

Cappai, F., Amadeu, R.R., Benevenuto, J., Cullen, R., Garcia, A., Grossman, A., Ferrao, L.F.V., Munoz, P., 2020. High-resolution linkage map and QTL analyses of fruit firmness in autotetraploid blueberry. Front. Plant Sci. 11, 562171. https://doi.org/10.3389/fpls.2020.562171

Donahue, D.W., Work, T.M., 1998. Sensory and textural evaluation of Maine wild blueberries for the fresh pack market. J. Texture Stud. 29, 305- 312. https://doi.org/10.1111/j.1745-4603.1998.tb00172.x

Ehlenfeldt, M.K., Martin, R.B., 2002. A survey of fruit firmness in Highbush blueberry and species-introgressed blueberry cultivars. Hortscience 37, 386-389. https://doi.org/10.21273/HORTSCI.37.2.386

Ferraz, A.C.O., Sargent, S.A., Fox, A.J., 2000. Delays to cooling and postharvest quality of fresh Florida blueberries. Proc. Fla. State Hortic. Soc. 113, 268-271.

Ferraz, A.C., Sargent, S.A., Fox, A.J., 2001. A nondestructive method for measuring firmness of blueberry fruit. Proc. Fla. State Hortic. Soc. 114, 138-142.

Gilbert, J.L., Olmstead, J.W., Colquhoun, T.A,, Levin, L.A., Clark, D.G., Moskowitz, H.R., 2014. Consumer-assisted selection of blueberry quality traits. HortScience 49(7), 864-873. https://doi.org/10.21273/HORTSCI.49.7.864

Giacosa, S., Torchio, F., Segade, S.R., Giust, M., Tomasi, D., Gerbi, V., Rolle, L., 2014. Selection of a mechanical property for flesh firmness of table grapes in accordance with an OIV ampelographic descriptor. Am. J. Enol. Viticult. 65, 206-214. https://doi.org/10.5344/ajev.2014.13115

Giongo, L., Poncetta, P., Loretti, P., Costa, F., 2013. Texture profiling of blueberries (*Vaccinium* spp.) during fruit development, ripening and storage. Postharvest Biol. Technol. 76, 34-39. https://doi.org/10.1016/j.postharvbio.2012.09.004

Jordan, R.B., Loeffen, M.P.F., 2013. A new method for modelling biological variation using quantile functions. Postharvest Biol. Technol., 86, 387-401. https://doi.org/10.1016/j.postharvbio.2013.07.008

Li, M., Rivera, S., Franklin, D., Nowak, E., Hallet, I., Kolenderska, S., Urbanska, M., Vanholsbeeck, F. East, A., 2021. Use of optical coherence tomography and light microscopy for characterisation of mechanical properties and cellular level responses of 'Centurion' blueberries during weight loss. J. Food Eng. 303, 110596. 10.1016/j.jfoodeng.2021.110596

Lobos, G.A., Hancock, J.F., 2015. Breeding Blueberries for a changing global environment: A review. Front. Plant Sci., 6, 1-14. https://doi.org/10.3389/fpls.2015.00782

Lobos, G.A., Bravo, C., Valdes, M., Graell, J., Ayala, I.L., Beaudry, R.M., Moggia, C., 2018. Within-plant variability in blueberry (*Vaccinium corymbosum* L): maturity at harvest and position within the canopy influence fruit firmness at harvest and postharvest. Postharvest Biol. Technol. 146, 26-35. https://doi.org/10.1016/j.postharvbio.2018.08.004.

Lobos, T.E., Retamales, J.B., Luengo Escobar, A., Hanson, E.J., 2021. Timing of foliar calcium sprays improves fruit firmness and antioxidants in "Liberty" blueberries. J. Soil Sci. Plant Nutr. 21, 426-436. https://doi.org/10.1007/s42729-020-00371-2.

Moggia, C., Graell, J., Lara, I., Schmeda-Hirschmann, G., Thomas-Valdes, S., Lobos, G.A., 2016. Fruit characteristics and cuticle triterpenes as related to the postharvest quality of highbush blueberries. Sci. Horticul. 211, 449-457. http://dx.doi.org/10.1016/j.scienta.2016.09.018

Moggia, C., Graell, J., Lara, I., Gonzalez, G., Lobos, G.A., 2017. Firmness at harvest impacts postharvest fruit softening and internal browning development in mechanically damaged and non-damaged Highbush blueberries (*Vaccinium corymbosum* L.). Front. Plant Sci. 8. https://doi.org/ 10.3389/fpls.2017.00535.

Moggia, C., Gonzalez, C., Lobos, G. A., Bravo, C., Valdes, M., Lara, I., Graell, J., 2018. Changes in quality and maturity of 'Duke' and 'Brigitta' blueberries during fruit development: postharvest implications. Acta Horticul. 1194, 1495-1501. https://doi.org/10.17660/ActaHortic.2018.1194.209

Ochmian I., 2012. The impact of foliar application of calcium fertilizers on the quality of highbush blueberry fruits belonging to the "Duke" cultivar. Not. Bot. Horti. Agrobot. Cluj-Napoca 40,163–169. https://doi.org/10.15835/nbha4028058

Olmedo, P., Zepeda, B., Rojas, B., Silva-Sanzana, C., Delgado-Rioseco, J., Fernandez, K., Balic, I, Arriagada, C., Moreno, A., Defilippi, B.G., Campos-Vargas, R., 2021. Cell wall calcium and hemicellulose have a role in the fruit firmness during the storage of blueberry (Vaccinium spp). Plants 10, 553, 1-13. https://doi.org.10.3390/plants10030553

Paniagua, A.C., East, A.R., Hindmarsh, J.P., Heyes, J.A., 2013. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. Postharvest Biol. Technol. 79, 13-19. https://doi.org/10.1016/j.postharvbio.2012.12.016

Paniagua, A.C., East, A.R., Heyes, J.A., 2014. Interaction of temperature control deficiencies and atmosphere conditions during blueberry storage on quality outcomes. Postharvest Biol. Technol. 95, 50-59. https://doi.org/10.1016/j.postharvbio.2014.04.006

Piazza, L., Giovenzana, V., 2015. Instrumental acoustic-mechanical measures of crispness in apples. Food Res. Int. 69, 209-215. http://dx.doi.org/10.1016/j.foodres.2014.12.041

Proctor, A., Miesle, T.J., 1991. Polygalacturonase and pectinmethylesterase activities in developing highbush blueberries. HortScience, 26(5), 579-581. https://doi.org/10.21273/HORTSCI.26.5.579

Prussia, S.E., Tetteh, M.K., Verma, B.P., NeSmith, D.S., 2006. Apparent modulus of elasticity from FirmTech 2 firmness measurements of blueberries. Trans. ASABE 49, 113-121. https://doi.org/10.13031/2013.20219

Rosenthal, A.J., 2010. Texture profile analysis - How important are the parameters. J. Texture Stud. 41, 672-684. https://doi.org/10.1111/j.1745-4603.2010.00248.x

Saftner, R., Polashock, J., Ehlenfeldt, M., Vinyard, B., 2008. Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. Postharvest Biol. Technol. 49, 19-26. https://doi.org/10.1016/j.postharvbio.2008.01.008

Silva, J.L., Marroquin, E., Matta, F.B., Garner, J.O., Stojanovic, J., 2005. Physicochemical, carbohydrate and sensory characteristics of highbush and rabbiteye blueberry cultivars. J. Sci. Food Agr. 85(11), 1815-1821. https://doi.org/10.1002/jsfa.2083

Slaughter, D.C., Rohrbach, R.P., 1985. Developing a blueberry firmness standard. Trans. ASAE 28(3), 986-992. https://doi.org/10.13031/2013.32374

Vicente, A.R., Ortugno, C., Rosli, H., Powell, A.L.T., Greve, L.C., Labavitch, J.M., 2007. The temporal sequence of cell wall disassembly events in developing fruits 2 Analysis of blueberry (Vaccinium species). J. Agric. Food Chem. 55, 4125-4130. https://doi.org/10.1021/jf063548j