THE EFFECT OF NITROGEN FERTILISER ON THE SENSOMETABOLOMIC PROFILE OF STEAMED POTATOES

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Abstract

For growers of high-value potatoes (*Solanum tuberosum* L.), flavour is an important product characteristic to consider, as a driver of consumer purchasing behaviour. Recent potato flavour research has focused on combining sensory and analytical techniques to better understand flavour. However, there is a lack of understanding around the relationship between consumer flavour preferences and flavour-related composition. In addition, there is very little known about the impact of agronomic factors on these aspects combined. For nitrogen, a nutrient of concern in New Zealand because of its environmental implications, understanding on how different rates of nitrogen fertiliser affect flavour is limited. Therefore, this study used a combined approach of consumer sensory evaluation with metabolite testing (sensometabolomics) to determine if nitrogen fertiliser affects the perception of steamed gourmet potato flavour and composition.

Two potato varieties, 'Annabelle' and 'Andean Sunside', were treated with three different rates of nitrogen fertiliser during crop growth: 0 kg N ha^{-1}, 150 kg N ha^{-1}, and 300 kg N ha^{-1}. One hundred and eleven regular potato consumers assessed steamed potato samples for liking, using a labelled affective magnitude scale, and flavour, using rate-all-that-apply, across 14 flavour attributes. The levels of key flavour-related metabolites and variables were measured in the same potatoes, including dry matter, sugars, glycoalkaloids, polyphenols, umami amino acids, total nitrogen, nitrate, and volatile compounds. Principal component analysis was used to assess the association between changes in composition and flavour.

Nitrogen fertilisation significantly affected composition and flavour in Annabelle and Andean Sunside, and ranked liking in Andean Sunside. In both Annabelle and Andean Sunside, total nitrogen, asparagine, and glutamine levels significantly increased with the rate of nitrogen fertiliser applied, from 0 kg N ha^{-1} to 300 kg N ha^{-1}. In Annabelle alone, nitrate, 2-butanone, 2-methyl-2-(*E*)-butenal, methional, and benzaldehyde increased with nitrogen, while dry matter and both α-solanine and α-chaconine concentrations decreased. The effect of fertiliser on β-ocimene was variable. In Andean Sunside, α-solanine, β-ocimene, and phenethyl alcohol all increased with nitrogen supply, while glucose and fructose concentrations significantly decreased. Levels of (*Z*)-hex-3-en-1-ol, linalool, and benzyl alcohol significantly increased between 0 and 150 kg N ha^{-1} treatments. The effect of nitrogen on quercetin-3-rutinoside was inconsistent in Andean Sunside.

While average liking was not significantly affected by nitrogen, Andean Sunside 0 kg N ha^{-1} samples were ranked significantly higher in liking compared to 300 kg N ha^{-1} samples. Overall, nitrogen fertilisation appeared to slightly, but significantly, increase the intensity of attributes with more negative associations. Annabelle 300 kg N ha^{-1} samples contained significantly higher intensities of nutty, musty, and sour attributes, while Andean Sunside 0 kg N ha^{-1} samples were significantly more buttery, and less cardboard and green grass-flavoured, compared to 150 kg N ha^{-1} samples. Therefore, for Annabelle, either 0 and 150 kg N ha^{-1} treatments were recommended to reduce the intensity of off-flavoured attributes. For Andean Sunside, decreasing the rate of nitrogen applied was recommended to reduce the intensity of off-flavours and increase the intensity of buttery flavour, as observed in 0 kg N ha^{-1} samples. Changes in composition could not be associated with changes in perceived flavour, within the varieties and treatments used in this study.

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Chapter 1

Introduction

1.1 Background

One of the most popular vegetables in New Zealand, potatoes (*Solanum tuberosum* L.) are a dietary staple prepared and enjoyed by ~94% of New Zealanders at least once a week (Potatoes New Zealand, 2013). Global production of potatoes is estimated at just under 390 million tonnes per year (FAO, 2019). The popularity of potatoes can be partly attributed to the number of preparation methods available to consumers, in combination with their mild flavour (Dresow and Böhm, 2009; Maga, 1994). In addition, the historically cheap prices of potatoes, given their traditional status as a commodity crop, is also a large driver for the high rates of potato consumption in New Zealand (Horticulture New Zealand, 2017).

However, in recent times, the flavour and taste of potatoes has emerged as an important factor influencing consumer purchasing decisions, along with appearance, convenience, texture, and price (Morris and Taylor, 2019; Sharma, 2019). Many consumers are also shopping for specific varieties and believe potatoes are a nutritious food (identified in a USA consumer study, Sharma (2019)), compared to over 10 years ago in New Zealand, where they were regarded as unhealthy (Potatoes New Zealand, 2013). Therefore, in order to differentiate their products and respond to changing consumer drivers, growers have started to develop gourmet or speciality fresh potato products, priced higher and marketed as superior-tasting (Jacobs, 2019). For example, there is now a large market for small gourmet new potatoes, specially marketed at Christmastime, sold at a premium price. Flavour and quality is now a very important consideration in potato breeding (Sharma, 2019), when traditionally breeding focused on yield and resistance to pests, diseases, and other plant stresses (Bradshaw and Ramsay, 2009). For example, novel varieties and subspecies of *S. tuberosum* are now being investigated for their potentially superior sensory qualities (e.g. *S. phureja*) (Ducreux et al., 2008; Morris et al., 2007), when previously they were investigated for disease resistance (Bradshaw and Ramsay, 2009). With a continued and increasing focus on flavour as an aspect of potato quality (Morris and Taylor, 2019), consumer acceptance and

consumption of potatoes could be further encouraged. This is also beneficial to growers because it helps to increase overall profit - survival as a potato grower is dependent on developing successful differentiated products sold at a premium, or by having a large scale of production (personal communication, B. Hart, February 3, 2020).

To maintain consistency, or even improve the flavour of their potatoes, some growers are also becoming interested in how flavour can change with variation in growing location or agronomic practices (e.g. irrigation or fertiliser). For example, there is an expanding body of literature comparing the quality and flavour of potatoes grown under conventional and organic systems (Gilsenan et al., 2010; Hajšlová et al., 2005; Lombardo et al., 2012; Maggio et al., 2008). This focus is the response to an increasing trend around the perceived environmental and health benefits of organic foods, over conventional production (Hajšlová et al., 2005). In New Zealand, the excessive use of fertiliser in the agricultural industry has become a large issue and is a growing concern for many consumers (Gorman, 2019). High rates of soluble nitrogen application is an environmental concern as leftover nutrient, not taken up by plants, is left to leach from the soil profile, contaminating groundwater and surrounding waterways (Davenport et al., 2005). However, adequate applications of nitrogen fertiliser is an essential part of the potato crop production system, encouraging high yields especially in process potato production (Koch et al., 2019). Therefore, if growers must make changes to their nutrient applications in response to increasing public pressure, the change in growing conditions could impact the flavour of their high-value potato products. Unfortunately, there is currently limited understanding around how changing levels of nitrogen and other nutrient fertilisers actually affect perceived potato flavour. There is only a small body of research suggesting that heightened levels of nitrogen fertiliser could increase the perception of off-flavours in cooked potatoes (Fischer, 1991).

1.2 General project aims

Given the importance of flavour to growers of high-value potatoes and the need to understand how growing practices may affect the flavour of their products, this project aims to investigate the effect of varying rates of nitrogen fertiliser on the flavour of steamed gourmet potato varieties. A combination of metabolite analysis on compounds that contribute to potato flavour, in addition to consumer sensory evaluation, will be undertaken to investigate this, called 'sensometabolomics'. By using sensometabolomics, any treatment effects on the metabolic profile of potatoes can be tested by sensory analysis to determine if any changes are large enough to impact flavour perception. In addition, because consumers are the target audience for gourmet potato growers, this project will apply consumer sensory testing methods, instead of using a trained panel. The effect of nitrogen fertiliser on potato flavour will be assessed in two gourmet potato varieties, an *S. tuberosum* variety and another with *S. phureja* parentage. As *S. phureja* varieties have been previously reported

to exhibit superior flavour to *S. tuberosum* varieties (Morris et al., 2007), they represent a potential opportunity for gourmet potato growers to develop new, flavour-differentiated potato products. Furthermore, by using a wider range of genetics, the effect of nitrogen fertiliser on potato flavour will be better understood for a wider range of varieties than if just *S. tuberosum* was tested. The results of this project will add to the understanding of how potato production practices affect potato flavour. Therefore, if changes need to be made to their production systems in the future, growers will be equipped with the knowledge on how their actions may be affecting the flavour of their products. This could lead to an increased consistency in the flavour of gourmet products, or potential improvements to flavour, depending on the findings of this project.

1.3 Project overview and scope

To introduce the main themes discussed as part of the project background, a comprehensive literature review has been undertaken, first introducing potatoes as a crop, outlining the key aspects of flavour, the potential impacts of nitrogen fertiliser, and the metabolic and sensory analyses that could be employed to capture flavour changes. This leads to a precise statement of this project's key research questions and hypothesis (see Section 2.6). In Chapter 3, the methods of this project are described in detail, including the field trial treatments, sensory evaluation techniques used, and the metabolic analyses conducted. Following this, Chapter 4 outlines the challenges and limitations experienced during the project, and how these may influence the results obtained. Chapter 5 focuses on the results and an initial discussion of how nitrogen and variety has impacted potato flavour, ending with a correlation analysis between sensory and analytical variables. An extended discussion on the impact of the trial's treatments on flavour will be presented in Chapter 6, ending with the project's conclusions and suggestions for future work (Chapter 7). Throughout this document, any discussion of existing research will relate to boiled or steamed potatoes, unless otherwise specified, given this project focuses on the impact of nitrogen on steamed potatoes.

Chapter 2

Literature review

2.1 Introduction

A comprehensive literature review has been conducted to introduce the research area, review the existing literature on potato flavour and the potential impact of nitrogen fertiliser on flavour, and develop specific research questions relating to the background and general aims of this project. An overview of potatoes as a cultivated crop is first provided, with information relevant to the genetic difference between *S. tuberosum* and *S. phureja* potato varieties. The relevance and reception of potatoes in New Zealand is also briefly covered. Following this, the flavour of potatoes is described, as an important consideration for growers of high-value potato crops in New Zealand. The contributions of aroma and taste compounds contributing to flavour are discussed, followed by a brief description of the reported flavour differences between *S.tuberosum* and *S. phureja* varieties. This leads into a discussion on how nitrogen fertiliser could affect flavour-related composition and sensory-perceived flavour in potatoes, as the main research question in this study. The effect of nitrogen fertiliser on the flavour of other horticultural crops is also shortly described. The final section of the review provides a summary of the main analytical and sensory techniques that could be used to measure any changes to potato flavour, as a result of varying the rate of nitrogen fertiliser applied. This leads onto the main research focus and questions this project will attempt to answer.

2.2 Potato origin, genetics, and production as a crop

2.2.1 Potato origin and early cultivation

The potato (*Solanum tuberosum* L.) is a domesticated tuber crop originating from the highlands of South America (Burton, 1989). No records of it as a wild plant exist - indeed, even the earliest estimations suggest potato is the result of millennia of cultivation. The centre of earliest potato cultivation is believed to be in modern-day south Peru and west Bolivia (Burton, 1989). In South America, potatoes were used as a main food source, the Andean people also preserving them to create the highly-prized dried 'chuño' potato food, still made and eaten today (Bradshaw and Ramsay, 2009). The export of potatoes out of South America and into Europe began when the Europeans discovered potatoes during the Spaniard expeditions to South America in the mid-16^{th} century. The first potatoes to reach Europe are suggested to have originated from Chile, a water-colour painted by Philippe de Sivry in Belgium (1588) the first certain record of their presence in Europe. Therefore, estimations suggest potatoes were first introduced into Europe around 1580 (Bradshaw and Ramsay, 2009; Burton, 1989).

While potatoes were initially regarded as a 'botanic curiosity', grown for medicinal purposes and general interest only, their potential as an agricultural food crop was discovered in Ireland in the 17^{th} century (Bradshaw and Ramsay, 2009; Burton, 1989). Landlords in Ireland were interested in agricultural improvement, a number documented to bringing over potatoes from England to grow as crops (Burton, 1989). Given the favourable growing conditions, and their continued success when other crops (e.g. maize) failed, potatoes became the most important food source in Ireland in the late 18^{th} century. In other parts of Europe, the spread of potatoes as a crop was slower, starvation and famine one of the main stimuli for their eventual cultivation and acceptance (Burton, 1989). However, the famine alleviation brought by potatoes became Ireland's downfall between 1845 and 1860, after the arrival of 'late blight' (*Phytophthora infestans*) and the Irish Potato Famine (Bradshaw and Ramsay, 2009; Burton, 1989). Their over-reliance on potatoes, in combination with the total crop decimation caused by late blight, saw the death of 1 million people in Ireland and migration of a further 1.5 million. Naturally, this reduced confidence in potatoes as a food crop, and the total area of cultivation halved in the 20^{th} century from its peak of 486,000 ha in Ireland (Burton, 1989).

Although the increase and then decline in potato production over the 19^{th} and 20^{th} centuries was mirrored across Europe, production overseas expanded and increased with the movement of European colonists and missionaries (Bradshaw and Ramsay, 2009; Burton, 1989). The first potato crops reached the Phillipines in the 17^{th} century; India in the 18^{th} (potentially earlier) century; and New Zealand and Australia in the 18^{th} century also (Burton, 1989). While European potato production has continued to decline since the 20^{th}

century, Chinese and Indian production has increased hugely. From the 19thth century, potatoes were grown worldwide (Bradshaw and Ramsay, 2009). Today, China and India lead total potato production and area cultivated, growing 99,205,580 and 48,605,000 tonnes in 2017 respectively, followed by Russia growing 29,589,976 tonnes (FAO, 2019).

2.2.2 Modern potato cultivation

Compared to early cultivation, potato production today has come a very long way. Since the last century, potato yield and quality has significantly improved in most countries (Bradshaw and Ramsay, 2009). This is due to a combination of factors. Growers now have a better understanding and availability of fertiliser to provide crops with sufficient nutrition, whilst balancing minimal leaching, to achieve high yields (Westermann, 2005). In 2017, New Zealand achieved the second highest potato yield worldwide after Kuwait, with an average yield of 49.3 t ha^{-1} (FAO, 2019). Potato breeding has often focused on developing resistance to major pests and diseases, such as late blight, powdery scab (*Spongospora subterranea*), and bacterial soft rot (*Erwinia* spp.) (Anderson et al., 2004). The use of potato subspecies has further improved the development of strong and resistant new varieties (Bradshaw and Ramsay, 2009). Growers have access to a wide range of agrichemicals, in combination with integrated pest management plans, to target and control threats to both yield and quality. Additionally, advances in understanding around the importance of clean seed and the transfer of viruses between seed generations has led to the development of seed certification schemes, ensuring seed and potato growers alike are growing crops from high-quality and healthy seed, further improving agronomic performance (Bradshaw and Ramsay, 2009).

2.2.3 Taxonomy and genetics

The modern, widespread, and most successful potato species is the tetraploid (4x) *Solanum tuberosum* L. *S. tuberosum* is part of the *Solanum* family, which includes other crops such as tomatoes (*S. lycopersicum*) and eggplant (*S. melongena*). While thousands of cultivars native to South America have been collected and documented, the taxonomy of *S. tuberosum* and its original genetic material is widely-disputed and often reassessed (Bradshaw and Ramsay, 2009). Hawkes (1997, in Burton (1989)) suggests that the potato's starting material may resemble the diploid (2x) species *S. cacasense*, which is found in the area (Chile) where cultivation was thought to begin. From this, *S. cacasense* (2x) was selected and mutated into *S. stenotomum* (2x), the beginning of the currently cultivated species, including *S. phureja* (mostly 2x), *S. chaucha* (3x), *S. andigena* (4x), and *S. tuberosum*) (4x). Fig. 2.1, from Bradshaw and Ramsay (2009), shows four different interpretations from four publications on how the *Solanum* subspecies eventuated. The theories mostly differ by their classification of the cultivated species as species on their own, or as subspecies/groups of *S. tuberosum*. Three other hybrid species have also been recognised by taxonomists: *S.* ×

curtilobum, *S.* × *juzepczukii*, and *S.* × *ajanhuiri*, these all originating from crosses between the former groups and earlier *Solanum* species. The hybrid species have been recognised as both groups and species by separate taxonomists (Bradshaw and Ramsay, 2009). As Fig. 2.1 suggests, classifying the many related species of potato is very complicated and still in dispute (Bradshaw and Ramsay, 2009).

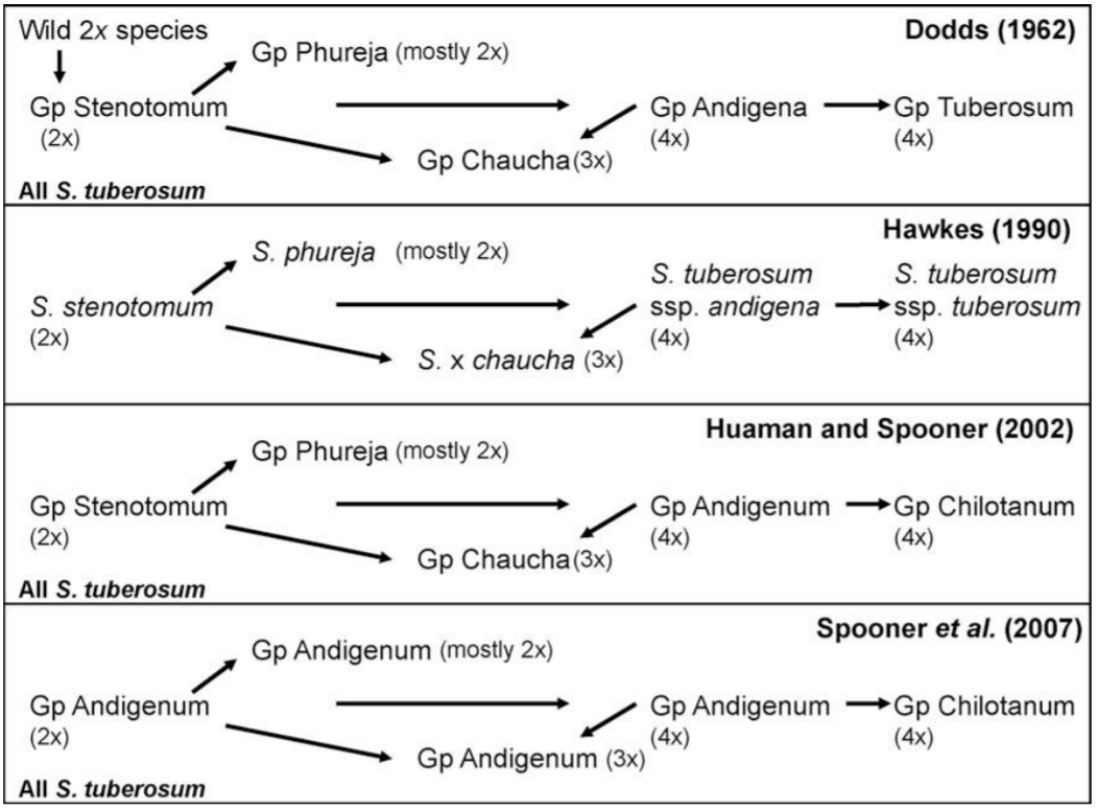


Figure 2.1: Potato taxonomical groups suggested by different authors. Used with permission from Bradshaw and Ramsay (2009).

2.2.4 Plant and tuber characteristics

Morphological characteristics

As an extremely wide range of potato varieties exist, developing a concise description of a potato's major morphological and physiological features is somewhat of a challenge. Potatoes are herbaceous perennials that can grow over 60 cm in height with an erect, semi-erect, or decumbent (lying on ground, angling upwards) growth habit (Burton, 1989). Plant stems can be simple or branched, round or angular, and coloured green, red-brown, purple, or a mixture of pigments. The number of stems can range from one to over ten (Burton, 1989). Like the stems, potato leaves also vary considerably between varieties, and can range from short (<25 cm) or long (>30 cm), simple to fully dissected into leaflets, with shapes including

elliptic, oblong, ovate, obovate, and orbicular. Flowering occurs at different times for different varieties, flower colours ranging from white, pink, red, blue, purple, yellow, and many hues in-between (Burton, 1989; Huaman et al., 1977). The flowers, if fertilised, may form potato fruit, which looks similar to a cherry tomato. Some varieties rarely fruit, others produce large amounts. Fruits are normally green, but can be white-spotted or striped/streaked (Huaman et al., 1977). The seeds within the potato fruits are referred to as the "true potato seed", and these seeds are used for the development of new varieties.

However, the distinctive tuber characteristics can help to more-easily differentiate between potato varieties. Firstly, the stolons connecting the tubers to the plant can range from short (10 cm) to long (40 cm) (Burton, 1989). Tubers can be small, medium, or large, and different varieties will set different numbers of tubers. A wide range of tuber shapes also exist, including round, ovate, oblong, or compressed (Huaman et al., 1977). A number of more unusual tuber shapes are also described in Huaman et al. (1977), including coiled, reniform (kidney-shaped), and digitate (fist-like) shaped potatoes. The depth, number, and distribution of eyes is another feature of potatoes that varies significantly between varieties (Burton, 1989; Huaman et al., 1977). The primary and secondary skin colour and texture of tubers, in addition to the primary and secondary flesh colour, are other major discriminating features. Primary skin colour can be white-cream, yellow, orange, brown, pink, red, purple-red, purple, and dark purple-black. The same colours can be present as a secondary skin colour, which could be scattered, stippled, or focused around the eyes (Huaman et al., 1977). Flesh colour can be white, cream, yellow-cream, yellow, red, violet, or purple. The same colours can be present as a secondary flesh colour, and are commonly patterned as scattered spots or areas, as a vascular ring, as pith, or all flesh excepting the pith (Huaman et al., 1977). Additionally, cooked texture is strongly influenced by tuber dry matter content, where loose mealy varieties will likely contain high dry matter (>24%) and waxy sticky potatoes will likely contain a lower dry matter content (18 - 20%) (van Dijk et al., 2002; Van Marle et al., 1997). Naturally, all other compositional and nutritional characteristics will also vary considerably between varieties and growing conditions (Bradshaw and Ramsay, 2009).

Physiological characteristics

One of the main methods of classification for potatoes is the grouping of varieties by time to maturity (Burton, 1989). In New Zealand, potatoes are categorised as 'early' and 'maincrop' - traditionally, early (or new) potatoes are harvested before Christmas, maincrop after Christmas. Early varieties (e.g. Ilam Hardy, Annabelle, and Jersey Bennes) have a shorter time to maturity (approx 70 - 100 days), whereas maincrop varieties generally take over 100 days minimum to reach maturity - 'Moonlight' takes 140 - 150 days (Anderson et al., 2004; Morton Smith-Dawe Ltd., 2019). As many early varieties are also harvested early (i.e. as smaller tubers), time to maturity is therefore more dependent on tuber bulking as opposed

to foliage death, although early-bulking potatoes will likely also die down sooner (Burton, 1989). In addition to maturity times, potato varieties also physiologically vary in their resistance to different potato pests and diseases (Burton, 1989; Huaman et al., 1977). For example, the variety Moonlight is more resistant to the *P. infestans* (late blight), compared to Ilam Hardy, which is moderately susceptible to infection (Anderson et al., 2004). These are just two examples of how potato varieties also vary physiologically.

For more detail on the characteristics of potatoes, the reader is directed to Burton (1989) and Huaman et al. (1977).

2.2.5 Potatoes and potato consumers in New Zealand

In New Zealand, potatoes (incl. frozen and processed) are the biggest vegetable export, earning $141 million NZD in 2018 (The New Zealand Institute for Plant and Food Research, 2019). While potatoes are grown all over New Zealand, production centres in Canterbury (46% by volume grown), Auckland (19%), and the Waikato (19%) (Horticulture New Zealand, 2017). In 2018, 527,190 tonnes of potatoes were grown on 10,344 planted ha, by a total of 171 growers (The New Zealand Institute for Plant and Food Research, 2019). To emphasise the scale of potato production in New Zealand, the total planted area is almost double the second largest vegetable crop, squash, with only 6,642 planted ha. Over 50 potato varieties are grown in New Zealand, the main ones of which include Russet Burbank, Nadine, Agria, Moonlight, Desiree, and Ilam Hardy (Potatoes New Zealand, 2019).

New Zealand consumers enjoy potatoes - an average of 22.90 kg was consumed per person in 2016 (Horticulture New Zealand, 2017). 94.5% of the population consumed potatoes in 2013, down from 97% in 2006. Core potato purchasers are generally aged 55+ in a 1 - 2 member household, with no children (Potatoes New Zealand, 2013). Survey results from 2013 suggest that shoppers under 35 perceived potatoes as difficult to prepare and extravagant, an “occasion” needed to consume them. However, interest around varieties, health benefits, and new recipe ideas has increased - in 2006, consumers were instead more interested in rice and pasta as alternative carbohydrate options (Potatoes New Zealand, 2013). Over the past three years, sales for potatoes within 5 kg to 10 kg weight categories has decreased, while the growth for below 1 kg (generally gourmet) potatoes has been strong (personal communication, R. Johnstone, December 21, 2019). This helps to show the changing purchasing habits of New Zealanders, away from larger bags and towards smaller, more-conveniently packaged potato products.

2.3 Defining potato flavour

In order to develop gourmet or speciality potato varieties that can command a higher price from consumers in supermarkets, especially given the change in purchasing behaviour towards smaller packaged speciality products, growers must seriously consider the flavour of their products, identified as a key aspect of successful potato varieties (Morris and Taylor, 2019). As discussed, this could be achieved through crosses with novel subspecies to develop varieties with superior flavour. Flavour could also be affected through the impact of agronomic practices. Therefore, first understanding what contributes to the flavour of potatoes is important to understand the changes that could occur if compounds contributing to flavour (e.g potent aroma volatiles) were affected. Therefore, this section will provide an overview of sensory-perceived potato flavour with a discussion on the compounds thought to be key contributors to overall flavour, including aroma and taste compounds. Unless otherwise stated, this section will focus on research concerning boiled and/or steamed potatoes in relation to the steaming method used for this study.

2.3.1 What is flavour?

Flavour is an extremely complex sensorial and synergistic combination of aroma (volatile compounds, easily evaporable) and taste (non-volatile compounds) (Auvray and Spence, 2008; Jansky, 2008; Shepherd, 2006). Basic taste (sweet, sour, bitter, salt, and umami) is perceived on the tongue (Noble, 1996) whereas aroma (or odour) is perceived by sensory cells in the olfactory epithelium (Shepherd, 2006). Odour perception can occur in two ways, either via orthonasal olfaction (sniffing through nostrils) or retronasal olfaction, where volatile compounds released at the back of the mouth during ingestion move through the nasopharynx to the olfactory epithelium (Shepherd, 2006). Retronasal olfaction especially is essential in flavour identification because of its interaction with many other brain pathways, forming a 'flavour system' (Shepherd, 2006). Along with the presence of flavour-characterising volatile compounds, mastication and food texture impacts the time taken for these chemicals to be released from food and delivered to receptors (Salles et al., 2010). Multimodal (or cross-modal) interactions, where texture and aroma signals overlap by sharing common neural pathways, are also known to impact flavour perception (Cook, 2003). However, while its role is acknowledged, texture will not be addressed any further in this review as this study solely focuses on flavours.

2.3.2 What is the flavour of cooked potatoes?

Describing the flavour of cooked (boiled) potato is certainly not as glamorous as wine or chocolate - the introductions to potato flavour research papers often use initial descriptors such as bland and weak, yet characteristic and typical (Maga, 1994; Ulrich et al., 2000). This

means potato works well as a food to cook in multiple ways and add condiments to (Maga, 1994). However, the flavour of cooked potatoes can be described in more detail than 'bland but typical'. Some of the most common flavour attributes used to describe cooked potato flavour in flavour research include 'typical/potato-like', 'earthy', 'bitter', 'buttery', 'sweet', and 'cardboard-like' (Mondy et al., 1971; Petersen et al., 1999; Seefeldt et al., 2011; Ulrich et al., 2000). Furthermore, the presence and intensity of particular flavour attributes can help to indicate whether the flavour is liked or not, especially if combined with acceptance testing, which is yet to be conducted with consumers.

Early flavour research focused on describing and analysing the cause behind flavour attributes of interest. For example, Buttery and Ling (1973) conducted metabolite testing to investigate the cause of 'earthy aroma' in potatoes. Mondy et al. (1971) assessed 'bitterness' and 'astringency' to determine the impact of polyphenols and glycoalkaloids on boiled mashed potato flavour. The discovery of 'musty' and 'earthy' flavoured Russet Burbank potatoes prompted a research investigation to examine why these flavours were occurring, instead of tasting 'pleasant [and] potato-like' (Mazza and Pietrzak, 1990).

After 2000, a wider range of attributes started to appear in the literature. Flash testing by Porcherot and Schlich (2000) was conducted for 16 steamed potato varieties, where panellists were asked to generate a sensory vocabulary capable of differentiating varieties, in a limited amount of time. Many flavour descriptors were generated, including pastry, sweet butter, chestnut, cereal, earthy, astringent, bitter, raw potato, metallic, artichoke, celery, and herbaceous. Around the same time, Ulrich et al. (2000) conducted a sensometabolomic investigation on steamed potatoes, using the attributes sweet-like, earthy, burnt, fodder, untypical, musty, fruity, and typical. 'Typical' and 'potato-like' are popular attributes used to describe potato flavour - Lombardo et al. (2012) also used typical to describe samples, as part of an investigation on organic and conventionally-grown potatoes. A 'cardboard-like' off-flavour, identified in boiled potatoes stored after cooking, has been the subject of many research studies because of the development of pre-cooked ready-to-eat foods (Blanda et al., 2010; Comandini et al., 2018; Petersen et al., 1999). Morris et al. (2007) investigated the impact of umami amino acids on flavour intensity, flavour creaminess, sweetness, and overall acceptability, with a follow-up flavour experiment also adding 'savoury' to the attribute list (Morris et al., 2010). Seefeldt et al. (2011) used a wide range of attributes in an exploratory flavour profiling, including potato taste, sweetness, saltiness, astringency, butter, reheated, earthy storage, green taste, bitterness, and off-taste.

More recently, Bough et al. (2020) included 'lemon' and 'woody' in a list of 12 aroma and flavour attributes as part of a sensometabolomic investigation, these more unusual flavours being agreed on by a trained panel. In Japan, *egumi* is special taste perceived in potatoes, a mix between bitterness and astringency - its cause is suggested to be linked to a tyrosine metabolite Sato et al. (2019). Sharma (2019) conducted extensive panel training to develop

a lexicon of 40 attributes relating to aroma and flavour, new attributes of which included 'cauliflower', 'mustard', 'eggy', 'nutty', and 'sweet potato', amongst others.

Assessing flavour differences between varieties or treatments with consumers, in combination with consumer acceptance testing, can help to indicate which flavour attributes increase or decrease product flavour perception. Many sensory evaluations include a "catch-all" trait, such as 'overall quality' or 'overall acceptance', to understand which attributes drive consumer acceptance. For example, Bough et al. (2020) included 'overall quality as part of an attribute list of flavours. Bough et al. (2020) found that while 'potato-like' clustered with overall quality on a PCA, bitter and off-flavours negatively correlated, suggesting these attributes are undesirable in cooked potatoes. A recent Check-all-that-apply (CATA) study (unpublished) found flavour acceptability (as assessed by consumers) was associated with sweet, roast potato, and buttery flavours (V. Corrigan, personal communication, February 17, 2020). Results from Morris et al. (2007) indicate similar clustering, where sweet and 'creamy flavour' was correlated with acceptability in trained panel analysis. Using penalty analysis on a consumer CATA dataset, Sharma (2019) illustrated how cooked potato flavour and aroma attributes had a positive effect on liking, whereas metallic decreased liking. Additionally, open-ended questioning grouped flavour attributes into several categories (Sharma, 2019). Some potatoes were described as "processed, metallic, disgusting, unnatural, chemicals, bitter, dull and strange tast[ing]" while others were "potatoey, flavorful, natural, light, [and] buttery tasting". Another group were "bland, flavourless, plain, [and] boring" (Sharma, 2019). The use of open-ended questions and emotive language by consumers allowed the grouping of flavour attributes into desirable and undesirable flavours. Interestingly, the attributes 'earthy' and 'bitter' have often sat in-between acceptability ratings, sometimes driving liking if present in significant quantities, otherwise often driving dislike (Bough et al., 2020; Sharma, 2019). This leads to a discussion, outside of this study's scope, on changing preferences based on the perceived intensity of an attribute within the expected range for that product (Sharma, 2019). However, it can be generally concluded that potatoes with buttery, savoury, sweet, and potato-like attributes will likely score higher in acceptability assessments compared to potatoes with bitter, off-flavoured, and metallic attributes.

2.3.3 The chemistry of potato flavour: Aroma

The presence of volatile compounds, producing food aroma, is believed to be the main determining factor in a food's perceived flavour (Whitfield, 1992, in Dresöw & Bohm (2009)). Taylor et al. (2007) dispute this claim for potatoes, suggesting potato flavour sensory data provide insufficient evidence to support this claim. Indeed, Solms and Wyler (1979) instead suggest that umami compounds chiefly characterise overall potato flavour. Whatever the answer for potatoes, a food's final aroma is not always the outcome of one characterising compound. Instead, it can be the result of a specific blend of volatile compounds (Dresow

and Böhm, 2009). In potatoes, these compounds include aldehydes, ketones, acids, esters, hydrocarbons, amines, furans, alcohols, lipids, and sulfur compounds (Dresow and Böhm, 2009). The many different methods of potato preparation and cooking cause a large amount of variation in the volatiles contributing to potato aroma. Each cooking method produces a new selection of aroma volatiles with changes in temperature and fat presence resulting in a range of volatile-forming reactions. This variation is reflected in the total number of identified volatile compounds across raw, boiled, and baked potatoes – 159, 182, and 392 compounds respectively (Dresow & Böhm, 2009).

Methods of volatile formation

Unlike many other vegetables, potatoes are different in that they are almost always consumed after thermal processing. However, methods of volatile formation will vary considerably with factors such as cooking time, temperature, and/or the addition of fat. Hence, the aroma of raw, boiled, baked, or fried potatoes originates from a wide range of compounds and volatile-forming reactions. These include: already being present in raw tissue (methoxypyrazines, terpenes), lipid oxidation, enzymatic degradation of fatty acids, thermal degradation of fatty acids, Maillard reactions and/or sugar degradation, and sulfur amino acid degradation (Dresow and Böhm, 2009; Duckham et al., 2001; Jansky, 2010; Oruna-Concha et al., 2002b).

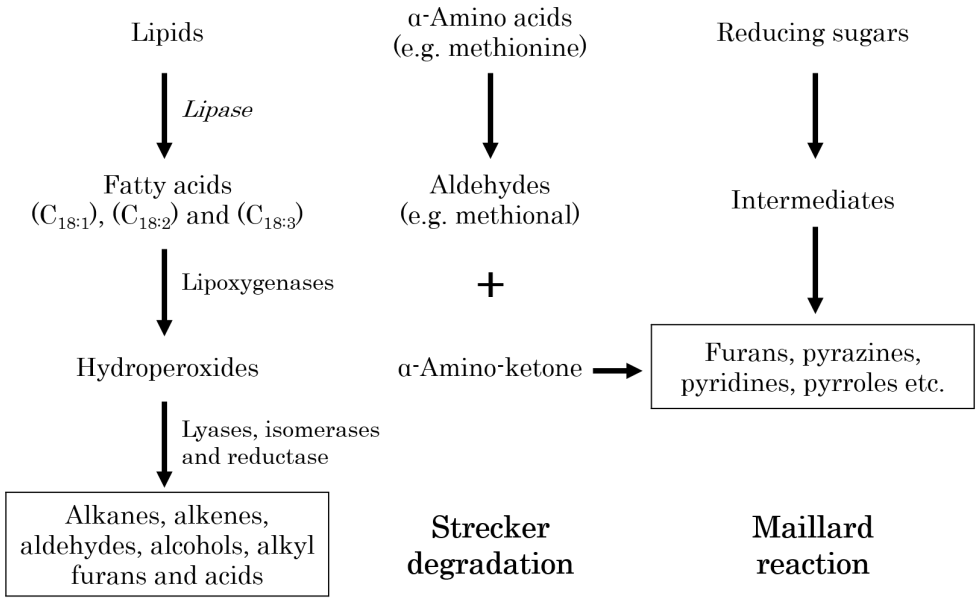


Figure 2.2: Origin of flavour volatiles in cooked potatoes. The main products from boiling are provided in the boxes. Used with permission from Taylor et al. (2007).

Only a limited body of research exists on the biogenetic pathways of raw potato volatile compounds because potatoes are generally consumed cooked (Dresow and Böhm, 2009). The volatiles produced, before the tuber is peeled or cut, are simply from the plant's own photosynthetic and metabolic processes. Examples include methoxypyrazines, early studies on raw potato identifying the presence of 3-isopropyl-2-methoxy-pyrazine and 3-isobutyl-2-methoxy-pyrazine, both of which have earthy aromas (Buttery and Ling, 1973; Murray and Whitfield, 1975). Sesquiterpenes have also been identified in raw potatoes (Desjardins et al., 1995).

When potato tubers are subjected to thermal processing, the number of different volatile compounds produced increases, from 159 in raw potatoes to at least 182 compounds in boiled potatoes (Dresow and Böhm, 2009), although Petersen et al. (1998) identified a higher number and concentration of volatiles in raw potatoes (when compared to boiled tubers). The increase in temperature allows thermal degradation (Maillard) and Strecker degradation reactions to arise. Maillard reactions occur when compounds containing carbonyl groups, such as reducing sugars, react with free amino groups (Fig. 2.2) (Taylor et al., 2007). Strecker degradation involves the conversion of an α-amino acid into its related aldehyde (e.g. methionine to methional), the products of which react with the compounds formed in Maillard reactions, generating volatile pyrazines, oxazoles, and furans, amongst other compounds (Dresow and Böhm, 2009; Taylor et al., 2007). The products of the Strecker degradation of amino acids do not necessarily react further – they are often important contributors to aroma themselves, as methional is to boiled potato aroma (Petersen et al., 1998). With boiling, thermal lipid degradation reactions will also occur, and the products of raw potato lipoxygenase-catalysed reactions, such as 2,4-decandienal, will be broken down into (*E*)-2-octenal and hexanal (Josephson and Lindsay, 1987; Petersen et al., 1998).

As cooking time and temperature increases, the number of different volatiles identified increases, and the general origin and method of volatile formation also changes (Oruna-Concha et al., 2002b). The Maillard rate of reaction and the reactivity between sugar and amino groups increases with temperature, resulting in the faster production of volatiles (Martins et al., 2000). Furthermore, the possibility of additional secondary reactions may also increase. This provides some explanation as to why baked potatoes, in comparison to boiled potatoes, are generally regarded as having a more complex aroma and overall flavour (Maga, 1994).

When comparing the relative abundance of volatile compounds between boiled, microwaved, and baked potatoes, Oruna-Concha et al. (2002b) found the ratio of volatile compound yield originating from Maillard/sugar degradation and lipid-derived reactions changed considerably between the different cooking methods, from 8.5:9.1 (lipid : sugar/-Maillard derived) in boiled potatoes to 0.4:1.1 in conventionally baked potatoes. The higher temperatures used in baking reduce the activity of lipoxygenase, and as baked potatoes are

cooked in their skin without any peeling or cutting, this further limits the opportunity of lipoxygenase, or autoxidation, to break down fatty acids (Oruna-Concha et al., 2002b).

In fried potatoes (e.g. crisps or French fries), the very high-temperature frying process allows lipids to further react with other potato constituents to produce additional flavour compounds, contributing to the enticing aroma of fried potatoes (Maga, 1994). van Loon et al. (2005) found 85% of odour-active compounds in French fries were Maillard/sugar-derived, compared to only 15% for lipid-derived compounds, further illustrating how cooking method has a very large impact on the resulting aroma and flavour of potatoes.

Volatile profile of boiled and steamed potatoes

Boiling is a simple yet popular method to prepare potatoes and thus has subsequently been well-documented within the literature on potato flavour (Blanda et al., 2010; Josephson and Lindsay, 1987; Mondy et al., 1971; Mutti and Grosch, 1999; Oruna-Concha et al., 2002b; Petersen et al., 1998; Self et al., 1963; Ulrich et al., 2000). Overall, boiled potato flavour is the result of at least 140 volatile compounds (Ulrich et al., 2000), in combination with other taste compounds, discussed in the following section. The thermal impact of boiling results in the formation of volatiles from Maillard reactions, Strecker degradation, and thermal lipid degradation (Fig. 2.2). An increase in the number of methods of volatile formation means boiled potato aroma is very different from its original raw potato aroma (Comandini et al., 2011). Steamed potatoes are cooked similarly to boiled potatoes, with no addition of fat and exposure to the same maximum temperature (i.e. 100 °C) (Descours et al., 2013). Therefore, flavour research on boiled and steamed potatoes in this study will be used interchangeably, although the difference in cooking method is acknowledged as a potential influence on flavour.

Methional, an aldehyde formed from the amino acid Strecker degradation of methionine, is one the most important and characterising compounds in boiled potatoes (Thybo et al., 2006; Ulrich et al., 2000). Its 'boiled potato' aroma is one of the most odour active volatiles within the aroma profile of boiled potatoes, where odour activity is the ratio of a volatile's concentration to its threshold (the point at which the odour is perceived) (Grosch, 1993; Mutti and Grosch, 1999). The large majority of volatile compounds identified in boiled potatoes still originate from lipid degradation (Oruna-Concha et al., 2002b; Salinas et al., 1994), resulting in the production of aldehydes such as hexanal, 2,4-decandienal, nonanal, and heptanal (Josephson and Lindsay, 1987; Mutti and Grosch, 1999; Petersen et al., 1998; Ulrich et al., 2000). Many of these aldehydes have fatty green odour qualities with the capacity to contribute to off-flavour production in potatoes (Mutti and Grosch, 1999; Petersen et al., 1999). tr-4,5-epoxy-(*E*)-2-decanal was also identified as another highly odour active volatile, which has a metallic odour quality (Mutti and Grosch, 1999). A range of pyrazines and methanethiol have also been noted as other character impact volatiles (Mutti

and Grosch, 1999; Ulrich et al., 2000), as well as c4-heptenal, which was shown to enhance the 'earthy potato-like flavour' of potatoes tested (Josephson and Lindsay, 1987). More recently, Bough (2017) identified positive 'buttery' flavours in peeled boiled (and baked) potatoes, which clustered with the volatiles 1-nonanal, pentanal, (*E*)-2-heptenal, (*Z*)-2- methyl-2-penten-1-ol, benzaldehyde, and 2-phenylacetaldehyde - these volatiles were suggested as potential buttery flavour biomarkers. However, it is worth noting that while potatoes will contain many volatiles, not all will contribute to aroma (Mutti and Grosch, 1999) - the odour threshold and potency within the potato aroma profile must first be determined before concluding whether a compound significantly contributes to potato aroma and flavour.

2.3.4 The chemistry of potato flavour: Taste

In addition to aroma volatiles, potatoes contain compounds that contribute to four basic tastes, umami (savoury), bitterness, sweetness, and sourness. Evidence of saltiness in potatoes is not prevalent, therefore will not be discussed further. Unlike aroma compounds, which are detected in the olfactory epithelium, taste compounds are perceived on the tongue (Noble, 1996; Shepherd, 2006). Taste compounds, in combination with aroma compounds, help contribute to the final perceived flavour of potatoes.

Umami

Early studies from Solms and Wyler (1979) suggest potato taste and overall flavour is chiefly characterised by umami compounds developed during cooking. Umami is a basic taste originating from glutamate compounds. In combination with a 'savoury' or 'meat-like' taste, umami compounds also amplify flavour pleasantness (McCabe and Rolls, 2007; Yamaguchi and Ninomiya, 2000). In potatoes, umami taste originates from the natural combination of the major umami amino acids glutamate and aspartate with 5'-ribonucleotides, including GMP (guanosine monophosphate) and AMP (adenosine monophosphate) (Morris et al., 2007; Solms and Wyler, 1979). In general, umami taste is produced during cooking, where temperature increases also increase RNA degradation, leading to the release of more ribonucleotides which then react with amino acids (e.g. glutamate) to form umami compounds (Taylor et al., 2007). The reactions that occur between umami amino acids and ribonucleotides have a synergistic effect on overall umami taste (Yamaguchi et al., 1971). This relationship was applied by Morris et al. (2007) when investigating the difference in umami taste between *S. tuberosum* and *S. phureja* potato cultivars, the concentrations of umami compounds used to calculate an equivalent umami concentration (EUC) value. Interestingly, Morris et al. (2007) found *S. phureja* cultivars exhibited a significantly higher EUC compared with *S. tuberosum* cultivars, in addition to discovering strong positive correlations between EUC and flavour intensity and acceptability.

Bitterness

Bitter taste in potatoes is traditionally attributed to the presence of glycoalkaloids, phenolic compounds, and potentially several amino acids (Davids et al., 2004; McKenzie and Corrigan, 2016; Sato et al., 2019). Glycoalkaloids (TGA), steroidal nitrogenous glycosides, are produced by many species within the *Solanaceae* family, and are concentrated in the cortex, just under the skin (Valkonen et al., 1996). α-solanine and α-chaconine make up approximately 95% of all TGA compounds in potatoes (Slanina, 1990), therefore most research around TGA and bitterness generally focuses on these two compounds alone. At levels over 200 mg kg^{-1}, TGAs can make potatoes intensely bitter and potentially toxic, and may also induce persistent throat burning and scratchiness (Sinden et al., 1976). However, at the levels found in mature conventional varieties, which approximately range from 5 - 120 mg kg^{-1} (Patchett et al., 1977), the contribution of TGAs to potatoes' bitter taste is conflicting. Sinden et al. (1976) found potatoes needed to contain over 140 mg kg^{-1} to generate perceptible bitter responses in participants. When testing a range of Maori potato cultivars, all under 105 mg kg^{-1} TGA content, Savage et al. (2000) failed to identify a correlation between TGA content, bitterness, and sample preference. Additionally, Amer et al. (2014) found that an increase in crisp TGA content after storage, up to approximately 100 mg kg^{-1} across four varieties, was highly correlated with chip flavour and acceptance, with no mention of any bitter tastes. A recent Japanese study investigating *egumi*-taste (Japanese for 'a bitter and astringent taste') also found no correlation between TGA content and *egumi* across several potato cultivars. Overall, this suggests that while TGA compounds may produce a bitter taste in potatoes at in high concentrations, it is often not correlated with bitterness, because the TGA contents measured are often below 140 mg kg^{-1}.

A small amount of research has also linked phenolic acids to bitterness, although like glycoalkaloids, the literature contains contradictory information. Mondy et al. (1971) found significant links to total phenolic content and bitterness and astringency in potatoes, using a trained panel. A later study found that peeled potatoes, with less phenolic acids, tasted less bitter (Mondy and Gosselin, 1988). In comparison, Sinden et al. (1976) did not find any significant links between phenolic acid content and bitterness. However, even if phenolic acids to contribute to bitterness, bitterness may only be perceived if phenolic acid levels are above the perception threshold. For example, Work and Camire (1996) found that in reconstituted mashed potatoes, the threshold for chlorogenic acid was 82 ppm (82 mg kg^{-1}). Therefore, levels will need to be perceptible before the level of phenolic acid could influence overall taste.

More recent research points to several amino acids playing a major role in bitter potato taste (Davids et al., 2004; Sato et al., 2019). The amino acids phenylalanine, tyrosine, tryptophan, leucine, isoleucine, and valine are well-known to elicit a bitter taste in foods (Nishimura and Kato, 1988; Solms, 1969), Kirimura et al. (1969) also listing histidine, methionine, and

arginine as bitter amino acids. In a storage and protease trial measuring changes in amino acid concentration, Davids et al. (2004) observed the resulting potato juice (after protease digestion) tasted extremely bitter. This was thought to be linked to increased concentrations of leucine, isoleucine, phenylalanine, and lysine. In the recent Japanese study investigating *egumi*-taste (Sato et al., 2019), the cultivar with the second-highest *egumi*-taste score (cv. 'Snowden') contained the highest levels of bitter phenylalanine, tryptophan, histidine, and arginine compared to three other potato cultivars, and the highest overall amino acid content. However, in addition to a strong bitter and astringent taste, 'Snowden' also scored the highest for 'potato flavour' and 'taste', positive potato sensory attributes. The second strong *egumi*-tasting cultivar ('Kitahime') did not have significantly higher levels of amino acids (Sato et al., 2019). While the results from Davids et al. (2004) were only informal, and Sato et al. (2019) provides conflicting results, there is some evidence to suggest that amino acids should be considered, just as much as glycoalkaloids, when assessing bitterness in potatoes.

Sweetness

In contrast to the desirable presence of savoury taste, with a slight amount of bitterness, sweet taste is traditionally an unwanted trait of potatoes, especially for tubers stored and processed into chips and crisps (Muttucumaru et al., 2013). Sweet taste is mainly attributed to the presence of soluble sugars, including glucose, fructose, and sucrose (Magwaza and Opara, 2015). High levels of glucose and fructose can develop as starch is mobilised into sugars during in-ground or postharvest storage (Muttucumaru et al., 2013). These sugars can cause browning and blackening during baking or frying, which is extremely undesirable for chip processors (McKenzie and Corrigan, 2016; Muttucumaru et al., 2013). In Katundu et al. (2007), an increase in sugar content during traditional storage (mud floors in thatched houses) reduced preference rank scores in South Africa, compared to potatoes stored in traditional, ambient conditions. However, this reduction in preference appeared to be more closely-linked to corresponding changes in starch content (Katundu et al., 2007). Overall sweet flavour in potatoes could also be linked to certain volatile compounds, where positive correlations between sweetness and 2-butylfuran, 2-pentylfuran, 1-penten-3-one, and 2,4-heptadienal were identified in Morris et al. (2010). The amino acids glycine and alanine also impart sweet taste (Nishimura and Kato, 1988), which may also contribute to sweetness. It appears no study to date has directly investigated causes of sweetness in potato - only Vainionpää et al. (2000), using canonical correlation analysis on potatoes for a nitrogen and storage experiment, concluded that sweetness is "far from a direct consequence of sugar accumulation".

While potatoes are not traditionally regarded or desired as sweet-tasting, several positive correlations and apparent links between quality perception, likeability, and sweetness have recently been recorded for both processed and table potatoes (Arvanitoyannis et al.,

2008; Jansky, 2008). Strong positive correlations between sensory-evaluated 'sweetness' and 'deliciousness' have been published on steamed potatoes, suggesting sweetness could be a desirable attribute for table potatoes (Jitsuyama et al., 2009), up to a point. Additionally, results from Arvanitoyannis et al. (2008) show increased overall acceptability of potato samples with increased sweetness scores. Such results could be linked to the overall increase in sugar consumption in developed countries (McKenzie and Corrigan, 2016). In general, sugars are also important precursors in the production of volatile compounds (Whitfield and Last, 1991, in Comandini et al. (2011), therefore, higher levels of sugar could be important in aroma development (Oruna-Concha et al., 2001). As such, notions around potatoes and sugar may begin to move away from the traditional perception that sweetness is an unwanted taste within a potato flavour profile. It must also be noted that a preference for possibly sweeter BBQ chips (Maier et al., 2007) does not necessarily indicate a consumer preference shift to sweeter baked or boiled potatoes. In addition, sugar has recently been regarded as something to avoid because of its adverse health effects (Yang et al., 2014). Therefore, potatoes marketed with higher sugar levels or a sweeter taste are unlikely to increase product demand.

Sourness

While not a defining taste, sourness (acid taste) in potatoes is thought to be caused by the presence of organic acids. These compounds could include ascorbic acid, chlorogenic acid, citric acid, glutamic acid, malic acid, and quinic acid (Maga, 1994; Sinden et al., 1976; Zhang and Peterson, 2018). Although glutamate and aspartate are characteristic umami-tasting amino acids, in their dissociated state, they exhibit a sour taste (Nishimura and Kato, 1988). Heightened levels of phenolic acids, which include chlorogenic acid and quinic acid, may also increase the perception of astringency (Mondy et al., 1971). Sinden et al. (1976) found that the addition of 120 mg 100 g^{-1} chlorogenic acid to cooked potato tissue resulted in some panellists reporting a slight sourness. The limited recent literature available on sourness in potatoes is related to crisp flavour. Zhang and Peterson (2018) found with increasing frying time, sourness in potato crisps decreased, this correlated and likely caused by a 35% degradation and reduction in chlorogenic acid concentration. When testing different crisp pre-treatments, Mestdagh et al. (2008) found that pre-treating potato crisps with 0.025 mol L^{-1} citric acid significantly increased sourness, in addition to significantly reducing 'taste acceptability' and 'overall acceptability', as evaluated by a panel. Although the pre-treatment citric acid concentration is difficult to link to citric acid potato tissue concentration, these results indicate that both chlorogenic acid and citric acid have been found to contribute to sourness in potatoes, and that sourness may not be a positive taste attribute.

2.3.5 Varietal flavour variation: *S. tuberosum* and *S. phureja*

In addition to the growing environment, storage conditions, and cooking method, the flavour of a potato is strongly influenced by the potato's genetic makeup and varietal origins (Jansky, 2010). With increasing consumer awareness of flavour, and its role in influencing purchasing decisions, flavour is becoming a focus for potato breeders. Because of this, aspects of flavour research have been directed into analysing and investigating the metabolic and flavour profile of novel varieties, especially those from different different genetic groups of *S. tuberosum*, such as *S. phureja* (Bártová et al., 2015; Dobson et al., 2004, 2008, 2010; Ducreux et al., 2008; Morris et al., 2007). Additionally, the unique flesh and skin colour (e.g. yellow and purple) of tubers from other potato genetic groups make them an attractive choice for specialist food products. As this study involves the flavour analysis of a potato variety with *S. phureja* parentage (ex. Phureja), the metabolic and sensory differences in flavour between *S. tuberosum* (Tuberosum) and *S. phureja* (Phureja) potatoes will be briefly covered.

Perceived flavour

One of the biggest attractions of commercial Phureja varieties is their potentially superior flavour and acceptability over standard Tuberosum varieties, based on existing flavour research. Morris et al. (2007) and Morris et al. (2010) investigated the variation in several flavour attributes across steamed Phureja and Tuberosum varieties, using a trained panel. In Morris et al. (2010), the evaluation was part of a wider study on storage. In both studies, the Phureja varieties had a stronger savoury flavour and a more intense flavour overall. In most cases, the Phureja varieties also had more 'flavour creaminess' and greater overall acceptability compared to Tuberosum varieties (Morris et al., 2007, 2010). In a CATA consumer study, the Phureja variety Mayan Gold was associated with sweet and savoury flavours (V. Corrigan, personal communication, February 17, 2020). Therefore, the limited literature available indicates Phureja varieties may have a more desirable taste over Tuberosum varieties, given the combination of increased intensity, sweetness, and savouriness with increased overall acceptability (Morris et al., 2007).

Flavour-related composition

Given Phureja varieties have a slightly different genetic makeup to Tuberosum varieties (see Section 2.2.3), the presence and concentration of flavour metabolites will be different, contributing to the different flavour attributes as described above. To determine the influence of umami compounds on flavour, Morris et al. (2007) calculated 'equivalent umami concentration' (EUC) for Phureja and Tuberosum varieties, EUC an equation that takes into account the relative contributions of glutamic acid, aspartic acid, and 5'-ribonucleotides, as major contributors to umami flavour. Phureja samples contained significantly higher

concentrations of glutamate, 5'GMP, and 5'-AMP in steamed samples at harvest maturity, compared to Tuberosum samples. These significant differences were linked to the sensory evaluation results, determining that potato EUC was strongly correlated with savouriness, flavour intensity, and overall acceptability (Morris et al., 2007).

Phureja cultivars may also contain higher levels of fatty acids. Dobson et al. (2004) found that Phureja samples contained consistently higher levels of fatty acids (e.g. linolenic acid), on average 37% higher than Tuberosum samples. Fatty acid content has tentative links to the production of off-flavour aldehydes in stored cooked potatoes (Petersen et al., 1999). Therefore, Phureja samples could be more likely to develop cardboard-like off flavours after cooking, although this remains to be investigated. According to Ramsay et al. (2005), total glycoalkaloid content between Phureja and Tuberosum samples (which could impart bitter flavours) remained similar as their genetic makeup is not too dissimilar. In comparison, wild types (e.g. *S. canasense*) contained over 10 times the glycoalkaloid content of Phureja and Tuberosum samples. Finally, Ducreux et al. (2008) examined the tuber gene expression between Tuberosum and Phureja cultivars to investigate any genes and corresponding metabolites that may be important in flavour. Differences in gene expression were related to cell-wall synthesis (texture), potential 5'-ribonucleotide formation (umami compounds) and α-copaene, a volatile compound. The role of α-copaene was further investigated using transgenics (Morris et al., 2011). However, it was found to have no effect on the rest of the volatile profile or sensory perceived flavour. More thorough comparative metabolic profiling between *S. tuberosum* and other subspecies like *S. phureja* is required to better understand how breeding with novel germplasm will influence the final flavour profile, thus helping to create flavourful varieties that increase consumer product acceptance.

2.4 How does nitrogen fertiliser affect flavour?

Supplying a potato crop with a sufficient supply of nutrients is essential to achieve economical yields. One of the three key macronutrients (NPK) to consider when growing potatoes is nitrogen fertiliser. For table potatoes, the rate of nitrogen applied to crops is approximately 120 - 150 kg N ha^{-1} (personal communication, B. Hart, February 8, 2020). Nitrogen is principally applied to increase vegetative production in the crop haulm (tops), increasing plants' photosynthetic capability and yield potential (Belanger et al., 2002; Kumar et al., 2007). The benefits of fertiliser are especially evident when growing on low fertility soils (Davenport et al., 2005). However, excessive nitrogen application can achieve the opposite – high rates applied during tuber bulking could reduce tuber yield (Rens et al., 2015). High rates of soluble nitrogen application are also an environmental concern as leftover nutrient, not taken up by plants, is left to leach from the soil profile, contaminating groundwater and surrounding waterways (Davenport et al., 2005). Therefore, careful management of nitrogen inputs is an essential aspect of commercial potato production.

However, despite the key role and importance of nitrogen fertiliser in potato production, almost no research has directly investigated the impact of varying rates of nitrogen fertiliser on potato flavour. This can be partly attributed to a general lack of attention on flavour overall - potato breeding efforts have mainly focused on improving yield and disease-resistance, flavour only a minor concern (Morris and Taylor, 2019). Existing research has addressed other effects of nitrogen fertiliser on quality, including dry matter content, nitrogen and nitrate content, protein content, and crisping quality (Belanger et al., 2002; Kumar et al., 2007; Lin et al., 2004; Muttucumaru et al., 2013). Comparing the effect of organic and conventional growing on flavour, often with different overall levels of nitrogen input, have also been investigated (Gilsenan et al., 2010; Lombardo et al., 2012; Maggio et al., 2008). To date, no research has assessed both sensory and compositional changes with varying rates of nitrogen fertiliser. However, with flavour becoming a major factor in consumer purchasing decisions and a focus for growers of high-value potatoes (Morris and Taylor, 2019), understanding the potential impact of varying rates of nitrogen fertiliser on flavour is critical. In addition, with increasing societal pressure around the environmental impact of food production, an understanding on how the flavour of a high-value potato product may change, if fertiliser inputs change, is important to understand. Therefore, this section will collate and review any research conducted around the impact of fertilisers, on aspects relating to potato flavour, to build a better understanding of what a study directly focusing on fertiliser and potato flavour might reveal.

2.4.1 Potatoes

Effect on potato off-flavour

Increasing rates of nitrogen fertiliser may negatively impact flavour, according to a small body of mostly German research from 30-40 years ago (Fischer, 1991; Hunnius et al., 1978; Mondy and Koch, 1978; Nitsch and Klein, 1983). Early work, focusing on the effect of changing quantities of nitrogen fertiliser on compounds like starch and vitamin C, suggested high nitrogen application on potatoes would almost certainly lead to bad tasting potatoes (Hunnius et al., 1978; Nitsch and Klein, 1983). However, the point at which this bad taste occurs is ill-defined, Hunnius et al. (1978) suggesting taste will remain unaffected up 130 kg N ha^{-1} applied fertiliser. Furthermore, the soil's fertility and potentially available nitrogen was not discussed, this also playing a role in the total nitrogen available to the potato crop.

Later work from Fischer (1991) investigated the impact of changing nitrogen fertiliser quantities on the concentration of potato volatile compounds. Fischer (1991) conducted a pot experiment using quartz and clay, nitrogen additions ranging from 0.75 g to 4 g N per pot. Concentrations of pentanol, hexanol, heptanal, (*E*)-2-hexenal, (*E,E*)-2,4-decadienal, and (*E,Z*)-2,4-decadienal significantly increased with increasing nitrogen input. Increased concentrations of aldehydes like 2,4-decadienal and (*E*)-2-hexenal may increase fatty and

green odours, negatively impacting potato taste and flavour, as suggested by Hunnius et al. (1978) and Nitsch and Klein (1983). Furthermore, unsaturated fatty acids (e.g. linoleic acid), the precursor molecules to these aldehydes, have previously been found to also increase with increasing nitrogen input (Mondy and Koch, 1978). Therefore, through the potential increase in unsaturated fatty acids and resulting off-flavour aldehydes, increasing nitrogen input may negatively impact flavour. However, the level of nitrogen fertiliser required to achieve this is difficult to establish given a lack of conversion from pot to standard units. Additionally, sensory evaluations were not carried out to support these results and claims of bad taste - further research is required.

Increases in tuber nitrate and total nitrogen

In addition to potential increases in off-flavour aldehyde concentrations, an increase in nitrates and total tuber nitrogen content, caused by high nitrogen fertilisation, may also negatively impact flavour. It is well-established that there is a strong positive correlation between the rate of nitrogen applied, total tuber nitrogen (N) content, and nitrate content (NO_3^-) (Belanger et al., 2002; Carter and Bosma, 1974; Lin et al., 2004). Furthermore, Thybo et al. (2006), when carrying out a sensory and chemical investigation on wound healing and storage on pre-peeled potatoes, identified highly significant negative correlations between total N and nitrate with the sensory term 'potato flavour' (assumed to refer to typical potato flavour). Highly significant ($p < 0.001$) positive correlations were also identified between both total N and nitrate with potato 'off-flavour'. In support of this, Cieslik (1997) suggested high nitrogen decreases sensory quality, with the production of bitter amides and organic acids, such as chlorogenic acid. Therefore, while no investigation linking nitrogen fertiliser, nitrate, and total-N content with flavour can be found, the literature suggests that high nitrate and total N content, as a result of high nitrogen fertilisation, may decrease desirable potato flavours and increase off-flavour production. Whether or not the production of nitrates and total N is linked to the production of off-flavours, produced by the aldehydes discussed above, is unknown.

Impact on glycoalkaloid content

Changing tuber glycoalkaloid content with the addition of nitrogen fertiliser also has the potential to influence potato flavour. According to Love et al. (1994), across three potato varieties, increasing the nitrogen application from 0 to 168 and 336 kg N ha^{-1} significantly increased α-solanine content from 37 mg kg^{-1} FW to 45 and 46 mg kg^{-1} FW. Total glycoalkaloid content (TGA) is known to be strongly positively and significantly correlated with bitterness (Sinden et al., 1976), more recent research also finding strong negative correlations between α-solanine and α-chaconine content with potato flavour intensity, savouriness (umami), and flavour creaminess (Morris et al., 2010). However, at similar α-solanine

levels as measured by Love et al. (1994), Sinden et al. (1976) found less than 20% (of 9 panellists) actually detected bitterness. Instead, glycoalkaloid content needed to measure over 140 mg kg^{-1} to generated perceptible bitter responses in participants (Sinden et al., 1976). In support of this, a small New Zealand study on Maori potatoes using 21 panellists found no relationships between TGA content, sample preference, and bitterness, the TGA content for all cultivars measuring less than 105 mg kg^{-1} (Savage et al., 2000). Therefore, any potential bitterness flavour response to nitrogen may only be observed in varieties with existing high levels of total glycoalkaloids (e.g. over 140 mg kg^{-1}), in addition to conducive environmental conditions, which have a stronger influence on TGA content compared to nitrogen fertiliser (Love et al., 1994).

Potential increases in umami amino acids

Alternatively, increasing the rate of nitrogen fertiliser applied to potato crops may instead positively influence potato flavour by increasing the perception of savoury flavour and overall flavour intensity in potatoes. Total free amino acid content, including the umami amino acids glutamate and aspartate (Morris et al., 2007), has been found to increase with nitrogen fertilisation, in a pot experiment conducted by Eppendorfer (1996) across a range of potato varieties. Morris et al.(2007) found that heightened concentrations of umami amino acids glutamate and aspartate in *S. phureja* contributed to increased potato flavour intensity and overall acceptability, supporting much earlier but similar conclusions drawn by Solms and Wyler (1979). By linking these studies, there is a suggestion that increasing nitrogen fertiliser content, and thus the concentration of umami amino acids, may play a central role in enhancing desired potato flavour.

2.4.2 Other horticultural crops

Investigating the effect of nitrogen on the flavour of other vegetables or *Solanum* species may provide a better understanding of how the flavour and composition of potatoes may change with increasing rates of nitrogen fertiliser application. Although different crops will have different nitrogen requirements and produce different metabolites, a common effect could be identified.

In carrots (*Daucus carota* L.), nitrogen does not appear to affect flavour perception. While Hogstad et al. (1997) found high (100 - 196 kg N ha^{-1}) nitrogen applications significantly decreased total sugar content (1990) and total soluble solids (1989), in two different trial years, compared to low (0, 40 - 80 kg N ha^{-1}) nitrogen applications, the only accompanying effects on flavour was a significant decrease in 'total flavour strength' in 1990, as part of a sensory assessment, where attributes fruity, sweet-taste, bitter taste, and off-flavour were also measured. Seljåsen et al. (2012) found increasing nitrogen fertilisation, from 0 to 180 kg N ha^{-1}, significantly decreased dry matter % and increased root nitrate content.

However, these compositional changes did not affect flavour perception, where no significant differences between nitrogen treatments were recorded for carrot odour, taste intensity, earthy flavour, and the tastes sweet, bitter, and acidic, using a 12-membered trained panel.

Perhaps higher applications of nitrogen were needed to achieve differences in flavour perception - additions of 400 kg N ha^{-1} (400 N) to nitrogen-hungry globe artichoke crops (*Cynara cardunculus* var. *scolymus* (L.) Fiori) significantly reduced flavour intensity (of 'artichoke' and 'herbaceous' flavours) and increased off-flavour intensity, compared to 0 kg N ha^{-1} (0 N) treatments (Lombardo et al., 2017), these results recorded in a combined nitrogen and storage trial using a semi-trained panel of 11. With these changes in perception, sugar content also varied, with fructose significantly decreasing from 14.0 g kg^{-1} DM (0 N) to 5.9 g kg^{-1} DM (400 N). Somewhat shockingly, 400 kg N ha^{-1} is not reflective of the upper nitrogen rate used for artichokes - in the past, growers have been known to apply up to 700 kg N ha^{-1} in Italy (Magnifico, 1987, in Lombardo et al. (2017)).

Focusing in on the effects of nitrates specifically, Heeb et al. (2005) identified flavour differences in tomato fruit (*Lycopersicum esculentum* Mill cv. 'Armada') when varying the ratio of nitrate (NO_3^-) and ammonium (NH_4^+) in the nutrient solution fed to boxed plants. Using a 4:1 ratio of NO_3^-:NH_4^+ ions (to supply 650 mg N $week^{-1}$) significantly decreased fruit sweetness and acidity, compared to a 1:4 (NO_3^-:NH_4^+) ratio. Overall acceptance and 'flavour duration' scores also decreased when using a higher ratio of NO_3^- (Heeb et al., 2005). While these results do not address the effect of increasing overall nitrogen on flavour, the study offers some insight into the influence of increased nitrate content on flavour. And in this case, a higher nitrate ratio significantly reduced the overall flavour intensity and acceptance of tomatoes when nitrogen was not limiting.

Therefore, from the limited literature available on the effect of varying nitrogen fertiliser rates on flavour, it appears that at varying levels, higher rates of nitrogen (i.e. 400 kg N ha^{-1}) and/or nitrate may reduce overall flavour intensity and potentially introduce off-flavours in artichokes and tomatoes. In carrots, applications ranging from 0 to 200 kg N ha^{-1} appear to have no consistent effect on flavour, the year and variety more important factors affecting quality (Seljåsen et al., 2012).

2.5 Sensometabolomics: Flavour and composition analysis

Understanding sensory-perceived potato flavour, in combination with the implications of potato composition on flavour, is important in growing and selling a gourmet potato product or variety. Also understanding whether any changes in flavour affect potato preference or liking is valuable information for understanding how treatments could affect a product's performance or reception. Previous literature clearly indicates that changing levels of nitrogen fertiliser can affect both flavour-related composition and perceived flavour. Therefore, using a combined approach of descriptive sensory testing and composition analysis of flavour contributors may be an effective way to capture flavour changes with changing levels of nitrogen fertiliser. Many different tests and analyses can be used to achieve this in potatoes. This section will cover composition and sensory analyses options separately, then discuss the benefits of a 'sensometabolomic' approach, and how this has been previously applied to potatoes.

2.5.1 Flavour compound analysis

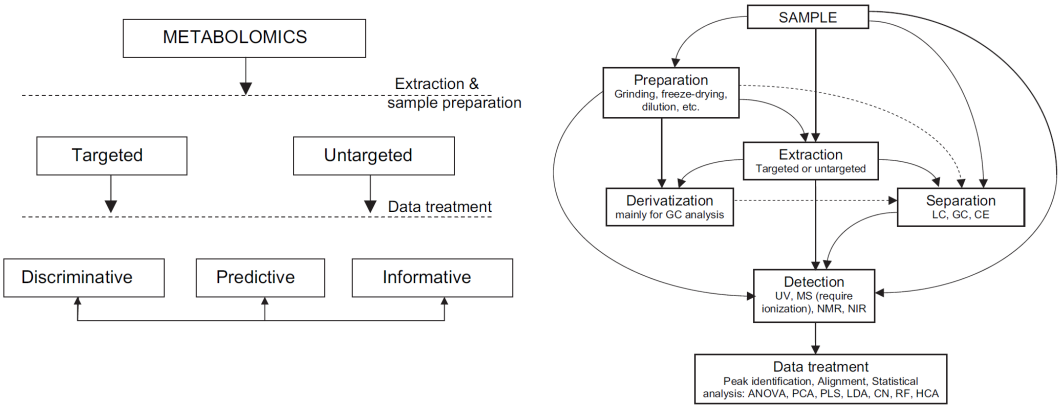
Overview

Metabolomics is the measurement and study of compounds (metabolites) in an organism. Many metabolites within an organism's metabolic profile may relate to, and affect flavour. Flavour compound analysis, using metabolomics, is a valuable tool to describe and understand the many flavour compounds within a product. In recent times, metabolomics has become increasingly popular as a tool for food quality, processing, and assessing safety (Cevallos-Cevallos et al., 2009). Monitoring the changes that may occur to compounds in a metabolite profile can help to improve understanding on how treatments, such as changing levels of nitrogen fertiliser applied to potatoes, may affect the final product. When paired with sensory analysis (sensometabolomics), the treatment effects on a metabolite profile can be tested to determine whether the changes are significant enough to affect flavour perception or preference. Additionally, flavour compound and metabolite analysis can help to elucidate the cause behind changes in flavour perception.

Using metabolomics to characterise the flavour compounds within potatoes, especially boiled and baked potatoes, has been a popular area of research. The earliest metabolite analyses focused on describing the metabolome of cooked potatoes and identifying the many volatile aroma compounds in potatoes (Buttery et al., 1973; Coleman et al., 1981; Nursten and Sheen, 1974). Other research aimed to characterise certain aromas, such as earthiness (Buttery and Ling, 1973) or musty-flavoured potatoes (Mazza and Pietrzak, 1990). Research also focused on taste attributes, such as umami taste (Solms and Wyler, 1979) and bitterness relating to glycoalkaloids and phenolic acids (Mondy and Gosselin, 1988; Sinden et al., 1976). For flavour research pre-2000, Maga (1994) provides a comprehensive

summary. At the turn of the century, a UK research group released several papers comparing the volatile aroma profile between potato varieties, amongst different cooking methods (Duckham et al., 2001; Oruna-Concha et al., 2001, 2002b,a). These papers are some of the most comprehensive references for flavour aroma compounds. Research on volatile flavour metabolites since then has focused on using metabolomics to describe differences between potato genetic groups (e.g. *S. tuberosum* and *S. phureja*) (Dobson et al., 2010) and investigating off-flavour development in stored boiled potatoes (Blanda et al., 2010; Petersen et al., 1999). For non-volatile metabolites, the effect of cultivar and storing boiled potatoes on the concentration of amino acids and organic acids has also been an area of research (Thybo et al., 2006), in addition to investigating the differences in flavour compounds between organic and conventionally-grown potatoes (Shepherd et al., 2014). Measuring changes in umami amino acid concentration, in relation to genetic differences and crisp frying time, has also been researched by Morris et al. (2007) and Zhang and Peterson (2018) respectively.

The process of flavour compound analysis, undertaken by all of the aforementioned studies, involves many steps. Fig. 2.3 helps to summarise the key planning decisions and key analysis steps. To begin, the analysis direction must be defined - targeted, or untargeted (Cevallos-Cevallos et al., 2009). Targeted analysis focuses on a specific group of flavour-related compounds (e.g. sugars), assessing any changes that occur between treatments. In untargeted analysis, as many compounds as possible are identified before looking for patterns or changes across groups of compounds between treatments, not necessarily involving individual compound identification (Cevallos-Cevallos et al., 2009). Additionally, when looking for changes between treatments, the analysis can be classed as discriminative, common in potato metabolite testing (Fig. 2.3). Other classifications include informative and predictive testing. Informative testing aims to collect sample-specific details for the purpose of updating metabolite databases (for example), while predictive testing is used to analyse samples to 'predict' a metabolite (or variable) that cannot be identified or quantified using standard techniques (Cevallos-Cevallos et al., 2009). The purpose of the analysis drives the direction and steps taken in the analysis process, which will include a selection of sample collection, preparation, extraction, derivatisation, separation, detection, data processing, and statistical analysis (Fig. 2.3). Using previous research conducted on potatoes, the methods available for each analysis step, when analysing key groups of volatile and non-volatile potato flavour compounds, will be discussed.



(a) Classification of metabolomics.

(b) Process of metabolomic analysis.

Figure 2.3: Classifying a metabolomic analysis process (a) and the key steps in the process of metabolomics analysis (b). Used with permission from Cevallos-Cevallos et al. (2009).

Aroma volatile analysis

Aroma volatiles significantly contribute to a food's final perceived flavour (Whitfield, 1992, in Dresow and Böhm (2009)). As discussed in Section 2.3, many volatile compounds contribute to potato aroma and flavour, such as methional, a key 'boiled potato'-smelling compound. Identifying and measuring the relative concentration of the aroma compounds present in potato samples is an important part of flavour compound analysis. Additionally, the influence of treatments, such as changing the quantity of nitrogen fertiliser applied, could be quickly determined by comparing volatile profiles between different treatments.

Sample preparation

Conducting analysis of volatile flavour compounds first begins with effective sample preparation (Kim and Verpoorte, 2010). A range of preparation methods has been previously used for potato aroma analysis. To compare the baked potato volatile profiles between different cultivars, Oruna-Concha et al. (2001) and Duckham et al. (2001) separated the cooked skin and flesh, before simply dicing and placing the samples directly into a flask for extraction. Shepherd et al. (2007) used a similar method, mashing the samples after cooking before placing in a sealed flask ready for extraction. Both of these studies continued onto extraction and separation immediately after cooking - in many cases, this may be difficult to achieve in a busy lab or if coupled with sensory evaluation. Alternatively, Mutti and Grosch (1999) froze chopped boiled potatoes in liquid nitrogen, added anhydrous sodium sulfate, then homogenised samples using a blender. Freezing samples after cooking reduces the urgency to commence the rest of the analysis immediately. Blanda et al. (2010) used a similar method, first homogenising slices of boiled potato with sodium chloride solution before

extraction, as part of an investigation on off-flavour development. The addition of a drying agent, such as anhydrous sodium sulfate, can be beneficial because varying levels of moisture in the sample can affect metabolite identification and quantification (Cevallos-Cevallos et al., 2009). Moisture also allows any enzyme-mediated reactions to continue occurring, potentially leading to metabolite decomposition before extraction (Kim and Verpoorte, 2010). Proper grinding and homogenising may also help to enhance volatile release during extraction (Cevallos-Cevallos et al., 2009). Additionally, if the metabolite data will be analysed and linked to sensory data, the samples must come from, or be subjected to, the exactly same cooking conditions as was used for the sensory analysis. This ensures the flavour data accurately reflects sample composition at the time of sensory analysis.

Extraction

After sample preparation, the next phase for aroma compound analysis is extraction. The extraction phase is the most important or critical phase of any metabolomics analysis process because the procedure is used to maximise the concentration and amount of flavour compounds being targeted (Cevallos-Cevallos et al., 2009). As no solvent can dissolve all metabolites, choosing the correct solvent is very important, depending on whether a key group of compounds, or the total metabolome, needs to be investigated (Kim and Verpoorte, 2010). Most early potato metabolomic analyses used liquid solvent-based extraction techniques for further volatile analysis. These were conducted using a vacuum steam distillation continuous extraction (SDE) apparatus, where potatoes are placed in distilled water and heated over an extended period (e.g. 3 hours), using alkanes such as hexane as the extraction solvent (Buttery and Ling, 1973; Buttery et al., 1973; Nursten and Sheen, 1974). More recent studies by Mutti and Grosch (1999) and Blanch et al. (2009) utilised a similar SDE technique, both using dichloromethane as a solvent and extracting for 8 and 4 hours, respectively. However, SDE can be a less-reliable extraction technique because long extraction periods may increase the chance of side-reactions occurring (Kim and Verpoorte, 2010).

To avoid the shortcomings that accompany steam distillation extraction, several other extraction methods have become more popular to use for potato volatile analysis. These include dynamic headspace (DH) extraction (purge and trap), and more recently, headspace solid-phase micro-extraction (SPME) (Blanda et al., 2010; Bough et al., 2020; Duckham et al., 2001; Mazza and Pietrzak, 1990; Morris et al., 2011; Oruna-Concha et al., 2001; van Loon et al., 2005). In headspace (HS) extraction, the samples are first placed into a vial with free volume above the sample (the headspace). After this, the sample is incubated and stirred (agitated) for a period, encouraging highly volatile metabolites to escape the solid (or liquid) phase of the sample and enter the vial HS. DH extraction is carried out by 'purging' or flushing the vial HS with an inert gas (e.g. N_2), carrying the volatile compounds out to adsorb

onto a 'trap', extracting and concentrating volatiles (Blanda et al., 2010). Tenax TA (a polymer resin) is commonly used for the trap. Alternatively, SPME can be used for extraction. After heating and agitation, a needle-like fused silica fibre, coated with an adsorbent material (e.g. polydimethyl-siloxane), is injected through the vial septum into the sample HS (Elmore et al., 1997). Volatiles are extracted from the sample through their adsorption onto the fibre coating - this process is based on the establishment of an equilibrium between the sample, HS, and fibre coating (Povolo and Contarini, 2003). Both DH and SPME extraction techniques have been used for aroma volatile extraction in potatoes. There is a large body of research dedicated to comparing DH and SPME across a range of food products, including tomatoes, dairy products, and cola (Beltran et al., 2006; Contarini and Povolo, 2002; Elmore et al., 1997; Povolo and Contarini, 2003). In general, both methods appear to produce comparable results. While DH may yield a higher quantity of volatiles, SPME is faster and cheaper to run (Contarini and Povolo, 2002; Povolo and Contarini, 2003). Both methods can achieve effective compound discrimination between treatments or different samples. A direct method comparison is yet to be carried out on cooked potatoes.

Separation and detection

Once extraction is complete, the volatiles must be separated out and detected, these two stages instrumentally coupled. For potato volatile aroma compounds, gas-chromatography coupled with mass spectrometry (GC-MS) is the most popular method used. All chromatography methods separate compounds in a sample based on variation in their affinity to the mobile phase and stationary phase (GMU, 1998). In GC, the sample is carried in the mobile phase (an inert carrier gas) through a coiled column coated on the interior with a highly polar substance (the stationary phase). Variation in chemical properties and affinity to the stationary phase will determine how quickly or slowly compounds exit the column. The amount of time between the injection of the sample into the column and a compound's exit is referred to as the retention time (RT) (Clark, 2016a). As GC is the accepted technology for volatile separation, it has been used for nearly all volatile analyses in potato flavour research, including profiling key volatile compounds (Mutti and Grosch, 1999; Shepherd et al., 2007), characterising the differences in volatile compounds between different cultivars (Duckham et al., 2001; Oruna-Concha et al., 2001, 2002a), and investigations on potato off-flavour (Blanda et al., 2010; Petersen et al., 1999).

Detection is carried out after separation by GC. As discussed, GC coupled with mass spectrometry (GC-MS) is the most popular separation and detection method for analysing volatile compounds. Once a compound exits the GC column, it enters the coupled mass-spectrometer. The MS ionises the compound with a stream of electrons, breaking it apart into charged ion fragments (GMU, 1998). Fragments are detected using their mass to charge ratio, producing a mass spectrum, which is used in combination with the GC-output to

identify and quantify each compound. Alternatively, GC can be coupled with a sniffing port, referred to as gas-chromatography olfactometry (GC-O). Assessors sniff and describe the odour, intensity, and duration of the compounds as they elute from the GC coil (Delahunty et al., 2006). GC-O is a valuable detection method because it can be used to determine the odour activity of compounds, which includes a compound's odour threshold, intensity, and quality (Delahunty et al., 2006). GC-O has been used a number of times for potato volatile analyses, in Mutti and Grosch (1999), Petersen et al. (1999), Shepherd et al. (2007), Ulrich et al. (2000), and van Loon et al. (2005) (for French fries). To note, flame ionisation detectors (FID) can also be coupled with GC-O to aid in compound identification (Ulrich et al., 2000). The final step, after separation and detection, is compound identification - this can be achieved by comparing the data with existing compound databases or with authentic standards run at the same.

Non-volatile compounds

The analysis of non-volatile flavour compounds in potatoes includes the important compounds contributing to taste, as discussed in Section 2.3. Examples include glycoalkaloids, phenolic acids, umami amino acids, and carbohydrates. Analysing the taste compounds, in addition to the aroma volatiles, is essential in understanding the entire flavour profile - umami taste compounds have been suggested as major flavour contributors by both Solms and Wyler (1979) and Morris et al. (2007). Similar to the standard use of GC-MS for volatile compound analysis, high performance liquid chromatography (HPLC), coupled with MS, is commonly used for the analysis of non-volatile compounds. The standard and recommended techniques for each step of non-volatile flavour analysis in potatoes will be discussed below.

Sample preparation

For the analysis of non-volatile flavour compounds in potatoes, careful sample preparation is important, given the same prepared sample could be used for a wide range of different tests. In general, one sample preparation can be used for all non-volatile metabolite testing. After cooking, early researchers investigating glycoalkaloid content did not prepare the samples any further - samples were immediately blended with a solvent to begin extraction (Mondy and Gosselin, 1988; Zitnak and Johnston, 1970). However, as discussed earlier, drying is essential for accurate metabolomics analysis (Kim and Verpoorte, 2010). If the samples are not dried, enzyme-mediated reactions will continue, leading to the potential degradation of the metabolites targeted. Additionally, if any ^{1}H-NMR spectroscopy is used, water molecules will affect the resulting spectra resolution (Kim and Verpoorte, 2010). Therefore,

the recommended preparation technique for plant material is to first freeze samples in liquid nitrogen (avoiding any further metabolite changes), following by freeze-drying to remove all water present (Kim and Verpoorte, 2010). Freeze-drying also helps to concentrate the sample (Cevallos-Cevallos et al., 2009). All recent potato metabolite analyses, investigating a range of compounds, have used this preparation method. Morris et al. (2007), Morris et al. (2011), Shepherd et al. (2007), and Thybo et al. (2006) froze all samples immediately after cooking in liquid nitrogen, followed by freeze-drying and homogenising to obtain a dry powder. Shakya and Navarre (2006) and Maggio et al. (2008) first ground the frozen potato samples before freeze-drying. Grinding is also an essential sample preparation step as it increases the available sample surface area, improving the extraction efficiency (Kim and Verpoorte, 2010). Many studies also sieve the final ground sample (Morris et al., 2011; Shepherd et al., 2007).

Extraction

While only one sample preparation process is required for nearly all metabolite testing (excluding volatiles), options for non-volatile liquid extractions are more complex and time-consuming. Given that no solvent will dissolve all compounds, it is likely several different solvents are required to extract all target metabolites (Kim and Verpoorte, 2010). In addition, the ratio of sample to solvent is important to perfect, given compounds with limited solubility may require more solvent to dissolve in (Kim and Verpoorte, 2010). For untargeted metabolomics, where many compounds may be unknown, a range of solvents and methods should first be tested and compared (Cevallos-Cevallos et al., 2009). However, as many metabolite analyses have been previously conducted on potatoes, existing literature can help to determine the best solvent and extraction process to apply, depending on the target group of compounds.

A wide range of solvents and extraction conditions have been used for the extraction of non-volatile flavour compounds, such as amino acids, carbohydrates, glycoalkaloids, phenolic acids, and fatty acids. The Scottish potato research group (Morris et al., 2007, 2010, 2011) used a methanol/water/acetic acid solvent [49:49:2 v/v/v] for the extraction of umami amino acids, while Maggio et al. (2008) used ethanol/water [40:60 v/v]. Sato et al. (2019) also used ethanol and water [60:40 v/v] for the extraction of amino acids in a study on *egumi* flavour in potatoes. However, Sato et al. (2019) prepared a second extraction for glycoalkaloid analysis, using methanol instead of ethanol as the solvent. In Morris et al. (2010), a second extract using ethanol/water [80:20 v/v] was prepared for HPLC carbohydrate analysis. For the analysis of glycoalkaloids, ascorbic acid, and phenolics, Shakya and Navarre (2006) performed one extraction with using 50% methanol, 2.5% metaphosphoric acid, and 1 mM EDTA. Dobson et al. (2004) used chloroform (a hydrophobic solvent) as part of a solvent to extract lipid material (i.e. fatty acids) from the potato samples. After the addition of

a solvent, the sample is generally homogenised, rested, potentially chilled, then centrifuged and filtered, some extractions also requiring evaporation or heating to remove any residual solvent after extraction is complete. The key outcome of this step is to ensure the method and solvent used maximises the extraction of the targeted compound groups.

Derivatisation

When GC-MS is used for the separation and detection of non-volatile taste compounds, derivatisation is required. Chemical derivatisation increases the volatility and thermal stability of the samples (Kim and Verpoorte, 2010). Any active hydrogen atoms on polar groups are replaced with trimethylsilyl groups, which reduces dipole-dipole interactions, allowing the sample to become more volatile (Cevallos-Cevallos et al., 2009; Kim and Verpoorte, 2010). Derivatisation is normally achieved by first adding an *O*-alkylhydroxylamine, creating an oxime, followed by trimethylsilylation. Derivatisation (and following GC-MS analysis) can be used for amino acids, organic acids, fatty acids, sugars, and sugar alcohols (Kim and Verpoorte, 2010). Phenolics and other secondary metabolites are too unstable when derivatised to allow measurement using GC-MS.

Dobson et al. (2010) used derivatisation to volatilise potato samples analysed by GC-MS for fatty acid content, comparing the different genetic groups within *S. tuberosum* (e.g. Phureja, Andigena). Methoxylamine hydrochloride in anhydrous pyridine, followed by silyation with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), was used to volatilise the polar sample extract. The same method was used by Shepherd et al. (2007) as part of a study profiling metabolite flavour compounds in potatoes. Beckmann et al. (2007) also used the same derivatising agents for GC-MS analysis as part of a potato metabolome fingerprinting study.

The most important part of derivatisation is ensuring the sample remains dry (Kim and Verpoorte, 2010). The presence of water will affect the stability of the trimethylsilyl-derivatised samples, as well as potentially disrupting the instruments. Therefore, all samples must be evaporated to dryness before derivatisation (Dobson et al., 2010), with analysis soon after to avoid any moisture absorption from the surroundings.

Separation and detection

Unlike volatile flavour analysis, where GC-MS is the most common separation and detection method used, a number of options are available to separate and detect non-volatile flavour compounds. Separation is most often achieved for potato metabolites using liquid chromatography (LC), including HPLC and ultra performance liquid chromatography (UPLC) (Cevallos-Cevallos et al., 2009). As with GC, separation is achieved by the difference in compounds' attraction to the stationary phase within the column, and the solvent. Retention times will vary with column pressure, the nature of the stationary phase, and the column

temperature (Clark, 2016b). HPLC is commonly used to analyse sugars, amino acids, glycoalkaloids, and phenolic acids in potatoes. Maggio et al. (2008), Morris et al. (2007), Sato et al. (2019), and Zhang and Peterson (2018) all used HPLC to separate samples for amino acid analysis. Morris et al. (2010) used HPLC to assess the sugars present in the potato samples. Alternatively, if derivatised, separation can be achieved using GC, as carried out by Beckmann et al. (2007), Dobson et al. (2010), and Shepherd et al. (2007). Capillary electrophoresis (CE) is another separation technique that has been previously applied to potatoes, however mostly in the context of pesticide presence Chicharro et al. (2008).

A wide range of detection methods are available to couple with separation for non-volatile compounds. LC-MS, LC-UV, HPLC-DAD (diode array detectors), and GC-MS separation and detection couplings are popular for analysing non-volatile potato flavour compounds. LC, coupled with MS, works in the same way as GC-MS coupling - once a compound exits the column, it enters into the mass spectrometer, which breaks the compound into charged ion fragment for detection (Clark, 2016b). In general, MS coupling is helpful when wanting to identify a large number of peaks (Cevallos-Cevallos et al., 2009; Shakya and Navarre, 2006). UV detectors work by detecting the amount of light absorbed by the sample, which can used to determine the quantity of the compound present (Clark, 2016b). Lombardo et al. (2012) used UV-vis spectroscopy to determine the difference in phenolic content between organic and conventionally-grown 'early' potato cultivars. Diode array detectors (DAD) are also commonly coupled with HPLC for the identification of glycoalkaloids (Amer et al., 2014; Morris et al., 2010), or other secondary metabolites such as carotenoids (Andre et al., 2007). For derivatised non-volatile samples, GC-MS is most commonly used for separation and detection.

Using coupled LC technologies (e.g. LC-MS) can potentially identify the biggest proportion of a metabolic profile compared to GC-MS (Wishart, 2008), which may be beneficial for untargeted exploitative analyses. However, GC-MS provides superior compound separation over LC-MS and has larger databases to compare data to, aiding identification (Wishart, 2008). Alternatively, nuclear magnetic resonance (NMR), another detection method, is much faster than both LC-MS and GC-MS, with the added benefit of being non-destructive when used as an imaging technology. While it is much less sensitive than other separation and detection techniques, NMR has been successfully used for dry matter and texture assessments in potatoes (Hansen et al., 2010) and carotenoid identification in tomatoes (Tiziani et al., 2006, in Wishart (2008)), indicating it could become more widely used in the future.

2.5.2 Sensory analysis: Measuring changes in attribute perception and liking

Descriptive analysis

Descriptive analysis is a well-known and well-used sensory technique, applied to evaluate "the nature and magnitude of sensory characteristics" in a sample (Kemp et al., 2018). The evaluation of flavour, using descriptive analysis, is an established and objective evaluation method because the use of trained panels and/or consumers minimizes the influence of personal opinions within well-defined and structured testing conditions. Many different descriptive analysis methodologies exist. One of the key decisions to make is choosing between trained panellists and untrained assessors (i.e. consumers) (Kemp et al., 2018). With anywhere from 2 weeks to 12 months training time, a small (e.g. 10-membered) trained panel can use methods such as 'Quantitative Descriptive Analysis' (QDA®, Tragon) or the 'Spectrum Method™' to assess products (Muñoz et al., 2018). Very broadly, these methods apply or develop a lexicon of sensory terms which panellists use to describe and rate the intensity of in samples, generating detailed descriptive quantitative data (Issanchou, 2018).

In potatoes, many flavour evaluations have used a form of trained panel descriptive analysis. Most evaluations use a QDA-style approach to assess the strength of very broad flavour attributes, such as 'taste', 'typical flavour', or 'odour-intensity', using an intensity scale (Brazinskiene et al., 2014; Hajšlová et al., 2005; Morris et al., 2010; Thybo et al., 2006). Other studies have targeted specific flavour attributes to investigate treatment effects. For example, QDA®was used by Blanda et al. (2010), with a trained 12-membered panel, to investigate the change in intensity of 10 off-flavour attributes in stored boiled potato slices. Significant and meaningful variation in flavour intensity, especially for 'cardboard-like off odours', was recorded using this method. The same research team later conducted another study using QDA®, but with a wider range of attributes (e.g. sweetness, 'typical flavour') to characterise the entire potato sensory profile (Comandini et al., 2018). Gilsenan et al. (2010) also used QDA®to train and use a 10-membered panel to evaluate the appearance, aroma, texture, and taste of baked potatoes subjected to organic and conventional growing conditions, specifically focusing on 'mustiness', 'earthiness', 'sweetness', and 'aftertaste' for flavour assessments. More recently, Sato et al. (2019) used 14 trained panellists to measure the intensity of a special bitter astringent taste, known as *egumi*, in four potato varieties.

Similar descriptive quantitative sensory insights could also be collected using consumers and rapid sensory testing methods, removing the need for time-intensive and expensive panel training. For example, techniques such as Check-all-that-apply (CATA) provide assessors with a list of predetermined attributes (e.g. sweet, bitter, nutty) to tick and apply to a product (Muñoz et al., 2018). Using chocolate milk desserts, Ares et al. (2010) found that the CATA and trained panel data generated similar configurations for the sensory terms used. Rate-all-that-apply (RATA), an extension of CATA, gives assessors the opportunity to tick

and also rate the intensity of any selected attributes (Buck and Kemp, 2018). Previous studies have used a 3 or 5-point intensity scale for rating (Vidal et al., 2018). RATA was developed to improve sample discrimination as CATA only produces binary data. This improvement could be valuable for mild-flavoured foods, like potato. However, while some studies report increased term usage and a greater number of significant results using RATA, other studies believe RATA does not provide any large improvement over CATA (Ares et al., 2014a; Vidal et al., 2018). Other more 'flexible' methods are also available for consumer studies, such as free choice profiling (FCP), where consumers identify and rate the intensity of any perceivable attributes in a sample. Porcherot and Schlich (2000) conducted a FLASH profile of steamed potatoes, where 14 participants were asked to write down, characterise, and rate the sensory differences between products.

However, despite the range of rapid testing options now available, most previous consumer evaluations on potatoes have been hedonic-based (discussed later). Only a handful of descriptive-based consumer evaluations have been undertaken, many of these conducted alongside trained panel descriptive analysis with broad sensory attributes. Gilsenan et al. (2010) used 80 consumers to assess differences between organic and conventional potato 'aroma', 'texture', and 'taste', employing a trained panel to investigate more specific attributes, such as 'earthiness'. After using a trained panel to develop a large potato sensory lexicon with 66 attributes, Sharma (2019) compared open-ended questions and CATA using 12 different potato varieties. While Sharma (2019) suggested the open-ended questions generated more "richness of responses" compared to CATA, only four flavour and aroma attributes were included for CATA (cooked potato, metallic, raw potato, and earthy). Additionally, 67% of participants found the open-ended questions difficult, compared to 31% for CATA (Sharma, 2019). Corrigan and McKenzie (unpublished) is likely the most comprehensive consumer flavour study conducted thus far on potatoes, with 22 flavour attributes included as part of a CATA evaluation. To date, no RATA evaluation has been conducted on potatoes - only CATA.

Deciding on a method and the use of a trained panel or consumers to conduct descriptive analysis depends on many factors, including time, funding, study objectives, and the level of product detail required (Muñoz et al., 2018). Trained panels can discern small changes to a product's sensory profile and may be more effective for potatoes if targeting a particular attribute, like *egumi* taste in Sato et al. (2019). Trained panel descriptive analysis has been the most popular choice in previous potato flavour literature. However, panel training is very expensive and time-consuming - where possible, costs and time are reduced if the same panel can conduct multiple studies (Muñoz et al., 2018). In rapid consumer testing, many more assessors must be recruited because consumers are not trained to produce reliable or reproducible results (Buck and Kemp, 2018). The data will also be less detailed. For CATA and RATA methods, a minimum of 50 assessors is required, with 60-80 consumers

recommended to achieve stable descriptive configurations (tested on fruits, bread, yogurt, crackers, juice, milk desserts, and anti-ageing cream) (Ares et al., 2014b; Buck and Kemp, 2018). However, despite the recruitment time and cost, consumer testing is significantly faster and participants may only need to return for several sessions, if any. Additionally, acceptance tests can be included in descriptive analysis consumer testing to gain insights into consumer preferences about the overall product and sensory attributes. This may be important for projects with a commercial interest. If consumers are used, then a suitable evaluation method must be selected. Open-ended and profiling-style evaluations may provide more detailed insights into consumer thinking and perception about a product. However, the evaluation style is more difficult and tiring for participants, and the statistical analysis is complex (Muñoz et al., 2018; Sharma, 2019). While CATA and RATA-style evaluations are fast and generally easier for participants, responses are limited to the single list of attributes supplied, which consumers may interpret in different ways. The final choice ultimately depends on the study objectives and product tested.

Acceptance testing

Consumer acceptance testing is conducted to understand consumers' preferences, opinions, and perceptions about a product (Kemp et al., 2009). When used in combination with descriptive testing, acceptance testing may also help to understand product attributes consumers like or dislike. Measuring consumer preference for a food product is commonly carried out using one of two scales: the 9-point hedonic scale, or an adapted category ratio scale (e.g. a labelled affective magnitude scale [LAM]) (Kemp et al., 2009). The Peryam and Giradot 9-point hedonic scale is one of the most popular hedonic scales used to assess preference. It consists of 9 liking statements, ranging from dislike extremely to like extremely (Fig. 2.4). The response positions are converted into a numerical value for data analysis, assuming equal intervals between the categories (Kemp et al., 2009; Lim, 2011). When carrying out consumer preference testing, a minimum of 100 participants (representative of the target market) is generally recommended if the results are to be extrapolated to all consumers (Kemp et al., 2009).

The hedonic scale is a very popular method to evaluate overall consumer liking of potatoes, in addition to assessing liking of broad attributes (e.g. texture or taste) (Gilsenan et al., 2010; Lundgren et al., 1979; Maier et al., 2007; Pardo et al., 2000; Sharma, 2019). Gilsenan et al. (2010) used a 9-point hedonic scale to assess colour, aroma, texture, and overall taste-acceptability between organic and conventionally-grown baked potatoes for 80 consumers. Using this method, organically-grown potatoes were found to significantly affect texture preference, but not taste and overall acceptability. More recently, Sharma (2019) also used a 9-point hedonic scale to assess overall liking and broad sensory characteristics (appearance, aroma, taste, flavour, texture) of mashed potatoes from 12 different cultivars, as part

of a wider potato sensory profiling study. The liking results were combined with CATA data for penalty analyses.

While the 9-point hedonic scale has been the preferred test choice in previous potato acceptance testing, the scale has several limitations that other tests (e.g. LAM scales) have adjusted for (Lim, 2011). The assumption of equal intervals between each label on a 9-point scale (Fig. 2.4) has been disputed - intensity is instead thought to increase superlinearly (Moskowitz, 1970). This means that parametric statistical tests (e.g. ANOVA) should not be applied to the data, because the intervals are not equal (Kemp et al., 2009). Additionally, the two extreme ends of the scale are often underutilised, potentially creating a central tendency effect (rating in the middle of the scale) and limiting discrimination for well-liked or disliked samples (Schutz and Cardello, 2001). Adapted category-ratio scales, such as the LAM scale, are alternative options to the hedonic scale to evaluate consumer preference. The LAM scale spaces labels along a visual scale, based on the magnitude of their affective meaning when in relation to food liking (see Fig. 3.7). Two additional labels, 'greatest imaginable like' and 'greatest imaginable dislike', are added onto the scale ends (Lim, 2011). As potatoes have a subtle flavour that may be less-polarising than other food products, a LAM scale could improve preference discrimination between samples, especially given the opportunity to select a position on the scale in-between labels, unlike categorical 9-point hedonic scales.

Figure 2.4: Example of a 9-point hedonic scale used to assess consumer food preference. Used with permission from Kemp et al. (2009).

2.5.3 Sensometabolomics: A combined approach

While metabolite and sensory testing alone provides valuable data on the flavour of food, including potatoes, a combined approach can generate more information on how compositional changes may affect sensory-perceived flavour. For example, by correlating metabolite profiles with consumer quantitative descriptive analysis results, it could be possible to predict consumer-perceived flavour, using analytical measurements alone (Morris and Taylor, 2019). Here, sensometabolomic testing involves extensive volatile and/or non-volatile testing (with several compound groups) with quantitative descriptive sensory testing, where the flavour attributes assessed are more specific than 'typical flavour' (i.e. sweet or bitter).

In potatoes, there is very limited published literature on the potato sensometabolomic profile and metabolites that play a key role in overall flavour perception (Morris and Taylor, 2019). Many studies have instead focused on the metabolic cause or origin of a specific flavour attribute, such as 'cardboard-like' off-flavour (Blanda et al., 2010; Petersen et al., 1999). However, the development of a clear understanding around key flavour metabolites may be difficult because many flavour attributes are likely caused by an interaction of multiple metabolites or compounds (Morris and Taylor, 2019).

Targeted flavour analysis with metabolite profiling

The combined use of sensory and metabolite testing has been utilised in potato flavour research for a long time. The earliest studies investigated the effect of glycoalkaloids and phenolic acids on bitterness perception, using trained panels (Mondy et al., 1971; Mondy and Gosselin, 1988; Sinden et al., 1976). Josephson and Lindsay (1987) investigated the effect of adding c4-heptenal to mashed potato aroma and flavour - they found that at high concentrations, added c4-heptenal reduced flavour freshness and intensity. Petersen et al. (1999) linked the development of 'cardboard-like' off-flavour in potatoes with lipid-derived aldehydes (e.g. pentanal, hexanal, 2,4-nonadienal) using a panel trained in identifying off-flavours, GC-MS, and GC-O. The additional use of GC-O (GC-sniffing) is valuable because it helps to confirm whether the volatiles detected are above the threshold for human detection. Further off-flavour research has been conducted by Blanda et al. (2010), where QDA®was used to rate samples with typical potato and off-flavours. The quantitative sensory data were linked to aroma data, captured using HS-SPME-GC-MS. Strong (and significant) correlations were found between off-flavoured samples and 2-pentenal, 2-hexenal, 2-heptenal, 2-pentylfuran and 2-decenal (Blanda et al., 2010). The influence of umami amino acids and 5'-ribonucleotides on overall flavour intensity and acceptability was investigated by Morris et al. (2007). And more recently, Sato et al. (2019) combined quantitative trained panel evaluation with glycoalkaloid and amino acid metabolite testing to investigate the cause of bitter and astringent *egumi* taste.

Through analysing a specific aspect of flavour and composition, these studies have improved understanding and elucidated how particular metabolites can generate flavour attributes in potatoes. However, as only individual metabolite groups and specific flavours were targeted, the effect of the metabolites on other flavour attributes, and any flavour synergy or interaction, is unknown. Additionally, targeted flavour and metabolite analysis has rarely focused outside the effect of cultivar, cooking method, and storage - only a few papers have investigated the influence of organic versus conventional growing techniques (Gilsenan et al., 2010; Hajšlová et al., 2005; Lombardo et al., 2012).

Extensive descriptive sensory analysis with metabolite profiling

A handful of studies have attempted to link extensive metabolite testing with descriptive sensory analysis between different cultivars and genetic material (Bough et al., 2020; Morris et al., 2010, 2011). To date, there is no existing sensometabolomic research on the effect of growing treatments or agronomic conditions on potato flavour. The volatile and sensory profile of peeled boiled potatoes was assessed by Ulrich et al. (2000), this study appearing to be the first attempt at correlating a comparatively large range of attributes with volatile data. Ulrich et al. (2000) assessed the variation in sensory perception, across 3 varieties and a trained 15-membered panel, with eight attributes: sweet-like, earthy, burnt, fodder, untypical, musty, fruity, and typical. GC-PND (nitrogen specific detector), GC-MS, and GC-O were used to analyse the volatile fraction after SDE (Ulrich et al., 2000). However, no clear conclusions or predictions between sensory and volatile data were drawn, data instead considered individually with no statistical support.

Several years later, the Scottish potato research group (Morris et al., 2010) applied sensometabolomic techniques to characterise the flavour and metabolomic differences between steamed *S. tuberosum* and *S. phureja* potato varieties. The non-volatile metabolite fraction (amino acids, equivalent umami concentration, glycoalkaloids, and carbohydrates) was assessed using HPLC and the volatile fraction was assessed using SPME-GC-MS. Descriptive analysis was conducted by a trained panel (10 assessors) rating attributes aroma, flavour intensity, flavour-sweetness, flavour savouriness, flavour creaminess, and flavour–off-flavours on a 0-10 linear scale. Significant differences between *S. tuberosum* and *S. phureja* were evident in the descriptive analysis results, *S. phureja* samples exhibiting greater flavour savouriness and the highest scores for creamy flavour. Correlation analysis was used to link sensory results with the metabolic profile. A later study by Morris et al. (2011) investigated the over-expression of α-copaene in potatoes, once again using a sensometabolomic approach, to determine whether α-copaene played an important role in the increased flavour intensity and acceptability of *S. phureja* cultivars. Morris et al. (2011) found that α-copaene did not affect the flavour of the transgenic samples.

Perhaps the most comprehensive sensometabolomic study to date was conducted by Bough et al. (2020). Using 12 flavour attributes (in addition to texture and appearance attributes), 15 different boiled and baked cultivars were assessed by a trained panel across two growing seasons. In addition, non-targeted volatile analysis using HS-SPME-GC-MS was carried out on all cooked cultivars. Across both years, bitter and other off-flavours significantly varied between cultivars, these attributes also the most consistent undesirable attributes. Using multivariate analysis, Bough et al. (2020) suggested a number of compounds that could be used as breeding biomarkers for flavour attributes such as buttery, aroma intensity, and woody. However, as results varied considerably between seasons and several correlations disagreed with those recorded in Morris et al. (2010) and Ulrich et al. (2000), further work is still required to fully understand how perceived flavour links to composition in potatoes, and why different correlations have been discovered by different researchers. Further findings, linking consumer sensory data (from CATA analysis) and both volatile and non-volatile metabolite testing, is expected from Corrigan and McKenzie (unpublished).

2.6 Conclusions and statement of research

2.6.1 Summary of literature review

Potatoes, with its origin as a crop in the South American highlands, is the largest vegetable crop in New Zealand. Potatoes are popular with consumers, New Zealand's purchasing habits trending towards smaller packaged speciality products, potentially differentiated by superior flavour. The flavour of potatoes is the result of a complex blend of aroma volatiles, taste compounds, and human perception. The evolution of character-impact aroma compounds, in combination with flavour-enhancing umami compounds and bitter glycoalkaloids, help to contribute to overall potato flavour, which is often described as typical/potato-like, earthy, bitter, buttery, and sweet. Aroma-producing reactions, such as lipid oxidation, Maillard/sugar degradation, and amino acid degradation, are significantly influenced by preparation and cooking methods. Flavour will also vary with genetics, *S. phureja* cultivars regarded as more flavourful and savoury in comparison to *S. tuberosum* cultivars.

Nitrogen fertilisation has the potential to affect the flavour of cooked potatoes. Previous research has linked increases in nitrogen fertiliser application to an increase in potato off-flavour aldehydes, potentially leading to the development of cardboard off-flavours (Fischer, 1991; Petersen et al., 1999). In addition, total nitrogen and nitrate content has been linked to the perception of general off-flavours (Cieslik, 1997; Thybo et al., 2006) - as increasing nitrogen fertiliser increases the total nitrogen and nitrate content, this could further contribute to a deterioration in flavour. While glycoalkaloids have been found to increase with the rate of nitrogen fertiliser applied, if levels remain below the perception threshold for bitterness, flavour will likely remain unaffected - the growing environment will have a greater

effect on glycoalkaloid content (Love et al., 1994). Alternatively, flavour could improve with increasing nitrogen fertiliser rates as the levels of umami amino acids may also increase, contributing to flavour intensity and savouriness (Eppendorfer, 1996; Morris et al., 2007). However, to date no research has assessed the influence of nitrogen fertilisation on a wide range of contributors to potato flavour.

The potential changes to potato flavour, with the addition of nitrogen fertiliser, could be measured using a range of techniques. After cooking, effective sample preparation is essential to ensure the material remains stable and can be used for a wide range of testing. For volatile aroma analysis, samples should be frozen immediately and ground, with a dehydrator added, while for non-volatile analysis, samples should be frozen, freeze-dried to remove water, ground, then frozen again before analysis. Dynamic headspace extraction and SPME are the most popular extraction methods for volatile analysis, and GC-MS is commonly used for separation and detection, while GC-O can be used to determine the odour threshold of targeted compounds. As many different compound groups could be targeted for non-volatile analysis, solvents for extraction should be optimised for the key compounds of interest. Many different options could be used for separation and detection, HPLC-MS, or LC coupled with UV or DAD common options for analysing non-volatile compounds in potatoes.

For the sensory analysis of potato flavour, trained panel analysis or rapid consumer methods could be employed. Trained panel analysis may be more sensitive for discriminating subtle differences in samples whereas consumer testing is valuable to gain insight into whether regular consumers can perceive any changes to flavour. A combined approach of sensory testing and metabolite analysis (sensometabolomics) has only been employed a handful of times for potatoes (Ulrich et al., 2000; Morris et al., 2010; Bough et al., 2020). Sensometabolomics can be used to investigate the link between composition and flavour, helping to determine whether changes to the metabolite profile of a potato will affect sensory-perceived flavour.

2.6.2 Statement of research

Combining the project background and initial aims in Chapter 1 with the information gained in this literature review, five research questions have been developed to direct and fulfil the purpose of this project.

1. How does the supply of nitrogen during crop growth affect steamed tuber composition, with a focus on flavour-related metabolites?
2. How does the supply of nitrogen during crop growth affect consumer-perceived flavour and acceptance of steamed potatoes?
3. How does variety impact cooked tuber composition, with a focus on flavour-related metabolites?
4. Does variety influence how the supply of nitrogen during crop growth affects consumer-perceived flavour, acceptance, and composition of steamed potatoes?
5. Is it possible to link variation in composition to differences in consumer-perceived flavour or acceptance?

From the research questions, the following objectives were outlined:

1. Determine if nitrogen fertiliser, applied to Annabelle and Andean Sunside crops, can affect flavour-related tuber composition by measuring the following variables, after the application of three levels of nitrogen fertiliser (0, 150, and 300 kg N ha^{-1}) during crop growth:
    - Dry matter content
    - Total nitrogen and nitrate content
    - Carbohydrate content: glucose, fructose, and sucrose
    - Umami amino acids
    - Glycoalkaloids: α-chaconine and α-solanine
    - Polyphenols: chlorogenic acid and related isomers
    - Volatile compounds

2. Determine if nitrogen fertiliser, applied to Annabelle and Andean Sunside crops, can affect the consumer acceptance and perceived flavour of steamed tubers by using the following sensory methods, after the application of three levels of nitrogen fertiliser (0, 150, and 300 kg N ha^{-1}) during crop growth:

    - Capture any differences in flavour by conducting rapid consumer sensory evaluation using Rate-all-that-apply (RATA).
    - Capture any differences in consumer acceptance using a Labelled Affective Magnitude (LAM) scale.
    - Capture and identify any consumer segmentation in sample acceptance, as related to the level of nitrogen fertiliser applied.

3. Determine whether flavour-related composition and consumer flavour perception can be associated, in relation to the nitrogen fertiliser treatments applied:

    - Assess the presence of any associations on the combined RATA and compositional data sets by conducting Principal Component Analysis .

Chapter 3

Materials & methods

3.1 Field experiment

3.1.1 Site location and growing conditions

The field trial was located on a single site, Kala Farm, within potato crops grown by A.S. Wilcox & Sons (Wilcox), at 320 Taihoa Road South (37° 51' S, 175° 47' E), approximately 8 km south of Matamata, in the Waikato region. An aerial image of Kala Farm is provided in Fig. 3.1. The soil type was Piarere silt loam (Landcare Research, 2019a), a moderately well-drained allophanic orthic soil with a high moisture availability (Landcare Research, 2019b). Crops planted in the previous season included broccoli (*Brassica oleracea* var. *italica*) and black oats (*Avena strigosa*). The trial site terrain was flat with gently rolling surrounds. Pre-planting soil tests were carried out by A.S. Wilcox & Sons in August 2018 and submitted to Hill Laboratories in Hamilton for analysis. The soil from the two trial sites allocated contained 44-48 kg ha^{-1} potentially available N (to 15 cm depth) (100-150), Olsen P of 53-61 mg L^{-1} (30-60), total CEC 17 me 100 g^{-1} (12-25), and the following minerals in MAF QuickTest units: K at 12-17, Ca at 6-8, Mg at 24, and Na at 2-3. The numbers in the brackets represent the average range of values reported for potato soil, as recorded by Hill Laboratories. The soil pH was 6.5, which is higher than the average range of 5.4-5.8. Appendix A contains a copy of both soil test reports from each allocated trial site.

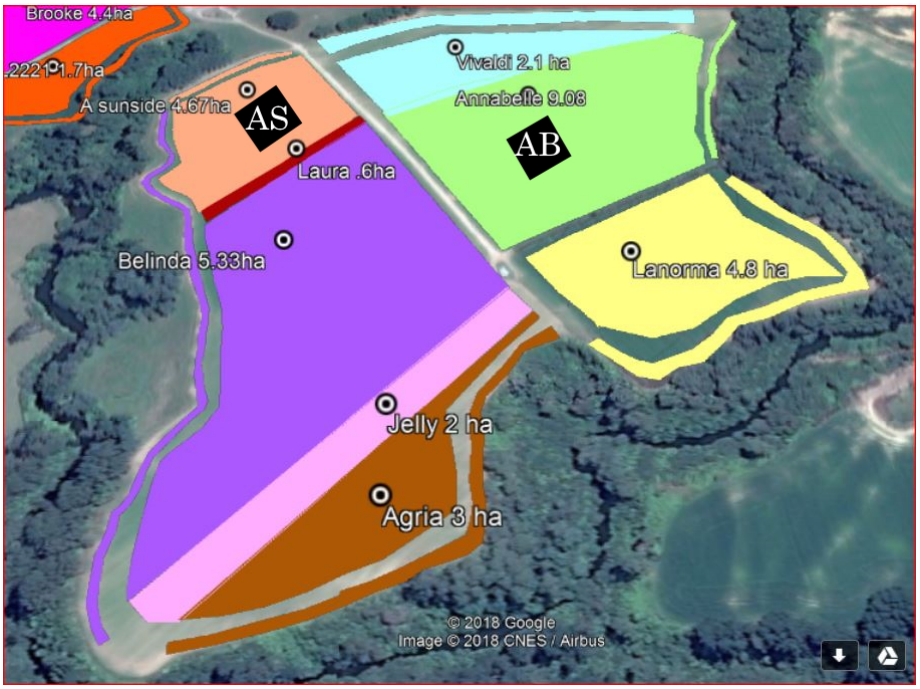


Figure 3.1: An aerial photograph of Kala Farm in Matamata. The coloured areas and labels represent Wilcox's commercial potato crops for 2018/19 growing season. The labelled black squares indicate the approximate position of the trial plots for this study, where AS = Andean Sunside and AB = Annabelle (Google Maps).

3.1.2 Trial design

Trial layout

Two potato varieties, 'Annabelle', (*S. tuberosum*, Nicola × Monalisa, HZPC) and 'Andean Sunside' (*S. tuberosum*, ARD 89-1402 × ARD 88-883 (ex Phureja), Agrico UK) were selected for the trial. The whole seed was grown in Canterbury, New Zealand, and supplied to Wilcox in April 2018. Annabelle and Andean Sunside were planted in separate plots, approximately 200 m apart (Fig. 3.1). A pre-planting base fertiliser mix was applied on 10 September 2018, which included 34 kg P ha^{-1}, 250 kg K ha^{-1}, and 120 kg Mg ha^{-1}, using granulated CalMag (Ballance Agri-Nutrients Ltd., Tauranga), Serpentine Super 25K (Ballance Agri-Nutrients Ltd., Tauranga), boron, zinc sulphate, and Selcote Ultra (Nufarm, Otahuhu). A split-plot design was planned for both plots (Fig. 3.2 and Fig. 3.3). Eight blocks (field reps) were allocated for each variety and treatment. Each block within each varietal trial plot measured 6.5 m by 1.72 m; a standard planting bed width formed into 2 rows. A guard of 0.5 m was left between treatment blocks. As the trial had to fit within commercial crops, field reps 1 and 2 for Andean Sunside and field reps 4 and 5 for Annabelle had to be positioned next to the wheel track (Fig. 3.3 and Fig. 3.2). This was noted for potential effects on the yield results.

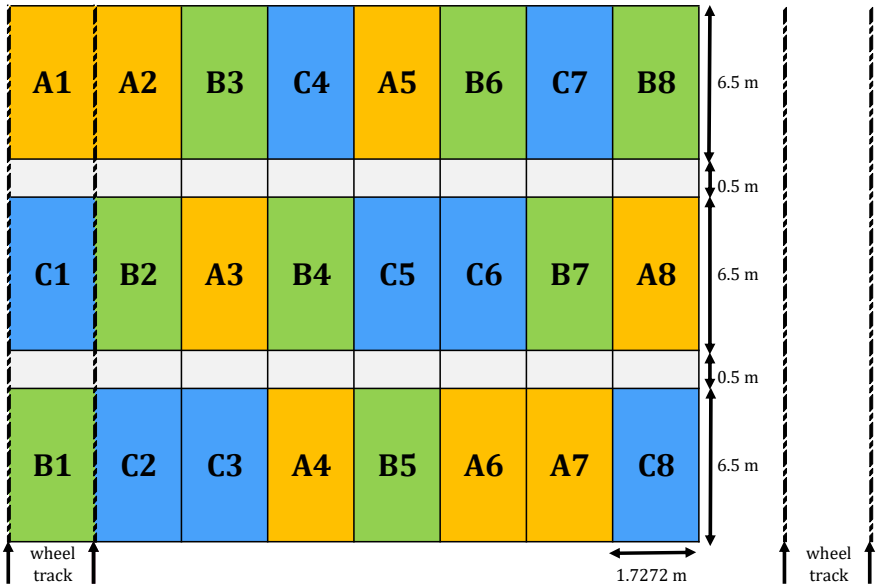


Figure 3.2: Split plot design trial map of the Andean Sunside planting, where A = 0 kg N ha^{-1}, B = 150 kg N ha^{-1} and C = 300 kg N ha^{-1}. Numbers represent field replicates and "wheel track" indicates the position of the spray row.

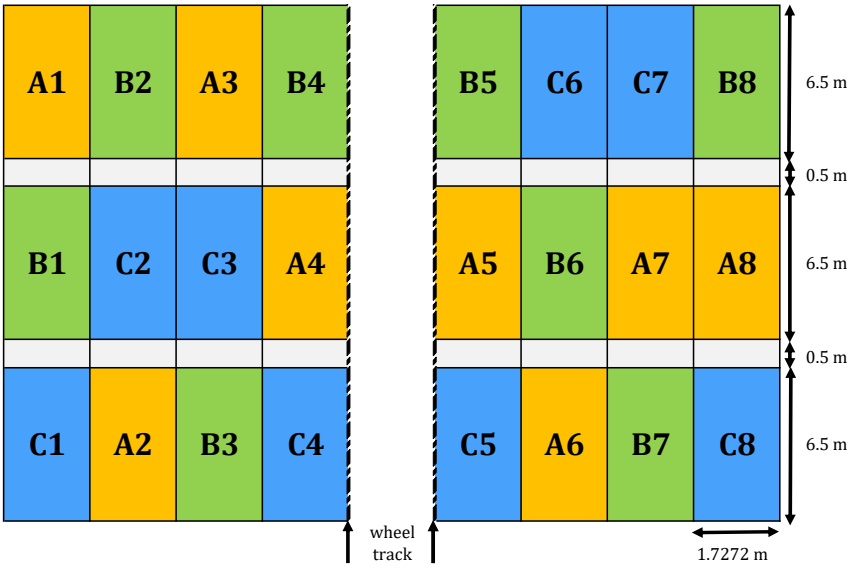


Figure 3.3: Split plot design trial map of the Annabelle planting, where A = 0 kg N ha^{-1}, B = 150 kg N ha^{-1} and C = 300 kg N ha^{-1}. Numbers represent field replicates and "wheel track" indicates the position of the spray row.

Nitrogen treatments

Three nitrogen (N) treatments were selected for the trial, 0 kg N ha^{-1}, 150 kg N ha^{-1}, and 300 kg N ha^{-1}. 150 kg N ha^{-1} is the same commercial N input for Annabelle, Andean Sunside crops generally receiving 120 kg N ha^{-1} (personal communication, B. Hart, February 8, 2020). The application of nitrogen was split across a base fertiliser at planting and a urea side-dressing. The nitrogen rates are summarised in Table 3.1. Other macronutrients, phosphate (153.8 kg ha^{-1}), potassium (105 kg ha^{-1}), and sulfur (42.5 kg ha^{-1}) were kept consistent across all treatments. A mix of Triple Super (0-20.5-0, 10.5 Ca), Sulphate of Potash (SOP, 0-0-41, 17 S), and Nrich Urea (46-0-0), all sourced from Ballance Agri-Nutrients Ltd, Tauranga, New Zealand, was hand-weighed, mixed, and bagged for the treatment applications. The quantity of each fertiliser applied per block is provided in Table 3.2.

Table 3.1: Total nitrogen rates applied to Andean Sunside and Annabelle trial treatment blocks, separated into a base fertiliser and side-dressing application.

Treatment code	**Base input** (kg N ha^{-1})	**Side-dressing input** (kg N ha^{-1})	**Total N input** (kg N ha^{-1})
0 N (A)	0	0	0
150 N (B)	69	81	150
300 N (C)	69	231	300

Table 3.2: Quantity of base fertilisers and urea, applied to each treatment block (3 m × 0.86 m), on both Andean Sunside and Annabelle trial plots. Treatment A = 0 kg N ha^{-1}; B = 150 kg N ha^{-1}; and C = 300 kg N ha^{-1}.

Treatment code	**Base fertiliser** (kg $block^{-1}$)			**Side-dressing** (kg $block^{-1}$)
	Triple Super	SOP	Urea	Urea
0 N (A)	0.842	0.280	0	0
150 N (B)	0.842	0.280	0.168	0.198
300 N (C)	0.842	0.280	0.168	0.564

3.1.3 Crop planting, establishment, and management

Andean Sunside

On 14 November 2018, the Andean Sunside trial plot was planted by hand at 200 mm spacing and 200 mm depth, the same parameters Wilcox uses for commercial Andean Sunside crops. 66 seed tubers were planted per block, totalling 1584 tubers for the entire plot. Some rot

was observed in the seed during planting. The base fertiliser was applied by broadcast, with applications of fungicides Monceren FS (Bayer New Zealand Ltd., at 33.7 kg ha^{-1}) and Gem (Adama, Nelson, at 3 L ha^{-1}) and the insecticide Actara (Syngenta New Zealand, at 4 g 100 m^{-1}) before re-mounding the row moulds. The trial site was managed similarly to the surrounding commercially grown Andean Sunside crop, which was planted approximately 3 weeks earlier (Fig. 3.1).

On 26 December 2018, the urea side-dressing was applied to treatment blocks B (150 N) and C (300 N) by hand-broadcast, 43 days post-planting (DPP) (Fig. 3.2). The trial was also hand-weeded. Crop checks and petiole/leaf tests for nitrogen (data not included) were conducted on 18 December 2018, 14 January 2019, and 5 February 2019, along with another hand-weeding on 23 January 2019 due to high weed pressure from redroot (*Amaranthus powellii*), willow weed (*Persicaria maculosa*), and groundsel (*Senecio vulgaris*) (Fig. 3.4. The trial was dessicated on 4 March 2019 (111 DPP) using a mixture of Reglone (Syngenta, 2 L ha^{-1}), Nail (NuFarm, 200 ml ha^{-1}), Karate Zeon (Syngenta, at 100 ml ha^{-1}), and Peptoil (Etec Ltd., Pakuranga, at 1 L ha^{-1}).

Figure 3.4: Illustration of weed pressure in the Andean Sunside trial plot. Photo taken 97 DPP on 18 February 2019 (Photograph by author).

Annabelle

On 12 December 2018, the Annabelle trial plot was planted by hand at 130 mm spacing and 200 mm depth, the same parameters Wilcox uses for commercial Annabelle crops. 100 seed tubers were planted per block, totalling 2400 tubers for the entire plot. The same fertiliser and agrichemical procedure, as described above for Andean Sunside, was applied at

planting to the Annabelle trial. After planting, the trial site was managed similarly to the neighbouring commercial Vivaldi potato crop, planted at a similar time.

On 29 January 2019 (49 DPP), the urea side-dressing was applied to treatment blocks B (150 N) and C (300 N) by hand-broadcast. Crop checks and petiole/leaf tests for nitrogen (data not included) were conducted on 3 January, 21 January, and 5 February 2019. Unlike the Andean Sunside trial plot, Annabelle had little weed pressure, except for a large amount of willow weed growing in the empty bed in the middle of the trial plot (Fig. 3.5), labelled 'wheel track' in Fig. 3.3. The trial was dessicated on 26 February 2019 (77 DPP) using a mixture of Reglone (Syngenta, 2 L ha^{-1}), Nail (NuFarm, 200 ml ha^{-1}), Karate Zeon (Syngenta, 100 ml ha^{-1}), and Peptoil (Etec Ltd., 1 L ha^{-1}).

Figure 3.5: Willow weed growing in the empty bed in the middle of the Annabelle trial plot (right of photo). Photo taken 69 DPP on 18 February 2019 (Photograph by author).

3.1.4 Harvest and post-harvest handling

All tubers from both trial blocks and varieties were hand-harvested on 1 April 2019, 139 DPP (Andean Sunside) and 111 DPP (Annabelle). A sample dig measuring 3 m × 0.86 m was harvested from each treatment block, within each replicate. All digs were stored in 10 kg netted onion sacks. In addition, for both varieties two reps (blocks) were selected for further sensory testing. For Andean Sunside, field reps 4 and 5 were selected as these were in the middle of the entire plot with no edge effects or neighbouring wheel tracks (Fig. 3.2). For Annabelle, field reps 3 and 6 were selected, given the potential spraying and edge effect of the centre wheel track (Fig. 3.3). From these selected replicates, an extra 6 m × 0.86 m sample dig was harvested from each block to have sufficient potatoes for sensory testing.

Immediately after harvest, the tubers were transported to Union Road in Pukekohe (A.S. Wilcox home site) and stored in a field cool store set to 3-4 °C. All samples were held at this temperature until field data assessments were completed. Three days later, the tubers were transported down to Palmerston North (4 April 2019) in a small car. Before transportation to Palmerston North, 180 tubers from each treatment, field replicate, and variety were selected for sensory testing, based on a clean skin finish, absence of greening, regular shape, and a suitable eating size (approximately 40 - 50 mm diameter). After transportation, the tubers were stored in 20 kg netted onion sacks in cool cabinets set to 10 °C at The New Zealand Institute for Plant and Food Research in Palmerston North until use for sensory testing.

3.1.5 Field data collection: Yield, tuber count, and tuber size

The tubers were removed from the field cool store and held at ambient temperature for a maximum of 4 hours for the collection of field data measurements. Yield (t ha^{-1}), average tuber mass (g), average tuber diameter (mm), and tuber count was determined for each treatment, field replicate, and variety using digital scales (accurate to ± 0.2) and digital callipers (accurate to ± 0.01), where diameter was measured at the widest point of the tuber held flat. The standard yield assessment procedure used by A.S. Wilcox & Sons was applied to estimate field yield in t ha^{-1}, where the total mass of one 3 m sample dig is multiplied by 3.3 for Andean Sunside, and 3.8 for Annabelle.

3.2 Sensory analysis

3.2.1 Participants

One single consumer study was completed by a total of 111 participants of both genders (79 females and 32 males). Ethical approval was sought before recruitment using the Massey University Research Information Management System (RIMS). The project was evaluated as low-risk and approved by peer review. Participants were recruited using the following channels: use of the Massey Food Experience and Sensory Testing (FEAST) consumer database, email circulation around Massey University department administrators, email circulation around Plant and Food Research, social media interaction and posts using Massey University, MUSA, Potatoes NZ, and Plant and Food Research, an online article on Stuff.co.nz, a notice on the Massey University staff portal, and advertising through a local church. As this study used varieties (Annabelle and Andean Sunside) marketed as speciality potatoes by A.S. Wilcox & Sons, the initial recruitment process was targeted towards consumers of gourmet potatoes, flyers including the questions: “Think yourself a potato connoisseur? A shopper of gourmet spuds?”. However, when insufficient ‘gourmet’ eaters were available, recruitment was widened to include all regular cooked potato consumers.

Potential participants were asked to complete an online questionnaire about their availability for the sensory trial dates. All participants were required to be aged between 18 and 65 years old and regular consumers of cooked potatoes. For this study, 'regular consumption' connotated fresh potato consumption at least once every 2-3 weeks. Participants were also required to read an information sheet about the study and sign a consent form prior to participating. All participants attended both sessions and received compensation for their participation and time.

3.2.2 Sensory tests, terms, and data collection

Session scheduling

The sensory evaluation was conducted from 9 - 12 April 2019. Annabelle was served for the first two days and Andean Sunside was served for the last two days (Table 3.3). The evaluation was arranged like this to reduce complexity and potential errors when cooking and serving, also allowing more flexibility if any crop or harvesting problems had arisen. Six 1-hour sessions were scheduled per day, at 8.00 am, 9.30 am, 11.00 am, 12.30 pm, 2.00 pm, and 3.30 pm. All participants were required to attend two 1-hour sessions. Annabelle was served in the first session and Andean Sunside was served in the second session.

In each session, the participants evaluated one sample from each nitrogen treatment twice, the first time for liking, and the second time for flavour. Each session held a maximum of ten participants. All participants were instructed to avoid consuming any food or beverages (except water) within one hour of their allocated session time.

Table 3.3: Order of variety and field replicate serving in the sensory evaluation. Consumers were required to attend two sessions, ensuring both varieties were evaluated.

Date	9 April	10 April	11 April	12 April
Variety	Annabelle	Annabelle	Andean Sunside	Andean Sunside
Field rep	3	6	4	5

Before their first session, participants were instructed on the purpose of the research, the evaluations they would be conducting, and the sensory terms they would be using in evaluation (Table 3.4). Participants were given multiple opportunities to ask for clarification or question any aspect of the study. Therefore, participants were familiarised with the procedure and potato flavour evaluation methods.

Sample preparation and serving size

All sample preparation and cooking was conducted in a commercial-grade product development laboratory and kitchen at Massey University, Palmerston North. Tubers were collected from 10 °C storage and washed the night before evaluation using cold water and a sponge (Fig 3.6a). Clean and relatively blemish-free tubers were selected, sorted, and weighed into groups of 10-12 tubers (Fig 3.6b). Spare potatoes were included in case any were found to be undercooked or had internal defects. One Andean Sunside tuber had blackheart, observed during serving. There was a large amount of size and quality variation within and between varieties and treatments. Therefore, instead of randomly selecting and grouping tubers in preparation for cooking, similarly-sized tubers were grouped together for each session to maintain a consistent serving and sample size within a session. For Annabelle, the average tuber mass served per session ranged from 61.5 g to 114.9 g. For Andean Sunside, the average tuber mass served served per session ranged from 44.2 g to 135.8 g. The average tuber mass served for every session, across each variety and nitrogen treatment, is provided in Appendix B, Table B.1. After air-drying, tubers were bagged in plastic supermarket potato bags and stored at room temperature until evaluation the following day (Fig 3.6c).

Table 3.4: List of sensory terms and definitions distributed to participants and used in the RATA sensory evaluation.

Attribute	Definition
Bitter	Taste associated with caffeine[1], cocoa, or brussel sprouts.
Boiled potato	Flavour associated with the internal portion of a salad (boiling) potato that has been boiled in water until cooked through.
Buttery flavour	Flavour associated with butter (dairy, cow).[2]
Cardboard	An off-flavour associated with the flavour of cardboard or paper.
Earthy	The flavour associated with dirt or soil (unwashed potato skin).
Oily flavour	Flavour associated with vegetable oil.
Green grass	The flavour and aroma associated with freshly mown grass.[3]
Metallic	An oxidised metal-like taste (e.g. copper coins or blood in the mouth).[2]
Musty	The flavour and aroma of a damp, mouldy, or unventilated room.
Nutty	A sweet and roasted flavour associated with nuts.[4]
Raw potato	The flavour associated with uncooked potato.
Savoury	Taste (umami) associated with monosodium glutamate or a meat broth.[1]
Sour	Taste stimulated by acid.[1]
Sweet	Taste stimulated by sugar.[1]

1. Leksrisompong et al. (2012)
2. Booysen et al. (2013) (buttery flavour definition only partially used)
3. Partially from Lee and Chambers (2007)
4. Drake et al. (2005)

(a) Washed tubers. (b) Grouped tubers. (c) Bagged for evaluation.

Figure 3.6: Annabelle tuber washing and sorting for evaluation the following day (Photographs by author).

Six steamers, Sunbeam VitaSteam™ steamers (ST6650) with three steaming layers, were used to cook the tubers. Tap water was used to fill the steamers. Ten tubers were placed into the top steamer layer and any spare tubers placed into the second steamer layer. Preliminary experiments determined the fastest cooking time was achieved in the top steamer layer. Tubers were left whole and unpeeled. All tubers were steamed for 50 minutes. The steamers were then switched off and the tubers were allowed to cool in the steamer for 30 minutes before serving. Approximately 3-5 minutes before serving, the tubers were removed from the steamers and quartered lengthways, taking care to keep the skin intact. The serving time was scheduled to allow tubers to cool to an internal temperature of approximately 60-65 °C before evaluation (based on preliminary experiments).

Participants were served a single steamed potato quarter to evaluate, presented skin-side up. However, given the large amount of natural variation in tuber size, for very small tubers, half a potato (cut lengthways) was served to ensure participants had enough sample to evaluate. Based on the average tuber mass cooked, the average serving size (one quarter) was 22.5 g for Annabelle and 20.3 g for Andean Sunside.

3.2.3 Evaluation details

All evaluations were conducted using Compusense®Cloud (Version 19.1.7318.30605) on Apple iPads. All samples served were coded with randomly-generated 3-digit codes. For each session, participants consumed two quarters per nitrogen treatment, totalling six potato quarters. Each potato quarter served originated from a different potato tuber. For all evaluations, participants were asked to consume the entire potato quarter, including skin, before moving onto the next sample.

Demographic questions

Before beginning the sensory evaluation, all participants were asked to provide demographic information, including their full name, date of birth, and gender. Participants were also asked two additional questions: "How often do you eat boiled, mashed, steamed, or roast potatoes?" and "What category of potatoes do you consume? (tick all that apply)". The potato category options included 'gourmet', 'loose', 'bulk-bags (10-15 kg)', 'home-grown', or 'other'. To capture the participants that may purchase and consume potatoes based on flavour, which may not exclusively entail 'gourmet' potatoes, participants were instructed to select 'gourmet' if the desirable flavour of a specific variety (e.g. Agria) influenced their purchasing and consumption behaviour.

LAM test

After completing the demographic questions, participants were served three quarters at the same time, one from each nitrogen treatment (0 kg N ha^{-1}, 150 kg N ha^{-1}, and 300 kg N ha^{-1}). Participants were instructed to taste the samples from left to right. The treatment order was randomised between sessions, but not within sessions. A Labelled Affective Magnitude scale (LAM) was used to assess liking (Fig. 3.7). Participants were instructed to select anywhere on the scale that best represented how much they like the potato quarter, the scale ranging from greatest imaginable dislike to greatest imaginable like. All three samples were rated on the same LAM scale to allow consumers to visually rank samples. The sample scale positions were converted by Compusense into a continuous numerical data point. A compulsory one-minute break was enforced by Compusense in between samples and participants were instructed to cleanse their palate before and between samples with filtered water. After sampling all quarters, participants were allowed to re-taste quarters and move the position of samples on the scale if required. After completion of the LAM evaluation, participants were asked to return to the break room for approximately 5 - 10 minutes before beginning the RATA evaluation.

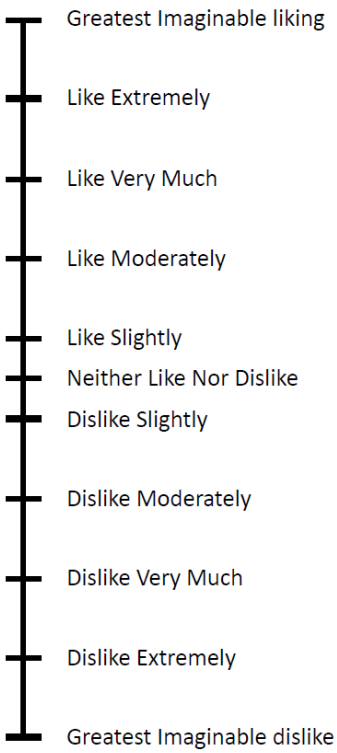


Figure 3.7: The labelled affective magnitude scale used by participants to indicate sample liking. The width of the axis ticks were identical on Compusense test (Schutz and Cardello, 2001).

RATA test

A Rate-all-that-apply (RATA) evaluation was used to gain insight into the different flavour attributes present in the potato samples. A pilot study in 2018 was used to finalise a list of 14 flavour attributes that could be used to describe potato flavour, initially compiled from previous literature on potato flavour. The list of attributes and definitions are provided in Table 3.4. The definition list was provided to participants one week before the trial to allow familiarisation with the terms. The terms and definitions were read out for all participants before the evaluation.

For the RATA evaluation, samples were served monadically with a compulsory one-minute break between samples, enforced by Compusense. Before and between samples, participants were instructed to cleanse their palate with filtered water. Each participant received three potato quarters, one from each nitrogen treatment (0 kg N ha^{-1}, 150 kg N ha^{-1}, and 300 kg N ha^{-1}). Like the LAM evaluation, sample order was randomised between sessions but served in the same order within a session. On receiving a sample, participants were first asked to select from the list of attributes which flavours they perceived in the sample. A definition list was provided in each booth for the participants' reference.

After selecting the attributes, a categorical scale from 1 (low) - 9 (high) was provided for each selected attribute, allowing participants to rate the intensity of the selected flavours perceived (Fig. 3.8). The order of the attribute list was randomised for each participant. An 'Other flavour' option was also provided in the attribute list to allow participants to record any attributes they perceived that were not listed. Additionally, a paper form was provided in each booth to provide participants with the opportunity to describe a perceived attribute that was not listed to minimise attribute dumping.

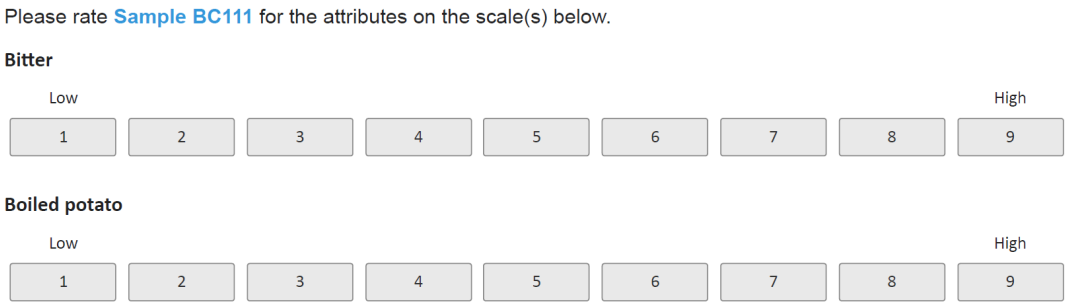


Figure 3.8: Screenshot of an example 9-point categorical RATA scale for 'bitter' and 'boiled potato'. Participants used this to rate the intensity of any perceived attributes.

3.2.4 Collection of analytical samples

After serving each quarter of a potato to the consumer, another quarter (including skin) was cut from the same potato and placed into a lidded stainless steel container sitting in a water bath set to 62 °C. At the end of each sensory test (LAM and RATA), the saved quarters were mashed into a bulk sample using a potato masher to create a homogenous mix of potato skin and flesh. From the bulk sample, two 70 mL polypropylene containers were filled with mashed potato and immediately frozen in liquid nitrogen. While most samples were frozen within 10 minutes of serving, overall, all samples were frozen within 30 minutes of serving and evaluation. Frozen samples were held in liquid nitrogen until the end of the final sensory session (approximately 4.30 pm). After this, the frozen samples were transported to Plant and Food Research and stored at -75 °C until analysis. As a bulk sample was saved from each session (24), nitrogen treatment (3), and potato variety (2), a total of 144 samples were collected and frozen for chemical analysis.

3.3 Composition analysis

3.3.1 Analytical sample preparation

From the sub-samples frozen for analysis, one pottle was freeze-dried (Labconco Freezone 2.5) and ground using a Sunbeam coffee grinder (Model EM0405). The ground freeze-dried samples were stored at room temperature for one month with desiccant, then stored in -20 °C until required for analysis. Sample mass before and after freeze-drying was recorded to calculate the percentage of dry matter. The other pottle of frozen sample was ground into a powder under liquid nitrogen using an IKA A-11 basic analytical mill, then stored at -75 °C until required for analysis.

3.3.2 Analysis of volatiles

2 g of the frozen ground potato powder and 2 g of sodium chloride (NaCl) were combined in a 20 mL glass headspace (HS) vial with a screw-top silicone septa cap in preparation for volatile analysis. All vials were stored at -75 °C until analysis. Immediately before analysis, samples were defrosted and brought to room temperature. The run order was randomised across all analytical samples.

Headspace solid phase microextraction gas-chromatography mass-spectrometry (HS-SPME-GCMS) analysis of volatile aroma compounds was carried out on a Shimadzu TQ-8050 GCMS (Kyoto, Japan) with a CTC PAL3 AOC-6000 autosampler (Zwingen, Switzerland) and GL Sciences B.V. Optic 4 Cryo-focuser (Eindhoven, The Netherlands). Prior to SPME sampling, the sample was allowed to equilibrate for 5 min at 60 °C in the CTC PAL3 incubator oven at an agitation speed of 250 rpm. Sampling was for 15 min at 60 °C temperature with a 65 μm DVB-PDMS SPME fibre (Supelco, Bellefonte, PA, USA). Splitless injections of 2.50 min were made at 240 °C onto a 20 m × 0.18 mm i.d. × 0.18 μm film Agilent J&W DB-WAX capillary column with a helium flow of 0.9 mL/min. The cryogenic trap was held at -120 °C for 165 sec before heating to 220 °C at 60 °C/sec. The GC oven temperature program was 2 min at 35 °C, 8 °C/min to 80 °C, and 20 °C/min to 240 °C, which was held for 8 min. Mass spectral analysis was carried out with an EI energy of 70 eV and a scan time of 0.1 s with a range of 40.5 to 500 m/z.

Targeted analysis on the resulting chromatograms was carried out, guided by a list of compounds known to be important in potato flavour compiled from previous published data. Volatile compounds were measured using selected ions and identified by comparison of mass spectra with those in the literature and in commercial mass spectral libraries (NIST Mass Spectral Search Program Version 2.0, John Wiley & Sons, Hoboken, NJ, USA) and comparison with alkane retention time standards. A total of 60 compounds were identified and integrated to determine area under peak for quantitative analysis. Three of these compounds were only tentatively identified. Due to changing retention times during the

run, the samples were processed in four separate batch files with slightly adjusted retention times. All identification and integration was carried out using Shimadzu Corporation GCMS Browser and Postrun LabSolutions GCMS solution (Version 4.11).

3.3.3 Analysis of amino acids and sugars

0.5 g of the freeze-dried samples were weighed out into plastic 50 mL Eppendorf Conical tubes and 25 mL of methanol/milli Q water/acetic acid [49:49:2 v/v/v] solvent was added. Samples were vortexed for 10 s, incubated overnight at 1 °C, then centrifuged at 3000 rpm for 10 min. For each sample, 20 μL of extract was pipetted individual 2 mL glass GC-MS vials. The vials were stored at -20 °C before GC-MS analysis. On the day prior to analysis, samples were first evaporated to dryness in a vacuum freeze-drier for 2 hours, then derivatised with 80 μL methoxylamine hydrochloride (MOX), made up at 20 mg mL^{-1} in anhydrous pyridine. The samples were then incubated for 1.5 hours while shaking, then 50 μL MSTFA (N-Methyl-N-(trimethylsilyl)trifluoroacetamide, Sigma-Aldrich) was added followed by further incubation with shaking for 30 min. Before analysis, the run order was randomised across all analytical samples.

Samples were separated and detected using GC-MS (Agilent 5977A). 1.1 μL of sample was injected into the GC using a PAL autosampler with a 10 μL syringe, cycle MACRO GC-Liq4-V3. The GC oven programme was set to the following: an initial temperature of 120 °C held for 0.5 min, then increased to 250 °C at 15 °C/min, with a further increase to 300 °C at 20 °C/min which was then held for 3 min. The carrier gas was helium at an inlet temperature of 250 °C. The injector mode was set to a 20:1 split, with a split flow of 24 mL/min, then after 6 min, 15 mL/min with a septum purge flow of 3 mL/min. The GC column was a Restek Rxi-5ms with 30 m × 0.25 mm internal diameter (ID) × 0.25 μm film thickness. The carrier gas constant flow was set to 1.2 mL/min and the Thermal Aux 2 transfer line between the GC and MS was held at 300 °C. The mass spec parameters were as follows: scan acquisition mode on MS, scan rate 9.6 scans/sec; solvent delay 4.2 min before scan start; scanning parameters between 33 and 500 m/z; MS source at 230 °C and MS quad at 150 °C.

Targeted identification and quantification of sucrose, glucose, fructose, citric acid, glutamine, glutamic acid, asparagine, and aspartic acid was carried out on the resulting chromatograms using authentic standards. Threonine, malic acid, GABA, quinic acid, myo-inositol, and galactose were identified by comparison with commercial mass spectral libraries (NIST Mass Spectral Search Program Version 2.0, John Wiley & Sons, Hoboken, NJ, USA). Targeted sugars and amino acids were quantified using the exact ion chromatograms and calibration curves created from standard reference sample run at the same time as the potato samples. Quantification was carried out using Agilent Technologies MassHunter Workstation Software Quantitative Analysis (Version B.06.00). Final concentrations were expressed as g kg^{-1} FW potato using the calculated dry matter results for each sample.

3.3.4 Analysis of polyphenols and glycoalkaloids

0.5 g of freeze-dried samples were weighed out into plastic 50 mL Eppendorf Conical tubes and 25 mL of methanol/milli Q water/acetic acid [49:49:2 v/v/v] solvent was added. Samples were vortexed for 10 s, incubated overnight at 1 °C, then centrifuged at 3000 rpm for 10 min before diluting for specific testing.

Polyphenols

A 1:9 dilution of extract and solvent was prepared by combining 900 μL of the same solvent (methanol/milli Q water/acetic acid) with 100 μL extract in a 2 mL plastic Eppendorf tube. Samples were vortexed and stored at -20 °C, then centrifuged for 10 min at 13,000 rpm. 800 μL of each extract was pipetted into individual 2 mL glass high-performance liquid chromatography (HPLC) vials which were stored at -20 °C until analysis. Before analysis, the run order was randomised across all analytical samples.

Samples were separated using HPLC. Separation was performed using a Phenomenex Luna Omega C18 column (2.1 × 100 mm, 1.6 μm), started in 100% acetonitrile, on a ThermoDionex Ultimate 300 equipped with a WPS-3000 TRS autosampler, a HPG-3400 RS pump, a SRD-3400 de-gaser and a TCC-3000 RS column oven. A multi-step solvent gradient was used, starting from 5% B (0 to 0.5 min), 5 - 15% B (0.5 to 4 min), 15 - 40% B (4 to 8 min), 40 - 95% B (8 to 11 min), 95% B (11 to 13 min), 95 - 5% B (13 to 13.2 min), 5% B (13.2 to 15 min), using a flow rate of 0.4 mL/min and an injection volume of 1 μL [solvent A, 0.2% formic acid, solvent B, acetonitrile]. The column oven temperature was held at 40 °C.

Electro-spray ionisation mass spectrometry (ESI-MS) was conducted using a micrOTOF-QII mass spectrometer (Bruker, ESI-Qq-TOF). The parameters were set to the following: dry temperature of 225 °C, drying N2 gas with a flow rate of 6 L/min, nebuliser N2 at 1.5 bar, endplate offset of -500 V, and a mass range of 100 to 1000 m/z. Negative ion electrospray mode was used with the capillary voltage set to +3500 V. The resulting chromatograms were calibrated using sodium formate, delivered by a syringe pump at the beginning of each run.

The data were processed and quantified using Bruker TASQ Version 2.1.22.1. Chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, and quercetin 3-rutinoside were quantified using exact ion chromatograms (EICs) from the HPLC-MS data of each sample and calibration standards. The concentration of chlorogenic acid and quercetin 3-rutinoside was calculated from calibration curves of authentic standards, neochlorogenic acid and cryptochlorogenic acid quantified using the calibration curve of chlorogenic acid. Final concentrations were expressed as mg kg^{-1} FW potato using the calculated dry matter results for each sample.

Glycoalkaloids

A 1:19 dilution of extract and solvent was prepared by combining 950 μL of the same solvent (methanol/milli Q water/acetic acid) with 50 μL extract in a 2 mL plastic Eppendorf tube. Samples were vortexed and stored at -20 °C, then centrifuged for 10 min at 13,000 rpm. 800 μL was pipetted into 2 mL glass HPLC vials which were stored at -20 °C until analysis. Before analysis, the run order was randomised across all analytical samples.

Samples were separated using high-performance liquid chromatography (HPLC). Separation was performed using an Agilent Zorbex SB-C18 RRHD column (2.1 × 150 mm, 1.8 μm), started in 100% acetonitrile using the same HPLC equipment as described for polyphenol analysis. A multi-step solvent gradient was used, starting from 25% B (0 to 0.5 min), 25 - 50% B (0.5 to 5 min), 50 - 100% B (5 to 6 min), 100% B (6 to 9 min), 100 - 25% B (9 to 9.2 min), 25% B (9.2 to 14 min), using a flow rate of 0.35 mL/min and an injection volume of 1 μL [solvent A, 0.2% formic acid, solvent B, acetonitrile]. The column oven temperature was held at 40 °C.

Detection was carried out using the same ESI-MS parameters and equipment as described for polyphenol analysis. However, positive ion electrospray mode was used instead, with the capillary voltage set to +3000 V. The resulting chromatograms were calibrated using sodium formate, delivered by a syringe pump at the beginning of each run. The data were processed and quantified using Bruker TASQ Version 2.1.22.1. α-solanine and α-chaconine were quantified using EICs from the HPLC-MS data of each sample and calibration standards and their concentrations were calculated from calibration curves of authentic standards. Due to slight variations in retention times during analysis, the samples were processed and quantified in two batches. Final concentrations were expressed as mg kg^{-1} FW potato using the calculated dry matter results for each sample.

3.3.5 Analysis of total nitrogen and nitrate

Analysis of total nitrogen and nitrate content was sub-contracted to Hill Laboratories in Hamilton due to time and resource constraints in Palmerston North. Additionally, only 72 out of 144 samples could be tested, therefore a random selection of ground freeze-dried samples from each variety and treatment were chosen for testing. Total nitrogen % was determined using Dumas combustion, with a default detection limit of 0.1%. Nitrate was determined using 2% acetic acid extraction followed by salicylate colorimetry (or Cd reduction) and NED colorimetry. The default detection limit for nitrate testing was 100 mg kg^{-1} sample.

3.4 Data analysis

The majority of the initial statistical analysis was outsourced to a statistical student consultant at Massey University. Initial analyses were conducted by the consultant in RStudio (Boston, MA, Version 1.1.447 for desktop), then further post hoc testing was carried out by the author where required, in both RStudio and XLSTAT by Addinsoft (Annual Version 2019.3.1 SENSORY 61027). Valuable statistical assistance was also provided by The New Zealand Institute for Plant and Food Research.

Given the split plot design of the field trial (Fig. 3.3 and Fig. 3.2), the varietal plots were analysed as separate trials. Therefore, generalised linear models (GLMs) and linear mixed-effect models were used for most of the analyses, instead of ANOVA. For the field data and sensory analysis, treatment effects were analysed within each variety. For composition analysis, the samples were analysed as six variety-nitrogen treatments, AN 0 N, AN 150 N, AN 300 N, AS 0 N, AS 150 N, and AS 300 N, where AN = Annabelle and AS = Andean Sunside. For all analyses where more than one variable was tested at a time, a Bonferroni-adjusted p-value was used at $\alpha = 0.05$ to correct for the family-wise error rate, unless otherwise specified. For any data summarising (e.g. Principal Component Analysis [PCA]), data from both varieties were included in analyses. Microsoft® Excel® for Office 365 (Version 1910) was used for spreadsheet management.

3.4.1 Agronomic performance and field data

ANOVA in XLSTAT (best model selection), using nitrogen treatment and field replicate as explanatory variables, was used to assess significant differences between the three nitrogen treatments, within each variety, for tuber count, average tuber mass (g), tuber diameter (mm), and predicted field yield (t ha^{-1}). Tukey's (HSD) method for multiple comparisons was used to assess the pairwise comparisons between treatments. The varieties were analysed separately for this data set because the grower was not interested in comparing varietal agronomic performance, only the effect of the treatments.

3.4.2 Nitrogen treatment effects on composition

Generalised linear models (`glm`) were used in RStudio to assess whether the various analytical variables had different average values for the varieties and nitrogen treatments (together), applying Bonferroni's adjustment to the p-value. Tukey's method for multiple comparisons (`glht`) was used to assess the pairwise comparisons between the treatments. PCAs (Pearson) were used to visually summarise the datasets for non-volatile and volatile variables. The missing data for nitrate were censored and any non-detected values were replaced with 0.5 × detection limit. Given ~75% of nitrate values for Andean Sunside had to be censored, nitrate was excluded as a variable from the PCAs conducted.

3.4.3 Variation in sample liking between nitrogen treatments

A scattergram-boxplot was generated in XLSTAT/Excel to display the differences in liking between nitrogen treatments, within each variety. Linear mixed-effects models (`lme`) in RStudio was used to assess significance between nitrogen treatments, within each variety. The continuous liking scores were converted to ranked data within each variety and a non-parametric Kruskal-Wallis test in XLSTAT was applied to assess significant differences between ranking scores for each nitrogen treatment within each variety.

3.4.4 Cluster analysis on consumer acceptance

In XLSTAT, agglomerative hierarchical clustering (AHC) was used to cluster the labelled affective magnitude scores (LAM) scores, using Ward's method, centring, reduction, automatic entropy truncation, and squared Euclidean distance for dissimilarity, on Annabelle and Andean Sunside separately. The cluster results were presented on a dendrogram and as bar charts.

3.4.5 Nitrogen treatment effects on flavour perception

The attribute intensity rating data were analysed using two different approaches. In the first approach, across all participants, any missing checks for an attribute were replaced as an intensity score of 0, as suggested in Meyners et al. (2016). In the second approach, the data were censored, whereby the data for participants that did not check an attribute across all three nitrogen samples were replaced with an 'NA' (for that attribute and variety only). If a participant checked and rated an attribute for one or two samples out of three, the non-checked samples were replaced with a 0 intensity score. Using these two approaches, linear mixed effects models (`lmer`) in RStudio were used to assess whether changing the rate of nitrogen applied significantly affected flavour attribute intensity in Annabelle and Andean Sunside. Least squares means (`ls_means`) was used to assess the pairwise comparisons between nitrogen treatments. The average intensity ratings, using the second method, were summarised using PCA biplots in XLSTAT (Pearson), including both varieties and all nitrogen treatments.

3.4.6 Assessing the associations between composition and flavour

PCAs (Spearman) in XLSTAT were used to display and observe the associations between composition and flavour, using both Annabelle and Andean Sunside data, presented as PCA biplots. Using averaged values, the PCAs were split into non-volatile and volatile variables. Only data from the second RATA data approach (discerning participants only) were used for PCA.

Chapter 4

Results & discussion: Part 1

Trial performance, agronomic effects, and challenges and limitations of sensory, analytical, and statistical methods

4.1 Field trial review

The field trial was managed by the Matamata staff from A.S. Wilcox & Sons. The student (author) carried out and controlled most major trial activities, including planting, fertiliser application, crop walks, and harvesting. However, given the trial's distance from Palmerston North and its location within a commercial horticultural setting, a number of factors could not be monitored as closely as desired. While both trial plots for Annabelle and Andean Sunside produced a successful crop, suitable for analyses in this study, several aspects of the trial that may have influenced later results need to be addressed.

4.1.1 Andean Sunside

The Andean Sunside trial plot was planted first, within a commercial Andean Sunside crop. Therefore, due to space limitations, half of field rep 2 (Fig. 3.2) lay in the centre pivot path - only half of the bed could be planted. This may have affected the yield of this replicate because the centre pivot damaged the edge of these plants. This also created a second edge effect, affecting row closure and creating an opportunity for weeds to grow. As the potato seed tubers were old, a portion of the Andean Sunside seed tubers were beginning to rot when planted. While the best seed was selected for planting, many gaps were observed after emergence, indicating that many seed tubers failed to sprout. Like the pivot row, this created gaps for weeds to proliferate, contributing to the weed pressure observed (Fig. 3.4, Chapter 3). The pre-emergence herbicide was also applied two weeks later than planned. Although the trial was hand-weeded on a couple of occasions, the weed pressure was significant. This very likely affected the crop's yield performance, tuber size, and tuber numbers

(Nelson and Thoreson, 1981). It may have also affected the uptake of the side-dressing fertiliser (applied 26.12.2018) as the weeds were already well-established. During harvest, the tubers were noted as very variable in size between replicates of the same treatment. A lot of tuber greening was also observed - this could have been due to the manual planting method. Machine planting generally places seed tubers deeper in the soil. Because of the large size variability, it was difficult to select consistently-sized tubers for sensory analysis.

4.1.2 Annabelle

The Annabelle trial plot was planted in early December, within a commercial Annabelle potato crop. The site was well-drained and on-top of a ridge, therefore ideally situated. The centre pivot did not run through the plot. As the surrounding crop was planted much earlier than the trial, the replicates on the edge (Fig. 3.3) did not have adjacent potato plants from approximately 45 DPP onwards. Tubers harvested from these plots may have been bigger as the plants were less shaded on one side, improving light interception and reducing competition for space and nutrients (Zhongmin and Guang, 1990). Although a gap was left in the middle of the trial, the large establishment of willow weed (Fig. 3.5) would have likely prevented a second edge effect from occurring in the two middle replicates. The Annabelle seed was very clean and healthy, and the trial emerged very well. However, due to uncontrollable circumstances, the side-dressing was applied six days late, 49 DPP instead of 43 DPP to match Andean Sunside. However, the potato crop managers at A.S. Wilcox & Sons, experienced with fertiliser trials, did not think this small delay would impact nitrogen uptake and ability to compare results between the two varieties (B. Hart, personal communication, January 23, 2019). During harvest, tuber moth (*Phthorimaea operculella*) damage was observed in several blocks, especially those with 0 kg N ha^{-1} applied. Pest damage is known to increase glycoalkaloid levels (Valkonen et al., 1996).

4.2 The effect of nitrogen on agronomic performance

Trial performance and the effect of nitrogen on agronomic parameters was gauged by measuring tuber count, average tuber diameter (mm), tuber mass (g), and predicted field yield in t ha^{-1} for each variety and nitrogen treatment, across 8 field replicates (Table 4.1). Overall, Annabelle yielded ~46 t ha^{-1} and Andean Sunside yielded ~29 t ha^{-1}. Varying the level of nitrogen fertiliser had no effect on any agronomic parameters for Andean Sunside. For Annabelle, tuber count and tuber mass (g) were significantly affected by the rate of nitrogen applied. The potatoes in treatment Annabelle 0 kg N ha^{-1} were significantly lighter in mass (by ~10 g) compared to the potatoes treated with 150 and 300 kg N ha^{-1}. Tuber count significantly decreased between 0 kg N ha^{-1} and 150 kg N ha^{-1}, from an average of 270 tubers per sample dig to 241 tubers.

Table 4.1: Predicted field yield (t ha^{-1}), average tuber count, tuber diameter (mm), and tuber mass (g) across variety and nitrogen treatments. The values provided are an average of 8 field replicates, using data collected from 3 m × 0.86 m (2.58 m^2) sample digs (one block). The units for the nitrogen treatment codes are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

Variable	**Annabelle**			**Sig.**[1]	**Andean Sunside**			**Sig.**[1]
	0 N	150 N	300 N		0 N	150 N	300 N	
Predicted field yield (t ha^{-1})	42.96	47.31	48.59	ns	26.74	30.12	30.81	ns
Tuber count	270^{b}	241^{a}	246^{ab}	*	168	203	209	ns
Tuber diameter (mm)	36.02	38.07	37.81	ns	36.82	36.25	36.49	ns
Tuber mass (g)	41.9^{a}	52.1^{b}	52.0^{b}	***	48.9	46.0	45.1	ns

[1.] Significance codes: ns - not significant, * p<0.05, ** p<0.01, *** p<0.001

^{ab} denotes significant pairwise comparisons using Tukey (HSD) at α level = 0.05.

Increasing the rate of nitrogen supply typically stimulates tuber growth, mass, and overall yield (Mack and Schjoerring, 2002). An increased nitrogen supply will initially increase yield and tuber mass because larger potato haulms (tops) can generate more photosynthates (Kumar et al., 2007), which are translocated to potato tubers for carbohydrate storage. Nitrogen supply between crop emergence and full cover is especially important, additional applications potentially speeding this process up, therefore extending the period of time the crop is at maximum light interception (Vos, 2009). For example, when Kumar et al. (2007) increased the level of nitrogen applied to Indian processing potatoes from 0 to 270 kg N ha^{-1}, total yield increased from 17.2 to 39.2 t ha^{-1}. However, yield does not increase linearly with nitrogen - in general, yields will only increase up to a point. In Zelalem et al. (2009), total yield for cv. 'Gabriella' increased up to 138 kg N ha^{-1}, while Ruža et al. (2013) found no further yield increase (for both early and late varieties) after only 120 kg N ha^{-1}. Excessive nitrogen rates instead stimulate vegetative growth in the potato tops at the expense of translocating photosynthates into the tubers (Goffart et al., 2008), thus diverting any further positive impact on tuber bulking and final yield.

In this study, increasing the rate of nitrogen fertiliser applied only affected the tuber mass and count of Annabelle samples - no other agronomic parameters were significantly affected. A large amount of variation between the field replicates may have resulted in the lack of statistical significance between treatments, for yield. Additionally, the seed 'misses' (failed seed) and weed pressure in the Andean Sunside trial would have reduced overall yield (Nelson and Thoreson, 1981), overwhelming any potential effects from the nitrogen treatments. This is supported by yield data from A.S. Wilcox: average commercial Andean Sunside yields in Matamata for the same season were 45 t ha^{-1}, approximately 15 t ha^{-1} more than this study (B. Hart, personal communication, February 3, 2020). In addition, the absence of a severe yield effect (for treatments with 0 kg N ha^{-1}) could be linked to the existing

quantity of nitrogen in the soil. Pre-planting soil tests indicated both plots had 44-48 kg ha^{-1} of potentially available nitrogen (Appendix A). This may have been sufficient for both crops, therefore contributing to the especially large yields for Annabelle, where commercial crops generally yield around 30 t ha^{-1} (B. Hart, personal communication, February 3, 2020).

A decrease in tuber count, with an increase in nitrogen application, was observed for Annabelle. This is supported by Mack and Schjoerring (2002), who found tuber number also decreased with increasing nitrate supply in a pot experiment. However, a greater number of studies suggest that increasing the rate of nitrogen applied may improve tuber counts. In Kumar et al. (2007), the number of tubers suitable for processing significantly increased between 0, 90, and 180 kg N ha^{-1} applied. Zelalem et al. (2009) found that increasing nitrogen applications from 0 to 207 kg N ha^{-1} approximately doubled the number of marketable 'Gabriealla' potato tubers in Ethiopia. Differences in variety or growing conditions (i.e. weed pressure) could affect the results obtained, especially for Andean Sunside, where no effect was observed for count.

4.3 Sensory trial participant profile

4.3.1 Consumer demographic information

A wide range of participants were recruited for the sensory evaluation, with the participant age range fairly balanced between 18 and 65 years old (Table 4.2). The average participant age was 39.6 years. The biggest age group was 18 - 28 years, likely due to the recruitment process being focused on Massey University staff and students. 71% of participants were female and 29% were male. No other demographic information was collected.

Table 4.2: The age range of participants involved in the sensory evaluation.

Participant age (years)	**Count**
18 - 28	30
28 - 38	24
38 - 48	20
48 - 58	24
58 - 65	13
Total participants	111

4.3.2 Potato consumption habits

Regularity of consumption

In addition to sampling potatoes for the sensory evaluation, participants were asked to answer two questions relating to their potato consumption habits. The first question, "How often do you eat boiled, mashed, steamed, and roast potatoes?" was asked to ensure the participants involved in the evaluation were regular consumers of home-cooked potatoes, as opposed to fried potato products (e.g. crisps). Fig. 4.1 shows that over half the participants (63 people) consumed cooked potatoes 2-4 times a week. Overall, 97% of participants consumed home-cooked potatoes at least once a week. While potato consumers were targeted for recruitment, data from Potatoes New Zealand (2013) suggests this statistic is reflective of nationwide potato consumption, where 94% of New Zealand households in 2013 prepared fresh potatoes at least once a week (more recent data are unavailable). This indicates nearly all participants were very familiar with consuming potatoes and therefore should have a good awareness of what flavours to expect in cooked potatoes.

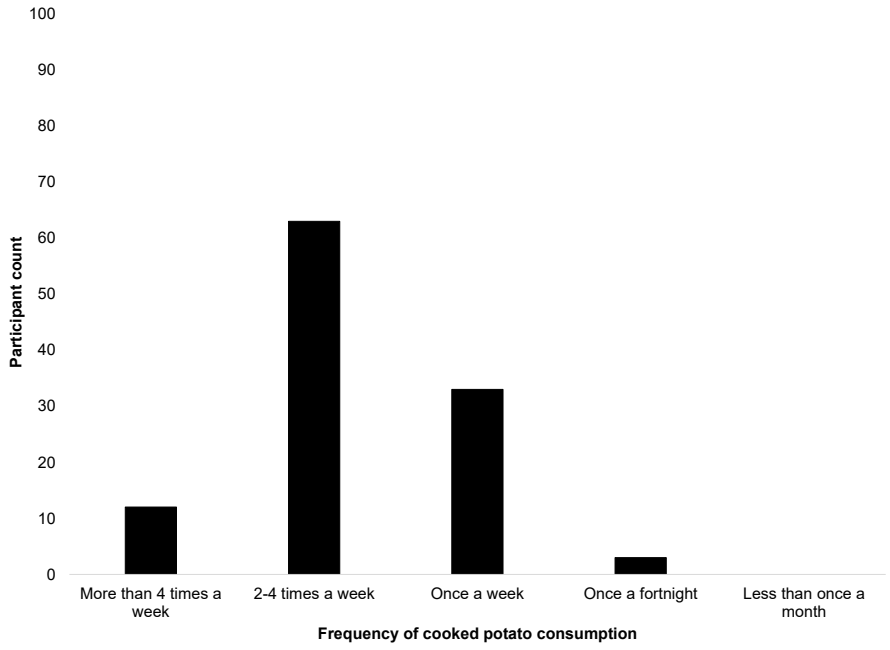


Figure 4.1: The regularity participants (n = 111) consumed home-cooked (boiled, mashed, steamed, and roast) potatoes. Participants that selected 'less than once a month' as an answer to the question were removed from the study.

What category of potatoes do participants consume?

Participants were also asked to indicate what category of potatoes they consume, using tick all options that apply. Participants were instructed to tick 'gourmet' if they purchased and consumed a particular product or variety based on its flavour, including the variety 'Agria', which is very popular in New Zealand. Fig. 4.2 indicates over 70 of the 111 participants consumed 'gourmet' or speciality potatoes, closely followed by 68 participants selecting 'loose' and 57 participants selecting 'bulk bags'. Given the varieties grown for this study are available as gourmet potato products in supermarkets, it was a success to see that over half of all participants were the target consumers for these products. A substantial number of participants consumed 'home-grown' potatoes, 33 in total (29.7% of participants) (Table 4.2). This result for home-grown potatoes was initially unexpected, as Palmerston North, while situated in a rural region, is an urban city. However, surveys conducted across households in Australia suggest approximately 39% of households grow their own vegetables (which may or may not include potatoes) (Wise, 2014). Similar figures could be applicable to New Zealanders. Unfortunately, survey data for New Zealand could not be found.

A relatively high proportion (60.8%) of the recruited participants consumed gourmet and loose potatoes over bulk bags. This could be reflective of the recruitment process initially targeting gourmet potato consumers. However, it may also indicate the shift in supermarket shopping and consumption habits, where consumers look to purchase smaller and more-convenient quantities of potatoes over the traditional 10 kg bags. Additionally, the largest participant age category was 18 - 28 years (Table 4.2), this group likely including many students and young professionals because the recruitment advertising was focused in and around Massey University. This age group may potentially shop for and consume potatoes in smaller quantities, instead of needing bulk potatoes to feed a big family. Consumer insights from Countdown New Zealand, presented at the Potatoes NZ 2019 conference (Christchurch, New Zealand), indicated that loose potatoes are often purchased with other loose root vegetables, and as part of a planned meal. In comparison, pre-packed (i.e. bulk bags) potatoes are often purchased with other budget-friendly supermarket items such as value meat products, perhaps shopping for a family-sized meal. These insights help to illustrate the ways different groups of consumers shop, and how different categories of fresh potato products are utilised.

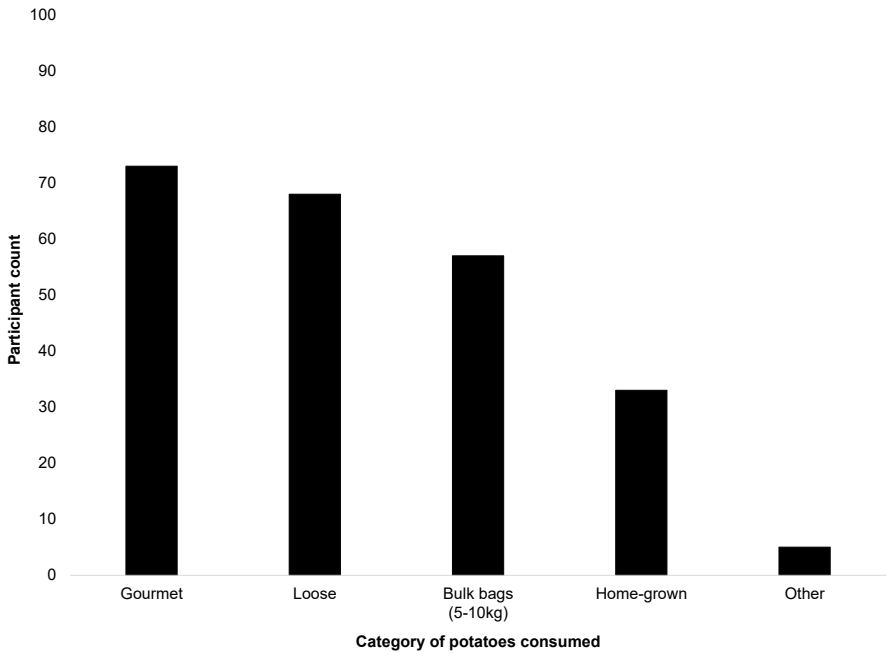


Figure 4.2: The categories of potatoes participants consumed (n = 111), where 'gourmet' potato products include speciality and boxed potatoes and/or flavour-differentiated varieties.

4.4 Sensory trial: Challenges and limitations

4.4.1 Trial design

For all participants, Annabelle was served in the first session, and Andean Sunside in the second session (Table 3.3). This design was decided on in case harvesting issues prevented one variety from being harvested. In that case, at least all participants would be served the same variety. Because of this field split plot design, as described in Chapter 3, variety, field replicate, and nitrogen effects could not be separated out as factors using ANOVA. Instead, any nitrogen treatment effects were analysed within each variety using linear mixed-effect models, the varieties treated as separate sensory trials. Any field replicate effects were disregarded for the rest of the trial. Therefore, any potential differences in flavour perception between Andean Sunside and Annabelle could not be compared.

4.4.2 Incorrect formatting in LAM scale

The sensory evaluation was set up and run using Compusense® on iPads. However, about half way through the second day of sensory testing (Wednesday), an error in the LAM scale design was observed. While the axis tick marks were correct, several of the labels were repeated on the scale (e.g. two 'like slightly' labels). The scale was fixed in time for the Wednesday 3.30 pm session and no further issues occurred for the rest of the evaluation. If the participants were very focused on selecting the sample liking position based off the scale labels, liking scores for Annabelle may have been affected. Therefore, the continuous liking data were converted into ranked data as it was assumed participants would have positioned samples on the scale in an order best reflecting their preference for the samples. When participants were told about the error at their second session, few appeared to have noticed it in their first session.

4.4.3 Inconsistent sample serving size

Due to the nature of working with a horticultural product with unavoidable variability, the sample serving size was inconsistent. This was especially prevalent for Andean Sunside, where many tubers with greening and blemishes had to be removed from the samples allocated for sensory testing. As discussed in Chapter 3, the average tuber mass used for serving was 90.2 g for Annabelle and 81.4 g for Andean Sunside. This equates to a serving size (one quarter) of 22.5 g and 20.3 g for Annabelle and Andean Sunside respectively. However, in some sessions, tubers weighed 44.4 g, half the average served tuber mass. This caused some indecision during the sensory evaluation around keeping the same serving presentation (i.e. a single quarter), or providing similar overall quantities of sample. In some cases, half a potato was served for the very small Andean Sunside tubers to ensure participants had sufficient sample to evaluate. Serving identically-sized samples at the same temperature and same shape is recommended practise for sensory evaluations (Issanchou, 2018). In this study, these recommendations were not met.

Additionally, Andean Sunside tubers from each treatment were grouped by size for each session because of the large variability in tuber size. For example, the smaller tubers from each treatment were allocated to the morning (8.30 am and 9.00 am) sessions and the larger tubers allocated to the lunchtime and afternoon sessions (12.30 pm and 2.00 pm). This decision was made to ensure participants received consistently-sized quarters within their session for each treatment. This was deemed to be preferable over serving a selection of random quarters where size may have varied considerably between treatments, potentially influencing perception. The average tuber mass was recorded for each treatment, and is available in Appendix B, Table B.1.

4.4.4 Serving temperature of potato quarters

For the sensory evaluation, a timed serving schedule was established to ensure the potato quarters were served around 60-65 °C. Using pilot experiments (data not included), this temperature was determined to provide the best balance of heat at a comfortable level and clear flavour perception. The samples also had to be cool enough to avoiding burning the participants. However, as participants were sometimes late, and not all participants proceeded through the evaluation at the same speed, the samples may have been cooler at the time of evaluation for several sessions. One participant commented on the variability in temperature of their samples. In a study found investigating panellist sensory perception and serving temperature in potatoes, there was no difference found in total impression and off-odour between peeled steamed potatoes served at 60 °C, 75 °C, and 90 °C (Lundgren et al., 1979). However, temperature in general is known to influence the perception of most basic tastes (Kapaun and Dando, 2017) - this very likely could have affected consumer-perceived flavour and the samples' flavour profiles. In future experiments of this nature, serving temperature must therefore be better-controlled.

4.4.5 Bitterness of some Annabelle samples

Two participants commented on the bitterness of their Annabelle samples served in their first session, indicating it created a lingering aftertaste that transferred onto the following samples. After this feedback, the participants (in this session only) were provided with water crackers to cleanse their palette in-between samples, in addition to the filtered water. The crackers were provided again for same participants in their second session. No other comments were received.

4.5 Limitations of analytical variables measured

4.5.1 Total nitrogen and nitrate content

As the testing for total nitrogen and nitrate content could not be carried out in Palmerston North, it was outsourced to Hill Laboratories in Hamilton. However, this meant only half the number of analytical samples could be tested - 72, instead of 144. While this was not an issue for investigating the significant differences between samples because each variety-nitrogen treatment corresponded to 12 analytical replicates, it created complications for data analysis. As the data for all other composition and metabolite testing were collected for 144 analytical samples, the nitrate and total nitrogen data could not be integrated into the models used for statistical testing. Additionally, nearly all Andean Sunside samples tested for nitrate were below the instrument detection limit. As the values had to be censored, this limited the use of the nitrate data in further analyses.

4.5.2 Time constraints for umami compound testing

Previous literature on potato umami flavour has determined the concentration of umami-flavoured compounds by using measuring both umami amino acids and 5'-ribonucleotides (Morris et al., 2007). According to Yamaguchi et al. (1971), a weighted equation for these compounds can help to determine equivalent umami concentration, based on the synergy between the amino acids glutamate (Glu) and aspartate (Asp), and the ribonucleotides guanosine 5'-monophosphate (5'-GMP) and adenosine 5'-monophosphate (5'-AMP). In addition to using the measured concentrations of each metabolite, relative umami weightings are also factored into the equation, where Glu = 1, Asp = 0.077, 5'-GMP = 2.3, and 5'-AMP = 0.18. Based off these weightings, 5'-GMP contributes the most umami taste in potatoes. However, due to time and resource constraints, the 5'-ribonucleotides could not be measured. This reduced the capacity to link umami-related sensory attributes (e.g. savoury, sweet flavour) to composition because the strongest contributor to umami flavour was not measured. Additionally, Morris et al. (2007) clearly shows how varieties from group Phureja exhibit a significantly higher equivalent umami concentration. As Andean Sunside has group Phureja parentage, this trial constraint is somewhat of a missed opportunity to further explore the association between umami-flavoured metabolites and sensory-perceived flavour.

Chapter 5

Results & discussion: Part 2

Nitrogen effects on analytical and sensory variables in Annabelle and Andean Sunside

5.1 Nitrogen and variety effects on composition

5.1.1 Total nitrogen and nitrate content

The total nitrogen (N) and nitrate (NO_3^-) content in ground and freeze-dried flesh and skin samples, for each treatment, is provided in Table 5.1. Each value is an average of 12 analytical samples. Tukey's method was applied for multiple pairwise comparisons between varieties and treatments. In Fig. 5.1, the change in total N and nitrate content (mg g^{-1} FW) for each variety was plotted, the error bars representing confidence intervals (2.5% and 97.5%). For Andean Sunside, as the large majority of the nitrate readings returned were below the instrument detection limit, these values were substituted with 0.5 × the detection limit, hence the very small effect size.

In general, both total N and nitrate content increased with the rate of nitrogen fertiliser applied, excluding the Andean Sunside nitrate content. For both Annabelle and Andean Sunside, the total N content of both 150 N and 300 N treatments were significantly higher than the 0 N treatments. At each nitrogen application level, total N was significantly higher in Andean Sunside compared to Annabelle - this is clearly visible in Fig. 5.1. At 300 kg N ha^{-1}, Annabelle and Andean Sunside samples contained 1.69% and 1.88% total N respectively. In Annabelle, the nitrate content in both fresh (FW) and dry (DW) samples significantly increased with each nitrogen application, 300 N samples containing over 3 times the nitrate content compared to the 0 N samples, at 252.83 mg 100 g^{-1} DW and 0.46 mg g^{-1} FW. After accounting for average tuber mass, the nitrate content between Annabelle 150 N and 300 N did not significantly vary. Increasing the rate of nitrogen fertiliser from 0 kg N ha^{-1} to 300 kg N ha^{-1} had no effect on the Andean Sunside tuber nitrate content.

Table 5.1: Average total nitrogen and nitrate content (in dry weight, fresh weight, and per tuber) in the ground skin and flesh analytical samples for each variety and nitrogen treatment. The units for the nitrogen treatment codes are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

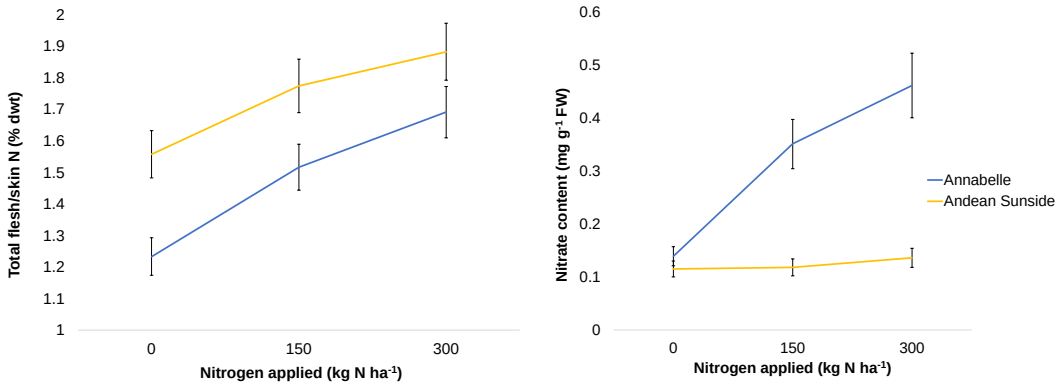
Variable	**Annabelle**			**Andean Sunside**			**Sig.**[2]
	0 N	150 N	300 N	0 N	150 N	300 N	
Total nitrogen (N) (%)	1.23^{a}	1.52^{bc}	1.69^{cd}	1.56^{bc}	1.78^{de}	1.88^{e}	V+N
Nitrate[1] (NO_3^-) (mg 100 g^{-1} DW*)	72.58^{b}	187.67^{c}	252.83^{d}	53.75^{a}	53.75^{a}	63.25^{ab}	V+N
Nitrate (NO_3^-) (mg g^{-1} FW*)	0.14^{a}	0.35^{b}	0.46^{c}	0.12^{a}	0.12^{a}	0.14^{a}	V+N
Nitrate (NO_3^-) (mg in average tuber (g))	12.03^{a}	31.47^{b}	43.33^{b}	9.87^{a}	10.51^{a}	9.13^{a}	V+N

^{abcde} denotes significant differences using Tukey's multiple pairwise comparisons using $\alpha = 0.05$.

1. Nitrate values (for Andean Sunside) below the instrument detection limit were substituted with 0.5 × detection limit.

2. At $\alpha = 0.05$, V - significant differences between varieties, N - significant differences between nitrogen treatments.

* DW denotes dry weight (freeze-dried sample) and FW denotes fresh weight (calculated back from dry matter %).



(a) Total nitrogen (%)

(b) Nitrate (mg g^{-1} FW)

Figure 5.1: Total nitrogen % (a) and nitrate (b) content in Annabelle and Andean Sunside, with increasing rate of nitrogen fertiliser applied. Error bars are confidence intervals (2.5% and 97.5%).

An increase in total tuber N and nitrate content with increasing nitrogen fertiliser application is commonly observed for potatoes. In Belanger et al. (2002), total N content in cv. 'Shepody' increased almost linearly up to 100 kg N ha^{-1} (approx 1.4%), then very slightly levelled off, reaching 1.7% N with 250 kg N ha^{-1} applied. The total N observed in Belanger et al. (2002) samples is similar to the values recorded in this study. There was also a significant cultivar difference between Shepody and Russet Burbank in Belanger et al. (2002), as observed in this trial between Andean Sunside and Annabelle. In Carter and Bosma (1974), a linear relationship between nitrogen applied and total N% was recorded. Augustin et al. (1977), Belanger et al. (2002), and Carter and Bosma (1974) all found that tuber nitrate content increased with nitrogen fertiliser application, as was observed for Annabelle (Fig. 5.1).

Using partial least squares regression, Thybo et al. (2006) found significant positive correlations between both total N and nitrate with off-flavour. The significant increases in total N and nitrate content for Annabelle especially could increase consumer perception of off-flavours (see Section 5.2). To note, concerns described in Section 2.4 about the influence of weed pressure on Andean Sunside side-dressing uptake appear unfounded, given the large and significant increases in total N observed (Fig. 5.1).

In general, potato nitrate levels range between 50 and 250 mg kg^{-1} FW (0.05 - 0.25 mg g^{-1}) (Gislason et al., 1984; Lachman et al., 2011; Lin et al., 2004; Mozolewski and Smoczynski, 2004). However, levels can be significantly affected by variety, growing region, and seasonality (Lachman et al., 2011). In this study, the nitrate levels of 150 N and 300 N Annabelle samples were comparatively quite high, especially compared to Andean Sunside (Fig. 5.1). This could be related to differences in nitrate reductase content. As most nitrate reductase is found in the leaves, an actively-grown haulm is required for nitrate assimilation (Westermann et al., 1988). A combination of varietal variation and stress on the Andean Sunside trial could help to explain the lack of detectable nitrate in Andean Sunside tubers. The average nitrate levels in these samples (Annabelle 150 N and 300 N) actually exceed the recommended maximum content (200 mg kg^{-1}) set by some countries (Gorenjak et al., 2013). However, the WHO recommended nitrate intake is based off body weight and overall daily consumption across all foods, and other vegetables such as beetroot can contain up to 2220 mg kg^{-1} (Thomson et al., 2007) - these potatoes should not pose a health risk.

5.1.2 Dry matter, sugars, and total sweetness

The key flavour-related carbohydrate metabolites in potatoes, in addition to dry matter content, were measured across all three nitrogen treatments for Annabelle and Andean Sunside (Table 5.2). In addition, a total sweetness index (TSI) was calculated to predict sample sweetness, based off the sweetness intensities of sucrose, glucose, and fructose (Magwaza and Opara, 2015). Fig. 5.2 is an example GC-MS chromatogram that was used for the integration and quantification of these metabolites. Significant varietal and nitrogen effects were recorded for dry matter, glucose, and fructose content, while sucrose and predicted sweetness (TSI) significantly differed between varieties (Table 5.2).

Nitrogen fertilisation significantly affected dry matter in Annabelle, but not Andean Sunside (Table 5.2). Dry matter significantly decreased between 0 kg N ha^{-1} and 300 kg N ha^{-1} treatments in Annabelle, from 19.26% to 18.30%. Andean Sunside samples contained approximately 2-3% more dry matter compared to Annabelle. In potatoes, a reduction in dry matter with increasing rates of nitrogen fertiliser is commonly observed (Belanger et al., 2002; Laboski and Kelling, 2007; Öztürk et al., 2010), although other research has also reported increases in dry matter with an increasing supply of nitrate (Mack and Schjoerring, 2002). Because nitrogen stimulates vegetative growth, at very high rates of nitrogen supply,

shoot growth is favoured over tuber growth (Mack and Schjoerring, 2002). In comparison, very low levels of nitrogen will limit canopy development and photosynthate accumulation in the tubers (Kumar et al., 2007). The crop must otherwise obtain its nitrogen from the soil (soil mineral N), which is normally insufficient to achieve targeted yields (Vos, 1999). In this case, because the absence of nitrogen fertiliser reduced tuber mass in Annabelle (Table 4.1), this may have contributed to the higher dry matter % overall. In Andean Sunside, because the nitrogen treatments had very little effect on the main crop agronomic and quality attributes, the lack of impact on dry matter content is not unexpected - it appears Andean Sunside tubers were unaffected by the changing nitrogen treatments.

Table 5.2: Dry matter and carbohydrate content identified and quantified in the variety and nitrogen treatments. The units for the nitrogen treatments are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

Variable	**Annabelle**			**Andean Sunside**			**Sig.**[2]
	0 N	150 N	300 N	0 N	150 N	300 N	
Dry matter (%)	19.26^{b}	18.76^{ab}	18.30^{a}	21.36^{c}	21.69^{c}	21.70^{c}	V+N
Glucose (g kg FW^{-1})	4.21^{c}	3.62^{c}	3.66^{c}	0.51^{b}	0.32^{a}	0.27^{a}	V+N
Fructose (g kg FW^{-1})	3.52^{c}	3.11^{c}	3.07^{c}	0.49^{b}	0.31^{a}	0.27^{a}	V+N
Sucrose (g kg FW^{-1})	1.60^{a}	1.45^{a}	1.50^{a}	4.14^{b}	3.93^{b}	3.83^{b}	V
TSI[1]	10.07^{b}	8.87^{b}	8.89^{b}	5.26^{a}	4.64^{a}	4.44^{a}	V

^{abc} denotes significant differences using Tukey's multiple pairwise comparisons at $\alpha = 0.05$.

1. TSI (total sweetness index) = (1.00 × [sucrose])+(0.76 × [glucose])+(1.50 × [fructose]) (Magwaza and Opara, 2015).

2. At $\alpha = 0.05$, V - significant differences between varieties, N - significant differences between nitrogen treatments.

An increase in nitrogen fertilisation resulted in significantly lower concentrations of glucose and fructose in Andean Sunside, but not Annabelle (Table 5.2). Nitrogen fertilisation had no effect on sucrose concentrations or TSI for both varieties. Andean Sunside 150 and 300 N samples contained approximately 0.2 g kg FW^{-1} less glucose and fructose compared to 0 N samples. The effect of fertilisation on the level of fructose and glucose, both reducing sugars, is a popular research area because high reducing sugar levels are linked to browning and acrylamide formation in potato crisps (Kumar et al., 2004). Previous literature indicates increasing the rate of nitrogen applied reduces the level of reducing sugars at harvest (Kumar et al., 2004; Mack and Schjoerring, 2002). This supports the trends and significant differences observed for both Andean Sunside and Annabelle fructose and glucose content (Table 5.2). In comparison, sucrose levels generally remain unaffected, as also observed (Kumar et al., 2004; Westermann et al., 1994), although Mack and Schjoerring (2002) reported a small increase in sucrose with increasing nitrate supply. However, a recent crisping study by Muttucumaru et al. (2013) suggested responses in sugar levels are more variety-dependent compared to the influence of nitrogen. TSI also remained unaffected, indicating that increasing the rate of nitrogen fertiliser is unlikely to affect potato sweetness perception.

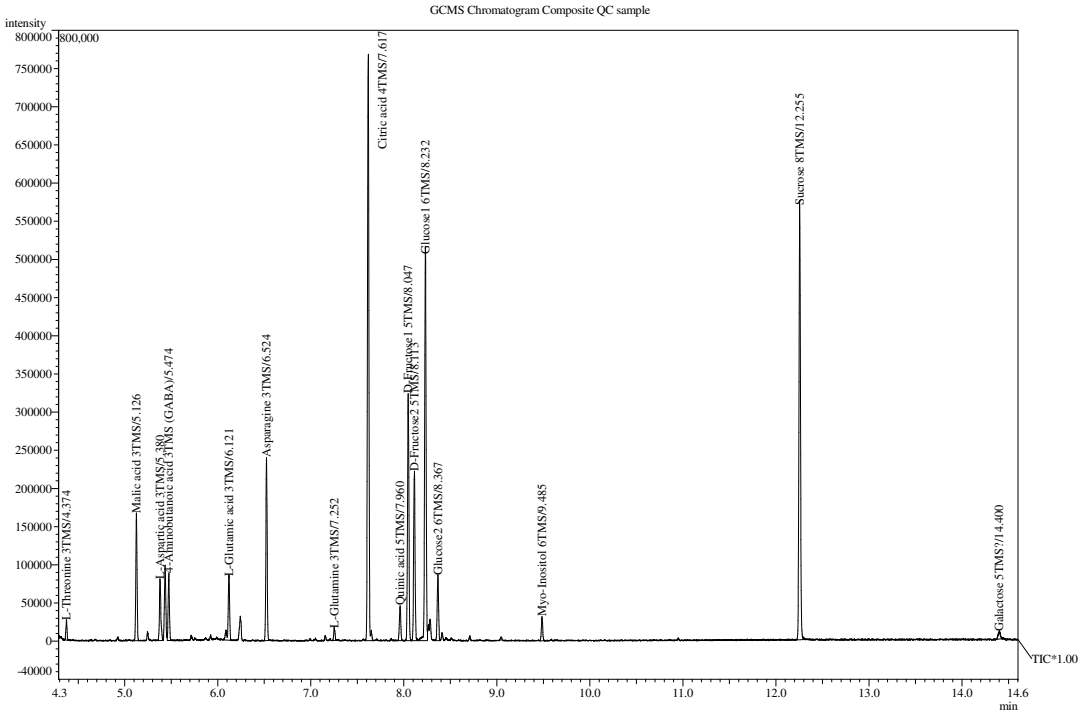


Figure 5.2: Chromatogram of sugars and amino acids identified using the Agilent GC-MS. The sample used for this chromatogram was a composite sample of all analytical samples. Compounds threonine, malic acid, GABA (gamma aminobutyric acid), quinic acid, myo-inositol, and galactose were tentatively identified using the NIST database but were not formally identified and quantified.

The significant differences in dry matter and sugars between Annabelle and Andean Sunside can be primarily attributed to varietal genetic differences. Varieties with *S. phureja* parentage are known to be more crumbly and floury compared to *S. tuberosum* potatoes, floury and mealy textures correlated with dry matter content (De Maine et al., 1993; Van Marle et al., 1997). In comparison, Annabelle potatoes, with a lower dry matter content, are firm-textured, suitable for early-harvested new potatoes (HZPC, 2018). In a metabolomics study on the subspecies groups within *S. tuberosum*, Dobson et al. (2010) found that fructose and glucose levels were significantly higher in *S. tuberosum* samples, compared to *S. phureja* samples. However, there was no difference in fructose and glucose between Mayan Gold (*S. phureja*) and Montrose (*S. tuberosum*) in Morris et al. (2010). Although Andean Sunside sucrose levels are over double those of Annabelle in this study, in Dobson et al. (2010) and Morris et al. (2010) levels measured shortly after harvest are comparative and not consistently different between Phureja and Tuberosum parentage. However, after storage for 3 months at 4 °C, both *S. phureja* varieties contained over double the sucrose content compared with both *S. tuberosum* varieties tested (Morris et al., 2010). A wider range of cultivars from both *S. tuberosum* and *S. phureja* parentage need to be tested at the same time to

determine whether the significant differences observed in this study relate to parentage or specific varietal characteristics. Additionally, Dobson et al. (2010) suggest harvest maturity may also affect carbohydrate levels - if one variety is more chemically mature at harvest, it may contain lower levels of reducing and non-reducing sugars. In this study, the harvest dates were planned to ensure harvest maturity occurred at a similar time - therefore, it is unlikely harvest times affected the samples' sugar content. And finally, given the total overall difference in TSI, Annabelle samples may be perceived as more sweet compared to Andean Sunside samples, although this contradicts previous research which suggests *S. phureja* varieties have higher intensities of (flavour) sweetness (Morris et al., 2007).

5.1.3 Polyphenols, glycoalkaloids, and umami amino acids

A combination of GC-MS and HPLC-MS was used to investigate the effect of variety and nitrogen treatment on polyphenol, glycoalkaloid, and umami amino acid content. Citric acid was also identified and quantified as a major potato metabolite. All compounds quantified were significantly affected by nitrogen treatment and/or variety (Table 5.3).

Table 5.3: Polyphenols, amino acids, citric acid, and glycoalkaloids identified and quantified in the variety and nitrogen samples. The units for the nitrogen treatments are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

Variable	**Annabelle**			**Andean Sunside**			**Sig.**[2]
	0 N	150 N	300 N	0 N	150 N	300 N	
Chlorogenic acid (mg kg FW^{-1})	186.38^{b}	175.91^{b}	178.06^{b}	38.76^{a}	41.42^{a}	37.73^{a}	V
Neochlorogenic acid[1] (mg kg FW^{-1})	40.85^{b}	41.47^{b}	40.28^{b}	17.51^{a}	17.74^{a}	14.91^{a}	V
Cryptochlorogenic acid[1] (mg kg FW^{-1})	90.42^{b}	90.79^{b}	88.77^{b}	30.41^{a}	31.39^{a}	28.02^{a}	V
Quercetin 3-rutinoside (mg kg FW^{-1})	40.82^{c}	41.73^{c}	42.11^{c}	7.71^{a}	10.82^{b}	7.26^{a}	V+N
α-chaconine (mg kg FW^{-1})	78.05^{c}	64.38^{b}	61.90^{b}	28.09^{a}	27.36^{a}	31.45^{a}	V+N
α-solanine (mg kg FW^{-1})	34.07^{d}	27.05^{c}	25.95^{c}	16.17^{a}	16.27^{a}	19.12^{b}	V+N
Aspartic acid (g kg FW^{-1})	1.01^{a}	1.05^{a}	1.11^{a}	2.21^{b}	2.36^{b}	2.43^{b}	V
Glutamic acid (g kg FW^{-1})	0.49^{a}	0.50^{a}	0.48^{a}	0.96^{b}	0.99^{b}	1.07^{b}	V
Asparagine (g kg FW^{-1})	1.87^{b}	2.67^{c}	2.95^{c}	1.33^{a}	1.83^{b}	2.11^{b}	V+N
Glutamine (g kg FW^{-1})	0.28^{a}	0.48^{bc}	0.55^{c}	0.28^{a}	0.36^{ab}	0.42^{bc}	N
Citric acid (g kg FW^{-1})	3.59^{a}	3.51^{a}	3.40^{a}	5.39^{b}	5.67^{b}	5.79^{b}	V

^{abcd} denotes significant differences using Tukey's multiple pairwise comparisons at $\alpha = 0.05$.

1. Neo- and cryptochlorogenic acid concentrations were calculated using the chlorogenic acid calibration curve.

2. At $\alpha = 0.05$, V - significant differences between varieties, N - significant differences between nitrogen treatments.

Polyphenols

Polyphenols are antioxidants and compounds of interest in potatoes because of their potential nutritional and health benefits (Andre et al., 2007; Brown, 2005). Chlorogenic acid (5-O-Caffeoylquinic acid, 5-CQA) is the main phenolic acid in potatoes (~90% of total polyphenol content) (Malmberg and Theander, 1985), mostly concentrated in the potato skin (Mondy and Gosselin, 1988). Neo- and cryptochlorogenic acid (3-CQA and 4-CQA respectively) are isomers of chlorogenic acid. Across all nitrogen treatments, Annabelle samples contained over 4 times the content of chlorogenic acid (~180 mg kg FW^{-1}) compared to Andean Sunside (~40 mg kg FW^{-1}) (Table 5.3). Annabelle also contained 2-3 times the content of neo- and cryptochlorogenic acid and approximately 4 times more quercetin 3-rutinoside (a flavanol) than Andean Sunside, ~41 mg kg FW^{-1} compared to ~8 mg kg FW^{-1}. All polyphenols measured were significantly higher in Annabelle, across all nitrogen treatments (Table 5.3). The only polyphenol affected by nitrogen treatment was quercetin 3-rutinoside - in Andean Sunside, the level increased from 7.71 mg kg FW^{-1} to 10.82 mg kg FW^{-1} between 0 and 150 N treatments, then decreased to 7.26 mg kg FW^{-1} with 300 kg N ha^{-1} applied. In Lugasi et al. (1999), chlorogenic content also remained unaffected by increasing rates of nitrogen (0 to 300 kg N ha^{-1}), with no consistent impact on total polyphenol content either. Therefore, while major polyphenol content remains mostly unaffected with increasing rates of nitrogen fertiliser, there is a large varietal difference.

In boiled and steamed potatoes, polyphenols have been linked to an increase in perceived bitterness. In Mondy and Gosselin (1988), the cortex (tissue under the skin) of potatoes without skin was evaluated to be less bitter than potatoes cooked with the skin intact (Mondy and Gosselin, 1988). This change in perception was linked to changes in total polyphenol content. Therefore, given the large difference in polyphenol content between Annabelle and Andean Sunside, Annabelle samples could be perceived as more bitter. Indeed, two participants vocally commented on strong bitter taste of their Annabelle samples when conducting the sensory evaluation (Section 4.4.5). More recently, chlorogenic acid has also been linked to sourness in crisps (Zhang and Peterson, 2018). However, the impact of these compounds on flavour depends on whether the difference in total polyphenol content is perceptible. In Sinden et al. (1976), a total phenolic content of ~300 mg kg FW^{-1} had no correlation with potato bitterness ratings. As the total chlorogenic acid content (including isomers) was also approximately ~300 mg kg FW^{-1} for Annabelle samples, it is unlikely consumer sensory testing will reveal significant differences in perceived flavour as a result of variation in polyphenol content.

Glycoalkaloids

Like polyphenols, glycoalkaloid content has also been linked to bitterness in potatoes (Sinden et al., 1976). In this study, α-chaconine (chac) and α-solanine (sola) content were both significantly affected by variety and nitrogen treatments (Table 5.3). The combined content of α-chaconine and α-solanine make up ~95% of the total glycoalkaloid (TGA) content in potatoes (Slanina, 1990). The glycoalkaloid content in Annabelle samples was significantly higher than Andean Sunside, with over double the content of α-chaconine. In addition, the ratio of α-chaconine to α-solanine differed between varieties. For Annabelle, chac:sola was ~2.3, compared to ~1.7 for Andean Sunside. This genetic-driven difference is supported by Ramsay et al. (2005), where *S. phureja* varieties had lower-measured chac:sol ratios (0.98), compared to 2.28 for a Chilean *S. tuberosum* variety.

Glycoalkaloids are secondary metabolites thought to be produced as part of the plant's defence mechanisms (Slanina, 1990). As they are concentrated in the cortex, just below the skin, peeling removes 50 - 90% of the total glycoalkaloid content (Valkonen et al., 1996). Potato TGA content generally ranges from 20 - 150 mg kg FW^{-1} (Patchett et al., 1977; Savage et al., 2000; Slanina, 1990), with a recent crisping study reporting TGA levels between ~25 and 100 mg kg FW^{-1} across four commercial varieties (Amer et al., 2014). A study by Bártová et al. (2015) on the nutritional value of cultivated South American potato species reported extremely high glycoalkaloid levels, 796 mg kg^{-1} FW for *S. phureja* samples. As these levels are over 10 times those in this study and singular in existing literature, values in Bártová et al. (2015) should be regarded with caution. The lower TGA content in Andean Sunside samples is supported by Morris et al. (2010), where *S. phureja* varieties were found to contain significantly lower glycoalkaloid concentrations compared to *S. tuberosum* varieties at harvest. However, for both varieties, the content of both α-chaconine and α-solanine is below the suggested level for bitterness perception, 140 mg kg FW^{-1} (Sinden et al., 1976). Therefore, like the concentration of polyphenols, while the varietal difference is significant, the total content may be too small to perceive in consumer sensory evaluations, if not trained panels as well.

For both Andean Sunside and Annabelle, increasing the nitrogen application rate from 0 to 300 kg N ha^{-1} significantly affected the content of α-solanine. α-chaconine content was only affected by nitrogen fertilisation in Annabelle samples (Table 5.3). However, for Andean Sunside, while the α-solanine concentration increased from 16.17 mg kg FW^{-1} to 19.12 mg kg FW^{-1}, in Annabelle, the concentration decreased from 34.07 mg kg FW^{-1} to 25.95 mg kg FW^{-1}. As glycoalkaloids are steroidal nitrogenous glycosides, nitrogen is essential for glycoalkaloid synthesis. Therefore, glycoalkaloid content will generally increase with nitrogen fertiliser compared to control (i.e. 0 kg N ha^{-1}) treatments (Love et al., 1994). However, as glycoalkaloid content is influenced by many other factors, such as variety or growing conditions (e.g. greening), the effect of high or low levels of nitrogen fertiliser can become

secondary to other influences (Love et al., 1994). For example, the pest pressure observed for Annabelle 0 kg N ha^{-1} tubers (see Section 4.1.2) could explain the significantly higher TGA content (Valkonen et al., 1996). In relation to impact on flavour, in Sinden et al. (1976), potato samples with 9, 20, and 44 mg kg FW^{-1} glycoalkaloid content all scored identical average bitterness scores in a sensory evaluation: an intensity rating of 0.1 out of 4. Therefore, the small (albeit significant) changes caused by a variation in the rate of nitrogen fertiliser applied are unlikely to influence perceived bitterness.

Umami amino acids

The major umami amino acids (aspartic acid [Asp] and glutamic acid [Glu]) (Morris et al., 2007), as well as asparagine and glutamine (nitrogen transporters) (Lea et al., 2007), were identified and quantified after sample derivatisation and GC-MS analysis. Both Asp and Glu were unaffected by nitrogen treatment (Table 5.3) but significantly varied between Annabelle and Andean Sunside. Andean Sunside samples contained ~2.3 g Asp kg FW^{-1} and ~1 g Glu kg FW^{-1}, these values both double that of Annabelle. Morris et al. (2007) and Morris et al. (2010) published similar results, where *S. phureja* varieties (Mayan Gold and Inca Sun) contained significantly higher levels of glutamate compared to *S. tuberosum* varieties (Pentland Dell and Montrose). Aspartate did not significantly differ between varieties in either study (Morris et al., 2007, 2010). Results from Dobson et al. (2010) are also similar to this study, where *S. phureja* samples contained higher levels of aspartic and glutamic acid compared to *S. tuberosum* samples. Therefore, the higher levels of Glu and Asp in Andean Sunside are most likely due to the variety's *S. phureja* parentage. While Glu and Asp are not the biggest contributor to umami flavour (compared to guanosine 5'-monophosphate), the increased level of these amino acids in Andean Sunside samples could indicate a higher savoury intensity and overall flavour intensity (Morris et al., 2007). However, as increasing the level of nitrogen fertiliser applied to plants had no effect on glutamic and aspartic acid content, nitrogen is unlikely to affect savoury and umami flavour.

The amino acids asparagine [Asn] and glutamine [Gln] were significantly affected by the level of nitrogen applied (Table 5.3). For Annabelle, Asn and Gln content significantly increased between 0 kg N ha^{-1} and 150 kg N ha^{-1} applied. For Andean Sunside, Asn content significantly increased between 0 N and 150 N, and for Gln, between 0 N and 300 N. In addition, for each nitrogen treatment, Annabelle samples contained significantly more Asn compared to Andean Sunside while there was no varietal difference in Gln levels (Table 5.3). Asn is reportedly the most dominant amino acid in potatoes and a major form of transported nitrogen in the plant because it has little charge and when soluble, does not participate in many reactions (Lea et al., 2007). Gln has also been linked to nitrogen transport, although more so in legumes (Lea et al., 2007). While there are few reports of these amino acids contributing directly to boiled potato flavour, Asn especially is a compound of interest

due to its role in toxic acrylamide formation in crisp production, negatively affecting crisp flavour (Lea et al., 2007). Additionally, due their roles as nitrogen transporters, both Asn and Gln content has been known to increase with increasing nitrogen fertilisation (Hippe, 1988). De Wilde et al. (2006) in Lea et al. (2007) found that free Asn content was strongly correlated with nitrogen availability (r^2 of 0.88) while Muttucumaru et al. (2013) reported significant nitrogen effects for both Asn and Gln with changing nitrogen fertiliser rates from 0 to 200 kg N ha^{-1}. The content of asparagine is also generally higher than glutamine, which was observed (Table 5.3). However, in contrast to most previous research, the dominant amino acid in Andean Sunside samples was Asp, and not Asn. This could be linked to the genetic variation caused by Andean Sunside's *S. phureja* parentage.

5.1.4 Volatile components

The volatile compounds identified in the potato samples, using SPME-GC-MS, are provided in Table 5.4, along with an example chromatogram in Fig. 5.3 displaying the biggest peaks, based on total ion current (TIC). A total of 57 compounds were confidently identified and integrated, with acetone, 2-ethyl-furan, and glutaric acid tentatively(*) identified in some, but not all samples. Several other compounds were identified in addition to these, but proved difficult to process and integrate, so were removed from this table. The average relative peak areas are provided for each variety and nitrogen treatment, for each compound, with the column retention time used for batch processing. The large majority of these compounds have been previously identified in volatile analysis of boiled and/or steamed potatoes (Bough et al., 2020; Dobson et al., 2008; Dresow and Böhm, 2009; Morris et al., 2010; Mutti and Grosch, 1999; Nursten and Sheen, 1974; Oruna-Concha et al., 2002b; Thybo et al., 2006). Tetrahydro-2-methyl-furan, ocimene, and 3-octen-2-one have been previously identified in baked and/or fried potatoes, but not boiled potatoes (Coleman et al., 1981; Dresow and Böhm, 2009; Duckham et al., 2001; Sanches-Silva et al., 2005). Ethyl formate has never been identified in the literature as it is likely a contaminant from formic acid. Cyclopentane and 2,4-dimethyl benzaldehyde appear to be new compounds identified within literature on potato flavour. Using generalized linear models, 26 compounds were identified to significantly differ between variety and/or nitrogen treatments.

Table 5.4: Volatile compounds identified and quantified as integrated peak areas, using mass spectrometry data and the NIST compound database. Relative average peak area is provided for each variety and nitrogen treatment. The units for the nitrogen treatments are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

Compound	**RT**[1] **(min)**	**Annabelle**[2]			**Andean Sunside**[2]			**Sig.**[3]
		0 N	150 N	300 N	0 N	150 N	300 N	
Cyclopentane	3.74	317202^{a}	336754^{ab}	363084^{ab}	474134^{ac}	581938^{c}	506431^{bc}	V
Methanethiol	3.79	249503	305346	341336	183310	183834	212354	ns
Dimethyl sulfide	3.88	15609^{a}	14565^{a}	14337^{a}	30596^{b}	31375^{b}	32867^{b}	V
Acetone*	4.03	721591	769736	787503	1180284	1284792	1277972	ns
Ethyl formate	4.06	62474	50772	69961	88237	88340	75416	ns
2-propenal	4.13	207363^{b}	230855^{b}	268291^{b}	127136^{a}	123156^{a}	147394^{a}	V
Tetrahydro-2-methyl-furan	4.23	22578	20722	21828	31439	35840	34485	ns
2-butanone	4.36	29407^{a}	34069^{ab}	36704^{bc}	39491^{bd}	40055^{cd}	43173^{d}	V+N
2-methyl-butanal	4.43	98826	107872	115308	92197	87123	95443	ns
3-methyl-butanal	4.45	37283	38523	42236	40966	40692	43921	ns
2-ethyl-furan*	4.66	18865	18051	17571	21749	22889	21924	ns
2,3-butanedione (diacetyl)	4.81	64143^{a}	67515^{a}	62898^{a}	92826^{b}	88533^{b}	90834^{b}	V
Pentanal	4.84	205459	211246	224003	195766	208808	204571	ns
α-pinene	5.21	17137^{a}	13140^{a}	14392^{a}	35977^{b}	36103^{b}	43723^{b}	V
Dimethyl disulfide	5.69	26575	36081	36760	15486	16031	15795	ns
Hexanal	5.80	2800000	2699302	2912748	3750219	3513749	3654245	ns
2-methyl-2-(*E*)-butenal	5.93	9990^{a}	12015^{ab}	14181^{bc}	17710^{d}	16347^{cd}	17741^{d}	V+N
β-pinene	6.05	20578	14233	17430	34671	33699	37900	ns
(*E*)-2-pentenal	6.35	6118	6078	6157	6764	6856	6696	ns
2-n-butyl furan	6.39	15902^{a}	13414^{a}	15841^{a}	24307^{b}	24365^{b}	24239^{b}	V
2-heptanone	7.07	28326^{a}	29456^{ab}	30754^{ab}	38625^{bc}	37140^{ac}	42421^{c}	V
Heptanal	7.10	45840	40994	47826	61496	60418	63177	ns
Limonene	7.28	6509^{a}	5128^{a}	6361^{a}	12193^{b}	16235^{b}	14876^{b}	V
Glutaric acid*	7.50	25248	24095	28479	82787	62220	53013	ns
(*E*)-2-hexenal	7.57	15714	15424	16150	19544	19047	19237	ns
2-pentyl furan	7.76	366566	297641	354932	492632	466977	489795	ns
β-ocimene-(*E**)	8.03	10731^{cd}	8407^{c}	12170^{d}	1410^{a}	2212^{b}	2250^{b}	V+N
1-pentanol	8.08	131752	132937	147123	101839	105414	111291	ns
p-cymene	8.26	7181^{a}	6436^{a}	7098^{a}	22874^{b}	27750^{b}	30177^{b}	V
Octanal	8.50	23613	21146	24966	29906	30797	31140	ns
1-octen-3-one	8.65	43393^{b}	46395^{b}	43138^{b}	27088^{a}	29313^{a}	28179^{a}	V
(*E*)-2-heptenal	8.89	106306	108656	106741	91604	91875	95568	ns
6-methyl-hept-5-en-2-one	9.04	38961^{a}	42412^{a}	47722^{ab}	53789^{b}	54513^{b}	57879^{b}	V
1-hexanol	9.23	33968	35356	36598	35138	31459	28311	ns
Dimethyl trisulfide	9.46	5420	13594	7732	3314	4656	2767	ns
(*Z*)-hex-3-en-1-ol	9.53	9319^{c}	11213^{c}	11842^{c}	1626^{a}	2342^{b}	1941^{ab}	V+N
Nonanal	9.59	95965	91736	99583	107577	116738	111864	ns
3-octen-2-one	9.72	5605	5166	5483	6918	6518	6829	ns
(*E*)-2-octenal	9.91	54839	54633	54048	44907	46193	48642	ns
1-octen-3-ol	10.08	175355	200945	170443	200438	225811	209615	ns

Table 5.4: Volatile compounds identified and quantified as integrated peak areas, using mass spectrometry data and the NIST compound database. Relative average peak area is provided for each variety and nitrogen treatment. The units for the nitrogen treatments are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

Compound	**RT**[1] **(min)**	**Annabelle**[2]			**Andean Sunside**[2]			**Sig.**[3]
		0 N	150 N	300 N	0 N	150 N	300 N	
Methional	10.12	883879^{b}	1170513^{bc}	1218913^{c}	604015^{a}	541676^{a}	588013^{a}	V+N
2-ethyl-hexanol	10.42	156944	136171	122827	213782	212614	196340	ns
(*E,E*)-2,4-heptadienal	10.44	102154	96677	96782	96381	95882	90436	ns
Decanal	10.48	20348	20911	21802	24471	24771	25973	ns
Benzaldehyde	10.67	362171^{a}	415470^{bc}	444670^{c}	390381^{ab}	410307^{ac}	438310^{bc}	N
(*E*)-2-nonenal	10.77	13606	12736	13668	14818	15382	15444	ns
Linalool	10.85	10275^{a}	9750^{a}	9799^{a}	12318^{ab}	18234^{c}	15158^{bc}	V+N
1-octanol	10.94	12741^{c}	12021^{bc}	13210^{c}	9371^{a}	10454^{ab}	10280^{ab}	V
Benzeneacetaldehyde	11.52	364394^{b}	420287^{b}	412496^{b}	220781^{a}	213661^{a}	214024^{a}	V
(*E,E*)-2,4-nonadienal	11.92	114080	109682	108550	90924	91353	94692	ns
(*E,E*)-2,4-decadienal	12.58	38953	36902	35805	27410	26958	27121	ns
2,4-dimethyl-benzaldehyde	12.63	20019^{c}	20236^{c}	18174^{bc}	12098^{a}	13005^{ab}	12317^{a}	V
Hexanoic acid*	12.82	298179	233816	237637	191567	197859	211152	ns
Benzyl alcohol	12.95	59794^{c}	58408^{c}	60200^{c}	31237^{a}	40481^{b}	34799^{ab}	V+N
Butylated hydroxytoluene (BHT)	13.13	102381^{b}	27363^{a}	24960^{a}	27071^{a}	34035^{a}	36951^{a}	N
Phenethyl alcohol	13.16	30720^{c}	33006^{c}	35679^{c}	4374^{a}	6879^{b}	6393^{b}	V+N
Phenol	13.63	22737	21061	19754	17582	17061	17984	ns
Nonanoic acid	14.50	206857^{b}	156628^{ab}	141554^{ab}	116600^{a}	113878^{a}	129665^{ab}	V
Glycerol	15.17	572036^{c}	618448^{c}	470043^{bc}	147030^{a}	235780^{ab}	197636^{a}	V
Vanillin	16.38	71594	68920	66959	75504	75381	78341	ns

1. RT stands for retention time in minutes in a Agilent J&W DB-WAX capillary column.

2. Values for all variety-nitrogen treatments are arbitrary integrated curve areas.

3. At $\alpha = 0.05$, V - significant differences between varieties, N - significant differences between nitrogen treatments, ns - not significant.

* Compound identified tentatively but not confirmed.

^{abcd} denotes significant differences using Tukey's multiple pairwise comparisons at $\alpha = 0.05$

Across all nitrogen treatments, the content of dimethyl sulfide, 2,3-butadione, α-pinene, 2-n-butyl-furan, and *p*-cymene was significantly higher in Andean Sunside compared to Annabelle. The content of cyclopentane, limonene, and 6-methyl-hept-5-en-2-one was significantly higher in most Andean Sunside samples, but not across all nitrogen treatments. The content of 2-propenal, 1-octen-3-one, and benzeneacetaldehyde was significantly higher in Annabelle compared to Andean Sunside across all nitrogen treatments, and the amount of 2,4-dimethyl-benzaldehyde and 1-octanol was higher in most Annabelle samples, but not across all nitrogen treatments. 2-butanone, 2-methyl-2-(*E*)-butenal, 2-heptanone, β-ocimene, (*Z*)-hex-3-en-1-ol, methional, linalool, benzyl alcohol, and phenethyl alcohol displayed significant differences caused both by variety and nitrogen treatment.

Many of the compounds with observed significant differences between Annabelle and Andean Sunside are known to both vary between cultivars and contribute to potato aroma and flavour. For example, Bough et al. (2020) found methional content to vary significantly with variety, methional being an aroma compound with a strong and distinct boiled potato odour quality (Mutti and Grosch, 1999). Oruna-Concha et al. (2002b) recorded varietal differences in α-pinene, benzeneacetaldehyde, 6-methyl-hept-5-en-2-one, and limonene content, as measured in this study. As Annabelle and Andean Sunside originate from different *S. tuberosum* parentage, significant differences in their aroma profile is expected. In the following section (Section 5.1.5), principal component analysis will help to illustrate which volatiles are more associated with each variety, and in Section 5.3, whether these can be associated with perceived flavour.

Only a small number of compounds were significantly affected by increasing applications of nitrogen fertiliser. Increasing rates of nitrogen fertiliser significantly increased benzaldehyde content for Annabelle (but not for Andean Sunside). Annabelle 0 N samples measured approximately 3 times in BHT content than all other variety and nitrogen treatments - this result is unusual and could be due to contamination. Therefore, analyses following this has BHT removed. 2-butanone, 2-methyl-2-(*E*)-butenal, 2-heptanone, β-ocimene, (*Z*)-hex-3-en-1-ol, methional, linalool, benzyl alcohol, and phenethyl alcohol displayed significant differences caused both by variety and nitrogen treatment. However, for these compounds, there was no consistent impact or pattern caused by nitrogen. Benzaldehyde, an almond-flavoured aroma compound, was the only volatile compound to significantly vary between nitrogen treatments (and not variety) for Annabelle and Andean Sunside (Table 5.4). 2-butanone (chemical), methional (potato-like), and 2-methyl-2-(*E*)-butenal (green fruit) significantly increased between 0 N and 300 N treatments in Annabelle, odour and flavour qualities provided in the brackets (The Good Scents Company, 2018). In Andean Sunside, (*Z*)-hex-3-en-1-ol (fresh green) and phenethyl alcohol (floral) significantly increased between 0 N and 150 N treatments. For linalool (floral, citrus) and benzyl alcohol (sweet floral, fruity), content significantly increased between 0 N and 150 N in Andean Sunside, then decreased with 300 kg N ha^{-1}. Inconsistent nitrogen effects were recorded for β-ocimene (floral, green), where levels decreased from 0 N to 150 N, then increased significantly up to 300 N (in Annabelle). However, for Andean Sunside, 0 N contained a significantly lower β-ocimene content compared to 150 N and 300 N treatments. Therefore, 2-butanone, methional, and 2-methyl-2-(*E*)-butenal for Annabelle, and (*Z*)-hex-3-en-1-ol and phenethyl alcohol for Andean Sunside, in addition to benzaldehyde for both, all displayed a pattern of increasing with increases in the rate of nitrogen fertiliser applied. However, the potential impact of these volatiles on perceived flavour is difficult to estimate, as there is little research on these compounds' odour thresholds in potatoes (aside from methional and benzaldehyde), and no quantification was carried out.

To note, no nitrogen effect was observed on the group of potato off-flavour (POF) aldehydes, reportedly linked to the development of cardboard and off-flavours in boiled potatoes (Blanda et al., 2010; Petersen et al., 1999). As discussed in Chapter 2, Fischer (1991) found that levels of pentanol, hexanol, heptanal, (*E*)-2-hexenal, (*E,E*)-2,4-decadienal, and (*E,Z*)-2,4-decadienal increased with nitrogen fertilisation in a potato pot experiment. Many of these compounds have been confidently linked to a cardboard-like off-flavour, especially prevalent in stored boiled potatoes (Blanda et al., 2010; Petersen et al., 1999). Therefore, it was predicted nitrogen could increase the perception of (cardboard-like) off-flavours in this study. Levels of these compounds remained unaffected with an increase to 300 g kg N ha^{-1} fertiliser. However, as fatty acid levels (e.g. linolenic acid) were not measured, fatty acids being the precursor compounds to POF aldehydes (Petersen et al., 1999), it is unknown whether the concentration of POF aldehydes would develop if the cooked samples were stored - the samples in this study were frozen after cooking. Therefore, further research is required to determine whether nitrogen fertiliser affects the levels of POF aldehydes in stored boiled potatoes, and thus the development of cardboard off-flavour.

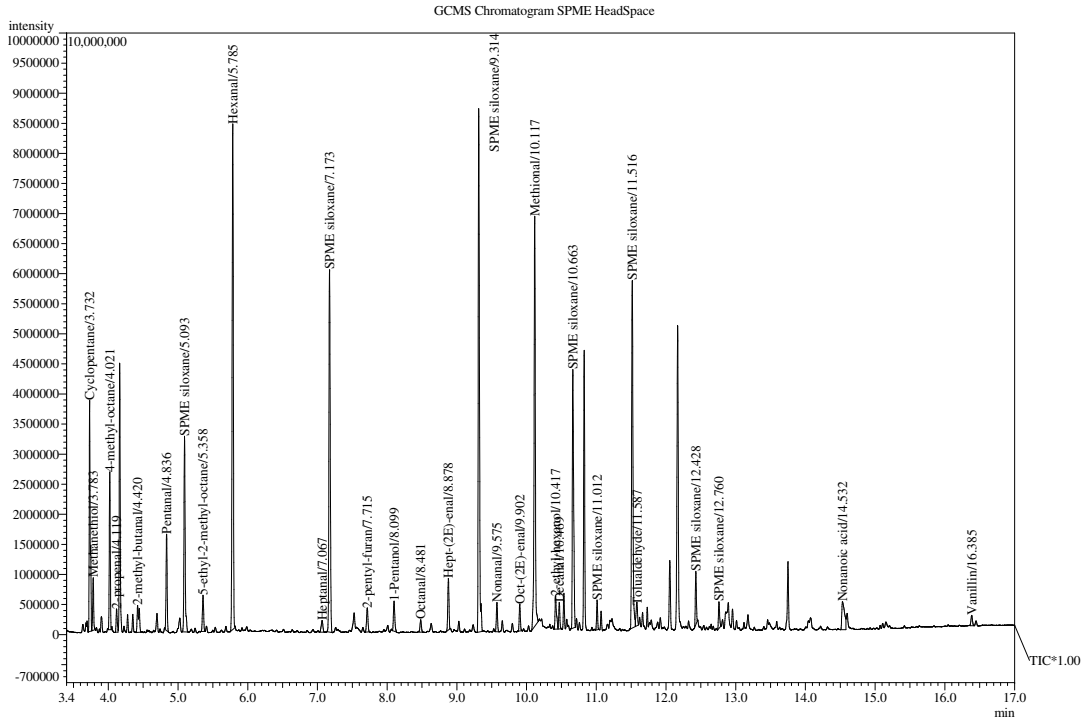


Figure 5.3: SPME-GCMS sample chromatogram of analytical sample MH63_089, using total ion current (TIC). MH63_089 is a sample of Annabelle, 150 N. The biggest peaks are labelled with their retention times in the Agilent DB-Wax column. The siloxane peaks are contaminants.

5.1.5 Principal component analysis

Principal component analysis (PCA) was used to condense and summarise the metabolite data. All variables measured and quantified have been reduced into PCA biplots, except for nitrate content (because of the censored Andean Sunside values) and BHT (a suspected contaminant). Fig. 5.4 summarises the non-volatile variables and Fig. 5.5 summarises the volatile variables. The F3 dimensions are not included as they explained only a very small proportion of the data (1% for non-volatile variables). In Fig. 5.4, the varietal separation is clear. Aspartic acid, glutamic acid, citric acid, sucrose, and dry matter (DM) were all found at significantly higher concentrations in Andean Sunside while all chlorogenic acid isomers, quercetin 3-rutinoside, glycoalkaloids, glucose, fructose, and TSI were all higher in Annabelle. The significant differences in these metabolites between Annabelle and Andean Sunside samples is represented by their association together, and separation on the F1 axis in Fig. 5.4. For both varieties on either side of the PCA biplot, the nitrogen treatments have been separated in order of 300 N, 150 N, and 0 N. This is likely caused by the variables total N, glutamine, and asparagine. These variables significantly increased with increasing rates of nitrogen fertiliser, and so being positioned near the top of the plot likely helped to separate the treatments on F2.

In Fig. 5.5, the volatile metabolites are plotted as variables in relation to variety and nitrogen treatment. The F1 and F2 dimensions explain 91.66% of the variability. As with Fig. 5.4, the variables have mostly been separated by F1 into two groups, the result of many significant differences in volatile content between Annabelle and Andean Sunside. Benzaldehyde, the volatile compound that increased with increasing nitrogen fertiliser rate (but did not differ between variety) is positioned near the top and middle of the PCA biplot, similar to asparagine and glutamine in Fig. 5.4. The PCA biplot does not show any clear clustering or close association between groups of compounds and the treatments, aside from the varietal separation on F1. The nitrogen treatments for both varieties are closely grouped together. However, Fig. 5.5 shows some associations between related compounds. For example, the aldehydes decanal, nonanal, hexanal, octanal, and heptanal are all closely associated on the right side of the graph. Similar clustering for steamed *S. tuberosum* and *S. phureja* potatoes can be seen in Morris et al. (2010). On the left side, both hexanoic and nonanoic acid are positioned together, as is dimethyl disulfide and dimethyl trisulfide. (*E,E*)-2,4-decadienal, (*E,E*)-2,4-nonadienal, and (*E,E*)-2,4-heptadienal are also positioned nearby on the left side of the biplot. The associations may indicate the similar origin of these compounds. However, as the nitrogen treatments did not significantly affect the content of many volatiles, this data visualisation is mostly variety-driven by Andean Sunside and Annabelle, which is expected, as they have different parentage and have been bred for different purposes. Using a greater range of varieties may have reduced this separation and aided in the observation of treatment-related associations.

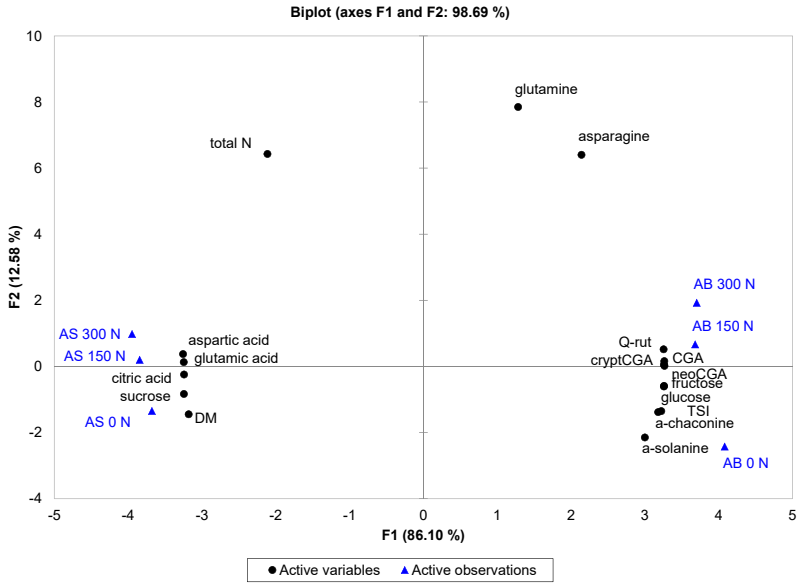


Figure 5.4: PCA biplot of non-volatiles variables with variety and nitrogen treatments, using averaged values. Together the F1 and F2 dimensions represent 98.69% of the total variation. The treatments are represented by blue triangles, where AB = Annabelle and AS = Andean Sunside. The units for the nitrogen treatments are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

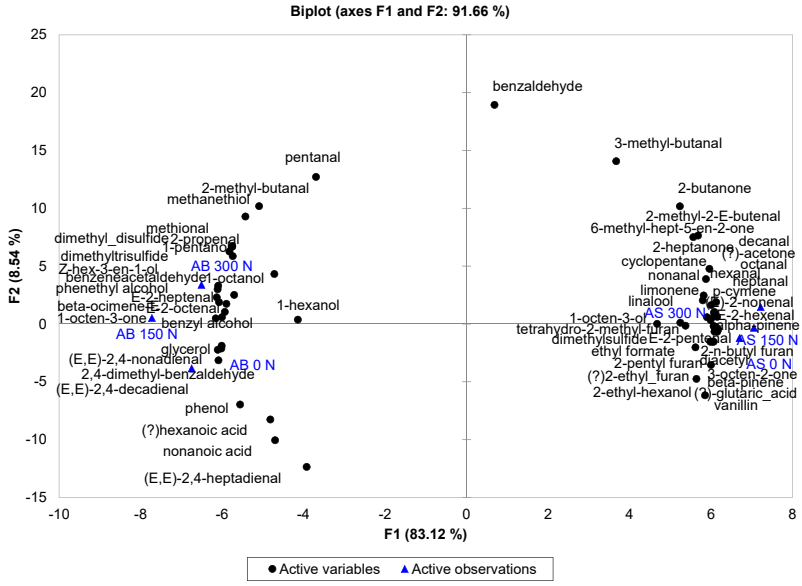


Figure 5.5: PCA biplot of all averaged volatile compounds identified in each variety and nitrogen treatment. Together the F1 and F2 dimensions represent 91.66% of the total variation. The treatments are represented by blue triangles, where AB = Annabelle and AS = Andean Sunside. The units for the nitrogen treatments are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied). Compounds with (?) are only tentatively identified.

5.2 Nitrogen effects on consumer sensory perception

5.2.1 Nitrogen effects on consumer sample liking

Liking scores

The liking score for each variety and nitrogen treatment was plotted in a scattergram-boxplot in Fig. 5.6. Overall, participants liked the steamed potato samples. Fig. 5.6 shows how both the mean and median for all treatments lie between 'like moderately' and 'like very much'. Andean Sunside 0 kg N ha^{-1} had the biggest spread of liking scores and the lowest rated sample out of all assessments, close to greatest imaginable dislike. Interestingly, this particular sample was rated as bitter (6/9), very earthy (7/9), and musty (4/9), flavour intensity provided in the brackets. No significant liking differences were found between nitrogen treatments, within each variety ($\alpha = 0.05$). Therefore, increasing the rate of nitrogen fertiliser did not affect consumer acceptance of the samples.

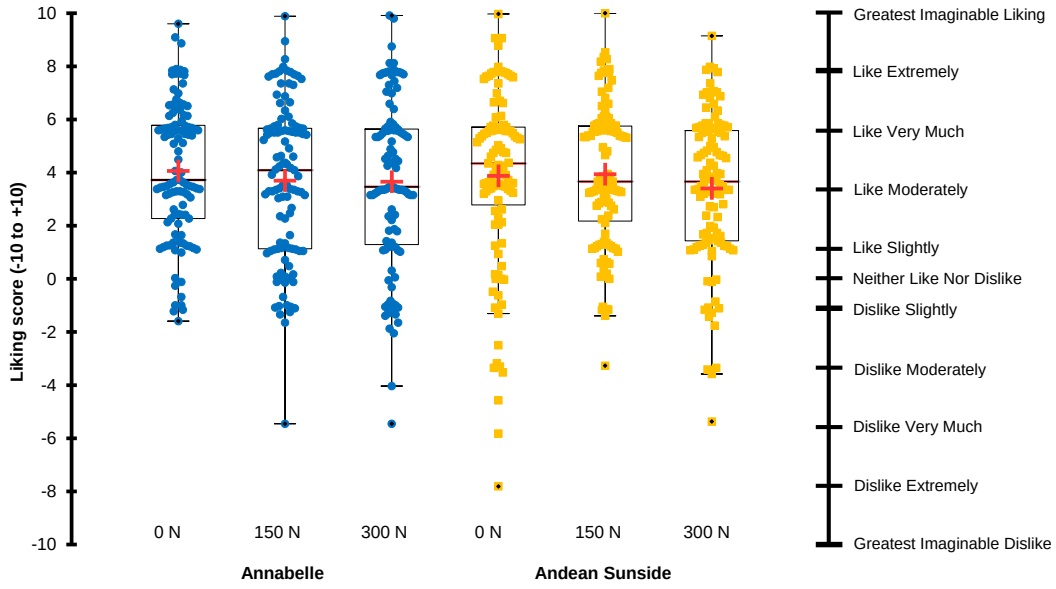


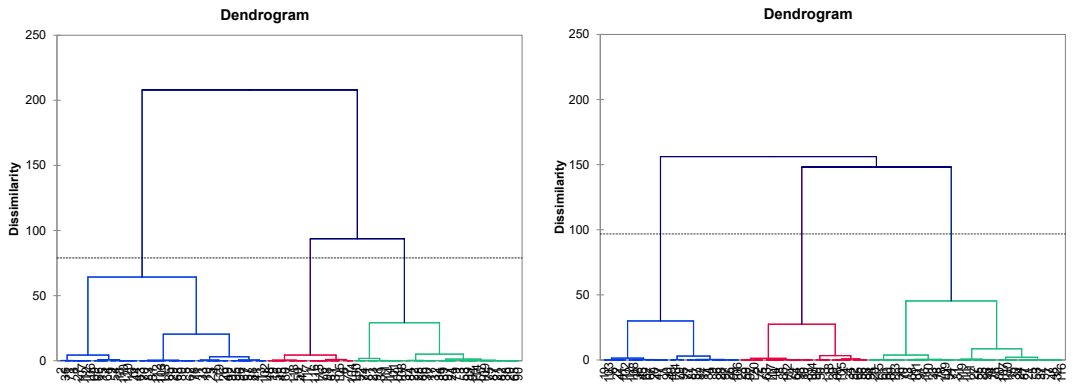
Figure 5.6: Scattergram-boxplot showing the variation in sample liking between nitrogen treatments, within each variety (Annabelle: blue • symbol, and Andean Sunside: yellow ■ symbol). The scattergram-boxplot shows maximum and minimum values, upper and lower quartiles, median (red line) and mean (red cross). The units for the nitrogen treatments are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

Cluster analysis on liking

To investigate any segmentation in liking, a post-hoc explorative agglomerative hierarchical clustering (AHC) analysis was carried out on the liking values. Fig. 5.7 shows a dendrogram of the clusters separately generated for Annabelle (a) and Andean Sunside (b), with participant code on the x-axis. The clustering analysis formed three clusters for both varieties. For Annabelle (a), cluster 1 (red) included 21 participants (19%), cluster 2 (blue) included 50 participants (45%), and cluster 3 (green) included 40 participants (36%). For Andean Sunside (b), cluster 1 (blue) included 33 participants (30%), cluster 2 (red) included 31 participants (28%), and cluster 3 (green) included 47 participants (42%).

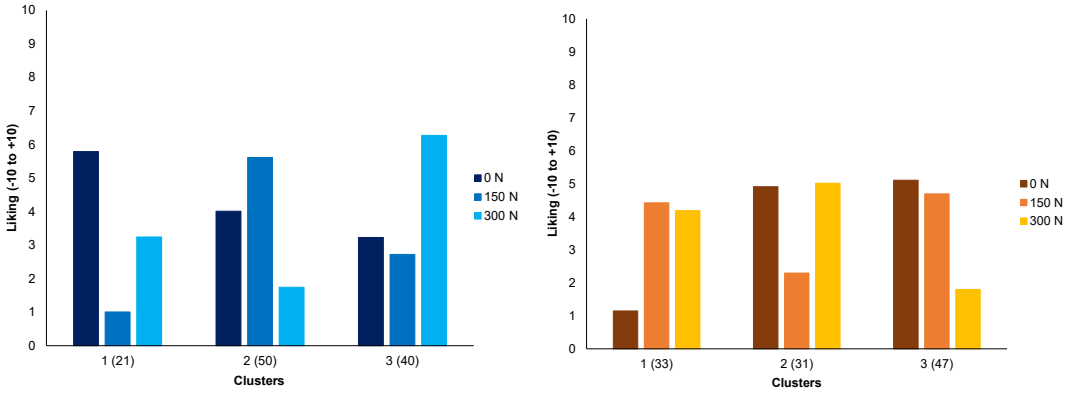
Mean liking per cluster for each nitrogen treatment, in Annabelle and Andean Sunside, was plotted in two bar charts (Fig. 5.8). For Annabelle, the AHC grouped participants by their highest average liking for 0 kg N ha^{-1} (cluster 1), 150 kg N ha^{-1} (cluster 2), and 300 kg N ha^{-1} (cluster 3). The largest cluster, Cluster 2, preferred samples with 150 kg N ha^{-1} applied. As each cluster of participants preferred a different level of nitrogen fertiliser, no fertiliser level can be recommended as being 'most-liked' in Annabelle. This is supported by the overall liking statistics, where liking did not significantly differ between treatments in Annabelle (Fig. 5.6).

For Andean Sunside, the cluster analysis split participants into three fairly evenly-sized clusters. Participants in cluster 1 liked 0 kg N ha^{-1} samples less than the samples with 150 and 300 kg N ha^{-1} fertiliser applied. This contrasts clusters 2 and 3, where participants liked the 0 kg N ha^{-1} nitrogen samples the best, close to 'like very much' (see LAM scale in Fig. 5.6). However, cluster 2 liked samples with 300 kg N ha^{-1} the same as those with no fertiliser, whereas cluster 3 liked samples with 300 kg N ha^{-1} the least. Across all three clusters, each nitrogen treatment level was liked the least (Fig. 5.8). Therefore, like Annabelle, no clear conclusion can be drawn about which nitrogen treatment was liked or disliked more in Andean Sunside. Further work with a larger number of consumers is required to investigate these differences further, or alternatively, a trained panel may be able to more-clearly discriminate the sample sensory properties driving these differences observed for each cluster.



(a) Annabelle (b) Andean Sunside

Figure 5.7: Dendrogram of three clusters generated by agglomerative hierachial clustering analysis (in XLStat) with participant code on the x-axis. For Annabelle (a), cluster 1 = red; cluster 2 = blue; and cluster 3 = green. For Andean Sunside (b), cluster 1 = blue; cluster 2 = red; and cluster 3 = green.



(a) Annabelle (b) Andean Sunside

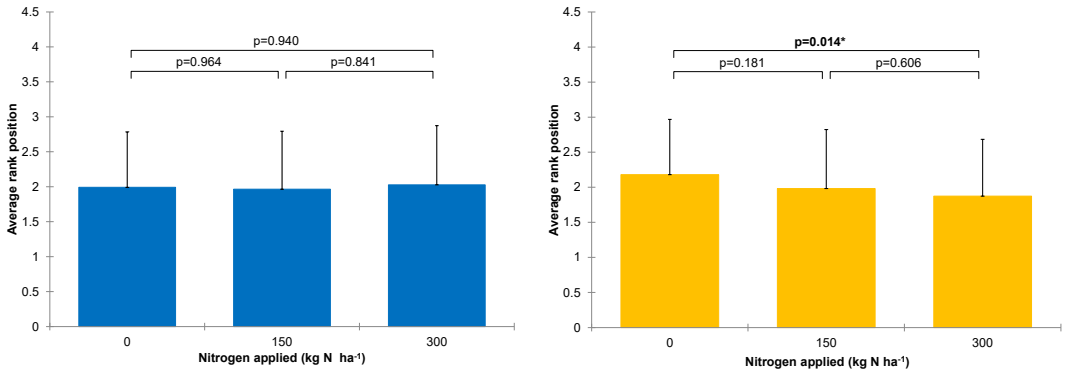
Figure 5.8: Bar graph of the clusters for each variety, with each bar representing the average liking score for each nitrogen treatment. 0 represents 'neither like nor dislike' and 10 represents 'greatest imaginable liking' for the equivalent LAM scale units. Numbers in brackets next to the cluster represents the number of participants in that cluster. The units for the nitrogen treatments are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

Ranked liking

The liking scores were converted into rank positions to further investigate any subtle differences in sample preference caused by varying levels of nitrogen fertiliser, where 3 = highest liking score, 2 = middle liking score, and 1 = lowest liking score. Using a Kruskal-Wallis test ($\alpha = 0.05$), any statistically signifiant differences in rank position were evaluated. Fig. 5.9 shows the change in ranking between nitrogen treatments for Annabelle (a) and Andean

Sunside (b). For Annabelle, there were no significant differences in rank position between nitrogen samples. For Andean Sunside, samples with 0 kg N ha^{-1} applied were ranked significantly higher than 300 kg N ha^{-1} samples, indicating that samples with 0 kg N ha^{-1} applied were more-liked compared to samples with the highest level of nitrogen applied. This indicates that increasing rates of nitrogen fertiliser affected consumer liking of Andean Sunside.

As this is the first study to assess the consumer acceptance of potatoes treated with different levels of nitrogen fertiliser, it is challenging to compare these results to existing literature. As increased rates of nitrogen fertiliser have previously been linked to increased concentrations of off-flavour volatiles (Fischer, 1991) (see Section 2.4), acceptance was thought to potentially decrease. In artichokes, high rates of nitrogen increased the perception of off-flavours (using a panel), and in tomatoes, a higher ratio of NO_3^-:NH_4^+ decreased acceptance (using consumers) (Heeb et al., 2005; Lombardo et al., 2017). However, in this study, average liking did not differ between nitrogen treatments, although Andean Sunside 0 kg N ha^{-1} was ranked higher in liking compared to 300 kg N ha^{-1} samples. Additionally, clustering indicated different consumer groups each preferred a different level of nitrogen fertiliser. Assessing the change in flavour between nitrogen treatments will help to determine whether any attributes could be driving the subtle differences observed.



(a) Annabelle (b) Andean Sunside

Figure 5.9: Average ranking scores for variety-nitrogen treatments, split by variety for Annabelle (a) and Andean Sunside (b). Scores are 3 = most liked and 1 = least liked ($n = 111, \alpha = 0.05$).

5.2.2 Using Rate-All-That-Apply to measure flavour changes between nitrogen treatments

Frequency of term selection

The number of times each attribute was perceived and checked by participants in the RATA test was totalled for each variety and treatment (Table 5.5). The attributes are ordered by the total times checked across all assessments (n = 666). The percentage presence of each attribute (based on attribute selection) in all samples is also provided in Table 5.5. Based on the total attribute selection, the most perceived attribute was 'boiled potato', checked 432 times. This was over 150 times more than the second most perceived attribute, 'buttery flavour' (277 times). Other attributes widely-perceived in over 25% of samples included earthy, savoury, nutty, and sweet. Musty, cardboard (for Annabelle), and green grass were only perceived in ~10% of samples, and raw potato was the least perceived attribute, with only 29 total checks over the entire evaluation. The effect of nitrogen fertiliser on flavour perception was assessed using the intensity data collected.

Table 5.5: Contingency table with frequency of term selection and usage across nitrogen treatments, within each variety. Total number of assessments is 666.

Attribute	**Annabelle**				**Andean Sunside**				**Total**[1]	**%**[2]
	0 N	150 N	300 N	Total	0 N	150 N	300 N	Total		
Boiled potato	74	67	72	213	72	71	76	219	**432**	**64.9**
Buttery flavour	50	50	45	145	54	37	41	132	**277**	**41.6**
Earthy	44	41	39	124	49	49	50	148	**272**	**40.8**
Savoury	39	34	39	112	32	34	35	101	**213**	**32.0**
Nutty	34	23	40	97	34	36	36	106	**203**	**30.5**
Sweet	35	39	34	108	33	26	35	94	**202**	**30.3**
Bitter	31	31	31	93	27	23	26	76	**169**	**25.4**
Oily flavour	22	25	14	61	26	19	21	66	**127**	**19.1**
Metallic	17	23	17	57	15	14	18	47	**104**	**15.6**
Sour	14	13	26	53	15	10	7	32	**85**	**12.8**
Musty	5	14	15	34	14	12	12	38	**72**	**10.8**
Cardboard	9	7	5	21	12	22	13	47	**68**	**10.2**
Green grass	13	12	6	31	9	15	12	36	**67**	**10.1**
Raw potato	5	1	3	9	6	8	6	20	**29**	**4.4**

[1.] Total number of times checked across all assessments (including both varieties) (n = 666).

[2.] Percentage attribute checked across all assessments (including both varieties) (n = 666).

While consumer studies have been previously conducted on potato flavour, many have used very broad attribute terminology (e.g. 'typical taste' or 'aroma') - this is only one of a few studies to have utilised a more descriptive range of attributes. A similar CATA (check-all-that-apply) study by Corrigan and McKenzie (unpublished), profiling 12 different potato varieties, has so far only presented a quick summary as a conference poster in New Zealand. Sharma (2019) conducted CATA analysis over 12 different potato varieties. However, only a limited number of flavour attributes were used, including cooked potato (292), metallic (64), raw potato (164), earthy (165), and bland (19), the total number of times each attribute checked provided in brackets. Interestingly, raw potato was selected the same number of times as earthy in Sharma (2019), indicating a very different flavour profile to these potatoes (or potentially many undercooked potatoes). However, a smaller range of flavour CATA terms were provided in Sharma (2019) because texture and aroma was also assessed at the same time. As cooked potato aroma and flavour were identified as drivers of liking in Sharma (2019), because all participants in this study liked (on average) the samples served (Section 5.2.1), this could be linked to the high checking of boiled potato flavour. Additionally, as discussed in Chapter 2, flavour acceptability has been associated with sweet, roast potato, and buttery attributes (V. Corrigan, personal communication, February 17, 2020) - similar terms (excepting roast potato) also checked the most times in this study. Therefore, the use and selection of terms is likely associated with the overall impression or perception of the sample. Furthermore, as CATA and RATA tests are rapid analyses based on fast thinking and consumers' immediate response to a product, they will be drawn to terms they are more familiar with (Buck and Kemp, 2018). Attributes like 'boiled potato' and 'buttery' will feel more natural to check compared to 'cardboard' or 'green grass', which are much less familiar, even if those flavours are perceptible in potatoes. While a consumer view was desired in this study, if an in-depth analysis into the treatment effects on each attribute was required, a trained panel would be more sensitive in perceiving those specific changes in flavour.

Changing flavour intensities between variety-nitrogen treatments

To assess nitrogen treatment effects on flavour perception, the rate-all-that-apply data were analysed using two different approaches. In the first approach, across all participants, any missing checks for an attribute were replaced as an intensity score of 0, as suggested in Meyners et al. (2016). Radar plots present the average intensity ratings for each attribute using this approach in Fig. 5.10, (a) and (b). In the second approach, the data were censored, whereby the data for participants that did not check an attribute across all three nitrogen samples were replaced with an 'NA' (for that attribute and variety only). If a participant

checked and rated an attribute for one or two samples out of three, the non-checked samples were replaced with a 0 intensity score. This approach allowed the inclusion of participants that appeared to discriminate attributes between samples whilst excluding participants that failed to perceive an attribute at all, which, in many cases, is genetically-driven (Nolden and Feeney, 2020). The average intensity ratings, using this approach, are presented in Fig. 5.10 (c) and (d), for Annabelle and Andean Sunside. Linear mixed effects models ($\alpha = 0.05$) were used to assess whether changing the rate of nitrogen applied significantly affected attribute intensity in Annabelle and Andean Sunside, across both approaches (Table 5.6).

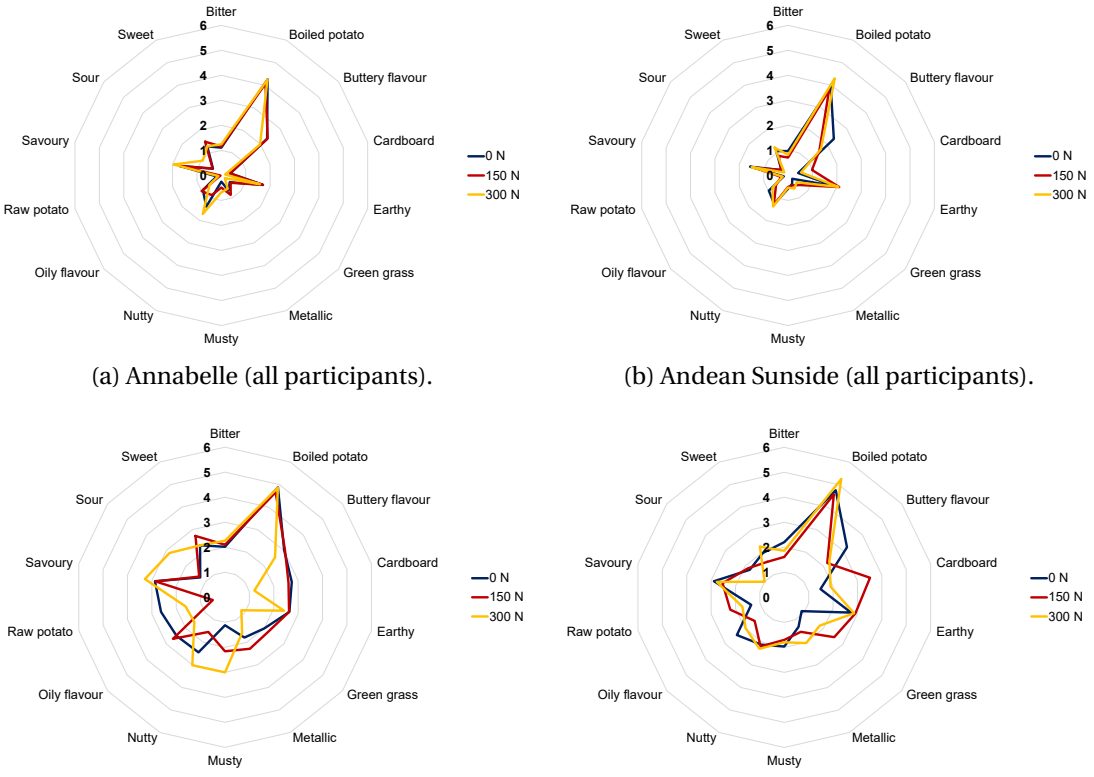
For both varieties and all nitrogen treatments, the flavour profile was dominated by 'boiled potato', with an average intensity rating of 4-5 out of 9, across both data approaches (Fig. 5.10). This is consistent with boiled potato also being the most-checked attribute for both varieties (Table 5.5). As discussed earlier, this is unsurprising, as these potatoes were steamed, which is a very similar cooking method to boiling (i.e. cooked to 100 °C, no other additions), and untrained consumers are drawn to terms they are more-familiar with (Buck and Kemp, 2018). Buttery flavour, earthy, nutty, and savoury were also perceived more-strongly on average (>2 intensity), compared to other attributes, for both varieties and all nitrogen treatments (using discerning participants). Therefore, in any future consumer studies using rapid methods like CATA and RATA, the inclusion of these attributes is recommended, as participants in this study appeared able to use these to rate and assess flavour.

Changing the level of nitrogen applied significantly affected flavour. Using data from all participants, nutty and sour in Annabelle, and buttery flavour, cardboard, and green grass in Andean Sunside, were significantly affected by nitrogen (Table 5.6). Using discerning participants only, the same attributes were significantly affected by nitrogen, with musty flavour also affected in Annabelle (Table 5.6). In Annabelle, 300 kg N ha^{-1} samples had the highest intensity of musty and sour, with a significant intensity increase of over 1.5 out of 9 between 0 kg N ha^{-1} and 300 kg N ha^{-1} samples (discerning participants). Musty especially is regarded as an off-flavour in potatoes (Mazza and Pietrzak, 1990). 300 kg N ha^{-1} samples were significantly more nutty than 150 kg N ha^{-1} samples in Annabelle. Different attributes were affected by nitrogen in Andean Sunside (Table 5.6). Buttery flavour significantly decreased between 0 kg N ha^{-1} and 150 kg N ha^{-1} samples. Cardboard flavour was the highest in 150 kg N ha^{-1} samples, with approximately double the intensity compared to 0 and 300 kg N ha^{-1} samples. The flavour of green grass significantly increased between 0 kg N ha^{-1} and 150 kg N ha^{-1} samples.

Table 5.6: Intensity of attributes that significantly varied between nitrogen treatments (α = 0.05) for Annabelle and Andean Sunside, using two different RATA data approaches, either including data from all participants, or discerning participants only. Maximum intensity rating = 9. The units for the nitrogen treatment codes are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

Variety	**Average intensity rating** (all participants)					**Average intensity rating** (discerning participants)				
	Attribute	0 N	150 N	300 N	**Sig.**[1]	Attribute	0 N	150 N	300 N	**Sig.**[1]
Annabelle	Nutty	1.39^{ab}	0.87^{a}	1.71^{b}	**	Musty	1.12^{a}	2.16^{ab}	3.00^{b}	*
	Sour	0.42^{a}	0.44^{a}	0.95^{b}	**	Nutty	2.44^{ab}	1.54^{a}	3.02^{b}	**
						Sour	1.27^{a}	1.32^{a}	2.84^{b}	**
Andean Sunside	Buttery flavour	2.34^{b}	1.60^{a}	1.68^{a}	*	Buttery flavour	3.21^{b}	2.20^{a}	2.31^{a}	*
	Cardboard	0.41^{a}	0.98^{b}	0.53^{a}	*	Cardboard	1.48^{a}	3.52^{b}	1.90^{a}	**
	Green grass	0.22^{a}	0.62^{b}	0.44^{ab}	*	Green grass	0.89^{a}	2.56^{b}	1.81^{ab}	*

[1]. Significance codes: ns - not significant, * p<0.05, ** p<0.01, *** p<0.001.
^{ab} denotes significant pairwise comparisons using Least Square Means at α level = 0.05.



(a) Annabelle (all participants).

(b) Andean Sunside (all participants).

(c) Annabelle (discerning participants).

(d) Andean Sunside (discerning participants).

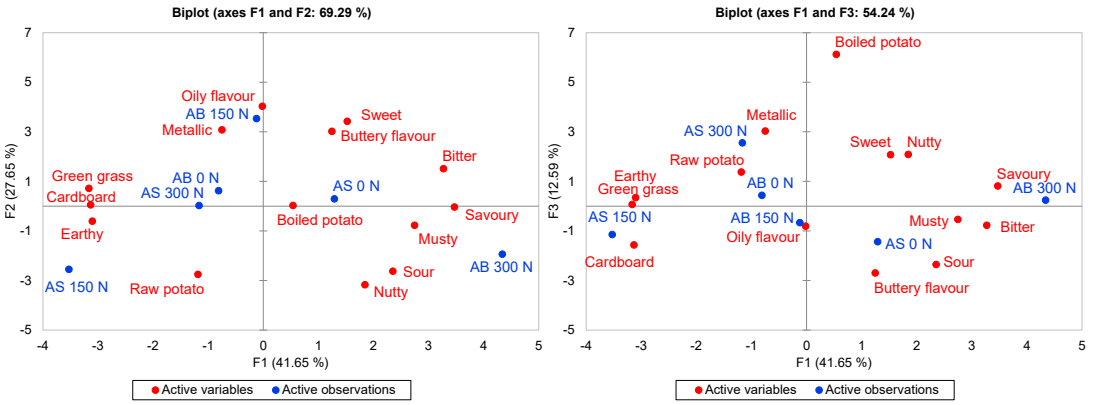
Figure 5.10: Radar plots of average RATA intensity ratings for Andean Sunside and Annabelle. 0 to 6 vertical axis represents average attribute intensity rating (with max. rating of 9). In (a) and (b), the average intensity ratings were calculated from all assessments, where non-selections replaced with 0. In (c) and (d), the average intensity ratings were calculated using data from participants that selected and rated at least one out of three samples for an attribute (discerning participants). The units for the nitrogen treatments are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

As with consumer acceptance, because this is the first comprehensive sensory evaluation measuring the effect of nitrogen fertiliser on flavour perception, there is little literature to compare these results to. In this study, flavour was both 'positively' and 'negatively' affected with increasing rates of nitrogen fertiliser, in relation to the attributes affected. With previous literature indicating that the concentration of compounds linked to off-flavour attributes (e.g. cardboard, musty, rancid) could increase (Fischer, 1991; Petersen et al., 1999; Thybo et al., 2006), there was an expectation that "less-desirable" flavour attributes, such as cardboard, musty, bitter, or sour, may increase with increasing nitrogen application rates (Section 2.4). For Annabelle, musty and sour did significantly increase with the application of 300 kg N ha^{-1}. However, nutty, described to consumers as "a sweet and roasted flavour" (see Table 3.4), increased with nitrogen, nutty assumed to hold more positive flavour associations, compared to musty and sour. None of these significant differences in flavour affected consumer liking in Annabelle (Fig. 5.6). For Andean Sunside, 0 kg N ha^{-1} had the highest buttery flavour intensity and the lowest green grass intensity. Interestingly, 0 kg N ha^{-1} samples were also ranked significantly higher in liking compared to 300 kg N ha^{-1} samples (Fig. 5.9) - this could be positively related to the significantly higher perception of buttery flavour, assumed to be a positive attribute in steamed potatoes. As cardboard flavour decreased between 150 and 300 kg N ha^{-1}, the cardboard off-flavours expected at high nitrogen levels were not observed.

Excluding the data from participants that failed to perceive an attribute across all three nitrogen treatments (i.e. discerning participants only) resulted in higher average attribute intensities and better visual separation between treatments within each variety (Fig. 5.10 and Table 3.3). In this study, converting all non-checks to a 0 intensity rating resulted in very low average intensity ratings, as seen in Fig. 5.10, where the average intensity of most attributes are concentrated in the middle of the plot, between 0 and 1. By including only discerning participants, the average intensity rating increased by ~1, allowing better visual separation between treatments (Fig. 5.10), good for foods like potatoes, where the overall flavour is already regarded as "weak" (Ulrich et al., 2000). Interestingly, despite the variation in average attribute intensities, the attributes which were significantly affected by nitrogen were mostly the same using both approaches. For Andean Sunside, the attributes were identical - using data from discerning participants only resulted in a lower p-value for cardboard. For Annabelle, using discerning participants resulted in a significant difference between 0 N and 300 N samples for musty, along with nutty and sour for both approaches. However, for further analysis using principal component analysis, the data set from discerning participants was used, given this slight improvement in sample discrimination. Additionally, if attempting to link compositional and sensory variables (Section 5.3), then data from participants that could perceive and discriminate attributes between samples will assist this.

Principal component analysis

The average RATA intensity ratings (using discerning participants) for Annabelle and Andean Sunside were analysed using principal component analysis (PCA, Pearson correlation). Fig. 5.11 (a) is a PCA biplot of the F1 and F2 dimensions, and Fig. 5.11 (b) is a PCA biplot of the F1 and F3 dimensions.



(a) F1 & F2 (b) F1 & F3

Figure 5.11: PCA biplot of average RATA intensity ratings (discerning participants) with Annabelle and Andean Sunside and all nitrogen treatments, using Pearson correlation. F1 & F2 explain 69.29% of the total variation and F1 & F3 explain 54.24% of the total variation. AB = Annabelle, AS = Andean Sunside. The units for the nitrogen treatment codes are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

In both biplots, there is little clustering between nitrogen treatments or samples from the same variety. Both 0 N treatments are positioned close to the origin in Fig. 5.11 (a) and (b), with Annabelle 0 N almost in the middle of Fig. 5.11 (b). Green grass, cardboard, and earthy are grouped on the left side of F1 with AS 150 N. AB 150 N is closely associated with oily flavour and metallic on F2, although these attributes did not significantly vary between treatments. On F2 and F3, sweet is associated with buttery flavour and nutty, respectively. Boiled potato sits in the middle of Fig. 5.11 (a), indicating it was not discriminated between treatments in both varieties. In Fig. 5.11 (a), nutty, sour, and musty are positioned closest to AB 300 N, which had significantly higher intensities of these attributes compared to samples with lower levels of nitrogen applied (see Table 5.6).

While the experiment was not planned to compare the sensory profiles of Andean Sunside and Annabelle, the lack of separation between varieties in Fig. 5.11 was unexpected. Andean Sunside, with *S. phureja* parentage, is likely to exhibit significantly higher flavour sweetness, creaminess, and intensity (Morris et al., 2007, 2010). Andean Sunside is marketed in New Zealand as tasting “uniquely sweet and buttery flavour[ed]” (A.S. Wilcox &

Sons, 2019). Therefore, the lack of clustering for attributes such as sweet, savoury, and buttery with Andean Sunside (AS) samples suggests these samples did not contain higher intensities of these flavours, compared to Annabelle. The differences in flavour between the two varieties may have been too subtle for this to reflect in the consumer attribute intensity ratings. Additionally, as the Andean Sunside trial plot was subjected to extensive weed pressure, this may have affected flavour, or even texture, which was informally noted to be more crumbly and less creamy than expected (B. Hart, personal communication, April 12, 2019). Therefore, further research is required to determine whether a consumer-perceptible flavour difference is present between Annabelle and Andean Sunside, as indicated in Andean Sunside's product marketing (A.S. Wilcox & Sons, 2019).

5.3 Associating sensory and compositional variables

To visualise the potential correlations and associations between the sensory and compositional variables, two PCA biplots (Spearman, $\alpha = 0.05$) were generated using averaged values for each variety and nitrogen treatment. Fig. 5.12 displays the non-volatile variables, plotted with RATA attribute intensity (for discerning participants) overlaid as supplementary variables. Fig. 5.13 displays the volatile variables, plotted with RATA intensity values (for discerning participants) overlaid as supplementary variables. The F3 dimensions were excluded for both PCAs as they only explained ~6% of the variation in the data. BHT and ethyl formate were removed from Fig. 5.13 as suspected contaminants.

5.3.1 Flavour and non-volatile variables

In Fig. 5.12, the biplot is characterised by the significant varietal separation on F1, with Annabelle and Andean Sunside samples positioned on opposite sides of the plot. As observed in Fig. 5.4, the significant differences between nitrogen treatments for total N, glutamine, and asparagine, have ordered the samples in increasing level of nitrogen fertilisation (i.e. 0 N, 150 N, and 300 N). While the non-volatile variables are placed at opposite ends of F1, the sensory variables are clustered in the middle of both plots. Earthy is closely associated with dry matter and sweet is associated with α-solanine. Additionally, sweet is more associated with glucose, fructose, and TSI, compared to sucrose (Fig. 5.12).

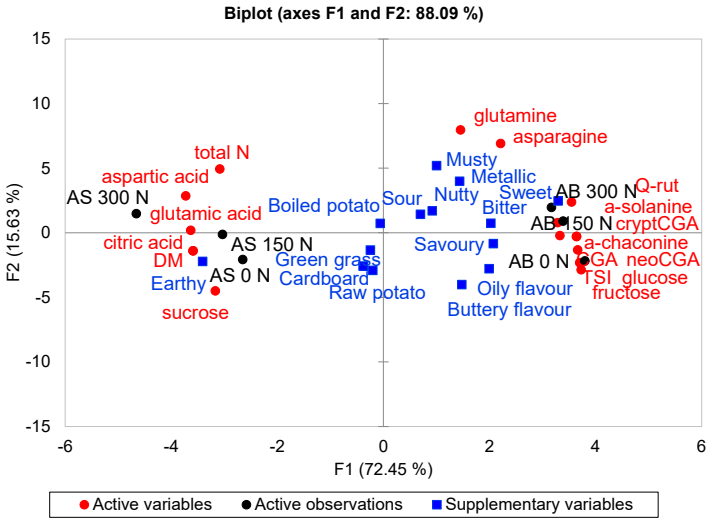


Figure 5.12: PCA biplot of non-volatile variables (active variables) with average RATA intensity ratings overlaid as supplementary variables (Spearman, α 0.05). F1 and F2 explain 88.09% of the variation in the data. AB = Annabelle, AS = Andean Sunside. The units for the nitrogen treatment codes are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

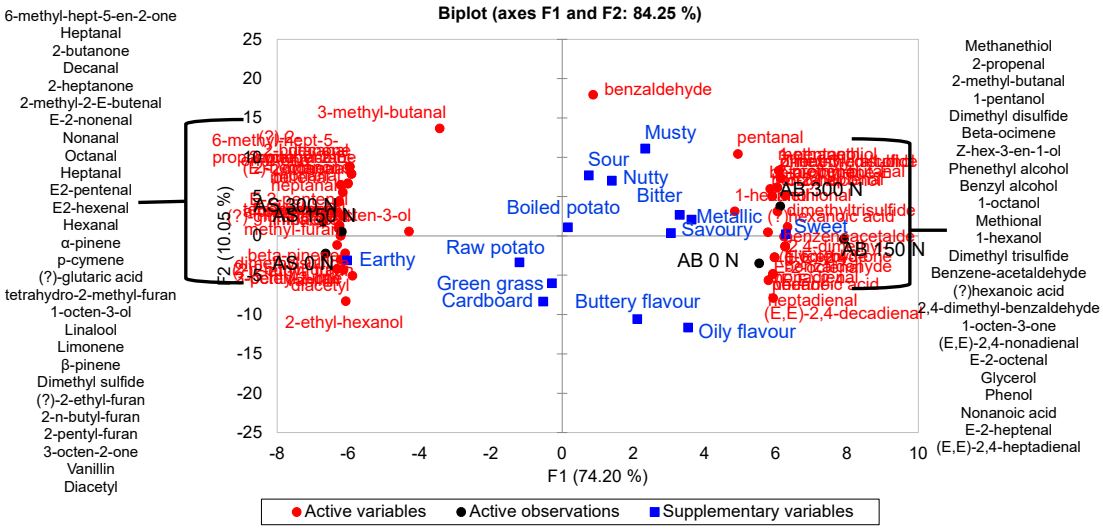


Figure 5.13: PCA biplot of volatile variables (active variables) with average RATA intensity ratings overlaid as supplementary variables (Spearman, α 0.05). F1 and F2 explain 84.25% of the variation in the data. AB = Annabelle, AS = Andean Sunside. The units for the nitrogen treatment codes are in kg N ha^{-1} (i.e. 150 N = 150 kg N ha^{-1} applied).

However, overall very few associations between composition and flavour are present, and many expected associations are absent. For example, glutamic acid has been previously associated with savoury flavour (Morris et al., 2007) - in these plots, savoury is positioned on the opposite side to glutamic acid. Sour and bitter flavours have been linked to chlorogenic acid content (Sinden et al., 1976; Zhang and Peterson, 2018), but no close associations between these variables were observed. In Thybo et al. (2006), total N was strongly correlated with potato off-flavour attributes - total N is not closely associated with any attributes. Additionally, no associations between flavour and composition can be observed for the flavour attributes that significantly varied with nitrogen fertiliser application (buttery flavour, cardboard, green grass, musty, nutty, and sour). Therefore, for the nitrogen treatments and varieties in this trial, changes in non-volatile composition were not reflected or associated with changes in consumer-perceived flavour.

5.3.2 Flavour and volatile variables

The PCA biplot of flavour attributes and volatile variables (Fig. 5.13 is characterised by two big clusters of volatile compounds, with most sensory attributes positioned near the middle of the biplot. The volatile variables have been separated by significant varietal differences between Annabelle and Andean Sunside, as observed in Fig. 5.12. The volatiles in the clusters have been listed on each side of the biplot in approximately the same order. As in Fig. 5.12, earthy and sweet have been separated on F1, earthy associating with β-pinene (herbal, woody, pine) and dimethyl sulfide (sulfurous) (The Good Scents Company, 2018). In a similar study, where trained panel descriptive analysis and volatile data from boiled and baked potatoes were analysed together, Bough et al. (2020) found pentan-1-ol was closely associated with earthy. In Fig. 5.13, sweet was closely associated with benzeneacetaldehyde, a honey and sweet-flavoured volatile (Mutti and Grosch, 1999). Bough et al. (2020) found benzeneacetaldehyde (2-phenylacetaldehyde) to be a 'bio-marker' for buttery flavour. The volatiles 3-methyl-butanal and benzaldehyde, whilst separate from the clusters in Fig. 5.13, are not closely associated with any sensory variables.

Like Fig. 5.12, many of the expected associations between flavour and volatile composition are not present, especially for the flavour attributes that significantly varied with nitrogen treatment. Many of the lipid-derived aldehydes (e.g. pentanal, (*E,E*)-2,4-heptadienal, (*E,E*)-2,4-nonadienal) have fatty, rancid, green, and marzipan odour qualities (Mutti and Grosch, 1999; Petersen et al., 1999). Altogether, these compounds can contribute a cardboard-like off-flavour, especially in stored boiled potatoes (Petersen et al., 1999). However, there were no close associations displayed in Fig. 5.13 between these aldehydes and similar attributes, such as oily flavour, green grass, or cardboard. Cardboard, musty, green grass, and sour, which were significantly affected by nitrogen, are positioned close to the centre of the biplot, and are not associated with any volatiles.

Interestingly, hexanal, the classic 'green' and cut grass-flavoured volatile generally present as the biggest chromatogram peak in potatoes (see Fig. 5.3), is not at all associated with the attribute green grass. However, according to Mutti and Grosch (1999), hexanal is not especially potent or odour active. Volatile aroma potency is measured by calculating the flavour dilution (FD) factor, the ratio of a compound's concentration in the initial extract to its concentration in the most dilute extract in which odour is detected by GC-O (Mutti and Grosch, 1999). Therefore, even if hexanal was present in high quantities (using integrated peak areas), it may not be strongly perceived or used as a discriminating attribute by participants. Instead, methional, with a characteristic 'boiled potato' aroma, has one of the highest FD factor scores measured in Mutti and Grosch (1999). Despite this, there is little association between boiled potato and methional in Fig. 5.13. Boiled potato was also one of the least discriminating attributes used in the sensory trial, even if it was present as the highest-intensity flavour attribute.

Overall, for both the non-volatile and volatile variables measured in this study, for the nitrogen treatments and varieties assessed, changes in composition were not reflected or associated with changes in consumer-perceived flavour observed. To a degree, this could be due to the nitrogen treatments only having a subtle impact on flavour - only 6 out of 14 attributes were significantly affected by treatment. However, the use of non-trained participants and rapid methods would have decreased discrimination and reduced the detail in flavour information obtained (Muñoz et al., 2018). While a consumer view was desired for this study to assess nitrogen fertiliser effects on consumer-perceived flavour, the use of trained panel descriptive analysis would have likely improved the flavour discrimination between nitrogen-treated samples (Muñoz et al., 2018). With a rigorous training programme, panels can provide very detailed qualitative and quantitative information on a product's flavour (Muñoz et al., 2018), also potentially improving the understanding of how composition can affect perceived-flavour.

Chapter 6

Extended discussion

6.1 Does nitrogen affect flavour-related composition?

Increasing the rate of nitrogen fertiliser (urea) (0 kg N ha^{-1} to 300 kg N ha^{-1}) applied to both Annabelle and Andean Sunside during crop growth significantly affected a number of metabolites in the steamed samples that have been previously linked to potato flavour. The percentage of total nitrogen and concentration of nitrate (in Annabelle only) significantly increased with the rate of nitrogen applied (Table 5.1). With increasing nitrogen, dry matter significantly decreased in Annabelle, and reducing sugar concentration (fructose and glucose) decreased in Andean Sunside (Table 5.2). The nitrogen-transporting amino acids (asparagine and glutamine) significantly increased with nitrogen for both varieties (Table 5.3). As noted in Section 5.1.3, free asparagine, especially, is associated with acrylamide formation during frying (Lea et al., 2007). While α-solanine increased with nitrogen in Andean Sunside, it decreased in Annabelle, as did α-chaconine concentrations in Annabelle with increasing rates of nitrogen applied. However, these concentrations never approached a harmful level. Analysis of the volatile compounds identified in samples found that 2-butanone, methional, and 2-methyl-2-(*E*)-butenal consistently increased with nitrogen fertilisation in Annabelle (Table 5.4). (*Z*)-hex-3-en-1-ol and phenethyl alcohol consistently increased with nitrogen fertilisation in Andean Sunside, with benzaldehyde increasing with nitrogen for both varieties.

The increase in total nitrogen, asparagine, and glutamine, indicate that for both varieties, the nitrogen treatment applications were correctly applied and successfully taken up by the plants during their growth, despite the potential of weed pressure in the Andean Sunside crop to interrupt this. These variables are well-known to increase with an increase in the rate of applied nitrogen fertiliser (Belanger et al., 2002; Hippe, 1988; Lea et al., 2007). The lack of nitrate variation in Andean Sunside samples may relate to a lower activity of nitrate reductase in the leaves compared to Annabelle, because the same effect was observed in this study's 2018 pilot experiment (data not included).

While the change in content or concentration of many variables were mostly consistent with existing research (i.e. dry matter, fructose, glucose), many of the nitrogen effects predicted to affect flavour, as discussed in Chapter 2, were not observed. For example, amino acid levels generally increase with nitrogen availability (Eppendorfer, 1996). Interestingly, this was not observed for aspartic acid and glutamic acid as suggested, these umami amino acids positively correlated with flavour intensity and savouriness in Morris et al. (2007). While the levels of α-chaconine and α-solanine in both Andean Sunside and Annabelle were comparable with existing literature (Patchett et al., 1977; Savage et al., 2000), the glycoalkaloid (TGA) content significantly decreased in Annabelle, inconsistent with previous reports of increases in TGA content with increased nitrogen application rates (Love et al., 1994). Observations of increased tuber moth damage in Annabelle 0 N blocks during harvest was suggested as a contributor to the heightened TGA levels measured. However, as the combined concentration of α-chaconine and α-solanine failed to reach the Sinden et al. (1976) TGA bitterness threshold of 140 mg kg FW^{-1}, these significant differences are unlikely to affect flavour - as Love et al. (1994) suggested, other environmental effects will have a larger effect on TGA content compared to nitrogen fertilisation.

A number of aroma compounds significantly increased with an increase in nitrogen application rate, although only one volatile significantly increased in both varieties (Table 5.4). As the aroma data were obtained from integrated chromatogram peaks and not quantified, the data are arbitrary and cannot be compared to previous volatile evaluations or odour thresholds - only the differences between the treatments can be assessed. This is a limitation of the volatile analysis. Additionally, there is limited existing literature that has evaluated the effect of nitrogen fertilisation on a large number of potato volatiles - only Fischer (1991) investigated the change in concentration of lipid-derived aldehydes with increasing rates of nitrogen added to potted potatoes. (*E*)-2-hexenal, (*E,E*)-2,4-decadienal, and (*E,Z*)-2,4-decadienal, all observed by Fischer (1991) to increase with nitrogen fertilisation, are linked to the development of potato off-flavours, especially cardboard-like flavours (Blanda et al., 2010; Petersen et al., 1999). In this study, these volatiles, and the other lipid-derived aldehydes in Fischer (1991), were unaffected by nitrogen fertilisation.

In Annabelle, the increase in methional content with nitrogen, known to be a potent volatile in cooked potatoes (Mutti and Grosch, 1999), could increase 'potato-like' flavour (Thybo et al., 2006). While the the other two volatiles, 2-butanone (chemical, fruity, green) and 2-methyl-2-(*E*)-butenal (The Good Scents Company, 2018), increased with nitrogen fertilisation, their potential impact on steamed potato flavour is unknown. van Loon et al. (2005) did not identify them as odour active compounds in an investigation on French fries flavour. In Andean Sunside, (*Z*)-hex-3-en-1-ol and phenethyl alcohol (floral) increased when nitrogen fertilisation increased (The Good Scents Company, 2018). However, neither of these compounds, in addition to benzaldehyde (sharp, almond), have been identified in

character impact assessments using GC-O (Mutti and Grosch, 1999; Petersen et al., 1998; Ulrich et al., 2000). Therefore, while the increase in methional may affect typical potato flavour perception, the nitrogen-related changes observed in the aroma profiles of Andean Sunside and Annabelle are unlikely to impact consumer-perceived potato flavour in this study. However, the lack of odour thresholds and volatile quantification is a limitation for this analysis - if these had been carried out, different results and nitrogen effects may have been observed.

6.2 Does variety impact flavour-related composition?

While only a few significant compositional differences relating to variation in nitrogen fertiliser applied were identified across both varieties, compositional variation between steamed Annabelle and Andean Sunside samples was considerable. In addition to containing a significantly higher dry matter content, Andean Sunside also contained significantly more total nitrogen than Annabelle, but undetectable concentrations of nitrate (Table 5.1). Annabelle samples contained significantly higher concentrations of reducing sugars, leading to a higher TSI (total sweetness index) compared to Andean Sunside, where samples were higher in sucrose instead (Table 5.2). Annabelle contained significantly higher levels of polyphenols and glycoalkaloids while Andean Sunside samples contained higher concentrations of aspartic acid and glutamic acid. Twenty four volatile compounds (out of 60) significantly varied between Annabelle and Andean Sunside (Table 5.4).

Given the different parentage and origin of Annabelle and Andean Sunside, the number of significant compositional differences between these two varieties is expected. Variety is known to significantly affect flavour-related composition and many compositional differences were identified between *S. tuberosum* and *S. phureja* varieties in Morris et al. (2010). In Oruna-Concha et al. (2002a), variation in the presence and levels of volatiles between eight varieties of microwaved potatoes was suggested to be linked to different levels of flavour precursors and different activities of lipid enzymes, a major source of volatile flavour metabolites. As discussed in Section 2.3.5, *S. phureja* varieties have different levels of gene expression for cell-wall biosynthetic enzymes that contribute to a crumbly and floury texture (De Maine et al., 1993; Ducreux et al., 2008). Although texture was not assessed, the higher dry matter value for Andean Sunside reflects this. Previous literature indicates *S. tuberosum* samples may contain significantly higher levels of reducing sugar compared to *S. phureja*, as observed (Dobson et al., 2010). This suggests the Annabelle samples could be perceived as sweeter than Andean Sunside. The significant difference in sucrose levels is less supported in existing literature (Dobson et al., 2010; Morris et al., 2010) - only after 3 months storage were *S. phureja* sucrose levels significantly higher than *S. tuberosum*. Annabelle also contained significantly higher levels of polyphenols and glycoalkaloids, which may result

in a higher intensity of bitterness compared to Andean Sunside. However, as consumer-perceived flavour was not compared between Annabelle and Andean Sunside due to the sensory trial design, these differences in composition can only be described, and cannot be discussed in relation to comparative flavour, in this case.

6.3 Was consumer acceptance affected by nitrogen fertilisation?

While the analysis of average liking alone determined no difference between nitrogen treatments for both varieties, using cluster analysis and converting the data into rank positions returned significant results, indicating that nitrogen fertilisation levels may have affected consumer liking. Using liking rank positions, Andean Sunside 0 kg N ha^{-1} samples were ranked significantly higher in liking compared to 300 kg N ha^{-1} samples. Although the LAM scale labels malfunctioned for many of the first sessions where Annabelle was assessed (Section 4.4.2), potentially affecting the liking assessments, very few consumers appeared to notice the error - therefore, it is unlikely this issue affected the data collected. For Annabelle, the three clusters formed from cluster analysis each preferred a different level of nitrogen fertiliser (Fig. 5.8). For Andean Sunside, three clusters were also formed, each cluster instead disliking a different level of nitrogen fertiliser. While average liking for each treatment, in each cluster, was not tested to see if the differences were significant, Fig. 5.8 clearly shows a large variation in preference for different rates of nitrogen fertiliser applied. Although further investigation into potential factors driving these preferences was not carried out, further work on this is suggested to determine whether a larger number of consumers display the same clustering and preferences for different levels of nitrogen. If this is true, it indicates consumer segmentation, which could be important for industry to consider.

Overall, limited research exists to indicate whether these results for the effect of nitrogen fertiliser on potato liking can be supported or disputed. The potential impact of nitrogen fertiliser on off-flavour development was extensively discussed in Chapter 2. Other evaluations have linked increases in off-flavour attributes to a decrease in sample preference and overall impression (Sharma, 2019). In Heeb et al. (2006), an increase in the ratio of nitrate to ammonium ions in tomato fertigation solution decreased consumer-perceived flavour intensity, sweetness, and overall acceptance of tomato fruit. Therefore, these results suggest that for preference to be affected, the treatments must have a large-enough impact on flavour that can be perceived by consumers. In this study, while changes in flavour were indeed perceived by the participants (Table 5.6), this did not have an impact on average liking, with only a subtle difference in ranking for Andean Sunside. Furthermore, as the rate of nitrogen applied to commercial Annabelle and Andean Sunside crops ranges from 120 - 150 kg N ha^{-1} (personal communication, B. Hart, February 8, 2020), the significant decrease in rank score for Andean Sunside 300 kg N ha^{-1} is unconcerning, assuming commercial nitrogen application rates either remain stable or decrease.

6.4 Has nitrogen affected consumer-perceived flavour?

Increasing the rate of nitrogen fertiliser applied to Annabelle and Andean Sunside crops slightly, but significantly, affected consumer-perceived flavour in both varieties. In Annabelle, 300 kg N ha^{-1} samples contained significantly higher intensities of nutty, musty, and sour, when using data from discriminating participants. In Andean Sunside, 0 kg N ha^{-1} samples were significantly more buttery and less green grass flavoured. Cardboard flavour was the highest in 150 kg N ha^{-1} samples, the intensity approximately double that of 0 kg N ha^{-1} and 300 kg N ha^{-1} samples.

In Chapter 2, the predicted impact of nitrogen fertiliser on perceived flavour, as related to previous compositional changes observed, is thoroughly discussed. In short, increasing rates of nitrogen fertiliser was predicted to increase off-flavours, especially cardboard-like off-flavours, bitterness (if above the threshold of TGA perception), and potentially savoury taste (Eppendorfer, 1996; Fischer, 1991; Hunnius et al., 1978; Love et al., 1994; Morris et al., 2007; Thybo et al., 2006). While the significant effects on flavour in this study could not be significantly linked to average liking (due to the lack of significant differences for sample acceptance), the positive and negative associations of the attributes impacted by fertiliser, as described, support the existing literature suggesting high rates of nitrogen increase the perception of off-flavours. In Annabelle this was observed, with an increase in musty and sour intensity at the highest nitrogen application rate. Musty is clearly described as an off-flavour in Mazza and Pietrzak (1990). In Andean Sunside, 150 kg N ha^{-1} samples were significantly more green grass flavoured compared to 0 kg N ha^{-1} samples. 'Green' is an odour quality linked to off-flavoured aldehydes (e.g. pentanal) (Petersen et al., 1999). Buttery flavour, a desirable sensory attribute, decreased between 0 and 150 kg N ha^{-1} Andean Sunside samples - this also points to a deterioration in flavour with increasing rates of nitrogen fertiliser. In Andean Sunside, cardboard flavour also increased between 0 and 150 kg N ha^{-1}. Only the increase in nutty flavour in Annabelle (between 150 N and 300 N) was a positive nitrogen effect, relating to an increase in "sweet and roasted flavour[s]" (Table 3.4). Therefore, based on the significant treatments effects, for Annabelle, either 0 and 150 kg N ha^{-1} treatments are recommended to reduce the intensity of off-flavoured attributes. For Andean Sunside, reducing the standard rate of nitrogen could improve flavour perception, with an increase in buttery flavour and a decrease in cardboard and green grass intensity observed in 0 kg N ha^{-1} samples.

However, despite these significant effects, the actual changes in intensities were very small. For example, buttery flavour decreased in intensity from 3.21 to 2.20 (using discerning participants), between 0 N and 150 N samples (in Andean Sunside). This is only a small change, when the maximum rateable intensity was 9. Therefore, while flavour was significantly affected, these differences were not large enough to influence average consumer acceptance.

Chapter 7

Conclusions

The flavour of high-value potatoes is an important consideration for potato growers, given the role of flavour in driving consumer preference and purchasing behaviour. However, while the components of potato flavour are mostly well understood, there is limited understanding around how growing practices or other aspects of production impact flavour, before the potatoes reach consumers. In New Zealand especially, there is growing concern around the use of nitrogen fertiliser. If the current application rates must be adjusted, in response to the introduction of council regulations for example, growers must understand how these changes could impact the flavour of their high-value potatoes, which are marketed as a product with premium flavour - this must remain consistent. Therefore, the impact of varying rates of nitrogen fertilisation on flavour-related composition was investigated for two high-value gourmet potato varieties, Annabelle (*S. tuberosum*), and Andean Sunside (ex *S. phureja*). Two varieties with different parentage were selected to investigate how effects on flavour could vary by variety. Consumer sensory testing was carried out to determine whether liking or perceived flavour was affected by nitrogen, as assessed by those who ultimately purchase and consume the products of interest.

7.1 Summary of main findings

Flavour and composition were significantly affected by nitrogen fertilisation

Increasing the rate of nitrogen fertiliser applied affected some flavour-related metabolites in both Annabelle and Andean Sunside. An increase in tuber total nitrogen and nitrogen transporting amino acids help to indicate the treatments were applied correctly and taken up by the plants, which was a concern for Andean Sunside with significant weed pressure at its crop site. Nitrate increased for Annabelle but not Andean Sunside - nitrate was undetected in ~75% of samples. This suggests Andean Sunside could be a low nitrate variety,

linked to nitrate reductase activity in the leaves. In Annabelle alone, 2-butanone, 2-methyl-2-(*E*)-butenal, methional, and benzaldehyde increased with nitrogen, while dry matter and both α-solanine and α-chaconine concentrations decreased. The effect of fertiliser on β-ocimene was variable. In Andean Sunside, α-solanine, β-ocimene, and phenethyl alcohol all increased with nitrogen supply, while glucose and fructose concentrations significantly decreased. Levels of (*Z*)-hex-3-en-1-ol, linalool, and benzyl alcohol significantly increased between 0 and 150 kg N ha^{-1} treatments. The effect of nitrogen on quercetin-3-rutinoside was inconsistent in Andean Sunside.

Based on existing flavour literature, it was predicted these changes would not result in large flavour differences, especially consumer-perceived flavour. This is because the changes, while significant, were likely too small to be perceptible. Furthermore, the concentrations measured for some compounds, such as α-solanine and α-chaconine, were below previously reported threshold values. Increasing the rate of nitrogen fertiliser did not affect the content of aldehydes linked to potato off-flavour (POF), one of the predicted effects of nitrogen fertilisation. However, off-flavour is more likely to develop when cooked potatoes are stored. Therefore, in future studies, the precursor fatty acids should also be measured to determine the influence of nitrogen on the POF aldehyde formation potential.

While average liking was not significantly affected by nitrogen, Andean Sunside 0 kg N ha^{-1} samples were ranked significantly higher in liking compared to 300 kg N ha^{-1} samples. Overall, nitrogen fertilisation appeared to slightly, but significantly, increase the intensity of attributes with more negative associations. Annabelle 300 kg N ha^{-1} samples contained significantly higher intensities of nutty, musty, and sour attributes, while Andean Sunside 0 kg N ha^{-1} samples were significantly more buttery, and less green grass-flavoured, compared to 150 kg N ha^{-1} samples. Therefore, for Annabelle, applying 150 kg N ha^{-1} will reduce the change of off-flavour formation. In Andean Sunside, reducing the rate of nitrogen further, from 150 kg N ha^{-1}, will also reduce the potential for off-flavour formation. Flavour may even improve with an increase in buttery flavour intensity. While these flavour changes may have impacted ranked liking in Andean Sunside, these results were not reflected in average consumer liking across both varieties. However, further work should be conducted on the consumer segmentation observed as clear preferences for different levels of nitrogen were distinctly present for both Annabelle and Andean Sunside.

Although both flavour-related composition and perceived flavour were significantly effected by the rate of nitrogen fertiliser applied, these could not be associated, within the varieties and treatments used in this study. As consumers are poorer discriminators and less-sensitive compared to trained panellists, repeating this study using trained panellists may reveal more insight into how changes in composition and perceived flavour are linked.

Composition significantly varied between Annabelle and Andean Sunside

As expected, many signifiant compositional differences were identified between Annabelle and Andean Sunside. The concentration of umami amino acids were significantly higher in Andean Sunside, suggesting these samples could contain higher intensities of savoury taste, as previously reported for varieties with *S. phureja* genetics. Annabelle samples contained significantly higher concentrations of polyphenols and glycoalkaloids (TGA). As both of these compound groups have been linked to bitterness, Annabelle samples could be perceived as comparatively more bitter. However, with respect to the (only limited and/or dated) information available on taste thresholds, at the levels measured in this study, overall taste was not expected to be impacted. The concentration of glucose, sucrose, and fructose significantly varied between the varieties. Annabelle contained a higher concentration of reducing sugar and Andean Sunside contained higher concentration of non-reducing sugar. Many volatile compounds varied significantly between Annabelle and Andean Sunside, however, as these were not quantified, it was difficult to determine whether the significant differences would be perceptible to consumers. However, as Annabelle and Andean Sunside were conducted as separate sensory evaluations, the consumer-perceived flavour of these varieties could not be compared.

Agronomic parameters were largely unaffected

Given the importance of agronomic parameters to growers, such as predicted yield and tuber size, these data were measured to ensure the impacts of nitrogen on flavour were also considered within the context of commercial potato production. For tuber count, diameter, mass, and predicted field yield, no significant differences were observed between nitrogen treatments for Andean Sunside. In Annabelle, tuber count significantly decreased between 0 and 150 kg N ha^{-1}, and tuber mass significantly increased between 0 and 150 kg N ha^{-1}. No other parameters were affected. Therefore, reducing the rate of nitrogen applied to Annabelle crops, from its standard application rate of 150 kg N ha^{-1} may increase the total number of tubers harvested, but decrease the average mass. While existing literature suggests yield will increase with nitrogen, from 0 kg N ha^{-1}, in this case, it had no effect. For Andean Sunside, the weed pressure during crop growth and the number of seed misses likely increased the variability between field replicates, resulting in the lack of significant variation between nitrogen treatments. Therefore, these results may be not be reflective of a commercial Andean Sunside crop, and should be regarded with caution.

Rate-all-that-apply methodology

As rate-all-that-apply is still a relatively new sensory evaluation technique, the data were approached in two different ways. By excluding the d ata from participants that failed to perceive an attribute across all three nitrogen treatments (i.e. using discerning participants), higher average attribute intensities were observed, in addition to better visual separation between treatments within each variety. For foods like potatoes, where their flavour has previously been regarded as weak or mild, this approach is beneficial in helping see differences between treatments. Additionally, compared to changing all non-checks to zero, using discerning participants resulted in a slight improvement in significant sample discrimination between flavour attributes.

7.2 Relevance of these findings

This study is the first comprehensive evaluation investigating the impact of varying rates of nitrogen fertiliser on both potato sensory-perceived flavour and composition. The results of this study help to update the existing body of literature related to this topic, as presented in the literature review. This is also the first completed study on consumer-perceived flavour and composition in potatoes. In addition, this is the first time the rapid consumer method, rate-all-that-apply, has been used to assess potato flavour.

The results of this study also have a number of implications for potato growers, as the funders of this research. This research was carried out to investigate how nitrogen fertiliser affects potato flavour, in relation to both the environmental implications of nitrogen, and growing high-value, flavourful potatoes, that can be sold at a premium price. This research indicates that keeping nitrogen application rates at 150 kg N ha^{-1}, or less, will reduce the potential for off-flavour formation, and even improve flavour, especially in Andean Sunside. However, flavour effects will vary between varieties, as observed. Additionally, varying the rate of nitrogen did not significantly affect yield, even at 0 kg N ha^{-1}. The increase in tuber number and decrease in tuber mass, as observed for Annabelle, is beneficial for their marketing and sale as small, gourmet potato product. The results of this project, recommending the application of reduced rates of nitrogen fertiliser, also have additional environmental and social benefits for the vegetable production industry as a whole.

7.3 Recommendations for future work

- As limited literature is available on the impact of agronomic factors on flavour in general, a similar study could be undertaken on the effect of other nutrient inputs (e.g. potassium), or irrigation, on flavour, because of the value this knowledge holds for potato growers.
- Because consumer testing is less sensitive than using a trained panel, if nitrogen-related impacts on flavour must be more clearly understood, this study should be repeated using a panel trained for quantitative descriptive analysis on steamed potatoes.
- Given the interesting preferences revealed in cluster analysis for different levels of nitrogen fertiliser, this study could be repeated with a larger number of participants, in combination with phenotyping, to determine the factors driving the consumer segmentation observed. If this segmentation is reflective of many regular potato consumers, these results have implications for growers and their ability to grow potatoes that can cater to different consumer preferences.
- To gain a better understanding of more subtle changes in flavour that could be occurring between the three application rates used in this study, the study could be repeated with a larger number of nitrogen fertiliser rates, for example, 0, 50, 100, 150, and 200 kg N ha^{-1}. However, if this was conducted, more replication will be required, as the treatments are closer together.

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Appendix A

Trial site soil tests

Certificate of analysis of soil tests for Andean Sunside and Annabelle planning planting locations, sampled on 10 August 2018. Low/Medium/High scale based off historical potato soil testing data and recommended levels selected by Hill Labs.

Andean Sunside

Sample Name: Kala 1
Sample Type: SOIL Potato (S64)
Lab Number: 2029816.6

Analysis		Level Found	Medium Range
pH	pH Units	6.5	5.4 - 5.8
Olsen Phosphorus	mg/L	53	30 - 60
Potassium	me/100g	1.04	0.50 - 1.00
Calcium	me/100g	8.0	4.0 - 10.0
Magnesium	me/100g	1.35	1.00 - 3.00
Sodium	me/100g	0.06	0.00 - 0.50
CEC	me/100g	17	12 - 25
Total Base Saturation	%	63	35 - 75
Volume Weight	g/mL	0.79	0.60 - 1.00
Sulphate Sulphur	mg/kg	37	20 - 50
Potentially Available Nitrogen (15cm Depth)*	kg/ha	48	100 - 150
Anaerobically Mineralisable N*	µg/g	41	
Organic Matter*	%	7.2	7.0 - 17.0
Total Carbon*	%	4.2	
Total Nitrogen*	%	0.43	0.30 - 0.60
C/N Ratio*		9.8	
Anaerobically Mineralisable N/Total N Ratio*	%	1.0	
Base Saturation %		K 6.3 Ca 48	Mg 8.1 Na 0.4
MAF Units		K 17 Ca 8	Mg 24 Na 2

Low Medium High

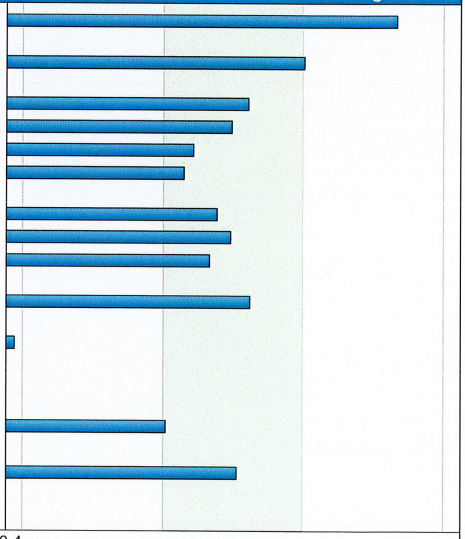
The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information. IANZ Accreditation does not apply to comments and interpretations, i.e. the 'Range Levels' and subsequent graphs.

Figure A.1: Hill Laboratories soil test results for Andean Sunside trial site, sampled on 10 August 2018.

Annabelle

Sample Name: Kala 2
Sample Type: SOIL Potato (S64)
Lab Number: 2029816.7

Analysis		Level Found	Medium Range
pH	pH Units	6.5	5.4 - 5.8
Olsen Phosphorus	mg/L	61	30 - 60
Potassium	me/100g	0.81	0.50 - 1.00
Calcium	me/100g	6.9	4.0 - 10.0
Magnesium	me/100g	1.43	1.00 - 3.00
Sodium	me/100g	0.07	0.00 - 0.50
CEC	me/100g	17	12 - 25
Total Base Saturation	%	54	35 - 75
Volume Weight	g/mL	0.73	0.60 - 1.00
Sulphate Sulphur	mg/kg	39	20 - 50
Potentially Available Nitrogen (15cm Depth)*	kg/ha	44	100 - 150
Anaerobically Mineralisable N*	µg/g	40	
Organic Matter*	%	7.2	7.0 - 17.0
Total Carbon*	%	4.2	
Total Nitrogen*	%	0.46	0.30 - 0.60
C/N Ratio*		9.1	
Anaerobically Mineralisable N/Total N Ratio*	%	0.9	
Base Saturation %		K 4.7 Ca 41	Mg 8.4 Na 0.4
MAF Units		K 12 Ca 6	Mg 24 Na 3



The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information. IANZ Accreditation does not apply to comments and interpretations, i.e. the 'Range Levels' and subsequent graphs.

Figure A.2: Hill Laboratories soil test results for Annabelle trial site, sampled on 10 August 2018.

Appendix B

Average tuber mass served in sensory analysis

Table B.1 includes the average tuber mass served per sensory session, variety, and treatment. Each mass is averaged across 10-12 potatoes.

Table B.1: The average tuber mass served in the sensory evaluation.

			Average mass of tuber for serving (g)						
Variety	**Field rep**	**Treatment**	8 AM	9.30 AM	11 AM	12.30 PM	2 PM	3.30 PM	**Average**
Annabelle	3	0 N	99.2	95.4	83.9	98.6	94.2	93.9	94.2
		150 N	73.2	80.4	90.2	90.2	87.1	88.4	84.9
		300 N	88.5	88.0	87.6	78.6	86.4	84.0	85.5
Annabellle	6	0 N	79.8	74.0	80.1	79.9	82.0	88.1	80.7
		150 N	94.7	95.6	104.8	99.2	92.0	88.4	95.8
		300 N	102.5	110.7	98.7	109.7	96.7	110.5	105.0
Andean Sunside	4	0 N	57.0	56.1	70.9	118.8	87.0	70.0	76.6
		150 N	58.6	70.2	89.2	130.1	94.0	62.9	84.2
		300 N	54.0	55.8	67.1	103.9	79.5	74.7	72.5
Andean Sunside	5	0 N	56.2	71.8	83.1	124.7	84.0	67.5	81.2
		150 N	52.2	69.5	96.1	121.4	102.1	63.1	84.1
		300 N	49.9	49.4	91.8	122.4	71.3	71.2	76.0
Average			70.8	76.4	87.0	106.5	88.0	80.2	84.9