Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Opportunities for the development of clean label probiotic ice cream

A thesis presented in partial fulfilment of the requirements for the degree of Master of Food Technology

Massey University
Albany, New Zealand

Reihaneh Feizi

2020

Abstract

In ice cream manufacture, stabilisers and emulsifiers are used as additives to produce products with desirable properties. Typically, mono-/diglycerides, polysorbate, and several gums such as guar gum, locust bean gum and carboxymethyl cellulose are used to emulsify and stabilise ice creams, respectively. Consumers have long been skeptical about the presence of additives with unfamiliar and complex chemical compounds with E-numbers on the label of food products and consider products with simple and natural ingredients as healthier. Therefore, there is need to identify potential ingredients to replace the commonly used additives in ice cream and produce products which may be more appealing to consumers. In this study chia seed gum (mucilage) (CSG) and sweet cream buttermilk (SCB) were the two ingredients identified to replace the commonly used stabilisers and emulsifiers in ice cream, respectively. The addition of a probiotic culture was also considered to produce functional ice cream with clean label as well as probiotic properties. The study commenced with the extraction of CSG, followed by four integrated phases which involved the production of prototype ice creams. The extraction of chia seed mucilage from chia seed using a seed:water ratio of 1:20 at 50°C/40 min, yielded 3.40±0.16% (w/w) of the dried CSG (9.9% moisture (w/w)). The pH of the extracted mucilage before drying was 6.14±0.07. In Phase I, the extracted CSG was added to ice cream at three concentrations (0.1%, 0.2% and 0.3% (w/w)) to replace the commercial stabilisers. Physico-chemical properties (pH, viscosity of the ice cream mix, overrun, fat globule size distribution, destabilised fat index, meltdown rate and hardness of ice cream) were determined in the prototype ice creams. The frozen desserts were also evaluated by a focus group of six sensory panelists. The most acceptable ice cream contained 0.2% CSG (w/w) which was characterised by an overrun of 103.10±0.47%, 41.00±5.35% destabilised fat index, 40.66±8.87% fat globule size distribution ($d_{4,3}$) and low meltdown rate (0.96±0.14 g/min) compared to the ice cream which contained guar gum (commercial stabiliser). The hardness of the ice cream was 40.06±7.23 N. The ice cream had desirable hardness, smooth texture and creamy mouthfeel without sensation of ice crystals and off-flavour.

In Phase II, the emulsifying ability of CSG was investigated in the ice cream formulation selected from Phase I (F2) in the absence of mono-/diglycerides (MDG) (commercial emulsifier used in Phase I). The absence of MDG from F2 did not change the viscosity of the ice cream mix (F7) whereas the overrun, destabilised fat index and fat globule size ($d_{4,3}$) of the ice cream decreased to 77.86±5.83%, 8.43±1.55% and 28.15±36.10%, respectively. The meltdown rate and hardness increased to 1.61±0.10 (g/min) and 93.88±15.5 (N), respectively. The sensory properties of F2 were more desirable to the focus group than F7. Sweet cream buttermilk was used in Phase III to improve the emulsification attributes of the ice cream (F7) due to the absence of MDG. Therefore, in this Phase the emulsifying properties of SCB was investigated when it partially substituted the skim milk powder and comprised 25% and 50% (w/w) of the nonfat milk solids (NMS) in ice cream. The addition of SCB did not affect the viscosity of the ice cream mix but it enhanced the overrun to 82.71±1.61% and 88.90±0.10% when constituted 25% (w/w) (F8) and 50% (w/w) of the NMS (F9), respectively. The increase in the concentration of SCB decreased the rate of meltdown to 1.57±0.05 (g/min) in F9 and 1.60±0.08 (g/min) in F8. The destabilised fat

index and fat globule size distribution ($d_{4,3}$) of the ice cream decreased to 8.03±1.31% and 6.74±0.30% in F8 and 4.43±3.84% and 22.53±2.12% in F9, respectively. The hardness of F8 and F9 increased to 102.17±41.00 and 101.85±33.00 N, respectively. The focus group selected F9 due to its smooth texture, desirable hardness, mouthfeel and melting attributes. F9 was selected as the best formulation based on physico-chemical and sensory results. In Phase IV, the effect of the addition of 10% milk fermented with probiotic culture (*Lactobacillus acidophilus* La-5) (w/w), to the ice cream mix (F10) after aging was investigated. The addition of the probiotic culture slightly enhanced the viscosity of the ice cream mix whereas the overrun and meltdown rate of the ice cream decreased to 88.49±0.54% and 1.24±0.04 (g/min), respectively. Compared to the rest of the formulations, the ice cream mix of F10 showed a high fat globule size distribution ($d_{4,3}$) (12.90±0.90%). Relative to F9, formulation F10 had a higher destabilised fat index (11.96±1.44%) but this ice cream had a lower fat globule size ($d_{4,3}$) (13.83±2.02%) and hardness (84.20±18.70 N). In this Phase, the pH, viable cell counts and the consumer acceptance (using a 9-point hedonic scale) of the ice cream (F10) were evaluated after hardening (48 h) and prolonged storage (30 days) at -20 °C. The pH, viable cell counts and overall mean sensory acceptability scores for the ice cream after hardening (48 h) were 6.27±0.01, 1.15×10^7 CFU/mL and 7.10±0.97 (n=24) whereas the respective values were 6.27±0.06, 1.07×10^7 CFU/mL and 7.27±1.14 (n=28) respectively, after 30 days of frozen storage. The sensory characteristics of the ice cream were stable during storage and the survival cell counts were above the recommended minimum limit (10^6 CFU/mL) required to confer probiotic health benefits. The overall results indicated that CSG can be used as stabiliser and emulsifier in ice cream but the overrun would be low. For producing ice cream with higher overrun, it would be desirable to add SCB. Also, results showed that a probiotic culture can be added to ice cream which improves the health attributes of the frozen dessert as well as decreases the meltdown rate of the ice cream. Further study is required to determine the contribution of CSG, SCB and the probiotic culture to the microstructure of ice cream using cryo-scanning electron microscopy.

Acknowledgments

I would like to express my sincere gratitude to my supervisor Dr. Tony Mutukumira for his continuous support, guidance, patience and motivation. I would also like to thank my co-supervisor, Associate Prof. Kelvin Goh who has been available whenever I needed his advice. My supervisors helped me throughout the research and writing this thesis with their constructive comments and encouraging me to bring the best out of my capabilities.

I would like to thank Associate Prof. Marie Wong and Associate Prof. Kay Rutherfurd-Markwick for their support. I also thank Negah Nikanjam, Rachel Liu and Begum Noorzahan for their help. I thank Dr. Charles Diako and Dr. Nihal Jayamaha for their guidance. I also thank the members of the focus groups and all the participants in the consumer sensory evaluation sessions.

I am grateful for the experience of working with Fonterra Brands (Tip Top) Limited, specifically Esraa El shall who was always happy to help and support me. I thank Chito Ramirez who kindly helped me whenever we needed to upscale in the pilot plant. It is also worth to note that working with the members of the research team in this company was a great experience for me.

Last but not least, I thank God for having my lovely family who have always supported me throughout my life.

Table of contents

Chapter 1 Introduction **1**

1.1 Aims and objectives 4

Chapter 2 Literature review **5**

2.1 Introduction 5

2.2 Global production and consumption of ice cream 6

2.3 Market and consumer trends 7
2.3.1 Demand for healthy food products 7

2.4 Manufacture of ice cream 13
2.4.1 Preparation of the mix 14
2.4.2 Pasteurisation 15
2.4.3 Homogenisation 15
2.4.4 Aging 16
2.4.5 Freezing 17
2.4.6 Hardening 21
2.4.7 Storage 22

2.5 Function of the constituents in ice cream 22
2.5.1 Milkfat 23
2.5.2 Nonfat milk solids (NMS) 24
2.5.3 Sweeteners 25
2.5.4 Stabilisers 26
2.5.5 Emulsifiers 28
2.5.6 Water and air 29

2.6 Opportunities to replace commercial additives in ice cream 29
2.6.1 Alternative stabilisers 29
2.6.2 Alternative emulsifiers 32

2.7 Functional properties of chia seed and sweet cream buttermilk for application in ice cream 34
2.7.1 Background 34
2.7.2 Chia seed 34
2.7.3 Sweet cream buttermilk 37

2.8 Analysis of ice cream and characterisation methods 40
2.8.1 Physical properties 40
2.8.2 Chemical and compositional properties of ice cream 44
2.8.3 Microbiological properties 45
2.8.4 Sensory evaluation 45

2.9 Conclusion 45

Chapter 3 Experimental background **47**

3.1 Organisation of the experiments 47

Chapter 4 Extraction of gum from chia seed **50**

4.1 Introduction 50
4.2 Materials and methods 51
4.2.1 Extraction of chia seed gum 51
4.2.2 Analysis of yield and moisture content 54
4.3 Results and discussion 54
4.4 Conclusion 56
Chapter 5 Optimisation of ice cream formulation containing chia seed gum and sweet cream buttermilk 57
5.1 Phase I Addition of chia seed gum as stabiliser to ice cream 57
5.1.1 Introduction 57
5.1.2 Materials and methods 58
5.1.3 Results and discussion 64
5.1.4 Summary 77
5.2 Phase II Addition of chia seed gum as stabiliser and emulsifier to ice cream 78
5.2.1 Introduction 78
5.2.2 Materials and methods 78
5.2.3 Results and discussion 79
5.2.4 Summary 86
5.3 Phase III Addition of sweet cream buttermilk as emulsifier to ice cream 87
5.3.1 Introduction 87
5.3.2 Materials and methods 87
5.3.3 Results and discussion 88
5.3.4 Summary 94
5.4 Phase IV Addition of CSG, SCB and probiotic culture to ice cream 95
5.4.1 Introduction 95
5.4.2 Materials and methods 95
5.4.3 Results and discussion 97
5.4.4 Summary 109
Chapter 6 General discussion 110
Chapter 7 Conclusion 112
Chapter 8 Recommendations 113
Chapter 9 References 114
Chapter 10 Appendices 137
10.1 Appendix A 137
10.1.1 Raw data 137
10.1.2 Calculation of ice cream mix formulations 150
10.2 Appendix B Data analysis 154
10.3 Appendix C Data analysis of sensory evaluation results 177

List of Figures

Figure 2.1 Clean label food categories experiencing the highest growth (Anonymous, 2017a).....8
Figure 2.2 Markets for food products containing probiotics (Bourianne, 2018) 9
Figure 2.3 Description of clean label (Rownan, 2017).....12
Figure 2.4 Ice cream processing (Goff, 1997).....14
Figure 2.5 Structure of ice cream mix and ice cream (Marshall, Goff, & Hartel, 2003).....18
Figure 2.6 Ice crystals in ice cream (Marshall, Goff, & Hartel, 2003).....19
Figure 2.7 A transmission electron micrograph showing the structure of partially coalesced fat, the freeze-concentration of casein micelles in the unfrozen phase and the interaction of fat at the air interface. 20
Figure 2.8 Partially coalesced fat globule aggregates and their associated protein membranes adsorbed to the surface of an air bubble interface (Goff, 1997).....21
Figure 2.9 Scanning electron micrographs showing the presence and absence of EPSs. White continuous line in image shows the network strands suggesting EPS structure (Dertli et al., 2016).....31
Figure 2.10 Chia seed (Muñoz, Cobos, Diaz, & Aguilera, 2013).....35

Figure 4.1 Extraction of chia seed gum 52
Figure 4.2 Sequential stages of the extraction of chia seed gum: (a) chia seed; (b) soaked chia seed in water; (c) hydrated chia seed after 40 min; (d) overhead stirrer; (e) Silverson mixer; (f) separated mucilage after using the silverson mixer; (g) centrifuged aqueous suspension; (h) seed residues; (i) extracted mucilage (gum); (j) Freeze-dried mucilage (gum) (The images were captured by iPhone 8, Apple Inc., USA).....53

Figure 5.1 Production of ice cream.....59
Figure 5.2 Apparent viscosity of ice cream mixes.....66
Figure 5.3 Effect of chia seed mucilage (0.25%) on storage (G′), loss (G″) moduli and tan δ as functions of frequency at constant temperature of 25°C. G′: full symbols; G″: empty symbols; tan δ: line. 67
Figure 5.4 Set up of meltdown rate test of an ice cream sample. 69
Figure 5.5 Weight loss of ice cream samples during meltdown 70
Figure 5.6 Average particle size distribution of ice cream mix 72
Figure 5.7 Average particle size distribution of ice cream melt 73
Figure 5.8 Hardness of ice cream samples.....76
Figure 5.9 Apparent viscosity of ice cream mixes.....79
Figure 5.10 Weight loss of ice cream samples during meltdown 82
Figure 5.11 Average particle size distribution of ice cream mix 83
Figure 5.12 Average particle size distribution of ice cream melt 84
Figure 5.13 Hardness of ice cream samples.....85

Figure 5.14 Apparent viscosity of ice cream mixes....88
Figure 5.15 Weight loss of ice cream samples during meltdown....90
Figure 5.16 Average particle size distribution of ice cream mix....91
Figure 5.17 Average particle size distribution of ice cream melt....92
Figure 5.18 Hardness of ice cream samples....93
Figure 5.19 Apparent viscosity of ice cream mixes....98
Figure 5.20 Weight loss of ice cream samples during meltdown....99
Figure 5.21 Average particle size distribution of ice cream mix....101
Figure 5.22 Average particle size distribution of ice cream melt....102
Figure 5.23 Hardness of ice cream samples....103
Figure 5.24 A schematic diagram of structure of ice cream, showing the principal structural elements of fat globules and clusters, air bubbles and ice crystals, all embedded within the freeze-concentrated unfrozen phase (Drawn using Microsoft Paint based on Goff, (2011))....104

List of Tables

Table 2.1 Approximate composition (%) of commercial ice cream (Goff, 2016) 23
Table 2.2 Relative sweetness index and freezing point depression of various sweeteners (Goff & Hartel, 2013) 26

Table 3.1 Description of variables used in each formulation 49

Table 5.1 The overrun, meltdown rate and pH of ice cream formulations 68
Table 5.2 Fat globule size distribution, destabilised fat index of ice cream mix and ice cream 74
Table 5.3 The overrun, meltdown rate and pH of ice cream formulations 80
Table 5.4 Fat globule size distribution, destabilised fat index of ice cream mix and ice cream 82
Table 5.5 The overrun, meltdown rate and pH of ice cream formulations 89
Table 5.6 Fat globule size distribution, destabilised fat index of ice cream mix and ice cream 90
Table 5.7 The overrun, meltdown rate and pH of ice cream formulations 98
Table 5.8 Fat globule size distribution, destabilised fat index of ice cream mix and ice cream 100
Table 5.9 Mean consumer sensory scores for commercial retail ice cream and F10 107
Table 5.10 Mean consumer sensory scores for commercial retail ice cream and F10 after storage (-20°C) for four weeks 108

List of Appendices

10.1 Appendix A....137

10.1.1 Raw data....137

Table 10.1 Mean value of hardness (N)....137
Table 10.2 Mean weight loss (g) of ice cream during meltdown rate test at 20°C....137
Table 10.3 Regression equations obtained from meltdown rate curves....138
Table 10.4 Overrun (%) of each formulation....138
Table 10.5 Destabilised fat index (DFI)....139
Table 10.6 Apparent viscosity of the ice cream mixes over the shear rate range of 0.1-100 s^{-1}....140
Table 10.7 The mean pH of each ice cream mix....145
Table 10.8 The mean pH of the cultured milk and the ice cream after storage for four weeks at -20°C....145
Table 10.9 Viable cell counts....145
Table 10.10 Weight of the chia seed gum (g) obtained from 240 g chia seed....145
Table 10.11 Consumer sensory scores for commercial retail ice cream....146
Table 10.12 Consumer sensory scores for clean label probiotic ice cream (F10)....147
Table 10.13 Consumer sensory scores for commercial retail ice cream after four weeks of storage at -20°C....148
Table 10.14 Consumer sensory scores for clean label probiotic ice cream (F10) after four weeks of storage at -20°C....149
Table 10.15 pH of the extracted mucilage from chia seed before drying....150

10.1.2 Calculation of ice cream mix formulations....150

Table 10.16 Ice cream mix formulations (Ingredients (g/100 g ice cream mix))....152

10.2 Appendix B: Data analysis....154
10.3 Appendix C: Data analysis of sensory evaluation results....177

List of Abbreviations

ANOVA	Analysis of Variance
BSG	Basil Seed Gum
CFU	Colony Forming Unit
CMC	Carboxymethyl cellulose
CSG	Chia Seed Gum (interchangeable with chia seed mucilage)
DE	Dextrose Equivalent
DFI	Destabilised Fat Index
EC	European Community
ESP(/s)	Exopolysacharide(/s)
FAO	Food and Agriculture Organisation
FGSD	Fat Globule Size Distribution
GRAS	Generally Recognised As Safe
HFCS	High-Fructose Corn Syrup
HHST	High-Heat Short-Time
HP treatment	High-Pressure treatment
HTST	High-Temperature Short-Time
LAB	Lactic Acid Bacteria
LTE	Low-temperature Extruder
LTLT	Low-Temperature Long-Time
MDG	Mono-/diglyceride
MFGM	Milk Fat Globule Membrane
MPa	Megapascal
NMS	Nonfat Milk Solid
SCB	Sweet Cream Buttermilk
SMP	Skim Milk Powder
UHT	Ultra-High Temperature
WHO	World Health Organization

Chapter 1 Introduction

Today's consumers have become increasingly conscious about the importance of food products on their health and well-being, due to the insurgence of food related illnesses (Soukoulis, Fisk, & Bohn, 2014). They tend to choose healthy food products that support healthy lifestyle and therefore, try to make informed purchase decisions by reading the ingredient list on the label of food products (Asioli et al., 2017). Consumers make strong association on the level of healthfulness of food products and claims such as "natural/All natural", "No artificial ingredient" and "No additives/E-numbers" (Anonymous, 2014).

Food additives are substances of natural or synthetic origin, which are added to foods to serve a certain technological and sensory function (Shim et al., 2011; Bearth, Cousin, & Siegrist, 2014). Depending on the respective food regulations, additives should be written by their specific names or E-numbers on the label of food products (Branen, Davidson, Salminen, & Thorngate, 2001). The E-numbering system was introduced in Europe in the sixties in which food additives were identified with an 'E' letter followed by 3 or 4-digits as shorthand for writing complicated chemical names or for the identification of permitted food additives (Tarnavolgyi, 2003). The E-numbers were later adapted in many other countries worldwide as an International Numbering System (INS) for food additives (Euromonitor International, 2016). The numbering system generated some challenges as they do not provide adequate information for consumers at the point of purchase (Dervisoglu & Yazici, 2006; Soukoulis, Lebesi, & Tzia, 2009). Consumers do not particularly know the purpose and origin of complex chemical compounds, scientific language and E-numbers on the label of food products. They are concerned about long lists of ingredients on food labels and have negative perceptions towards food additives and consider the additives as unnatural, unhealthy or even a public health risk (Campos, Ruivo, da Silva Scapim, Madrona, & Bergamasco, 2016). Therefore, there is strong desire for producing food products with a short and simple ingredient list without artificial or 'chemical-sounding' additives or E-numbers listed on food labels (Sloan & Adams Hutt, 2015).

On clean label perception, which provides a perceived healthier proposition (Berryman, 2014), consumers prefer minimally processed products containing familiar ingredients found in kitchens

and natural sounding ingredients that are easy to pronounce, with the knowledge of their origin (Asioli et al., 2017; Rownan, 2017).

Furthermore, consumers are increasingly seeking foods that provide additional health benefits that may reduce disease risk or promote health. Functional foods are foods or food components that may provide benefits beyond basic nutrition (Granato, Branco, Cruz, Faria, & Shah, 2010). The functional food market is dominated by foods containing probiotics or their ingredients (Soukoulis, Fisk, & Bohn, 2014).

Therefore, functional and clean label food products are currently among the major drivers in the global food industry (Tuormaa, 1994; Granato, Branco, Cruz, Faria, & Shah, 2010; Soukoulis, Fisk, & Bohn, 2014). The two trends are attracting the attention of both consumers and manufacturers (Euromonitor International, 2016; Aschemann-Witzel, Varela, & Peschel, 2019). Sales of natural healthy products increased by more than US$70 billion in 2008-2013 and fortified/functional products reached US$270 billion in 2013 (Bandy, 2013).

Food that contains probiotics is a common example of a functional food. To confer health properties, probiotics in foods should be at least 10^6 CFU/ml at the time of consumption (FAO/WHO, 2002). Therefore, the viability of probiotics must be maintained during production through to consumption (Mohan, Quek, Gutierrez-Maddox, Gao, & Shu, 2017). Compared with fermented milks as a vehicle for probiotics, ice cream provides an environment that can prolong the viability of microorganisms, due to its low temperatures during storage (Cruz, Antunes, Sousa, Faria, & Saad, 2009). Ice cream can be considered a suitable vehicle for the delivery of probiotics to a wider population due to its global popularity, consumer acceptability and appealing sensory attributes (Turgut & Cakmakci, 2009; Soukoulis, Fisk, & Bohn, 2014).

Over the years, there have been several attempts to develop ice cream with probiotic properties (Mohammadi, Mortazavian, Khosrokhavar, & da Cruz, 2011; Soukoulis, Fisk, & Bohn, 2014). In addition, finding potential alternative ingredients and processing methods which help to produce ice cream free from additives (stabilisers and emulsifiers) have generated increased interest (Segall & Goff, 2002b; Innocente, Comparin, & Corradini, 2002; Dervisoglu & Yazici, 2006; Goh, Nair,

& Matia-Merino, 2008; BahramParvar & Goff, 2013; Campos, Ruivo, da Silva Scapim, Madrona, & Bergamasco, 2016). Emulsifiers and stabilisers (e.g. mono-/diglycerides, polysorbate, and several gums such as guar gum, locust bean gum and carboxymethyl cellulose) are commonly used in the manufacture of conventional ice cream due to their desirable technological functions. To date, there is no published study on the development of clean label probiotic ice cream.

The overall aim of this study was to develop ice cream with a simple and short ingredient list using ingredients which are natural and familiar to consumers. Consequently, it was prompted to replace the commercial stabilisers and emulsifiers with natural ingredients that could impart similar or improved functionality as compared to ice cream produced commercially. Chia seed mucilage and sweet cream buttermilk were two key ingredients identified to replace the stabilisers and emulsifiers, respectively. The ingredients were selected based on their positive technological functions and desirable effects on processing and characteristics of the final product. The mucilaginous gel obtained from the hydrated chia seed contributes to the stabilisation of food structure through gel formation or thickening of the continuous phase due to its water-holding and water-absorption capacities (Capitani, Spotorno, Nolasco, & Tomás, 2012; De Falco, Amato, & Lanzotti, 2017). The water-holding capacity of chia seed gum is comparable to that of guar gum and nearly four times higher than that of gum Arabic (Timilsena, Adhikari, Kasapis, & Adhikari, 2016). Sweet cream buttermilk is another key ingredient in our study which imparts strong emulsifying properties to the ice cream which is similar to egg yolk due to the higher lecithin content (13 times) than that of skim milk (Whitaker, 1930).

In order to confer probiotic properties, a probiotic culture, *Lactobacillus acidophilus* La-5, was added to the ice cream mix. The developed ice cream can meet the expectation of consumers who seek after foods that are additive free, natural, and possess functional health benefits associated with the probiotic culture, chia seed and sweet cream buttermilk.

1.1 Aims and objectives

The study aimed to evaluate the potential of chia seed mucilage and sweet cream buttermilk as stabiliser and emulsifier in ice cream, respectively. The impact of a probiotic culture added to the ice cream mix was also investigated.

1.1.1 Specific objectives

- To extract the gum from chia seed and determine its moisture.
- To determine the optimum concentration of CSG as a stabiliser for the production of ice cream in place of commercial stabilisers by evaluating its physico-chemical and sensory properties.
- To evaluate the potential of CSG as an emulsifier in ice cream.
- To determine the optimum concentration of sweet cream buttermilk as emulsifier for the production of ice cream to replace the commercial emulsifiers.
- To evaluate the quality characteristics of the ice cream incorporated with *Lactobacillus acidophilus* La-5, by evaluating its physico-chemical properties, microbial analyses and consumer sensory evaluation.
- To examine the viability of the probiotics and sensory acceptance of the ice cream by consumer sensory evaluation after prolonged storage.

Chapter 2 Literature review

2.1 Introduction

Ice cream is a delicious, nutritious frozen dairy product which is widely consumed in different parts of the world due to its sweet flavour, smooth velvety texture, refreshing and delightfully palatable characteristics and glistening coldness (Marshall, Goff, & Hartel, 2003). Ice cream is also considered a nutritious product containing protein, minerals (calcium, phosphorus, iron) and vitamins (vitamin A, thiamine, and riboflavin) (Vivek, 2001). Historically, ice cream contained milk, sugar, cream and natural flavour in its formulation. As ice cream technology evolved over the years, additional ingredients known as additives spesifically stabilisers and emulsifiers have been added to contribute to the desirable smooth, creamy texture and mouthfeel without the sensation of large ice crystals (Lal, O'Connor, & Eyres, 2006; Goff, 2008; Alting & Van De Velde, 2012). These additives also impart desirable meltdown properties and improved storage stability during temperature fluctuation (Clarke, 2015).

Hydrocolloids are biopolymers commonly used in ice cream formulations as stabilisers (Freitas, Alves, & Reis, 2011) most of which are chemically or enzymatically modified to improve their rheological properties (Patel & Prajapati, 2013). Mono-/diglycerides and polysorbate 80 are common emulsifiers used in ice cream manufacture which are produced by chemical hydrolysis of vegetable fats. Stabilisers and emulsifiers have restricted use for food applications and are considered additives under European law (Patel & Prajapati, 2013; Clarke, 2015).

A previous study revealed that globally the number of people rating ingredient recognition as ‘very important’ is increasing from 78% in 2011 to 81% in 2013 (Anonymous, 2014). In the same survey, mono-/diglycerides, guar gum, carrageenan and carboxymethyl cellulose accounted for the ingredients not well understood and poorly accepted by consumers due to their chemical sounding and long names. Consumers prefer to avoid food additives especially synthetic, chemical and artificial sounding ones. This concern is related to a general fear of chemicals and their association with diseases (Blanchfield, 2000). The clean label proposition is generally considered to be seeking

natural alternatives to food additives, and listing named ingredients on labels rather than E-numbers (Berryman, 2014). Continuous attempts in finding new sources of emulsifiers and stabilisers over the years, represent the interest in substituting commercial additives and E-numbers with natural and cleaner ingredients. Currently, there are few published information on the subject of clean label and most of the present data seems to be provided by surveys and reports.

In this study, the attempt was to develop a probiotic clean label ice cream in the absence of commercial additives (stabilisers and emulsifiers) using natural alternatives. Chia seed gum and sweet cream buttermilk were used as stabiliser and emulsifier respectively identified for this study. In order to improve the functional properties of the ice cream, a probiotic culture was added.

2.2 Global production and consumption of ice cream

As a multi-billion dollar industry, ice cream trade is a big business as it serves to satisfy some of the most ardent cravings for food among humans (Marshall, Goff, & Hartel, 2003). New Zealand has one of the largest ice cream consumers in the world with 28 Liter (L) *per capita* compared to the US with 21 and Australia with 18 L *per capita*. In Western Europe and Eastern Europe, the reported ice cream volumes consumed *per capita* were 7-10 L and 3-4 L *per capita*, respectively. Although China is the largest manufacturer of ice cream in the world, it consumes only 2 liters *per capita*. The market for ice cream is highly segmented. The Asia pacific region makes up about 35% of the market by volume but only 25% by value which can be attributed to the Chinese market where there are a lot of products in the low end of the price. On the other hand, Western Europe even though accounts for 25% of the market by value, it only makes up about 18% of the volume which is associated with a lot more products with higher value per unit volume (Professor H.D Goff, personal communication, 31 January, 1500 h, 2019).

2.3 Market and consumer trends

2.3.1 Demand for healthy food products

The value of the dairy sector in New Zealand grew by an average of about 5% per annum, from about NZD8 billion in 2001 to about NZD17 billion in 2016 (Anonymous, 2018a). Among dairy products, ice cream and frozen desserts experienced a strong growth in this country in 2018 with over NZD400 million, which is almost double that of the previous period (2016/2017). The growth was attributed to the warmest summer in recorded history of New Zealand. The category is projected to reach nearly NZD500 million by 2023 (Euromonitor International, 2018). The global ice cream market is estimated to reach around USD80 billion to USD90 billion in global sales sometime between 2023 and 2025 (Hosch, 2018).

Consumers desire healthy food products, and ice cream is no exception. This concept is known as "permissible indulgence". In Mexico and Central America, ice cream with health and wellness claims increased from 15% in 2013 to 20% in 2017. A majority of European and American consumers are looking for healthier food products with natural and authentic ingredients (Anonymous, 2014; Hosch, 2018). This ever-growing need provides the food industry with an opportunity to connect with the issues that are driving the purchasing habits of consumers (Berryman, 2014). Fairlife Ultra Filtered Milk is one example, which is now the fastest growing among the top 10 milk brands in the US. The company capitalised on the clean label opportunity through providing a healthier halo for the product by utilising processing techniques to position the product as less processed with improved nutritional quality (Anonymous, 2018b). Another example is the Heinz tomato ketchup which was reformulated to remove high fructose corn syrup from the ingredient list and was renamed as simply Heinz (Asioli et al., 2017).

The market for products viewed as "clean label" has experienced a substantial growth in the recent years, and is estimated to rise from over USD160 billion in 2015 to about USD180 billion by 2020 (Anonymous, 2017b). As shown in Figure 2.1, the two categories which experienced the largest sales growth due to clean label products in 2017 were salty snacks (nearly USD200 million, or 1.9%) and candy (more than USD150 million, or 11.7%). The sales of clean label ice cream in 2017 grew by 26.5% which led to driving an incremental USD106 million to the ice cream

category in 2016. Other clean label food categories which experienced sales growth include beverages, liquid coffees, baked breads, frozen entrées, “wholesome” snacks, yogurt and cookies (Anonymous, 2017a).

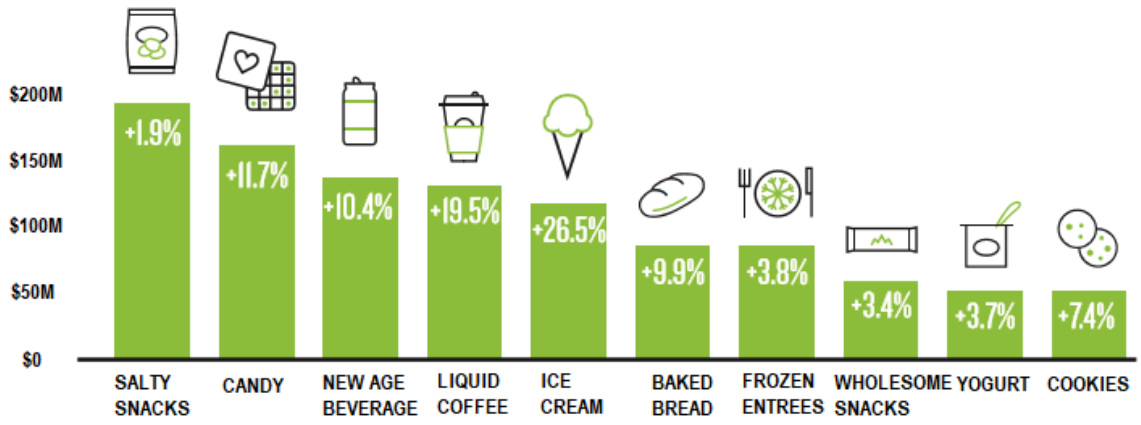


Figure 2.1 Clean label food categories experiencing the highest growth (Anonymous, 2017a)

Most people believe that good diet and nutrition play an important role in prevention of illness and disease, and consequently over 65% have made dietary changes to maintain good health (Peirce, 1997; Asioli et al., 2017). Therefore, there is an increasing demand for “clean label” food products with functional properties.

2.3.1.1 Functional food products

Functional foods are foods that may provide health benefits beyond basic nutrition (Siro, Kápolna, Kápolna, & Lugasi, 2008). Marketing of functional foods is dominated by probiotics. Probiotics are live microorganisms that, when ingested in adequate amounts, exert a health benefit on the host (FAO/WHO, 2002). More than 6,400 food products are being produced with probiotic properties due to a long history of the safe use of probiotics in food products (Bourianne, 2018).

Probiotic ice cream

The commercial application of probiotics has been adopted by almost all major food manufacturers. The dairy sector which comprises the largest functional food market is strongly linked to probiotics (Granato et al., 2010). After yogurt, drinks and bakery products, ice cream has undergone developments for incorporation of probiotics for therapeutic effects (Figure 2.2).

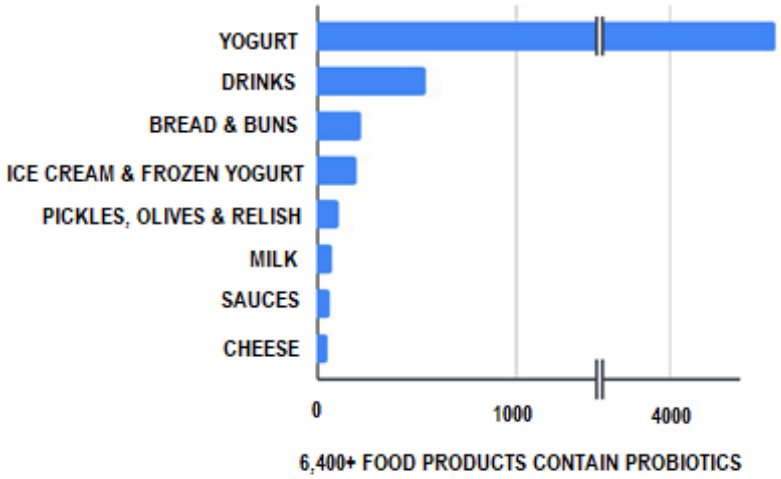


Figure 2.2 Markets for food products containing probiotics (Bourianne, 2018)

Previous studies have shown the efficacy of probiotics in prevention and management of gut diseases, such as inflammatory bowel disease, diarrhea, and potentially reducing the risk of colon cancer (Liong, 2011; Erejuwa, Sulaiman, & Wahab, 2014). Other health benefits associated with the probiotic cultures include reduction of serum cholesterol levels, improvement of lactose tolerance, calcium absorption, vitamin synthesis, and stimulation of the immune system (Turgut & Cakmakci, 2009; Soukoulis, Fisk, & Bohn, 2014).

Low processing and storage temperatures as well as the high consumer acceptability and appealing sensory attributes, render ice cream a suitable carrier for the delivery of bioactive compounds such as probiotic cultures (Cruz, Antunes, Sousa, Faria, & Saad, 2009; Turgut & Cakmakci, 2009; Granato, Branco, Cruz, Faria, & Shah, 2010; Mohammadi, Mortazavian, Khosrokhavar, & da Cruz, 2011). *Lactobacillus* and *Bifidobacterium* are the most common probiotic bacteria that

have been used in ice cream production (Alamprese, Foschino, Rossi, Pompei, & Savani, 2002; Di Criscio et al., 2010) with cell counts of over 10^6 CFU/g after six months of frozen storage (Haynes & Playne, 2002; Turgut & Cakmakci, 2009; Di Criscio et al., 2010; Granato, Branco, Gomes Cruz, Fonseca Faria, & Shah, 2010; Mohammadi, Mortazavian, Khosrokhavar, & da Cruz, 2011; Soukoulis, Fisk, & Bohn, 2014).

Probiotic ice cream can be produced by direct inoculation of the mix with freeze-dried or harvested cells or blending the ice cream mix with various concentrations of cultured milk (4-50%) at different stages of production depending on whether the ice cream is fermented or non-fermented (Salem, Fathi, & Awad, 2005; Magariños, Selaive, Costa, Flores, & Pizarro, 2007).

There have been some discrepancies on the survival rate of freeze-dried and harvested cells in frozen ice cream (Nousia, Androulakis, & Fletouris, 2011; Zhang et al., 2017). While the addition of the probiotic strains in form of cultured milk affected the overrun and meltdown rate, the ice cream samples received high sensory scores (Salem, Fathi, & Awad, 2005). The addition of harvested cells did not affect the physical properties of ice cream but changed the pH and sensory properties of the product (Alamprese, Foschino, Rossi, Pompei, & Corti, 2005; Abghari, Sheikh-Zeinoddin, & Soleimanian-Zad, 2011).

Viability of probiotics in ice cream

Losses in the viability of probiotic bacteria in aerated frozen dairy desserts occur during processing, storage and melting. During these processing steps probiotics are subjected to several intrinsic (species/strain, morphological characteristics of the bacteria and ability to produce intracellular cryoprotectants) and extrinsic (food substrate composition, exposure to heat, mechanical treatments, oxygen toxicity and pH) parameters (Soukoulis, Lyroni, & Tzia, 2010; Mohammadi, Mortazavian, Khosrokhavar, & da Cruz, 2011). Populations of 10^6–10^7 CFU/g viable cells in the final product are considered as therapeutic quantities of probiotic cultures in processed foods (Cruz, Antunes, Sousa, Faria, & Saad, 2009). Relatively, probiotic ice cream with higher pH (5.5 - 6.5) provides an increased survival of the probiotic cultures during storage as well as higher consumer acceptance (Cruz, Antunes, Sousa, Faria, & Saad, 2009; Mohammadi, Mortazavian, Khosrokhavar, & da Cruz, 2011). The lethality of probiotics throughout freezing is

primarily associated with the osmotic and mechanical stresses. Rapid freezing of the probiotic ice cream mix minimises sublethal injuries of the bacterial cells due to producing smaller ice crystals (Mohammadi, Mortazavian, Khosrokhavar, & da Cruz, 2011; Soukoulis, Fisk, & Bohn, 2014). Although some sugars such as sucrose and lactose show a cryoprotective effect on probiotics, a relatively high sugar content can adversely affect the viability of probiotics due to excessive increases in osmotic pressure (Mohammadi, Mortazavian, Khosrokhavar, & da Cruz, 2011).

A further possibility in improving the survival of probiotics is the use of prebiotics such as inulin which are selectively metabolised by probiotics and enhance their growth and activity (Akin, Akin, & Kirmaci, 2007; Di Criscio et al., 2010). The physical protection of probiotics by microencapsulation is another approach to improve the probiotic survival which helps to isolate the bacterial cells from the effects of the hostile environment and gastrointestinal tract (Homayouni, Azizi, Ehsani, Yarmand, & Razavi, 2008; Soukoulis, Fisk, & Bohn, 2014). Ahmadi et al. (2014), showed that the viable counts of free probiotics decreased from ~9.55 to ~7.3 log CFU/g after 60 days of frozen storage however encapsulated cells decreased by less than 1 log cycle.

2.3.1.2 Clean label food products

Consumers prefer products with simple ingredient lists which contain kitchen-type ingredients which can be easily recognised (Sloan & Adams Hutt, 2015, Euromonitor International, 2016). The concept which is called ‘clean label’ is driven by the consumer’s need to understand food labels, desire for transparency, and increasing interest in healthfulness of the diet (Sloan & Adams Hutt, 2015; Anonymous, 2018c). Generally, clean label involves the replacement of chemical sounding ingredients, artificial additives and E-numbers with natural alternatives (Blanchfield, 2000; Berryman, 2014). Figure 2.3 describes some attributes of clean label food ingredients and/or the foods.

Figure 2.3 Description of clean label (Rownan, 2017)

Most countries and regions have regulatory organisations for the approval of food additives (Anonymous, 2014; Aschemann-Witzel, Varela, & Peschel, 2019). Additives are natural or artificial substances added to food products to enhance food characteristics, processing, handling and storage (Shaheen Koyratty, Aumjaud, & Neeliah, 2014). In the 1960s the then European Community (EC) introduced the 'E' letter followed by a 3 or 4-digit number, which indicated the approved additives were safe for use (Tarnavolgyi, 2003; Saltmarsh, 2013).

The rapid expansion of the E-numbers in the food industry was viewed as an opportunity for manufacturers to hide the use of unnecessary components. The E-number generated a source of concern among consumers (Saltmarsh, 2013) and still E-numbers are surrounded by an atmosphere of mystery and distrust. In addition, in modern societies, the increasingly globalised markets and greater processing in the food chain have contributed to a perceived distance and knowledge gap between people and food manufacturers (Asioli et al., 2017).

Pollan (2008), suggested that consumers should not "...eat anything with more than five ingredients, or ingredients they can't pronounce". In 1994, Tuormaa estimated the growing use of food additives to over 200,000 tons per year with each person consuming an average 3.5-4.5 Kg (8-10 pounds) of food additives per year with some possibly eating considerably more. The report

linked food additive intolerance to various physical and mental disorders, particularly with childhood hyperactivity.

Problems often occur when additives are consumed in large quantities that could be harmful to the body (Campos, Ruivo, da Silva Scapim, Madrona, & Bergamasco, 2016). Consumers are concerned that the approvals process only assess additives consumed in normal and average quantities whereas some people may regularly exceed these amounts (Anonymous, 2017c). They are worried about long term serious health risks such as cancer which may currently be unknown or uncertain but could emerge years in the future (Altug & Elmaci, 1995; Tarnavolgyi, 2003; Williams, Stirling, & Keynes, 2004; Anonymous, 2017c).

Therefore, clean label positioning descriptions including "natural/All natural", "No artificial ingredient", "No additives/E-numbers" influence consumers switch their brands (Anonymous, 2014). Although the term clean label itself appeared for the first time during the 1980s, its importance in the food business has markedly grown (Saltmarsh, 2013). According to Rownan (2017), the market leader for clean label food is North America (USD67 billion), followed by Europe (USD59 billion) and China (USD23 billion). The global clean label category itself is dominated by retail packaged foods (USD 129 billion), soft drinks (USD 34 billion) and hot drinks (USD 3 billion) (Rownan, 2017).

2.4 Manufacture of ice cream

In order to provide uniform quality and meet legal standards, the mix composition needs to be balanced by performing mix calculation (Marshall, Goff, & Hartel, 2003). With increasing total solids in the mix composition, the proportion of water and the frozen water decreases (Sommer, 1951). Total solids contribute to the mix viscosity, the body and texture which range between 36-40% with sweeteners accounting for 12-16% of the formulation (Goff, 2016). High proportion of total solids may result in a heavy and soggy ice cream (Marshall, Goff, & Hartel, 2003).

The production of ice cream consists of preparation of mix, pasteurisation, homogenisation, aging, freezing, packaging and hardening followed by its storage (Figure 2.4) (Goff, 1997; Granger, Leger, Barey, Langendorff, & Cansell, 2005).

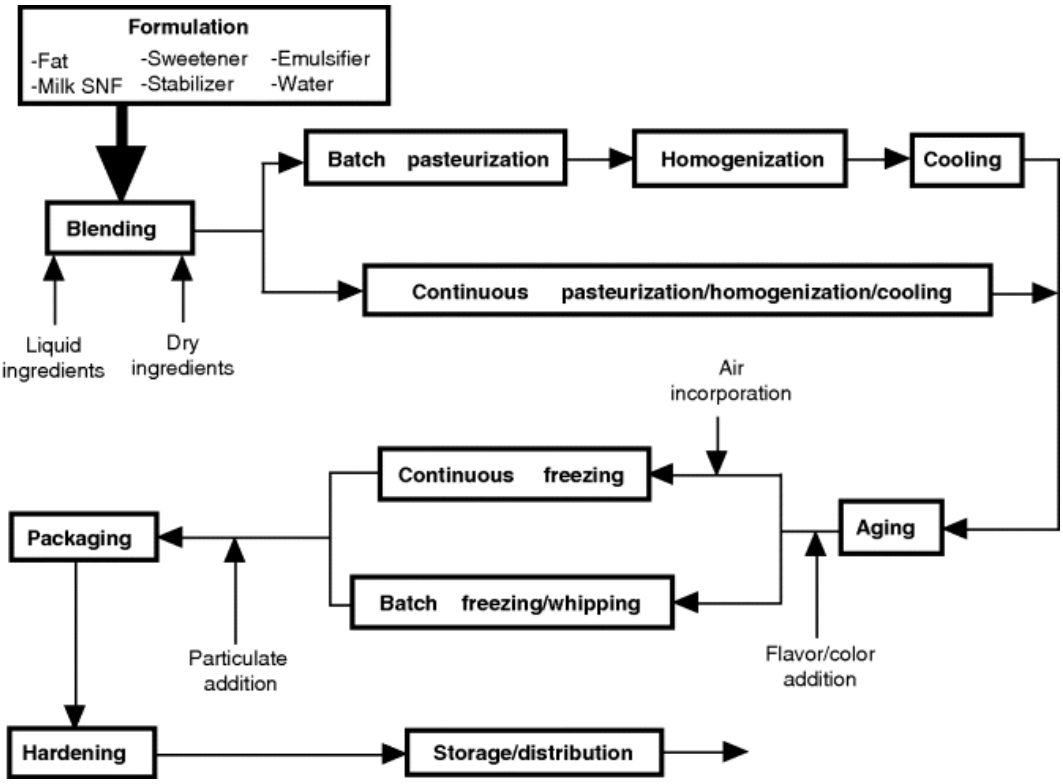


Figure 2.4 Ice cream processing (Goff, 1997)

2.4.1 Preparation of the mix

The ice cream mix is prepared by combining all the ingredients in which dry ingredients are added to the liquid materials which are being agitated before the temperature reaches 50°C. In this stage, agitation and high temperature help to dissolve the ingredients (Marshall, Goff, & Hartel, 2003).

2.4.2 Pasteurisation

Ice cream mixes are pasteurised to destroy potential pathogenic microorganisms and ensure the health and safety of consumers (Goff, 1997). Furthermore, pasteurisation eliminates the enzymatic activities of the raw materials which may affect the flavour and texture of the final product (Goff & Hartel, 2013). Heating the mix during pasteurisation helps to dissolve and hydrate the ingredients in the aqueous serum (Marshall, Goff, & Hartel, 2003). It also aids in homogenisation by melting the fat and decreasing the viscosity of the mix (Marshall & Arbuckle, 1996). Pasteurisation can be achieved by the batch (low-temperature long-time (LTLT) (69°C, 30 min)), and continuous (high-temperature short-time (HTST) (80°C, 25 s), higher-heat short-time (HHST) (90°C, 1-3 s), and ultra-high temperature (UHT) (138°C, >2 s)) (Marshall, Goff, & Hartel, 2003). Higher heat treatments increase the water-holding capacity of proteins, improve the body and texture and reduce the amount of required stabilisers by up to 25%. Moreover, the unfolded proteins resulted from high heat treatments act as additional reducing groups and increase the oxidation resistance (Pei & Schmidt, 2010; Goff, 2016).

2.4.3 Homogenisation

Homogenisation reduces the size of fat globules to less than 2 μm and increases the number and surface area of fat droplets by forcing ice cream mix through a small orifice under moderate pressure and temperature (~ 80°C) (Goff, 1997; Marshall, Goff, & Hartel, 2003). The applied pressure depends on the fat content of the mix. For a mix with 10% milkfat in a two-stage homogeniser the pressures are 13.8 MPa and 3.45 MPa on the first and second stages, respectively (Marshall, Goff, & Hartel, 2003). Homogenisation produces a stable and homogeneous fat emulsion without a buttery appearance and flavour (Tosaki, Kitamura, Satake, & Tsurutani, 2009). After homogenisation, surface active materials such as milk proteins (caseins, whey proteins and MFGM proteins), polar lipid materials native to the butterfat and added emulsifiers (mono-/diglycerides and polysorbates), are adsorbed to the newly bare surface of the fat globules to reduce the interfacial tension and stabilise the droplets (Goff & Jordan, 1989; Segall & Goff, 1999; Daw & Hartel, 2015).

The concentration of protein in a standard mix (10-14%, fat) is about 4%, which is sufficient to stabilise the fat droplets in an ice cream emulsion. The excess protein remains in the serum and contributes to overrun and texture (Oortwijn & Walstra, 1979; Tomas, Paquet, Courthaudon & Lorient, 1994). The fat droplets covered with proteins are very stable and therefore small molecule surfactants are required to displace the protein during aging and facilitate the partial coalescence of fat droplets (Segall & Goff, 1999).

2.4.4 Aging

Aging at 4°C for 4-24 h is necessary for an ice cream mix as it provides enough time for critical rearrangements in the fat globule membranes (Goff & Jordan, 1989; Segall & Goff, 1999) as well as full hydration of milk proteins and hydrocolloid stabilisers (Goff, 1997). Small molecule surfactants such as mono-/diglycerides or polysorbate 80 have a greater affinity towards the fat/water interface than the proteins and therefore displace the milk proteins from the interface (Segall & Goff, 1999; Daw & Hartel, 2015). This type of rearrangement in the fat globule is known as fat destabilisation or de-emulsification. Moreover, the fat phase of the mix begins to crystallise during aging (Berger & White, 1971; Goff & Jordan, 1989). Partially crystalline fat enhances fat destabilisation or partial churning due to shear forces during freezing (Goff Liboff, Jordan, & Kinsella, 1987).

Small molecule surfactants provide a thinner coating on the fat droplets than proteins with a reduced steric barrier which helps the occurrence of partial coalescence. Ice cream mixes which are not aged or formulated without small molecule surfactant do not undergo much partial coalescence and tend to be wet and lack body when frozen (Goff & Jordan, 1989; Segall & Goff, 1999). In the presence of emulsifiers, fat crystallisation and protein desorption are distinctly enhanced which result in increased agglomeration of fat globules during dynamic freezing deemed important for the formation and stability of the final product (Barford, Krog, Larsen, & Buchheim, 1991).

2.4.5 Freezing

The scraped surface freezers which are designed to remove heat from viscous liquids such as ice cream, consist of a cylindrical barrel equipped with a rotating stainless steel dasher. The dasher is equipped with scraper blades that fit tightly inside the barrel (Marshall, Goff, & Hartel, 2003). The ice cream mix is chilled in the freezer and whipped as the dasher rotates and subjects the mix to high shear (Clarke, 2015). The second function of the dasher is to scrape off the layer of ice crystals formed on the cold barrel wall. A typical temperature of drawing ice cream lies between -5 to -6°C (Hagiwara & Hartel, 1996). In a batch freezer the atmospheric air is incorporated in the aqueous phase due to a vigorous whipping process, whereas in a continuous freezer, the air is drawn into the mix by vacuum or injected under pressure (Pei & Schmidt, 2010). During freezing, at the same time that the ice crystals are forming and air is being incorporated, the partial coalesced fat globules contribute to the stabilisation of small incorporated air bubbles (Goff, Liboff, Jordan, & Kinsella, 1987).

2.4.5.1 Changes that take place during freezing

The aged ice cream mix is comprised of colloidal proteins as well as suspended fat globules with fat crystals inside the droplet and a mixed membrane of protein and surfactants (Figure 2.5). The rest of the components including carbohydrates, salts and proteins are dissolved in the water phase (Goff & Hartel, 2013).

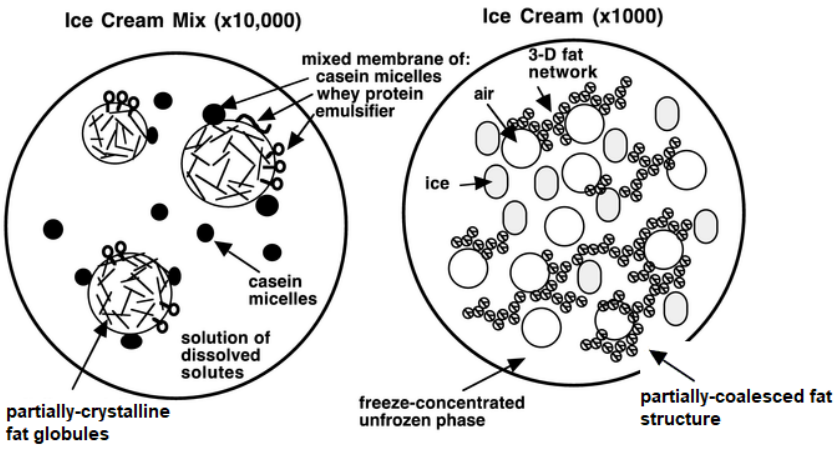


Figure 2.5 Structure of ice cream mix and ice cream (Marshall, Goff, & Hartel, 2003)

The structure of the ice cream mix changes markedly as it passes through the scraped surface freezers. Formation of ice crystals, destabilisation of the fat emulsion and incorporation of air account for substantial changes that occur during freezing (Figure 2.5) (Marshall, Goff, & Hartel, 2003).

I. Formation of ice crystals

During freezing, the water is frozen out of the solution to form ice crystals which results in an increase in the viscosity of the remaining fluid phase (unfrozen phase) followed by a decrease in the freezing point (Goff, 1997). The uniform size distribution of small ice crystals minimises the influence of thermal shock from high or fluctuating storage temperatures and enhances storage stability and shelf-life. The smooth surface of small ice crystals (preferably < 30 µm) (Figure 2.6) results in a smooth ice cream with creamy texture whereas the prevalence of large ice crystals (> 50 µm) yields a coarse (icy) textured ice cream (Marshall, Goff, & Hartel, 2003). Proper processing, storage condition and formulation account for the factors which contribute to the development of small ice crystals (Hagiwara & Hartel, 1996). Freezer temperatures as low as -30°C, result in rapid freezing and short residence times in the freezer that can promote ice nucleation and minimise the growth of ice crystals (Ruger, Baer, & Kasperson, 2002). Moreover, the type and amounts of ingredients in the ice cream mix can affect the characteristics of ice

crystals. In particular, sweeteners and stabilisers are the most important ingredients which influence the size of ice crystals (Hagiwara & Hartel, 1996).

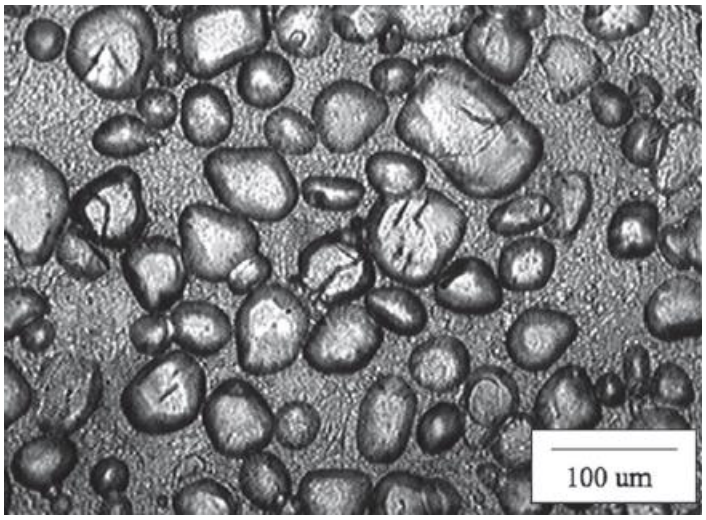


Figure 2.6 Ice crystals in ice cream (Marshall, Goff, & Hartel, 2003)

II. Destabilisation of the fat emulsion

Fat contributes to the structure of ice cream during freezing and whipping, by forming a partially coalesced three-dimensional network of fat globules (Bolliger, Goff, & Tharp, 2000). The semi-continuous network of fat clusters which stabilise the air cells, is responsible for the beneficial properties of stiffness and dryness upon extrusion, a smoother texture, creamy mouthfeel and resistance to meltdown during consumption (Pei & Schmidt, 2010). Partially coalesced fat globules are also vitally important in preventing collapse of the foam especially during storage (Marshall, Goff, & Hartel, 2003).

The concomitant effect of freezing, aeration and mechanical agitation results in the formation of a semi-solid foam and causes the emulsion to be partially coalesced due to the decreased interfacial tension at the surface of destabilised fat globules (Gelin, Poyen, Courthaudon, Le Meste, & Lorient, 1994). The increased concentration of components in the unfrozen water phase coupled with the physical shape of ice crystals, contribute to shearing during freezing and enhance the fat

destabilisation (Marshall, Goff, & Hartel, 2003). Furthermore, needle-like fat crystals puncture the mixed protein emulsifier layer on the other droplet, allowing the droplets to coalesce. When the droplets partially coalesce, they form a cluster that retains some of their individual nature (Goff, Liboff, Jordan, & Kinsella, 1987) (Figure 2.7 and Figure 2.8). As mentioned earlier, emulsifiers play an important role in enhancing fat destabilisation in the frozen product (Goff & Jordan, 1989).

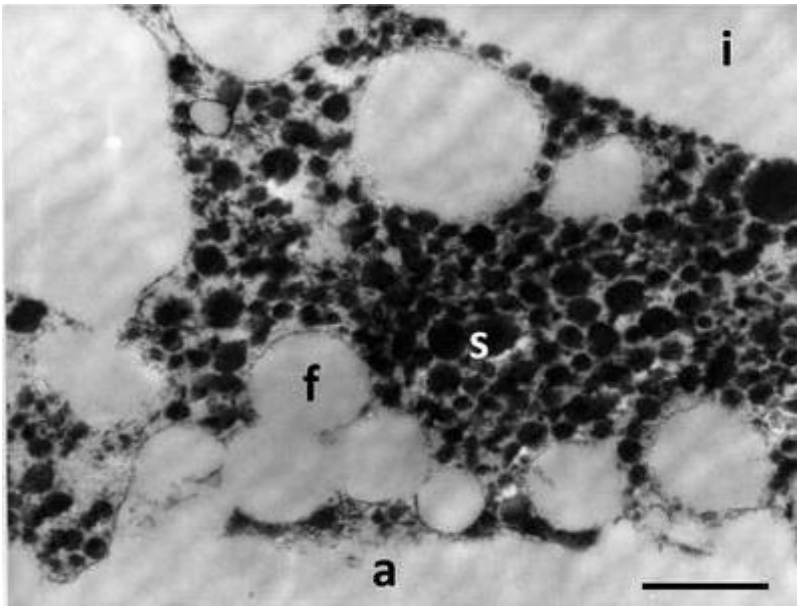


Figure 2.7 A transmission electron micrograph showing the structure of partially coalesced fat, the freeze-concentration of casein micelles in the unfrozen phase and the interaction of fat at the air interface.

Notes: a = air bubble, f = fat globule, i = ice crystal, s = serum phase packed with casein micelles, bar = 1 μm (Goff, 2016)

III. Incorporation of air

As the ice crystals are being formed and fat globules are coalescing during freezing, air is incorporated to form air cells. A typical ice cream consists of about 30% ice, 50% air, 5% fat and 15% matrix (sugar solution) by volume (Clarke, 2015). The air accounts for half of the volume of ice cream and must be stabilised as tiny bubbles in order to obtain ice cream with improved creaminess, optimal dryness and shape retention during melting as well as reduced rate of ice

recrystallisation (Pei & Schmidt, 2010). In ice cream the air cells have a range of sizes from a few microns to over 100 (Goff & Hartel, 2013).

The increased viscosity due to the freeze-concentration along with the shearing action of the dasher which breaks the large air bubbles into many smaller sizes enhance the stabilisation of air cells. Partially coalesced fat droplets come into contact with the air–matrix interface during freezing and help to immobilise and stabilise the air bubbles in ice cream (Figure 2.7, Figure 2.8) (Sofjan & Hartel, 2004; Pei & Schmidt, 2010; Bahramparvar & Mazaheri Tehrani, 2011).
In addition to the partially coalesced fat globule aggregates, a continuous layer of surface active milk proteins plays an important role in stabilising the air bubbles (Figure 2.8) (Goff, 2008).

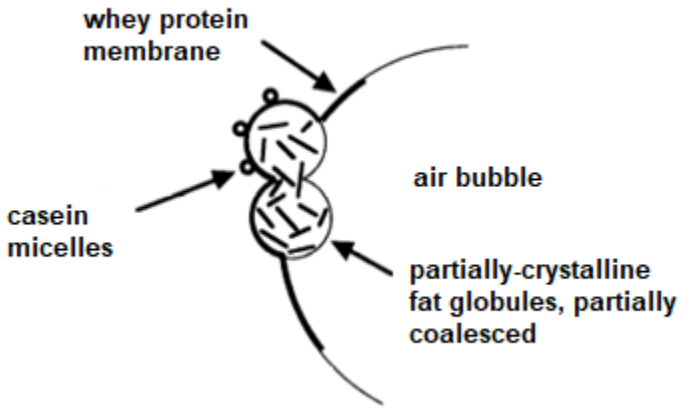


Figure 2.8 Partially coalesced fat globule aggregates and their associated protein membranes adsorbed to the surface of an air bubble interface (Goff, 1997)

2.4.6 Hardening

When ice cream leaves the freezer at about -5 ºC, it still contains between half to two thirds of its water in unfrozen state and is not stiff enough to maintain its shape (Hyde & Rothwell, 1973; Hagiwara & Hartel, 1996). Therefore, the packaged and labeled ice creams are transferred to a hardening room for further freezing (Hartel, 1996). During hardening, the products continue

freezing in air-blast freezer at −25 to −30°C until the temperature of the center of the product reaches to -18°C and 75-80% of the available water is frozen (Hartel, 1996; Marshall, Goff, & Hartel, 2003; Goff, 2016). The conversion of unfrozen water to ice and further concentration of the serum phase surrounding the ice crystals during hardening help to give ice cream its ability to be scooped and chewed at freezer temperatures (Goff, 2016).

In order to preserve the small ice crystals and air cells obtained during the initial freezing step, quick hardening is necessary to produce a smoother product because slow hardening results in a coarse textured ice cream (Ruger, Baer, & Kasperson, 2002). In general, no new ice crystals are formed during this process as the volume of the ice phase increases only through the growth of existing crystals (Hartel, 1996). Particulate flavoring ingredients, such as nuts, fruits, candy pieces, or ripple sauces, can be added to the semi-frozen product prior to hardening (Pei & Schmidt, 2010; Goff, 2016).

2.4.7 Storage

Following rapid hardening, ice cream storage should occur at low, constant temperatures, usually -25°C (Goff, 2016). Growth of ice crystals during storage or recrystallisation can affect the textural quality of ice cream (Miller-Livney & Hartel, 1997). The uniform size distribution of many small crystals is necessary to minimise the influence of thermal shock and enhance storage stability (Hagiwara & Hartel, 1996). Therefore, subsequent distribution and storage temperatures must be maintained to protect the structure and textural quality of the frozen dessert (Goff, 2011; Goff, 2016).

2.5 Function of the constituents in ice cream

Ice cream mix is a mixture of milkfat, nonfat milk solids (NMS), sweeteners, stabilisers, emulsifiers and flavours (Vivek, 2001). Mix formulations are defined as proportions of the constituents which can affect the quality of the mix and resultant ice cream (Goff & Hartel, 2013).

2.5.1 Milkfat

The milkfat content of ice cream varies from <0.5% in non-fat ice cream to ~18% in super premium ice cream (Table 2.1) (Goff, 2016). The fat component of ice cream mix carries flavour, gives body and contributes to a smooth and creamy texture. It aids the stabilisation of air bubbles, thereby contributing to desirable melting properties (Granger, Leger, Barey, Langendorff, & Cansell, 2005). Milkfat derived from cream, condensed milk blends, sweet (unsalted) butter, or anhydrous milk fat is the widely used fat source in the production of ice cream. Due to the association of high intake of saturated fat with public health, the use of vegetable fats and fat replacers in development of low-fat and non-fat products has gained popularity (Karaca, Guven, Yasar, Kaya, & Kahyaoglu, 2009; Choo, Leong, & Henna, 2010; Zulim Botega, Marangoni, Smith, & Goff, 2013).

Partial crystallisation of saturated fats in milkfat during aging contributes to the partial coalescence of fat droplets during freezing which is necessary in setting up the structure and texture in ice cream (Goff & Jordan, 1989; Granger, Leger, Barey, Langendorff, & Cansell, 2005). The development of the structure of ice cream, characterised by its smooth texture and resistance to collapse during melting partly depends on the presence of solid fat during whipping and freezing (Méndez-Velasco & Goff, 2011; Zulim Botega, Marangoni, Smith, & Goff, 2013).

Table 2.1 Approximate composition (%) of commercial ice cream (Goff, 2016)

Product	Milkfat	NMS	Sweeteners	Stabilisers and emulsifiers	Total solids
Non-fat ice cream	<0.5	12-14	18-22	1.0	28-32
Low fat ice cream	2-5	12-14	18-21	0.8	28-32
Light ice cream	5-7	11-12	18-20	0.5	30-35
Reduced fat ice cream	7-9	10-12	18-19	0.4	32-36
Economy ice cream	10	10-11	15-17	0.4	35-36
Standard ice cream	10-12	9-10	14-17	0.2-0.4	36-38
Premium ice cream	12-14	8-10	13-16	0.2-0.4	38-40
Super premium ice cream	14-18	5-8	14-17	0-0.2	40-42

Note: NMS = Nonfat milk solids

2.5.2 Nonfat milk solids (NMS)

The NMS consists of protein, lactose, vitamins and minerals of the milk derived ingredients. The content of NMS used in ice cream mix varies from 6 to 14% (Arbuckle, 2013). In ice cream manufacture, fresh concentrated or spray dried skim milk and whole milk account for important sources of NMS. Other sources containing milk protein (e.g., dry or condensed buttermilk, sodium caseinate, dried or condensed whey, whey protein concentrate, whey protein isolate) and vegetable proteins can also be used (Goff, 2016). The NMS contribute to the texture and body of the finished product as well as the overrun of the mix (Marshall, Goff, & Hartel, 2003).

Lactose in high concentrations is prone to crystallisation due to its relatively low solubility and freeze-concentration during freezing which can cause a textural defect known as sandiness in ice cream (Early, 1998). Lactose also contributes to freezing point depression, therefore the concentration of this disaccharide in ice cream should be controlled (Clarke, 2015).

Proteins contribute to the development of structure in ice cream due to emulsification, whipping and water holding capacities. The latter ability affects the viscosity of the unfrozen serum and inhibits the growth of ice crystals during hardening and storage (Pei & Schmidt, 2010; Goff, 2011). After homogenisation, proteins are adsorbed on the surface of small fat globules to stabilise the emulsion (Segall & Goff, 1999; Segall & Goff, 2002b). However, in ice cream production, partial coalescence of fat droplets is necessary which is obtained by small molecule surfactants known as emulsifiers and is discussed in section 2.5.5. During freezing, proteins at the air bubble surface interact with various components of ice cream such as the emulsifier and partially coalescing fat at the air interface (Goff, Verespej, & Smith, 1999). This phenomenon, helps entrap air bubbles into a semi-solid matrix with increased viscosity during freezing, thereby increasing their resistance to collapse (Marshall, Goff, & Hartel, 2003). Proteins that are not adsorbed to either the fat or air interface remain dispersed or dissolved in solution, where they help to increase the unfrozen phase viscosity by providing structure (Goff, 2008).

2.5.3 Sweeteners

The main function of sweeteners is to increase the acceptance of the product by providing sweetness with sucrose as the main sweetener. The sugar can be used in combination with hydrolysed starch from corn and other sources (Arbuckle, 2013). The major considerations in using multiple sweeteners include relative sweetness, effects on total solids and freezing point depression which can affect hardness (Goff, 2011). The sweetness index and freezing point depression of different types of sweeteners relative to sucrose are shown in Table 2.2.

Sweeteners are low cost sources of total solid in the mix and improve the body and texture of ice cream (Marshall, Goff, & Hartel, 2003). Lower dextrose equivalent (DE) corn syrups produce a smoother ice cream with increased resistance to heat shock and improved melting properties (Wittinger & Smith, 1986). Dextrose being a monosaccharide, causes greater freezing point depression than sucrose, maltose and lactose. Therefore, with increasing the DE, the sweetness increases but freezing point decreases. The maltodextrins are only slightly sweet and contribute greatly to the body and meltdown of ice cream (Goff & Hartel, 2013). High fructose corn syrups (HFCS) enhance flavour and provide sweetness comparable to sucrose at a lower cost although they contribute to freezing point depression more than sucrose (Wittinger & Smith, 1986).

Sweeteners influence viscosity and glass transition temperature as well as inhibit the growth of ice crystals (Hagiwara & Hartel, 1996; Miller-Livney & Hartel, 1997). Apart from stabilisers, sweeteners are one of the most important determinants of ice crystal size. Sweeteners affect softness and scoopability primarily by depression of the freezing point and thereby, reducing the amount of ice (Wittinger & Smith, 1986).

Table 2.2 Relative sweetness index and freezing point depression of various sweeteners (Goff & Hartel, 2013)

Sweetener	Relative sweetness	Relative freezing point depression
Sucrose	100	1.00
Dextrose (glucose)	74	1.90
Fructose	173	1.90
Lactose	16	1.00
Maltose	32	1.00
HFCS		
90%	125	1.88
55%	98	1.85
42%	86	1.80
Corn syrups		
64 DE	68	1.15
42 DE	48	0.80
36 DE	42	0.72
32 DE	40	0.61
20 DE	23	0.38
Maltodextrins		
15 DE	17	0.29
10 DE	11	0.19
5 DE	6	0.10

Note: HFCS=High fructose corn syrup; DE=Dextrose equivalent

Due to increased consumer demands for low calorie foods, alternative sweeteners such as honey and non-nutritive or low caloric sweeteners have been used in ice cream (Ozdemir, Dagdemir, Ozdemir, & Sagdic, 2008; Ozdemir, Arslaner, Ozdemir, & Allahyari, 2015; Goff, 2016; Moriano & Alamprese, 2017).

2.5.4 Stabilisers

Stabilisers are a group of water soluble biopolymers used in small amounts (usually 0.1-0.5%) in ice cream formulations (Bahramparvar & Mazaheri Tehrani, 2011). Most stabilisers are polysaccharides of plant origin, e.g. alginates and carrageenans (from seaweeds), locust bean gum and guar gum (from tree seeds), pectin (from fruit) and sodium carboxymethyl cellulose (from cotton). Xanthan, a bacterial polysaccharide, and gelatin, a polypeptide of animal origin, can be

also used. Under European law, stabilisers are considered food additives and therefore they are designated as ‘E-numbers’ (Miller-Livney & Hartel, 1997; Alting & Van De Velde, 2012).

In ice cream, stabilisers are used to increase mix viscosity, improve smoothness and reduce the growth of ice and lactose crystals during storage, especially periods of temperature fluctuation known as heat-shock (Granger, Leger, Barey, Langendorff, & Cansell, 2005; Lal, O'Connor, & Eyres, 2006; Goff, 2011). Further, stabilisers aid the suspension of flavoring particles in the semi-frozen ice cream before hardening, retard moisture migration from the product to the package or the air and prevent shrinkage of the product volume during storage (Bolliger, Wildmoser, Goff, & Tharp, 2000; Goff, 2016). Thus, stabilisers contribute effectively to increasing the shelf-life of frozen product. They also create a stable foam with optimum stiffness at the barrel freezer. Hydrocolloids can significantly increase the melting resistance of ice cream due to water-holding capacity and the ability to enhance microviscosity (Marshall, Goff, & Hartel, 2003; Bahramparvar, Haddad Khodaparast, & Razavi, 2009; Bahramparvar & Mazaheri Tehrani, 2011). Stabilisers must have a clean, neutral flavour and readily disperse in the mix. Moreover, they should not lead to excessive viscosity, separation, foam or off-flavour (Bahramparvar & Mazaheri Tehrani, 2011).

Recrystallisation which leads to coarse textures and loss of shelf-life occurs during storage and regularly by the long distribution chains and temperature fluctuation. The major functionality of stabilisers is to retard the rate of recrystallisation due to the increased viscoelasticity that results from freeze-concentration of the polysaccharide in the unfrozen phase (Uzomah & Ahiligwo, 1999; Regand & Goff, 2003). This structure from stabiliser can affect the rate of water diffusion to the surface of a growing crystal during temperature fluctuations or the rate of solutes and macromolecules diffusion away from the surface of a growing ice crystal (Bahramparvar & Mazaheri Tehrani, 2011; Soukoulis & Fisk, 2016).

Retarding recrystallisation is also associated with the ability of stabilisers to interact with water, form cryo-gels or entangled networks during freeze-thaw cycles and thereby reduce migration of free water in ice cream (Wittinger & Smith, 1986; Goff, 2008). However, it was found that some non-gelling stabilisers were more effective in retarding recrystallisation than gelling ones. The key

factor appeared to be associated with the molecular interactions between polysaccharides and proteins (Regand & Goff, 2002).

2.5.5 Emulsifiers

Emulsifiers are food additives which are added to ice cream formulations for their desirable effects on the fat structure (Blanchfield, 2000). Mono-/diglycerides and polysorbates appeared to replace egg yolk due to delivering much greater functionality in promoting fat destabilisation at much less cost (Goff, 2008). Mono-/diglycerides which associate an E-number (E471) are produced by partial hydrolysis of vegetable fats, such as soybean and palm oil (Whitehurst, 2004; Alting & Van De Velde, 2012). Sorbitan esters of fatty acids, such as polyoxyethylene sorbitan monooleate (also known as polysorbate 80) are structurally similar to mono-/diglycerides whereas the fatty acid is attached to a sorbitol molecule instead of glycerol (Clarke, 2015). Polysorbate 80 is also designated as an E-number (E433) (Whitehurst, 2004). Most of the emulsifiers have long and often complex chemical names which do not appeal to consumers. In addition, the sustainability of palm oil which is the source of many emulsifiers has generated concern in the food market (Euromonitor International, 2016).

Emulsifiers are used in food systems to produce a stable suspension of two immiscible liquids (Lal, O'Connor, & Eyres, 2006). In ice cream, emulsifiers are mainly responsible for increasing the fat destabilisation whereas proteins emulsify the mix. Emulsifiers or small molecule surfactants lower the fat/ water interfacial tension, displace the proteins from the surface of fat globules and reduce the stability of fat globules to partial coalescence during whipping and freezing (Bolliger, Goff, & Tharp, 2000). Emulsifiers contribute to the formation of a three-dimensional aggregation of fat globules which provides structural integrity (Granger, Leger, Barey, Langendorff, & Cansell, 2005; Lal, O'Connor, & Eyres, 2006). Polysorbate 80, produces the maximum amounts of fat destabilisation due to its small molecular weight which contributes to lowering the interfacial tension. Mixes with lower surface tension values tend to produce higher overrun and smaller air bubbles (Goff, Liboff, Jordan, & Kinsella, 1987; Goff, 1997). It has been reported that polysorbate 80 produces undesirable off-flavours over time (Baer, Wolkow, & Kasperson, 1997).

Partially coalesced fat is mainly responsible for stabilising the air bubbles and the foam structure. Therefore, fat destabilisation has a significant influence on meltdown behavior of the ice cream (Goff, 1997; Granger, Leger, Barey, Langendorff, & Cansell, 2005). Optimal fat structure and air bubble size may also help to slow down ice recrystallisation (Marshall, Goff, & Hartel, 2003). A certain amount of fat destabilisation is required to obtain a dry product, good shape retention, slow meltdown and firmer texture (Berger & White, 1971; Goff & Jordan, 1989). Aging the ice cream improves the functionality of emulsifiers (Koxholt, Eisenmann, & Hinrichs, 2001).

2.5.6 Water and air

Water in ice cream exists as liquid, solid or as a mixture of the two physical states (Marshall, Goff, & Hartel, 2003). Water may be added or come from dairy and non-dairy ingredients (Goff, 2016). The amount of air in ice cream affects the organoleptic attributes and storage stability of the product. It also influences the economy through enhancing the volume (Arbuckle, 2013).

2.6 Opportunities to replace commercial additives in ice cream

2.6.1 Alternative stabilisers

In the last few years, there has been increased interest to use alternative sources of stabilisers and produce ice cream in the absence of commercial additives. Dervisoglu and Yazici (2006), substituted sabilisers in ice cream with citrus fiber (0.4 to 1.2% w/w). The fiber did not improve the overrun, viscosity and sensory properties of ice cream but had a positive impact on the melting resistance. Cody, Olabi, Pettingell, Tong, and Walker (2007), showed that rice flour constrained recrystallisation throughout storage, markedly lowered the perceived iciness and improved creaminess but imparted a flour off-flavour to the product. Hazelnut flour and skins improved the viscosity, overrun and melting resistance of the ice cream but negatively affected the colour and texture (Dervisoglu, 2006).

The application of high-pressure (HP) treatment (100-600 MPa) on ice cream mix was reported to be a promising strategy for the production of high quality ice cream free from stabiliser (Huppertz,

Smiddy, Goff, & Kelly, 2011; Huppertz, Smiddy, Kelly, & Goff, 2012; Soukoulis & Fisk, 2016). HP treatment enhanced the ice cream mix viscosity and meltdown resistance of the final product (Huppertz, Smiddy, Goff, & Kelly, 2011; Soukoulis & Fisk, 2016). The function of this method was attributed to its impact on the solid-liquid phase transitions of water and its ability to induce the partial denaturation of whey proteins (Soukoulis & Fisk, 2016). Moreover, the network of micellar fragments resulting from the calcium-induced aggregation of caseins on decompression was hypothesised to be responsible for the increased viscosity and meltdown resistance (Huppertz, Smiddy, Goff, & Kelly, 2011).

Exopolysaccharides (EPS) produced by Lactic Acid Bacteria (LAB), are potential sources of stabilisers of food systems including ice cream (Mollet, 2001; Folkenberg, Dejmek, Skriver, Skov Guldager, & Ipsen, 2006; Yilmaz, et al., 2015; Dertli et al, 2016). The generally Recognised as Safe (GRAS) status of LAB allows the *in situ* production of EPS in food products without labelling (Knoshaug, Ahlgren, & Trempy, 2007; Florencia, 2013). The beneficial properties of EPS are attributed to their unique rheological properties such as pseudoplastic nature and capability of forming viscous solution at low concentrations contributing to the texture and mouthfeel of food products (Cerning, 1990; Marshall & Rawson, 1999; Duboc & Mollet, 2001; Goh, Nair, & Matia-Merino, 2008). The functionality of microbial EPS is also attributed to their water-binding properties and interactions with the protein network (Mende, Rohm, & Jaros, 2016).

The addition of LAB which produce EPS (in the form of slime or ropy) to ice cream mix improved the meltdown resistance and firmness of ice cream (Christiansen, Madeira, & Edelsten, 1999; Knoshaug, Ahlgren, & Trempy, 2007; Goh, Nair, & Matia-Merino, 2008; Zhang et al., 2017). The *in situ* synthesis of EPS by the ropy strain (*Lactococcus lactis* ssp. *Cremoris)* had a protective effect on ice cream against temperature fluctuations and improved the body and texture (Christiansen, Madeira, & Edelsten, 1999). Various strains of ropy cultures have been added to the ice cream mix in the form of cultured milk in different concentrations and had different effects on overrun. The incorporation of cultured milk (20%) with EPS-producing starter cultures to the ice cream mix improved the overrun which was attributed to the ability of EPS to form a fine porous fabric matrix (Figure 2.9), which can retain more oxygen (Goh, Nair, & Matia-Merino, 2008; Dertli et al., 2016; Zhang et al., 2017). However, Christiansen, Edelsten, Kristiansen, and Nielsen

(1996), attributed the reduced air incorporation ability of the ice cream mixes containing up to 50% ropy milk fermented with *Lactococcus lactis* ssp. *Cremoris*, to protein aggregation resulted from the lower pH.

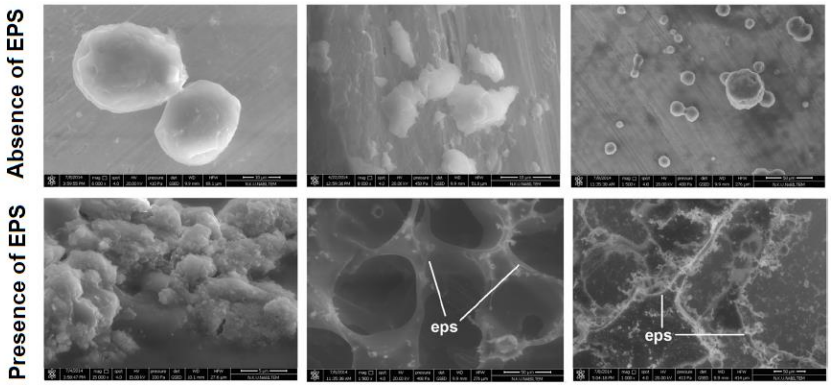


Figure 2.9 Scanning electron micrographs showing the presence and absence of EPSs. White continuous line in image shows the network strands suggesting EPS structure (Dertli et al., 2016)

Several seeds as the new sources of hydrocolloids with promising functional attributes comparable to commercial hydrocolloids in stabilising ice cream have been reported. The addition of gum extracted from achi seeds (*Brachystegea eurycoma*) produced ice cream with comparable overrun, viscosity, meltdown rate and shape retention to those of containing commercial stabilisers (Uzomah & Ahiligwo, 1999). *Acacia glomerosa*, *Enterolobium cyclocarpum* and *Hymenaea courbaril* are plants grown in Venezuela and yield gums with desirable structural characteristics. A mixture of the gums replaced the commercial stabiliser and provided suitable viscosity, overrun and texture for the ice cream with acceptable flavour and creaminess (Leon de Pinto, Rincón, & Beltrán, 2006). Cakmakci and Dagdemir, (2013) used the gumming liquid obtained from the roots and leaves of the *G. tournefortii* plant and produced ice cream with similar quality characteristics to those of formulated with commercial stabilisers.

The outer pericarp of basil seed swell into a gelatinous mass when soaked in water which has reasonable amounts of gum (Razavi et al., 2009). The gum can act as stabiliser (BahramParvar, Razavi, & Mazaheri Tehrani, 2012; BahramParvar & Goff, 2013), emulsifying agent (Osano,

Hosseini-Parvar, Matia-Merino, & Golding, 2014), fat replacer (Javidi, Razavi, Behrouzian, & Alghooneh, 2016) and film-forming material (Khazaei, Esmaiili, Djomeh, Ghasemlou, & Jouki, 2014) in food products. The function of the gum is attributed to its yield stress, high viscosity at low shear rates, pseudoplastic behavior and freeze-thaw stability (Hosseini-Parvar, Matia-Merino, Goh, Razavi, & Mortazavi, 2010; Zameni, Kashaninejad, Aalami, & Salehi 2015).

The desirable effect of basil seed gum on apparent viscosity of ice cream mix, draw temperature, meltdown behavior and total acceptance of ice cream revealed the ability of the gum in stabilising the ice cream (BahramParvar, Razavi, & Mazaheri Tehrani, 2012). BSG reduced the rate of ice crystal growth by 30-40% compared to commercial gums such as guar gum and carboxymethyl cellulose which did not have the ability to decrease recrystallisation rate (BahramParvar & Goff, 2013). In this previous study, relatively high viscosity of ice cream containing BSG may result in lower overrun. Moreover, BSG increased the particle size distribution which was attributed to the ability of the gum to lower the air and fat interfacial tensions however it was found that BSG itself forms large aggregates. In the same study, microstructure analysis of ice cream containing BSG showed relatively smaller air cells which was related to either its high viscosity or emulsification properties. This property was presumed to have positive impacts on the stability of the foam and the perception of creaminess during consumption. In addition to functional benefits, BSG as a pharmaceutical plant has shown health-promoting effects (BahramParvar, Razavi, & Mazaheri Tehrani, 2012).

Similar to BSG, the mucilage extracted from chia seed which is a functional seed with health-promoting attributes, has shown stabilising and emulsifying properties in food products (Capitani, Spotorno, Nolasco, & Tomás, 2012; Muñoz, Cobos, Diaz, & Aguilera, 2013; Timilsena, Adhikari, Kasapis, & Adhikari, 2016; De Falco, Amato, & Lanzotti, 2017). The characteristics of this seed will be discussed in section 2.7.2.

2.6.2 Alternative emulsifiers

Due to the increasing demand for food products free from synthetic additives, alternative strategies to replace commercial emulsifiers are being investigated. Egg yolk is an optimum clean label

ingredient which constitute 31% of emulsifier volumes used worldwide, however the technical and economical limitations influence its application (Euromonitor International, 2016).

A two-phase ice cream processing method was proposed to control the adsorbed proteins on the surface of milkfat after homogenisation and achieve optimal partial coalescence with no added emulsifiers (Segall & Goff, 1999; Segall & Goff, 2002a; Segall & Goff, 2002b). It was based on the fact that minimal amount of protein, specifically whey protein isolate leads to the production of an emulsion which is quiescently stable but has a protein surface concentration low enough to undergo substantial partial coalescence under shearing. The slow meltdown, moderate overrun and fat destabilisation, suggested the ability of the method in promoting the ice cream structure without added emulsifier however the processing steps may make the method, cost prohibitive.

Innocente, Comparin, and Corradini (2002), introduced the proteose peptone fraction of milk with similar characteristics to commercial emulsifiers in ice cream due to its ability in rapid reduction of interfacial tension. A study by Rinaldi et al. (2014), showed the emulsifying potential of soy and rice phospholipids in gelato however the sensory attributes were markedly affected.

Low-Temperature Extrusion (LTE) serves as a developed conventional freezing process where ice cream enters the LT extruder at -5°C and leaves it approximately at -12°C. The method resulted in higher extent of fat destabilisation compared to the conventional freezing process which translates into lower amounts of emulsifiers required. The technique also helps to control ice crystal growth due to the high shear forces, rapid freeze-concentration of the serum phase, reduction of the water diffusion rates and the hindrance of the collision of the adjacent ice crystals (Windhab & Wildmoser, 2002; Wildmoser, Scheiwiller, & Windhab, 2004).

Sweet cream buttermilk (SCB) and whey protein phospholipid concentrate as major sources of milk phospholipids have been reported to possess emulsifying properties (Levin, Burrington, & Hartel, 2016; Gnanasambandam, Torres-Gonzalez, Burrington, & Kapoor, 2017). SCB enhanced the emulsifying attributes in ice cream when it was used as a source of NMS (Rajor, Patel, Patel, & Gupta, 1901; Williams, Potter, & Hufnagel, 1950; Yanasugondha, 1951; Rajor, 1980; Hamilton,

1990; Shibu, Kumar, Narasımhan, & Pugazhenthi, 2000). The functionality and attributes of SCB will be discussed in detail in section 2.7.3.

2.7 Functional properties of chia seed and sweet cream buttermilk for application in ice cream

2.7.1 Background

The fear of artificial has created a demand for authentic ingredients that have a long, safe history of use. Ingredients inspired from ancient cultures, such as chia seed, have become more popular as their long and unprocessed history creates a sense of security (Anonymous, 2016). Chia seed is a well-known kitchen type-cupboard ingredient, consumed by different segments of consumers due to its beneficial health effects. The mucilaginous gel obtained from hydrated chia seed has been reported to contribute to stabilisation of food systems comparable to conventional stabilisers due to its water-holding and water-absorption capacities (Capitani, Spotorno, Nolasco, & Tomás, 2012).

Sweet cream buttermilk is a familiar ingredient, commonly used in home recipes. The composition of SCB is similar to skim milk, with increased content of phospholipids (Rajor, Patel, Patel, & Gupta, 1901). As a result, the substitution of skim milk with SCB has shown to improve emulsifying properties of the mix and impart richer flavour with smooth texture in ice cream (Hamilton, 1990; Shibu, Kumar, Narasımhan, & Pugazhenthi, 2000). To date, there is no recorded document about the development of a clean label ice cream exploiting the functionality of these two ingredients in the absence of commercial stabilisers and emulsifiers. Chia seed and SCB are therefore further discussed as the main experimental factors to achieve the objectives of the project.

2.7.2 Chia seed

The chia seed is increasingly being used in the manufacture of breakfast cereals, bars, snacks, fruit juices, cake, yogurt and bread especially in the United States, Canada, New Zealand, Chile, Mexico, Latin America, and Australia (Mohd Ali et al., 2012; Bochicchio et al., 2015). Chia is

also commonly consumed in salad as chia sprout, in beverages, and salad dressings. The culinary uses of chia seed have been as a whole seed, seed flour, seed mucilage, and seed oil. The seeds are small with an oval, flattened shape and ranged in colour from white to dark coffee and beige with small darker spots as shown in Figure 2.10 (Muñoz, Cobos, Diaz, & Aguilera, 2013; Felisberto et al., 2015). The seeds can be eaten raw or used in capsules to provide a nutritional supplement of omega-3 (Muñoz, Cobos, Diaz, & Aguilera, 2013). *Salvia hispanica L.* or chia is an annual plant belonging to the mint family (*Lamiaceae*), native to Mexico and Guatemala (Campos, Ruivo, da Silva Scapim, Madrona, & Bergamasco, 2016) which spread to other regions due to its high nutritional composition (Valdivia-López & Tecante, 2015). The name Salvia originates from the Latin word Salvare, meaning 'the healer' (Goren, Kiliç, Dirmenci, & Bilsel, 2006).

The seeds of chia, corn, beans, and amaranth formed the main components of the diet of the pre-Columbian peoples whose diets, met the dietary requirements established today by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (Muñoz, Cobos, Diaz, & Aguilera, 2013). The seed is a rich source of essential fatty acids, antioxidants, dietary fiber, and proteins (Valdivia-López & Tecante, 2015; Fernandes & Mellado, 2018).

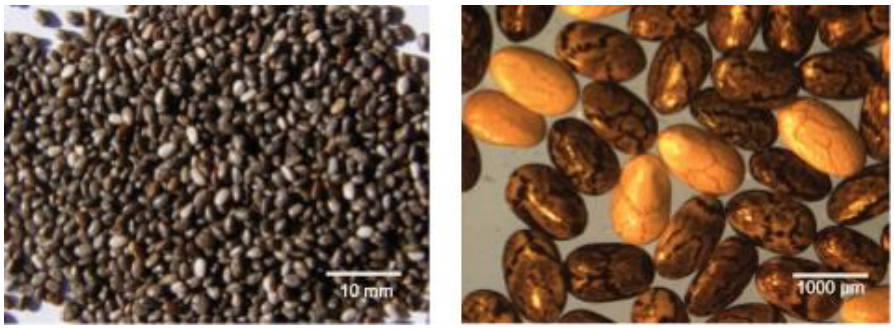


Figure 2.10 Chia seed (Muñoz, Cobos, Diaz, & Aguilera, 2013)

The protein content of chia seed is higher than any other grain (Timilsena, Adhikari, Kasapis, & Adhikari, 2016). Chia seed is gluten-free and is suitable for celiac patients (Muñoz, Cobos, Diaz, & Aguilera, 2013). The seed is an important source of minerals (calcium, phosphorus, potassium, magnesium, sodium, iron, and zinc) and vitamins (niacin, vitamins A and C) (Muñoz, Cobos, Diaz, & Aguilera, 2013; Orona-Tamayo, Valverde, & Paredes-López, 2017). According to Ulbricht et

al. (2009), chia has shown possible effectiveness for allergies, angina, athletic performance enhancement, cancer, coronary heart disease, heart attack, hormonal/endocrine disorders, hyperlipidemia, hypertension and stroke. Some evidences also suggest possible anticoagulant, antioxidant, and antiviral effects of *S. hispanica*. It helps to increase the satiety index, prevents inflammatory and nervous system disorders, and diabetes. Thus chia can be considered a functional food with excellent benefits for human health (Muñoz, Cobos, Diaz, & Aguilera, 2013; Orona-Tamayo, Valverde, & Paredes-López, 2017).

2.7.2.1 Chia seed mucilage

The seed has a significant amount of dietary fiber, compared to other fruits and seeds. In the presence of water, the chia seed absorbs 27 times its own weight of water (Bochicchio et al., 2015) and exudes a transparent mucilaginous gel, composed essentially of soluble fibers (Fernandes & Mellado, 2018). Hydrated seeds were traditionally used in beverages called “agua fresca” or “chia fresca” in Mexico (Bochicchio et al., 2015).

The seed has 5–6% mucilage (Muñoz, Cobos, Diaz, & Aguilera, 2013) which is a highly acidic branched tetrasaccharide, composed of a main chain constituted by units of (1→4)-β-d-xylopyranosyl-(1→4)-α-d-glucopyranosyl (1→4)-β-d-xylopyranosyl with branches of 4-O-methyl-α-dglucuronic acid at the O-2 position of β-d-xylopyranosyl in the main chain (Lin, Daniel, & Whistler, 1994; Chaves et al., 2018; Fernandes & Mellado, 2018). The gel is heat stable and has shown the characteristics of texture modifier, gelling agent, thickener, stabiliser, emulsifier (Timilsena, Adhikari, Kasapis, & Adhikari, 2016), bulking agent, encapsulant, and film/coating agent (Muñoz, Cobos, Diaz, & Aguilera, 2012; Timilsena, Adhikari, Kasapis, & Adhikari, 2016). The contribution of mucilage to the stabilisation of food systems is through gel formation or thickening of the continuous phase due to its water-holding and water-absorption capacities (Capitani, Spotorno, Nolasco, & Tomás, 2012; De Falco, Amato, & Lanzotti, 2017).

Therefore, the seed serves as a potential source of polysaccharide gum due to its exceptional mucilaginous properties at low concentration in aqueous solutions (Muñoz, Cobos, Diaz, &

Aguilera, 2012). Several studies have been reported on the use of chia seed and its mucilage for improving some properties in bakery and meat products (Coelho & de las Mercedes Salas-Mellado, 2015; Ding et al., 2018).

Few studies highlighted the possibility of using mucilage in ice cream formulations as stabiliser (Campos, Ruivo, da Silva Scapim, Madrona, & Bergamasco, 2016; Chavan, Gadhe, Sharma, & Hingade, 2017). Moreover, Maestrello et al. (2017), showed that replacing the commercial emulsifier in ice cream with chia mucilage resulted in appropriate chemical composition and sensory acceptance however the overrun obtained in this study was low.

The consumption of the mucilage makes digestion and the absorption of nutrients more efficient and provides more of a feeling of satiety (Capitani, Nolasco, & Tomás, 2013). The gel can act as prebiotic, regulate blood sugar and cholesterol levels (Capitani, Nolasco, & Tomás, 2013; Bochicchio et al., 2015). It can also be used to produce fiber-enriched foods (Muñoz, Cobos, Diaz, & Aguilera, 2013).

There is no evidence of adverse effects or allergenicity caused by whole or ground chia seeds. Thus chia seeds and derived products are promising sources of functional foods with multiple health benefits.

2.7.3 Sweet cream buttermilk

Buttermilk is widely used in the food industry due to its emulsifying property (Costa, Elias-Argote, Jiménez-Flores, & Gigante, 2010) and desirable impact on flavor and texture (Wong and Kitts, 2003; Sodini, Morin, Olabi, & Jiménez-Flores, 2006). Buttermilk is an aqueous by-product released during the churning of cream in butter manufacture. The composition of buttermilk is similar to that of skim milk with increased contents of fat and milk fat globule membrane (MFGM). Buttermilk encompasses the material derived from MFGM which is disrupted during churning and mostly migrates to the buttermilk fraction (Corredig & Dalgleish, 1997; Eyzaguirre & Corredig, 2011).

The MFGM, a lipid-protein complex, consists of 55% protein, 44% lipid, and a small amount of carbohydrate (Wong & Kitts, 2003), enzymes and other trace elements (Costa, Elias-Argote, Jiménez-Flores, & Gigante, 2010). Major proteins include mucins, the redox enzyme xanthine dehydrogenase/oxidase, glycoproteins, cluster of differentiation and butyrophilin, fatty acid-binding protein, and lipid binding protein. The proteose peptone fraction has also been associated with the MFGM as minor proteinaceous component. Mucin proteins present in buttermilk may exert a positive effect on the prevention of infections (Eyzaguirre & Corredig, 2011).

Phospholipids which are the native emulsifiers in milk (Rajor, Patel, Patel, & Gupta, 1901), constitute about one-third of the MFGM (Sodini, Morin, Olabi, & Jiménez-Flores, 2006). The content of phospholipids in buttermilk, is reported to be seven times more than whole milk with concentrations equal to 0.89 mg/g and 0.12 mg/g, respectively (Elling, Duncan, Keenan, Eigel, & Boling, 1996).

Buttermilk is considered a functional ingredient from both technological and nutritional perspectives due to the high content of phospholipids (especially phosphotidylcholine (lecithin), phosphatidylethanolamine, and sphingomyelin) (O'Connell & Fox, 2000; Jinjarak, Olabi, Jimenez-Flores, Sodini, & Walker, 2006). The dairy ingredient is commercially used to improve some properties in bakery and dairy products, sauces, chops, and chocolate products, cheese seasoning, ice cream mixes, yogurt and recombined milks (Wong & Kitts, 2003; Sodini, Morin, Olabi, & Jiménez-Flores, 2006; Eyzaguirre & Corredig, 2011; Kifah, Layla, & Baha, 2014). A unique functionality of buttermilk is its ability to increase the heat stability of recombined milks (Singh & Tokley, 1990), mainly due to phospholipid-protein interactions preventing protein coagulation during sterilisation (Sodini, Morin, Olabi, & Jiménez-Flores, 2006).

2.7.3.1 Utilisation of sweet cream buttermilk in ice cream

Sweet cream buttermilk can replace skim milk in ice cream mixes by contributing to about 80 per cent of the NMS. Ice cream containing buttermilk had better whipping properties and a richer,

creamier flavour than the ice cream which only contained skim milk (Williams, Potter, & Hufnagel, 1950). The addition of SCB to soft-serve ice cream mix improved the melting resistance and decreased both the coarseness and sandiness of frozen ice cream. SCB produced ice cream which was drier in appearance and had better consistency, structure and more stability than ice creams which only contained skim milk (Rajor, Patel, Patel, & Gupta, 1901). Superior emulsifying properties and rich creamy flavour were attributed to the ice creams containing buttermilk (Thomas & Combs, 1944; Williams, Potter, & Hufnagel, 1950; Yanasugondha, 1951; Rajor, 1980).

Whitaker (1930), found that when cream was used in ice cream formulation, the desired overrun was obtained faster compared to the mix which contained the butter obtained from churning the cream. The maximum overrun obtainable was also markedly increased over the ice cream which contained butter. The failure of butter mixes to whip as readily as cream mixes was attributed to the lack of the lecithin-protein complex or the buttermilk portion of the cream (Whitaker, 1930; Walts & Dahle, 1933). Addition of buttermilk to butter mixes enhanced the whipping ability of the ice cream mix as compared with mixes made with butter and skim milk (Walts & Dahle, 1933). The lecithin content of buttermilk and cream are 13 to 20 times higher than that of skim milk (Whitaker, 1930). Therefore, the beneficial effects of buttermilk on the whipping properties of ice cream mixes is, similar to egg yolk, due to the presence of lecithin-protein complex in relatively large amounts (Williams, Potter, & Hufnagel, 1950; Schoenfuss & Chandan, 2011).

Sweet cream buttermilk tends to impart richer and less cooked flavour in ice cream than skim milk powder (Rajor, 1980). Inferior whipping properties and distinct off-flavour were reported for the ice cream containing the buttermilk from sour cream (Williams, Potter, & Hufnagel, 1950).

2.7.3.2 Health benefits of sweet cream buttermilk

Buttermilk is produced on a large scale and is considered a low value product (Hickey et al., 2018). The U.S. Code of Federal Regulations does not have a standard of identity for buttermilk, other than the labeling requirement that buttermilk can be sweet cream buttermilk, concentrated sweet cream buttermilk, reconstituted sweet cream buttermilk, and dried sweet cream buttermilk (Schoenfuss & Chandan, 2011). Buttermilk consumption confers potential positive health effects

including reduction of hyperlipidemia and blood pressure. Further, phospholipids have anti-carcinogenic potential especially against colon cancer as well as protective effect against bacterial toxins and infection (Kuchta-Noctor, Murray, Stanton, Devery, & Kelly, 2016). They are also integral components of human cell membrane and account for the major constituents of brain, nerve tissue, heart, muscle, liver and sperm (Renner, Schaafsma, & Scott, 1989; Dewettinck, Rombaut, Thienpont, Messens, & Van Camp, 2008).

2.8 Analysis of ice cream and characterisation methods

Several factors such as chemical composition, air content, the size of ice crystals, air bubbles and fat globules contribute to the textural and structural quality of the final ice cream (Caillet, Cogné, Andrieu, Laurent, & Rivoire, 2003). Analysing the parameters which contribute to the quality of ice cream helps to optimise ice cream formulation and processing conditions. Analysing the quality attributes of ice cream is also necessary to meet the consumer expectation as well as the regulatory standards (Marshall, Goff, & Hartel, 2003).

Microscopic techniques are used to examine the microstructure (size, shape and location) of ice crystals, air bubbles and fat droplets. There is a mutual correlation between the microstructure and sensory properties of the ice cream and its rheological properties, hardness and meltdown rate (Clarke, 2015). Characterisation methods of ice cream which comprise physical, chemical, microbiological and sensory properties are further discussed.

2.8.1 Physical properties

2.8.1.1 Microscopic examination of ice cream

The microstructure of ice cream which is characterised mostly by the state of dispersion of the fat, the size of ice crystals and air bubbles is examined and characterised using optical and electron microscopy techniques (Marshall, Goff, & Hartel, 2003; Clarke, 2015). The temperature is controlled during these microscopic examinations so that ice crystals neither melt nor grow during imaging.

2.8.1.2 Overrun

Air is an important component in ice cream which affects both physical properties and storage stability. Overrun defines the structure of the final product since the presence of air gives the ice cream an agreeable light texture and influences the physical properties of melting and hardness (Sofjan & Hartel, 2004; Cruz, Antunes, Sousa, Faria, & Saad, 2009). The air content is measured and characterised by overrun, which is defined as the increase in volume or decrease in mass that occurs as a result of aeration. Frozen desserts can contain as high as 120–140% overrun. The overrun typically varies from 95 to 100% for the economy ice cream to about 30% for the super-premium ice cream while premium ice creams may be in the range of 60–90% overrun (Guinard, Zoumas-Morse, Mori, Panyam, & Kilara, 1996). Overrun is analysed by mass of ice cream (foam) compared to the ice cream mix of the same volume using equation 1 (Goff, 2016).

$$Overrun\ \% = \frac{(m_{mix} - m_{foam})}{m_{foam}} \times 100 \quad \text{.....(1)}$$

$\boldsymbol{m_{mix}}$: mass of mix

$\boldsymbol{m_{foam}}$: mass of ice cream (foam)

2.8.1.3 Meltdown rate

When ice cream is in the form of a cone or novelty stick, melting rate is of vital importance to the consumer. A fast-melting product is undesirable because it creates a messy situation and also tends to readily become heat-shocked (Bahramparvar & Mazaheri Tehrani, 2011). The meltdown test measures the ability of ice cream to resist melting when exposed to warm temperatures for a specified time (Clarke, 2015). Low freezing point is the primary cause of rapid melting (Marshall, Goff, & Hartel, 2003). The meltdown rate is affected by many factors, including the amount of air incorporated, the nature of the ice crystals, and the network of fat globules formed during freezing (Muse & Hartel, 2004; Goff & Hartel, 2013).

The meltdown rate of ice cream is determined by placing a sample of ice cream on a wire-mesh screen at warm temperatures and measuring the rate at which the fluid drips (melts). Accordingly, the mass of melted ice cream is recorded during melting and is plotted as a function of time

(Arbuckle, 2013). As the ice cream melts, heat transfers from the warm air surrounding the product into the ice cream to melt the ice crystals. The melted ice crystals then flow downwards (due to gravity) through the structural elements (destabilised fat globules, air cells, and remaining ice crystals) to drip through the screen (Muse & Hartel, 2004).

2.8.1.4 Hardness

Hardness is measured as the resistance of ice cream to penetration when an external force is applied using an appropriate probe placed on a texture analyser. The hardness of ice cream is affected by several factors such as the overrun, total solids, ice crystal size, ice phase volume, extent of fat destabilisation and, amount and type of stabiliser. The level of emulsifier or destabilised fat directly influences the hardness whereas overrun inversely affects the hardness (Tanaka, Pearson, & Deman, 1972; Goff et al., 1995; Wilbey, Cooke, & Dimos, 1998). Total solids contribute to depression of the freezing point therefore reducing the amount of ice and hardness (Marshall, Goff, & Hartel, 2003; Muse & Hartel, 2004). Moreover, monosaccharide sweeteners such as glucose lower the freezing point twice as much as does the disaccharide sucrose (Marshall, Goff, & Hartel, 2003). The ice phase volume and the amount of large ice crystals directly affect the level of hardness in ice cream (Muse & Hartel, 2004).

2.8.1.5 Fat destabilisation evaluation

The state of dispersion of fat has a large impact on the final structure of ice cream (Alvarez, Wolters, Vodovotz, & Ji, 2005). Fat undergoes partial coalescence during freezing and the extent of aggregation is associated with creaminess, dry appearance, shape retention and slow meltdown of the frozen product (Méndez-Velasco & Goff, 2012). Excessive fat destabilisation or churning results in poor whipping properties, fat and ice separation in the freezer, unsatisfactory extrusion and buttery texture (Berger & White, 1971). Measurement of fat globule size distribution and destabilised fat index are the two methods which can be used to evaluate fat destabilisation (Bolliger, Goff, & Tharp, 2000; Marshall, Goff, & Hartel, 2003).

I. Fat globule size distribution

The distribution of fat globule sizes can be determined by laser light scattering techniques using a Malvern Mastersizer. In a laser diffraction measurement, a laser beam passes through a dispersed particulate sample and the angular variation in intensity of the scattered light is measured (Malvern Instruments Ltd, 2013). Large particles scatter light at small angles relative to the laser beam and small particles scatter light at large angles. The angular scattering intensity data is then analysed to calculate the size of the particles responsible for creating the scattering pattern, using the Mie theory of light scattering (Anonymous, 2019a). The statistics of distribution are calculated from the results using the derived diameters of $d_{4,3}$ (volume weighted mean) and $d_{3,2}$ (surface weighted mean) (Malvern Instruments Ltd, 2013). Partial agglomeration of fat results in a bimodal distribution of particle sizes (Marshall, Goff, & Hartel, 2003).

II. Destabilised fat index

Destabilised fat index (DFI) is calculated comparing the absorbance of samples of mix (a_{mix}) and melted ice cream (a_{melt}) obtained from spectrophotometer. For a fix total amount of fat, large fat clusters have a smaller total surface area than small droplets. Therefore, they scatter less light so a lower absorbance results (Clarke, 2015). DFI is calculated using equation 2.

$$DFI = \frac{a_{mix} - a_{melt}}{a_{mix}} \times 100 \quad \text{.....(2)}$$

$\boldsymbol{a_{mix}}$ = absorbance of mix
$\boldsymbol{a_{melt}}$ = absorbance of melted ice cream

2.8.1.6 Viscosity

The most desirable attributes of ice cream such as smooth texture and cooling sensation are influenced by rheological properties of the mix (Dogan & Kayacier, 2007). Viscosity measurement is one of the most important tools for determining physical characteristics of food products (Dogan, Kayacier, Toker, Yilmaz, & Karaman, 2013). Knowledge of the flow behavior is also beneficial for process design and equipment selection, particularly for heat exchangers and pumps (Tabatabaee Amid & Mirhosseini, 2012; Dogan, Kayacier, Toker, Yilmaz, & Karaman, 2013).

In general, the viscosity directly affects the meltdown resistance and smoothness of ice cream but inversely influences the whipping ability (Marshall, Goff, & Hartel, 2003; Dogan & Kayacier, 2007). Viscosity (the resistance of a liquid to flow) is the internal friction that resists the sliding of one part of the fluid over another. It is defined by the relationship between shear stress and shear rate (Marshall, Goff, & Hartel, 2003). Ice cream mix is classified as pseudoplastic due to the inverse relationship between the viscosity and shear rate (Goff & Hartel, 2013). Various factors such as the type, quality and concentration of ingredients and composition of the mix (mainly fat and stabiliser), processing and handling play an important role on the textural and viscosity properties of ice cream (Dogan & Kayacier, 2007). The analysis of the individual or combined effects of these factors is important due to their impact on viscoelastic behavior and quality characteristics of ice cream (Capitani et al., 2015). The viscoelastic properties of the ice cream mix can be determined using stress-controlled rheometer equipped with a cone and plate at controlled temperature.

In order to analyse the effect of the mix composition or the stabilisers, on the viscoelastic properties of the final ice cream, the storage modulus (G′, Pa), loss modulus (G″, Pa) and tan δ (G″/G′) of the ice cream mix or aqueous dispersions of the stabiliser are measured as a function of angular frequency (ω, Hz). Strain sweeps are used to identify the linear viscoelastic region, where the shear stress varies linearly with the applied strain (Adapa, Dingeldein, Schmidt, & Herald, 2000; Capitani et al., 2015). A tan $\delta < 1$ indicates a predominantly elastic behavior, whereas tan $\delta > 1$ indicates a viscous behavior (Capitani et al., 2015). Ice cream mix is primarily a viscous system whereas ice cream is basically considered a viscoelastic system. However, in aqueous mucilage dispersion (as stabiliser), a prevalent elastic behavior is expected where G′ is larger than G″ (Marshall, Goff, & Hartel, 2003; Capitani et al., 2015).

2.8.2 Chemical and compositional properties of ice cream

The chemical composition is determined to ascertain the ice cream meets the regulatory standards. The ether extraction method is used to measure the fat content, the hot air oven method for total solids and the Kjeldahl test for measuring the protein content in ice cream (Vivek, 2001).

2.8.3 Microbiological properties

Microbiological analyses are used to ensure that adequate hygienic practices have been followed throughout the processing of ice cream and the regulated standards are satisfied. Addition of flavours, colouring agents, and ingredients such as fruits, nuts, and chocolate chips to the ice cream after pasteurisation can be sources of contamination (Varga, 2007). According to regulatory standards, 10^5 CFU Aerobic mesophilic, 10 CFU coliforms, 10 CFU *Staphylococcus aureus* are the permitted number of microorganisms per gram of ice cream. *Listeria monocytogenes* and *Salmonella* should be absent in 1 g and 25 g of ice cream, respectively (Harrigan, 1998).

2.8.4 Sensory evaluation

The choice of ingredients, handling and manufacturing processes influence the sensory properties of the final product. Hence, sensory evaluation tests are used to assess flavour, taste, body, texture, colour, appearance and overall acceptance of the frozen dessert (Homayouni, Azizi, Ehsani, Yarmand, & Razavi, 2008). It is common practice to use focus groups during the development of food formulations. Focus groups comprise of small numbers of people who may be familiar with the product. Focus groups are cost effective, convenient and help to collect useful data in a short period of time.

Once the formulation has been selected, consumer acceptance test can be used to evaluate the acceptance of the product. A 9-point hedonic scale is used to determine the degree of liking of the product by consumers with 1=dislike extremely to 9= like extremely, and 5= neither like nor dislike. This method is suitable for use with a large number of participants (Peryam & Pilgrim, 1957).

2.9 Conclusion

Ice cream is a palatable and wholesome frozen dessert consumed globally by a large number of people due to its desirable coldness and velvety texture. The structural and sensory properties of the frozen product depend primarily on the type and concentration of the constituents as well as the processing steps. There has been an increased interest to use alternative processing methods and ingredients which lead to improving the nutritional properties of ice cream that may be more

appealing to consumers. In this study, chia seed gum and sweet cream buttermilk were identified to have the potential to replace the commonly used stabilisers and emulsifiers in ice cream, respectively. The use of these two ingredients can contribute towards producing a product with cleaner label. Further, the addition of probiotic can boost the functional properties of the ice cream.

Chapter 3 Experimental background

3.1 Organisation of the experiments

This chapter describes the organisation of the experiments conducted in this study. The main focus of the research was to determine the potential of using chia seed gum and sweet cream buttermilk to replace commercial stabilisers and emulsifiers commonly used in ice cream. Chia seed gum has been reported to have a dual function in ice cream, as a stabiliser and emulsifier (Campos, Ruivo, Scapim, Madrona, & Bergamasco, 2016). Meanwhile, the emulsifying properties of buttermilk in ice cream are well documented (Williams, Potter, & Hufnagel, 1950; Schoenfuss & Chandan, 2011). As our overall goal was to produce a clean label ice cream with functional properties, a probiotic culture was also added to the formulation.

The study involved several variables which included CSG, MDG (commercial emulsifier), guar gum (commercial stabiliser), SCB, and a probiotic strain. The effects of the variables in the production of prototype ice creams were investigated in ten coded formulations. The experiments were conducted in Chapter 4 and Chapter 5.

The experiments commenced with the extraction of CSG as described in **Chapter 4.** Modified extraction of the CSG was based on previous studies which reported on the extraction of chia seed and basil seed gums (Hosseini-parvar, Matia-Merino, Goh, Razavi, & Mortazavi, 2010; Capitani et al., 2015; Zameni, Kashaninejad, Aalami, & Salehi, 2015). The efficiency of the extraction method was evaluated by measuring the yield. Moreover, the moisture content of the dried mucilage as well as the pH of the extracted mucilage before drying were measured.

The main goal in **Chapter 5** was to optimise the ice cream formulations containing CSG and SCB. Thus, the materials and methods used for the production and analysis of ice cream samples are described in this Chapter. Chapter 5 encompasses four sequentially integrated phases, with each phase described/discussed as stand-alone. The phases used in Chapter 5 are briefly introduced in below:

In **phase I,** several concentrations of CSG for stabilising ice cream in the absence of commercial stabilisers were examined. Various physico-chemical and structural parameters (viscosity, overrun, meltdown rate, fat globule size distribution, destabilised fat index and hardness) of the prototype ice creams were analysed to determine the optimum concentration of CSG to stabilise the ice cream. The prototype ice creams were also evaluated by a sensory focus group comprising of 5-7 experienced panelists.

In **Phase II,** the commercial emulsifier (MDG) was omitted from the optimised formulation obtained from phase I. In this phase, CSG was expected to act as both emulsifier and stabiliser (Campos, Ruivo, Scapim, Madrona, & Bergamasco, 2016). The ice creams produced in Phase II were examined using the same parameters mentioned in Phase I.

The main objective of **phase III** was to determine the optimum concentration of SCB as an emulsifier in ice cream. SCB has similar composition to SMP with increased content of phospholipids (especially lecithin) which are the native emulsifiers in milk (Rajor, Patel, Patel, & Gupta, 1901). To achieve this goal, based on the previous reports (Rothwell, 1974; Hamilton, 1990; Shibu, Kumar, Narasımhan, & Pugazhenthi, 2000) two different concentrations of SCB which constituted 25% and 50% of the NMS were used to partially substitute the SMP in the ice cream formulations. The same parameters mentioned in Phase I were examined in this Phase to select the optimum concentration of SCB in ice cream.

The **phase IV** consisted of four stages aimed to determine the effect of the addition of a probiotic culture to the ice cream formulation. Various attributes of the developed ice cream as described in Phase I were determined however the viable cell counts was also examined to determine the survival of the probiotic culture. In this Phase, instead of a sensory focus group, the overall acceptance of the frozen dessert was evaluated by consumer acceptance test using the 9-point hedonic scale. Moreover, the pH, viable cell counts and overall sensory acceptance of the ice cream were also examined after 30 days of storage (-20°C). Table 3.1 summarises the variables of the ten ice cream formulations studied in the four phases presented here.

Table 3.1 Description of variables used in each formulation

Formulation	Variables
F1	0.1% CSG (w/w) +0.15% MDG (w/w)
F2	0.2% CSG (w/w) +0.15% MDG (w/w)
F3	0.3% CSG (w/w) +0.15% MDG (w/w)
F4	No stabiliser+0.15% MDG (w/w)
F5	0.2% guar gum (w/w) +0.15% MDG (w/w)
F6	without any additive
F7	0.2% CSG (w/w) + No emulsifier
F8	SCB (25% NMS of mix (w/w)) + 0.2% CSG (w/w)
F9	SCB (50% (w/w) NMS of mix) + 0.2% CSG (w/w)
F10	SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w) +10% probiotic (w/w)

Notes: F1-F10 = Prototype ice cream formulations
CSG =chia seed gum; MDG = mono-/diglyceride; SCB = sweet cream buttermilk

Chapter 4 Extraction of gum from chia seed

4.1 Introduction

Seeds such as basil and chia seeds have the ability to hydrate and exude a mucilaginous gel which can be used in the manufacture of ice cream as the potential sources of hydrocolloids (BahramParvar, Razavi, & Mazaheri Tehrani, 2012; Campos, Ruivo, da Silva Scapim, Madrona, & Bergamasco, 2016). Various methods have been used to extract the crude mucilage from the seeds. In all the extraction methods, the seeds are hydrated using different water:seed ratios. The mixtures are stirred during soaking to facilitate hydration and the swelling of the seeds. Variable soaking time, temperature and pH are used depending on the type of the seed and the method used. Following the extraction of mucilage, the aqueous suspension can be directly dried or it can be either centrifuged or filtered before drying. The mucilage can be also scraped from the surface of the seeds using extractor before centrifugation and drying (Hosseini-parvar, Matia-Merino, Goh, Razavi, & Mortazavi, 2010; Capitani et al., 2015; Zameni, Kashaninejad, Aalami, & Salehi, 2015; Campos, Ruivo, da Silva Scapim, Madrona, & Bergamasco, 2016; Chavan, Gadhe, Sharma, & Hingade, 2017).

For extraction of the gum (gel) from chia seed, most studies used the optimised method described by Campos, Ruivo, da Silva Scapim, Madrona, and Bergamasco (2016) which includes heating the seed in water (ratio of 1:30) at 80°C for 4 h. In this method, the whole aqueous suspension is dried in an oven at 50°C. A study by Chavan, Gadhe, Sharma, and Hingade (2017), used the seed:water ratios of 1:20 and 1:40 with soaking time of 3 h accompanied by stirring without heating. The suspension was then centrifuged and the recovered gel was freeze-dried. Capitani et al. (2015) soaked the seeds in water (1:20) for 1 h at 22°C. The mucilage was scraped from the seeds by vacuum filtration and then concentrated on a rotavapour and freeze-dried (at -45°C, 0.06 mBar for 5 days).

A modified extraction method based on the preliminary trials and adapting the previous techniques used for chia seed as well as the extraction methods used for basil seed, which has similar physical properties to chia seed, has been used in this study. This chapter describes the extraction of chia

seed gum (mucilage), the techniques used for measuring its extraction yield and pH as well as the moisture content of the dried mucilage.

4.2 Materials and methods

4.2.1 Extraction of chia seed gum

The extraction of CSG was conducted based on the previous reports with some modifications (Hosseini-parvar, Matia-Merino, Goh, Razavi, & Mortazavi, 2010; Capitani et al., 2015; Zameni, Kashaninejad, Aalami, & Salehi, 2015). Chia seeds (*Salvia hispanica L.*) were soaked in warm distilled water (50°C) at a ratio of 1:20 (w/w) for 20 min. To facilitate swelling of the seeds, an overhead stirrer (IKA Labortechnik, RW 20.n, Malaysia) was used to stir the mixture for another 20 min at 1000 rpm. Figure 4.1 shows the procedures used for the extraction of chia seed gum.

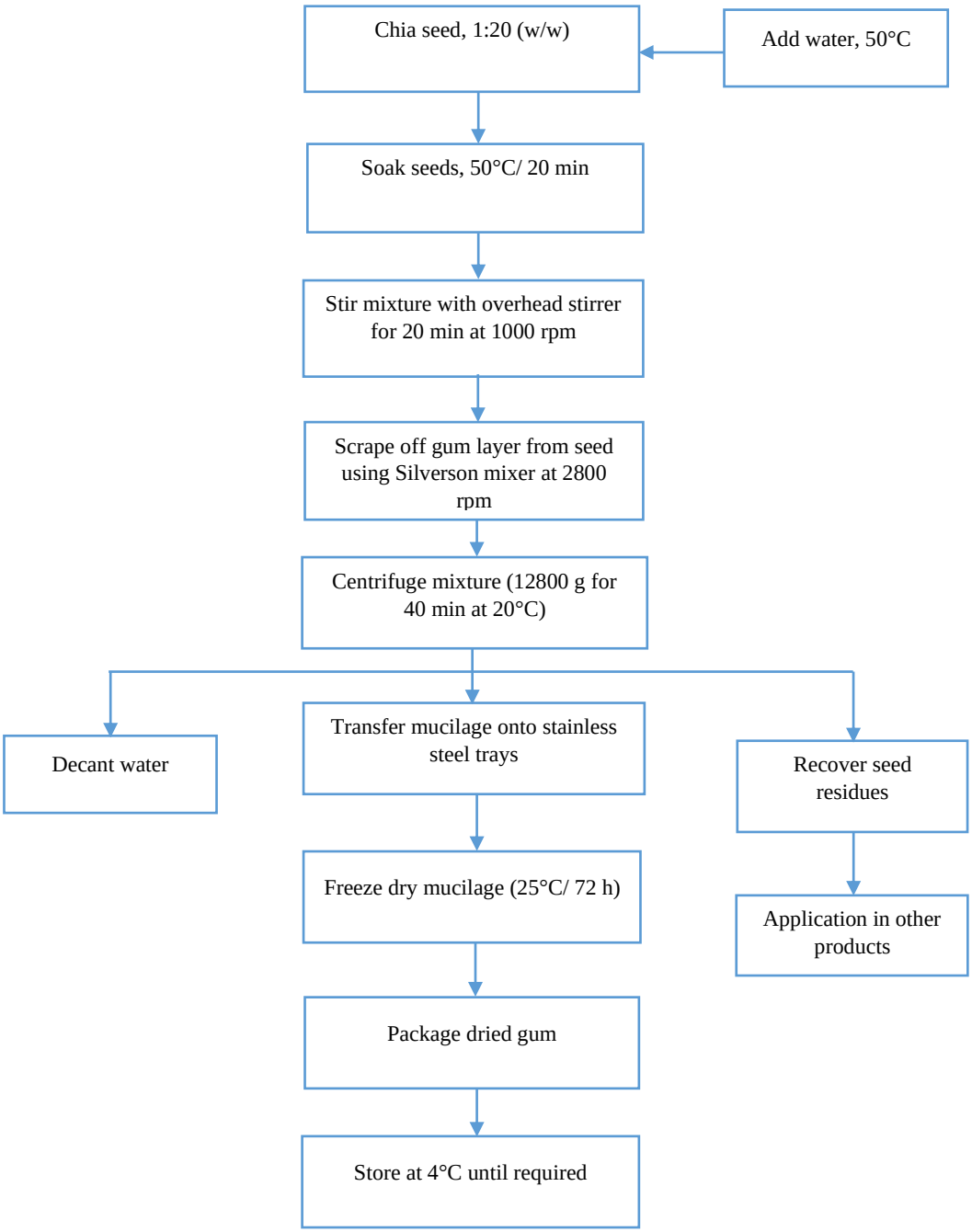


Figure 4.1 Extraction of chia seed gum

The gum layer was then scraped off the surface of swelled seeds using Silverson mixer (L4RT High Shear Mixer, England) at 2800 rpm. The aqueous suspension was centrifuged (12800 g/40 min at 20 °C) (Sigma 6-16 KS centrifuge) to separate the mucilage (gel). After centrifugation,

three discrete phases of water (top layer), mucilage (middle layer) and the seeds which settled at the bottom were obtained (Figure 4.2).

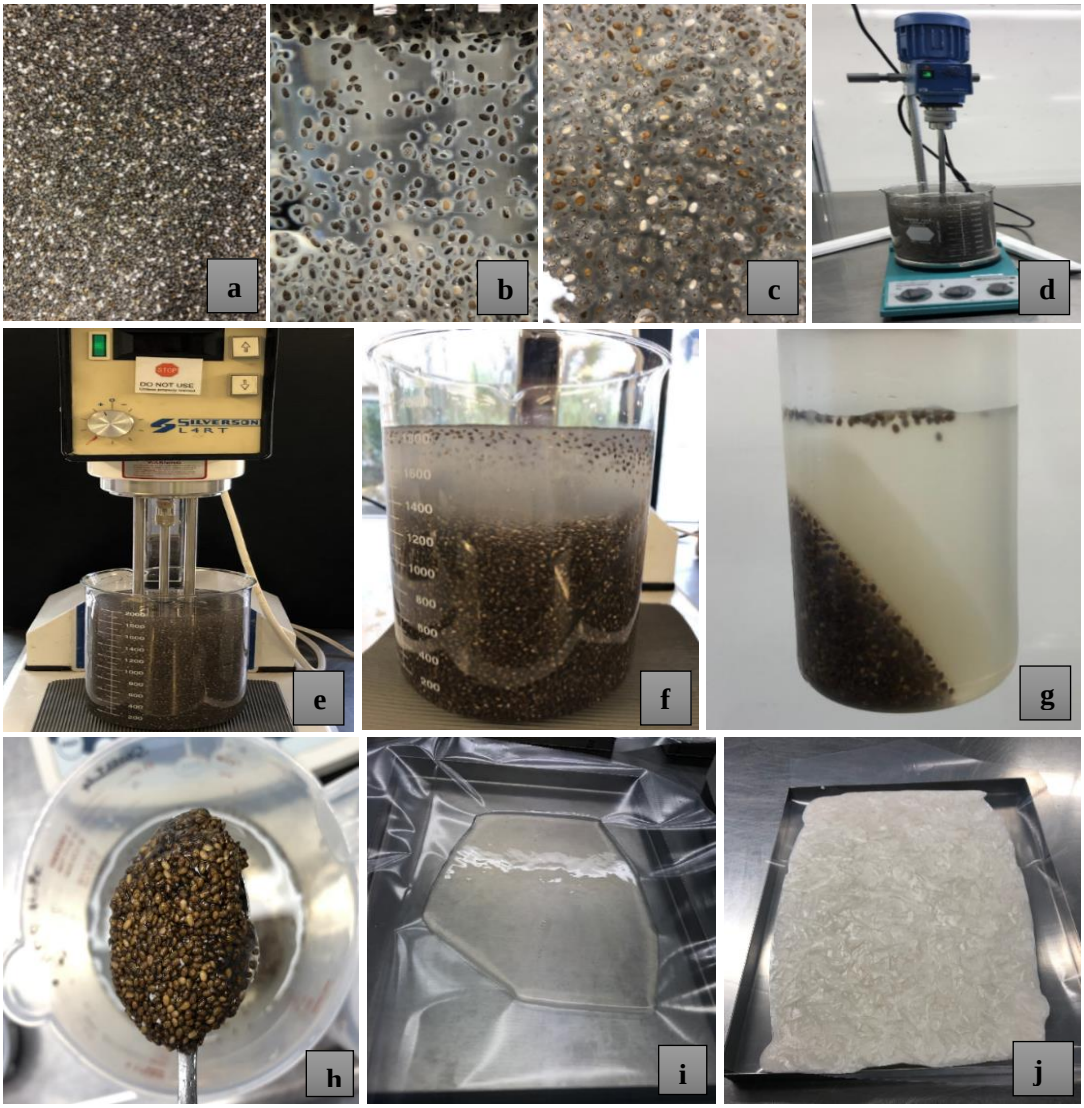


Figure 4.2 Sequential stages of the extraction of chia seed gum: (a) chia seed; (b) soaked chia seed in water; (c) hydrated chia seed after 40 min; (d) overhead stirrer; (e) Silverson mixer; (f) separated mucilage after using the silverson mixer; (g) centrifuged aqueous suspension; (h) seed residues; (i) extracted mucilage (gum); (j) Freeze-dried mucilage (gum) (The images were captured by iPhone 8, Apple Inc., USA).

The water phase was decanted and the gum layer was transferred onto stainless steel trays and dried in a freeze dryer (at 0.12 mBar with collector temperature of -50°C and tray temperature of 25 °C for 72 h) (Labconco freeze dryer, free zone 6, Kansas City, USA). The dried gum (9.9% moisture (w/w)) was packed in plastic bags and stored under cool and dry conditions until required for further use. The sequential stages of the extraction of the CSG are presented in Figure 4.2.

4.2.2 Analysis of yield and moisture content

Extraction yield

The yield of the extracted gum was determined by weighing the freeze-dried extracted gum obtained from a specified mass of chia seed as shown in equation 1.

$$yield = \frac{\text{Mass of extracted gum (g)}}{\text{Mass of chia seed (g)}} \times 100 \qquad \ldots\ldots\ldots\

and freeze-dried while in the second method, the seeds were soaked in seed:water ratio of 1:20 (w/v) accompanied by stirring to induce the mucilage exudation. Before freeze-drying, the mucilage was separated from the seeds using vacuum filtration and concentrated on a rotavapour.

At a constant extraction time, the highest yield was obtained at a lower water:seed ratio and higher temperature (Campos, Ruivo, da Silva Scapim, Madrona, & Bergamasco, 2016). At higher temperatures, the effect of time on yield was more pronounced. In the study by Campos, Ruivo, da Silva Scapim, Madrona, and Bergamasco (2016), the highest extraction yield was 5.09% (w/w) at 70°C with soaking time of 2.4 h and water:seed ratio of 14:1. In another study, a higher extraction yield was achieved (6.97% (w/w)) using an extraction temperature of 80°C with water:seed ratio of 40:1 and extraction time of 2h at pH 8 (Muñoz, Cobos, Diaz, & Aguilera, 2012). Using a lower temperature (27°C) resulted in a higher yield (8.46%) with the seed:water ratio of 1:20 and the pH adjusted at 8 (Tavares, Junqueira, de Oliveira Guimarães, & de Resende, 2018).

The variation in the results could be due to variable hydration rates which could be related to the application of different extraction conditions and variety of chia seeds. The hydration rate which affects the extraction yield depends on several factors such as ionic strength, pH, temperature, and seed:water ratio. For a seed:water ratio of 1:40, a maximum hydration rate occurs at low concentrations of added mono- and divalent salts (e.g., calcium, sodium, and potassium chlorides), pH of 6.5 or above pH 9, and temperature of 65 °C with best results at 80 °C (Valdivia-López & Tecante, 2015).

The average pH of the extracted mucilage from chia seed before drying was 6.14±0.07 which was probably in line with Rosa, alvarez-Ramirez, Vernon-Carter, Carrillo-Navas and Perez-Alonso (2015), who reported that the mucilage is a highly acidic branched polysaccharide. The pH of the mucilage was not measured in previous reports.

4.4 Conclusion

The extraction yield obtained in our study was 3.40±0.16% (w/w) which was relatively lower than those of previously reported. It could be due to attaining lower hydration rate of the seeds in our modified extraction condition in which the salts, high temperatures and adjusted pH were not applied. Optimisation was not the focus of the research therefore it was assumed that the application of a simple extraction method using water in the absence of chemicals and high temperatures would produce a more natural extract. The extracted gum (9.9% moisture) was colourless and had a clean, neutral flavour which could easily disperse in water.

Chapter 5 Optimisation of ice cream formulation containing chia seed gum and sweet cream buttermilk

5.1 Phase I Addition of chia seed gum as stabiliser to ice cream

5.1.1 Introduction

Phase I of this chapter investigated the effect of chia seed gum as a potential stabiliser of ice cream. The novel seed gum has received very little attention as a stabiliser in ice cream despite its popularity for health benefits (Orona-Tamayo, Valverde, & Paredes-López, 2017). As mentioned in the literature review, BSG has similar properties to CSG. Therefore, due to the absence of enough published data regarding the use of CSG in ice cream, the function of BSG as stabiliser in ice cream investigated in several studies, has been used for comparison in our research.

Javidi, Razavi, Behrouzian, and Alghooneh (2016), studied the stabilising properties of different concentrations of BSG, guar gum and their blends in low fat ice cream. Addition of BSG reduced the meltdown rate, coarseness, coldness and produced creamy ice cream. BSG and its blends with guar gum were suggested to be suitable fat replacers and stabilisers. A mixture of BSG and carboxymethyl cellulose (CMC) was used as stabiliser in ice cream and produced ice cream with acceptable meltdown, viscosity, overrun and destabilised fat index (BahramParvar, Razavi, & Mazaheri Tehrani, 2012). The presence of BSG in ice cream as stabiliser (0.1 and 0.2%), reduced the rate of recrystallisation compared to the ice creams stabilised with commercial stabilisers such as guar gum and CMC (BahramParvar & Goff, 2013). The gum also decreased the meltdown rate and increased fat particle size distribution suggesting that BSG produced a different structure possibly by lowering the air and fat interfacial tensions. In the current study, the potential of several concentrations of CSG as stabiliser in ice cream were examined.

5.1.2 Materials and methods

5.1.2.1 Description of selected ingredients

The ice cream formulations used in phases 1-4 consisted of 10% fat (w/w), 11% NMS (w/w), 12% sugar (w/w), 0.3% vanilla (w/w) and water. Other ingredients included Mono-/diglyceride (0.15% (w/w)), CSG (0.1, 0.2, 0.3% (w/w)), guar gum (0.2% (w/w)), SCB (3.04% and 6.08% (w/w) (25% and 50% of NMS (w/w), respectively)) and probiotic culture (10% cultured milk (w/w)) (Table 3.1). The details of the ice cream formulations made in the four phases are described in the appropriated sections. The fat content of formulations was derived from cream (37.3% fat) and SCB (5.8% fat). The cream, SMP, SCB and the probiotic cultured milk accounted for the sources of NMS in the ice cream formulations.

Low-heat skim milk powder (0.9% fat, 3.8% moisture) and sugar were supplied by Tip Top Ltd, Auckland, NZ and cream (37.3% fat) was purchased from a local supermarket. Sweet cream buttermilk powder (5.8% fat, 3.8% moisture) was supplied by Westland Milk Products, Hokitika, NZ. The commercial emulsifier (MDG 0069) was supplied by Hawkins Watts, NZ and organic chia seed was purchased from Aotearoad, Auckland, NZ. The freeze-dried probiotic culture, *Lactobacillus acidophilus* La-5 was supplied by Chr. Hansen Ltd, Hoersholm, Denmark and vanilla was obtained from Hansells, Alderley, Australia.

5.1.2.2 Preparation of ice cream

In phase I, three ice cream formulations (F1-F3) were prepared using different levels of CSG (0.1, 0.2 and 0.3% (w/w)) to replace the commercial stabilisers whereas the commercial emulsifier (MDG 0069) was used in the ice cream mixes. The concentrations of CSG used in our ice cream formulations were based on previous studies (BahramParvar & Goff, 2013; Chavan, Gadhe, Sharma, & Hingade, 2017). Two ice cream formulations (experimental controls), one with no added stabiliser (F4) and another with added guar gum (0.2% (w/w)) as commercial stabiliser (F5) were simultaneously prepared. The calculations and standardisation of the mix formulations are shown in appendix A. All ice cream mixes in phases 1-4 were prepared according to BahramParvar and Goff (2013) with some modifications (Figure 5.1).

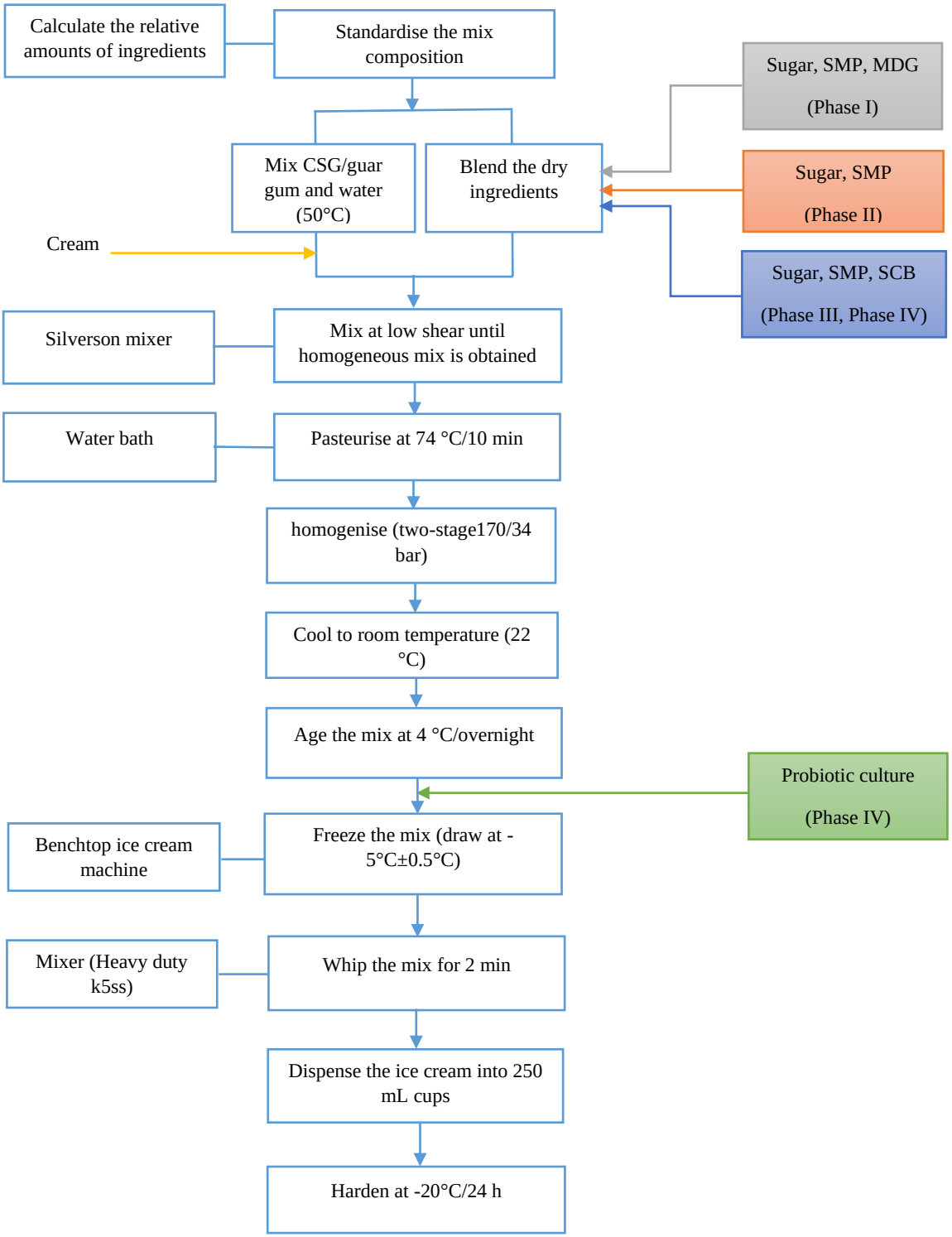


Figure 5.1 Production of ice cream

Notes: CSG =chia seed gum; MDG = mono-/diglyceride; SCB = sweet cream buttermilk; SMP=skim milk powder

Chia seed gum was dissolved in warm water (50 °C) and the cream was added. Pre-blended dry ingredients were then added and mixed by a Silverson Mixer (L4RT High Shear Mixer, England) at low shear prior to pasteurisation (74 °C/10 min using water bath) and two-stage homogenisation (170/34 bar) (APV-2000 homogeniser, Poland). The mixes were cooled and aged overnight at 4°C. After aging the ice cream mixes were frozen in a benchtop freezer (MagiMix). The draw temperature of ice cream measured by a thermocouple was -5.0°C ± 0.5°C. The ice cream was then whipped in a mixer (Heavy duty k5ss) equipped with a whisk for 2 min. The ice cream samples were collected in 250 mL laminated cardboard cups and hardened at -20°C for 24 h. All ice cream formulations were produced in duplicate.

5.1.2.3 Characterisation and analysis of ice cream

During ice cream making, samples were collected from the ice cream mixes and ice cream melts for determining various parameters. The overrun and destabilised fat index (DFI) were measured. Fat globule size distribution of the melted ice creams and ice cream mixes were analysed and the viscosity and pH of the ice cream mixes were determined. Further the meltdown rate and hardness of the ice cream samples were measured at 20.0±0.5°C after the samples were hardened at −20°C for at least 24 h. The physical and sensory attributes of the hardened ice creams were screened in a focus group consisting of six panelists.

Calculation of overrun

One of the major physical characteristics of frozen ice cream is weight per unit volume of product which is affected by the overrun developed in the product (Marshall, Goff, & Hartel, 2003). Overrun was calculated by comparing the weight of a known volume of ice cream (250 mL) to the weight of the same volume of unfrozen ice cream mix (Goh, Nair, & Matia-Merino, 2008). Overrun was calculated according to Equation 2.

$$Overrun\ \% = \frac{(m_{mix} - m_{foam})}{m_{foam}} \times 100 \quad \text{.....2}$$

$\boldsymbol{m_{mix}}$ = mass of mix
$\boldsymbol{m_{foam}}$ = mass of ice cream

Measurement of fat globule size distribution

Fat globule size distribution of the ice cream mixes and melted ice cream samples (melted at 4 °C for 3–4 h) were measured using the Malvern Mastersizer 2000 (BahramParvar & Goff, 2013). The tank of the sample suspension unit (DFI2001, Worcestershire, UK) was filled with 1000 mL Milli-Q water. The stirrer speed of the unit was set at 850 rpm while the water was being gently pumped into the Mastersizer unit. After the system was stabilised, the sample was added drop by drop until the laser obscuration of 10-20% was obtained. Dilution of the samples in the Mastersizer chamber was approximately 1:1000 (BahramParvar & Goff, 2013). Consequently, the measurement of the volume-average mean diameters ($d_{4,3}$) of the fat globules of the samples were done. The relative refractive index (n), i.e., the ratio of the refractive index of the emulsion droplets (1.456) to that of the dispersion medium (1.33) was 1.095. The absorbance of the fat globules was 0.001. The droplet size, $d_{4,3}$, is defined as:

$\Sigma n_i D^4_i / \Sigma n_i D^3_i$

Where n_i is the number of particles with diameter D_i (Goh, Nair & Matia-Merino, 2008). All measurements were carried out in duplicate.

Measurement of viscosity of the ice cream mix

Steady-state viscosity measurements of the aged ice cream mixes were performed using a controlled stress rheometer (TA Instruments AR-550, UK), with a cone and plate geometry (cone angle = 2 degrees, plate diameter = 60 mm) at 5°C. The temperature (5°C) was maintained using a water bath assisted with a cooler (Julabo FT200). The samples were loaded onto the lower plate of the rheometer, allowed to equilibrate (2 min) to ensure homogeneity of the samples. The samples were then subjected to pre-shear rate (1000 s^{-1} for 10 s) to erase thixotropy. The viscosity was measured over a range of shear rates (0.1 to 100 s^{-1}). The data obtained from the Rheology

Advantage program (TA Instruments Ltd) were plotted as viscosity versus shear rate in the logarithmic scale.

Meltdown test

Meltdown rate of ice creams were measured according to the methods described in previous studies with some modifications (Goh, Nair & Matia-Merino, 2008; Bahramparvar & Goff, 2013). ice cream samples (250 mL), which had been stored at -20°C were removed from containers and weighed on a top pan balance. The samples were suspended on a wire mesh screen (1 cm × 1 cm) and allowed to melt at 20.0±0.5°C (Figure 5.4). The weight of the melted material was recorded at 10-min intervals for 70 min. A plot of mass loss versus melting time was constructed. Meltdown rates were calculated from the slope of each meltdown curve. The regression equations are shown in the Appendix A (Table 10.3).

Determination of destabilised fat index

The destabilised fat index (DFI) in the melted ice cream samples was determined according to Segall and Goff (2002). Samples of ice cream mixes and melted ice creams (3 g) were diluted to 30 g with distilled water. One mL of this solution was then diluted to 50 mL in a volumetric flask giving a total dilution of 1:500. The diluted solutions were allowed to stand for 5 min and then the absorbance (a_{mix} and a_{melt}) of the solutions were measured at 540 nm using a UV-Visible Spectrophotometer (Shimadzu, UV-1601). Turbidity was used as an indicator of the amount of destabilised fat in the sample by applying Equation 3.

$$DFI = \frac{a_{mix} - a_{melt}}{a_{mix}} \times 100 \quad \text{.....3}$$

$\boldsymbol{a_{mix}}$ = absorbance of mix
$\boldsymbol{a_{melt}}$ = absorbance of melted ice cream

Texture analysis of ice cream

Frozen ice cream samples were analysed for their hardness using a Texture Analyser (TA- XTplus, UK) according to the method described by Goh, Nair, and Matia-Merino (2008). To determine the hardness of the ice cream samples using the Exponent software (Stable micro systems) a 10-mm-diameter cylindrical probe was applied to measure the maximum force required to penetrate a 44-mm thick sample at a test speed of 0.5 mm s^{-1} to a depth of 20 mm at 20.0±0.5°C. The samples were quickly measured during the experiment to minimise variability due to the environmental conditions. After attaching the probe and the loadcell (50 kg), the instrument was calibrated (force and height) before conducting the test. The data were recorded by the Exponent program in gravitational force (g) which were then converted to force (N) using equation 4 (Anonymous, 2019b).

$$Hardness\ (N) = \frac{Hardness\ (g)}{1000} \times 9.81 \quad \text{.....4}$$

Sensory evaluation

Focus group of six panelists comprising of staff members of the Department of Food Science and Technology, Massey University evaluated the ice cream samples. The hardened ice cream samples were removed from -20°C freezer and served in 60-mL cups labelled with random three-digit codes. The evaluation was carried out in sensory lab at ~22°C, Massey University. Different attributes of the ice creams comprising colour and appearance, texture and body, creaminess, coarseness and hardness, flavour and sweetness, melting quality, mouthfeel and overall acceptability were assessed on consensus. The panelists were asked to taste the ice cream samples and rinse their mouth with water between samples to refresh the palate. In addition to the experimental results, focus groups (Phase I, II and III) were used to evaluate the sensory properties of the developed ice creams, to select the best formulations.

The sensory properties of the final ice cream in Phase IV, were evaluated by conducting a consumer acceptance test in sensory booths under fluorescent white light at Massey University. The participants (n=52) who were announced by distributed flyers were recruited to taste two

coded ice cream samples including the final ice cream formulation and a commercial retail ice cream. The degree of liking of the flavour, body and texture, colour and appearance, melting quality, mouthfeel and overall acceptance were evaluated by participants on a 9-point hedonic scale from 1=dislike extremely to 9= like extremely, with 5= neither like nor dislike (Peryam & Pilgrim, 1957). The panelists rinsed their mouth with water between samples.

Statistical analysis

The experiments were repeated twice and data were analysed using the statistical software Minitab Version 19 (Inc. Pennsylvania, USA for Windows). One-way analysis of variance (ANOVA-ONE-WAY) was used to analyse the mean results of physical and chemical data to determine any significant differences ($p \leq 0.05$) between the formulations of each phase (Appendix B). Significant means were separated by Tukey's post hoc analysis (Appendix B).

Means of the scores obtained for the sensory attributes of the two samples (the final formulation and commercial retail ice cream) were analysed at days 2 and 30 during storage (-20°C) using the univariate of the general linear model (ANOVA) ($p \leq 0.05$) (Appendix C).

5.1.3 Results and discussion

Rheological properties

The most desirable attributes of ice cream, the process design and equipment selection are influenced by rheological properties of the mix (Dogan & Kayacier, 2007; Tabatabaee Amid & Mirhosseini, 2012; Dogan, Kayacier, Toker, Yilmaz, & Karaman, 2013).

The rheological properties of the ice cream mixes determined by apparent viscosity measurement are shown in Figure 5.2. All the ice cream mixes exhibited non-Newtonian pseudoplastic flow behaviour, with the apparent viscosity decreasing as the shear rate increased (BahramParvar & Mazaheri Tehrani, 2011). The addition of chia seed mucilage increased the apparent viscosity of the ice cream mixes compared to the formulation without added CSG (F4). Moreover, there

seemed to be a marked correlation between the viscosity of the ice cream mixes and the concentration of CSG in the formulations. Figure 5.2 shows that among the ice cream mixes which contained CSG, F3 had the highest viscosity across the entire shear rate range of 0.1-100 s^{-1}. Comparatively, F2 had the second highest viscosity followed by F1. There were no published data found on the viscosity of ice cream mix containing chia seed mucilage, therefore in the absence of such data the results were compared with ice cream mix containing BSG. Our results were consistent with BahramParvar, Razavi and Mazaheri Tehrani (2012) who reported increasing the BSG enhanced the apparent viscosity of the ice cream mixes. In this previous report, BSG resulted in higher viscosities at either 0.1% or 0.2% (w/w) compared to the combination of CMC and guar gum at equivalent concentrations. In our study, 0.2% guar gum in F5 produced lower viscosity than the same amounts of CSG in F2 at shear rate lower than 10 s^{-1} but the viscosity curves cross each other at high shear rates, indicating a more pseudoplastic behaviour of CSG (Figure 5.2).

The quality characteristics of ice cream such as meltdown rate, body and texture as well as sensory properties (Dogan, Kayacier, Toker, Yilmaz, & Karaman, 2013) are affected by stabilisers used to increase the apparent viscosity of the mix (Cottrell, Pass, & Phillips, 1980; BahramParvar & Mazaheri Tehrani, 2011). In previous studies the ability of hydrocolloids in enhancing viscosity and decreasing molecular mobility were correlated with control of ice crystal growth during storage (Cottrell, Pass, & Phillips, 1980; BahramParvar & Goff, 2013). Our results suggest that CSG provided high viscosity for the ice cream mixes and thereby sufficient rheological properties for ice cream samples.

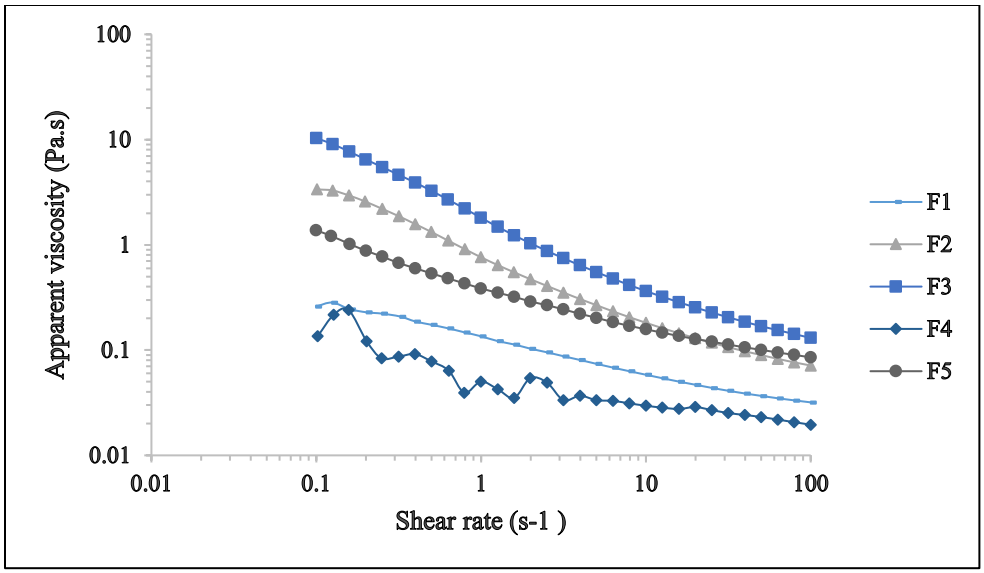


Figure 5.2 Apparent viscosity of ice cream mixes

Note: Ice cream formulations: F1 = 0.1% CSG (w/w) +0.15% MDG (w/w); F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F3 = 0.3% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F5 = 0.2% guar gum (w/w) +0.15% MDG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates. Only the variables that describe each formulation are shown.

The rheology of aqueous solutions of raw mucilage under different conditions have been investigated in previous studies (Capitani et al., 2015; Timilsena, Adhikari, Kasapis, & Adhikari, 2015; Tavares, Junqueira, de Oliveira Guimarães, & de Resende, 2018). Raw mucilage in the range of 0.25–1% (Capitani et al., 2015) and 1-5% (Tavares, Junqueira, de Oliveira Guimarães, & de Resende, 2018) and chia seed polysaccharide solutions (> 0.06%) (Timilsena, Adhikari, Kasapis, & Adhikari, 2015), exhibited non-Newtonian shear-thinning behavior at 25 °C. In addition to shear-thinning, the raw mucilage dispersion presented a weak elastic gel-like structure because the storage or elastic modulus (G′) was larger than the loss or viscous modulus (G″), indicating a prevalent elastic behavior of the samples, similar to that of psyllium gum solutions (Figure 5.3).

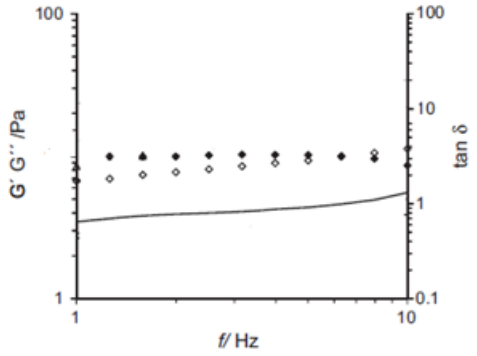


Figure 5.3 Effect of chia seed mucilage (0.25%) on storage (G´), loss (G″) moduli and tan δ as functions of frequency at constant temperature of 25°C. G´: full symbols; G″: empty symbols; tan δ: line.

Overrun

Air is an important component which contributes essentially to the final texture of ice cream and is measured by the overrun (Cottrell, Pass, & Phillips, 1979). In our study, the overrun decreased slightly with increasing CSG levels in the formulation. The highest and lowest overrun among the samples which contained CSG were obtained for F1 and F3, with 108.15±6.25% and 96.10±0.36%, respectively whereas the overrun for the ice cream containing 0.2% CSG (w/w) (F2) was 103.10±0.47% (Table 5.1). In a similar study, the reported overrun for the samples that contained BSG (0.1 and 0.2% (w/w)) were only 46.5 and 42.7% respectively (BahramParvar & Goff, 2013). Lower overrun of ice creams containing higher amounts of seed gums could be attributed to the rheological properties of the mixes and inefficiency of the freezer to incorporate as much air as in the samples with lower viscosity.

The viscosity of the mix has an important effect on overrun. An increase in viscosity can reduce the rate of whipping however a certain level of viscosity is essential for proper whipping and retention of air (Arbuckle & Frandsen 1966; Baer, Wolkow, & Kasperson, 1997). Therefore, the high and low viscosities in F3 and F4, respectively, compared to F1 and F2 may have contributed to their lower overruns possibly due to the inefficiency of the mixes to retain the incorporated air. The ice creams which contained CSG (0.1 and 0.2% (w/w)) had higher overrun relative to the unstabilised sample (F4). Similarly, Cottrell, Pass, and Phillips (1979), obtained lower overrun

(87%) for the unstabilised sample, whereas BahramParvar and Goff (2013), reported no significant difference among overrun of unstabilised samples and those stabilised with BSG (0.1 and 0.2%).

According to Goff, Verespej, and Smith (1999), formation of the destabilised fat during freezing, envelopes air cells and improves the overrun. Although in our study, this was not clearly observed in all formulations, the increased overrun in F1, F2 and F5 compared to F4 could be attributed to the increased DFI and volume-average mean diameter ($d_{4,3}$) of fat globules in these formulations.

The pH of ice cream mixes was examined using a pH meter (Sartorius PB-11). The pH of all ice cream formulations ranged from 6.50-6.59 and the presence of CSG did not significantly ($p > 0.05$) affect the pH. The pH is similar to another ice cream formulation with guar gum that has a pH of 6.7 reported in a previous study (Murtaza, Mueenuddin, Huma, Shabbir, & Mahmood, 2004).

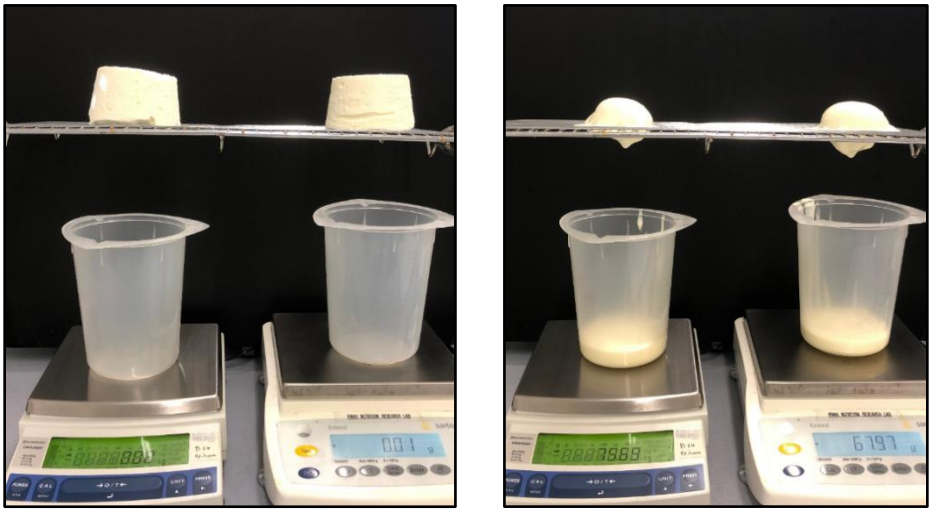
Table 5.1 The overrun, meltdown rate and pH of ice cream formulations

Formulation	Overrun (%)	Meltdown rate ($g.min^{-1}$)	pH
F1	$108.15 \pm 6.25^{a,b}$	1.34 ± 0.25^{a}	6.54 ± 0.06^{a}
F2	$103.10 \pm 0.47^{a,b}$	0.96 ± 0.14^{a}	6.55 ± 0.06^{a}
F3	96.10 ± 0.36^{b}	0.47 ± 0.32^{a}	6.59 ± 0.00^{a}
F4	$99.33 \pm 9.36^{a,b}$	1.15 ± 0.08^{a}	6.57 ± 0.02^{a}
F5	118.78 ± 2.77^{a}	1.26 ± 0.10^{a}	6.50 ± 0.00^{a}

Note: Ice cream formulations: F1 = 0.1% CSG (w/w) +0.15% MDG (w/w); F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F3 = 0.3% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F5 = 0.2% guar gum (w/w) +0.15% MDG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. ±= standard error of the mean value. Data were based on two replicates. Means with identical superscripts in a column are not significantly different at $P<0.05$.

Meltdown rate

The meltdown test of the ice cream samples was carried out at 20.0±0.5°C (Figure 5.4) after the samples were kept at -20°C for at least 24 h. The average mass of melted ice cream samples (weight loss) during 70 min are shown in Figure 5.5.



(a) (b)

Figure 5.4 Set up of meltdown rate test of an ice cream sample.

(a): time = 0; (b): time = 70 min

The rate of the meltdown was obtained from the slope of each meltdown curve using the regression equation (Appendix A, Table 10.3). The meltdown test determines the ability of ice cream to resist melting. The range of meltdown rate in our study was similar to other studies (Bollinger et al, 2000; Karaca et al, 2009; Bahramparvar & Goff, 2013). The addition of CSG reduced the rate of meltdown compared to the sample with no stabiliser (F4). Similar results were obtained for ice creams containing BSG as stabiliser (BahramParvar, Razavi, & Mazaheri Tehrani, 2012; BahramParvar & Goff, 2013; Javidi, Razavi, Behrouzian, & Alghooneh, 2016). Our results also showed that the ice creams containing higher concentrations of CSG were more resistant to melting (Table 5.1) resulting in F3 and F2 showing the lowest rates of meltdown (0.47±0.32 g/min and 0.96±0.14 g/min), respectively. The data are in agreement with other reports where high concentration of BSG resulted in high melting resistance which was attributed to high viscosity of the ice cream mix (BahramParvar, Razavi, & Mazaheri Tehrani, 2012; Bahramparvar & Goff, 2013). Previous studies showed that increase in apparent viscosity enhanced the resistance to meltdown and smoothness of the ice cream (Arbuckle & Frandsen, 1966; Dogan, Kayacier, Toker, Yilmaz, & Karaman, 2013). The increase in serum (unfrozen phase) microviscosity, results in longer time required for the water to diffuse into the concentrated serum phase before it begins to flow from the interior to the ice cream exterior (Muse & Hartel, 2004).

Other than the viscosity, meltdown rate is also affected by the fat structure formation, overrun and ice crystal size (Muse & Hartel, 2004). An inverse relationship exists between overrun and meltdown rate (Sakurai, 1996), which is attributed to the reduced rate of heat transfer due to a larger volume of air and also possibly due to the more tortuous path through which the melting fluid must flow (Hartel et al., 2003). This relationship was not observed in all formulations in our study which was similar to the results reported by Cottrell, Pass, & Phillips (1979) who obtained relatively higher meltdown rate for the ice cream containing guar gum with higher overrun. According to Figure 5.5, F1 showed the steepest slope although during the first 40 min higher mass of the melted ice cream dripped from F4. After F1, F5 which contained the commercial stabiliser exhibited the lowest resistance to meltdown even lower than that of the unstabilised formulation (F4) which was consistent with Cottrell, Pass, and Phillips (1979).

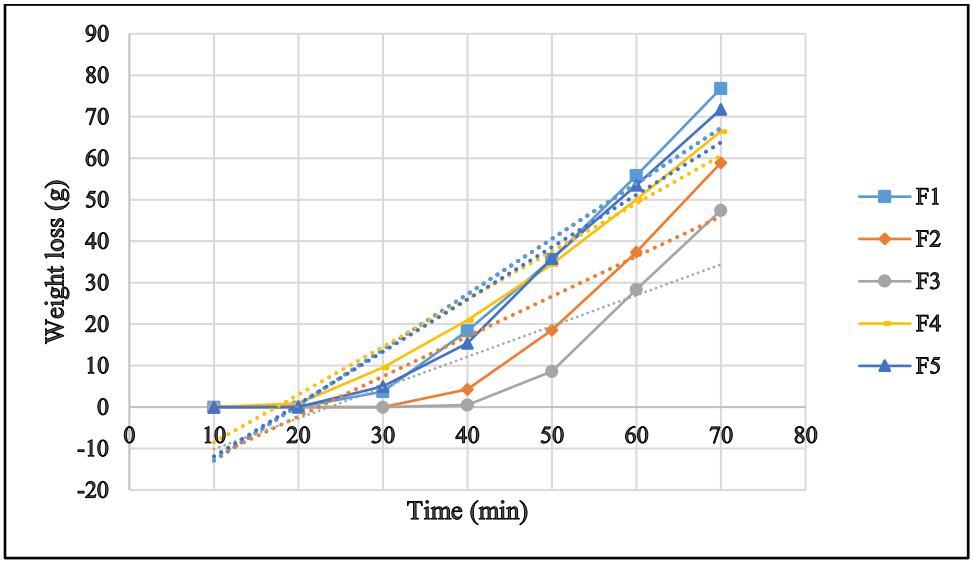


Figure 5.5 Weight loss of ice cream samples during meltdown

Note: Ice cream formulations: F1 = 0.1% CSG (w/w) +0.15% MDG (w/w); F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F3 = 0.3% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F5 = 0.2% guar gum (w/w) +0.15% MDG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates. Non-continuous lines represent the slope of each meltdown curve.

The formation of the network of partially destabilised fat is another factor which could help stabilise the air bubbles and thereby contribute to reducing the meltdown rate of ice creams (Muse

& Hartel, 2004). In our study, the effect of destabilised fat on overrun was not obvious but except for F5, increasing the destabilised fat in all formulations reduced the rate of meltdown. According to Goh, Nair, and Matia-Merino (2008), protein-protein and protein-polysaccharide at the fat globule/air cells interfaces and the dispersed components in the serum phase may also affect the meltdown properties.

Measurement of fat globule size distribution

Fat globule size distribution (FGSD) provides information on the degree of fat aggregation and/or partial coalescence in the emulsion systems such as ice cream. The distribution of small air cells stabilised by partially coalesced fat would contribute to a stiff ice cream with desirable creaminess, dry appearance, shape retention, smoother texture and slow meltdown (Pei & Schmidt, 2010; Méndez-Velasco & Goff, 2012). The average FGSD of the ice cream mix and melt samples are shown in Figure 5.6 and Figure 5.7, respectively. The $d_{4,3}$ values of the melt for all samples were higher than those of the mix (Table 5.2). The bimodal peaks in the particle size distribution of ice cream melts shown in Figure 5.7, could be attributed to the fat droplet coalescence and/or fat droplet aggregation upon whipping/freezing (Gelin et al., 1994; Goff, 1997; Bolliger et al., 2000; Relkin, Sourdet, & Fosseux, 2003). An increase in $d_{4,3}$ was observed with the incremental addition of CSG.

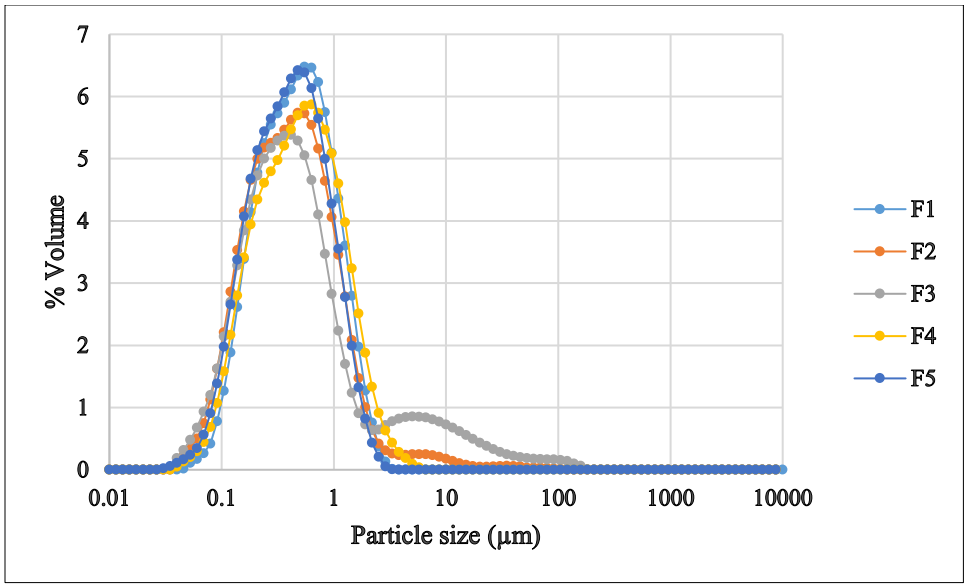


Figure 5.6 Average particle size distribution of ice cream mix

Note: Ice cream formulations: F1 = 0.1% CSG (w/w) +0.15% MDG (w/w); F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F3 = 0.3% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F5 = 0.2% guar gum (w/w) +0.15% MDG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates.

The higher viscosity in the continuous phase could also result in an increase in the emulsion droplet size (Dickinson & Wedlock, 1994). A molecular property of the CSG is its anionic behavior over a wide range of pH (≥1.8) which is attributed to the presence of uronic acids (Timilsena, Adhikari, Kasapis, & Adhikari, 2016). Also the presence of glucuronic acid in the repeating unit of the primary structure of chia seed mucilage results in polyelectrolyte behavior of the mucilage which is sensitive to external mono and divalent cations (Valdivia-López & Tecante, 2015). These suggest that the polysaccharide-protein interactions (via bridging flocculation) may contribute to the high $d_{4,3}$ values of the melt. The lowest $d_{4,3}$ values were obtained for the unstabilised ice cream (F4). A study by Bahramparvar and Goff (2013), which examined the $d_{4,3}$ values of the solutions of either 0.2% BSG or 0.2% BSG, 15% sugar and 11% SMP, found that BSG itself formed large aggregates. This previous study concluded that the BSG established a network structure in the ice cream perhaps due to interactions with fat or proteins leading to a larger particle size distribution, slower rates of meltdown and higher mix viscosity. BSG is a complex and acid stable gum composed of a major glucomannan and a minor xylan fraction, which contains acid side chains. The xylan fraction is more sensitive to pH and salt, which may contribute to the net charge of the

polysaccharide (Anjaneyalu & Gowda, 1979; Osano, Hosseini-Parvar, Matia-Merino, & Golding, 2014).

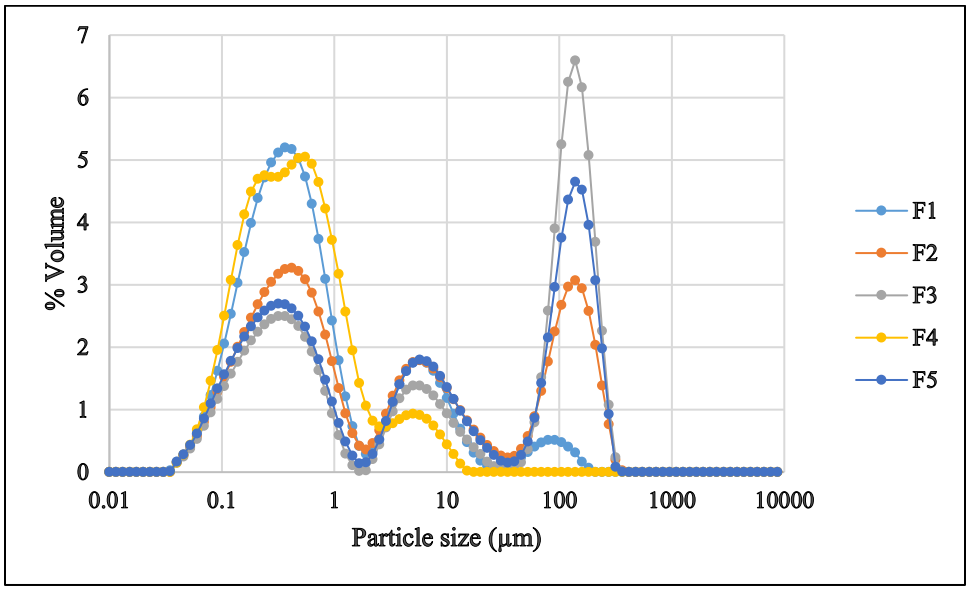


Figure 5.7 Average particle size distribution of ice cream melt

Note: Ice cream formulations: F1 = 0.1% CSG (w/w) +0.15% MDG (w/w); F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F3 = 0.3% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F5 = 0.2% guar gum (w/w) +0.15% MDG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates.

Destabilised fat index (DFI)

Turbidity is a relatively quick and easy measurement technique that gives less detailed information than light scattering. It is used to estimate the amount of destabilised fat rather than the particle size distribution (Clarke, 2015). Change in turbidity of diluted ice cream in comparison with the diluted mix is used to determine the extent of fat destabilisation (Goff & Hartel, 2004). Similar to FGSD, the DFI increased with the levels of CSG in ice cream formulations with 37.60±5.99, 41.00±5.35 and 47.91±3.76% for F1, F2 and F3, respectively (Table 5.2). F5 and F4 had much lower values for DFI relative to the ice creams which contained CSG. There was no report found on the DFI of ice cream mix containing guar gum.

The sample without added stabiliser (F4) had the lowest DFI (8.06±4.15%), although it contained the commercial emulsifier (MDG). The increased DFI in ice cream stabilised with CSG relative to the unstabilised one could be attributed to the surface active properties of CSG that caused increased fat destabilisation. The ability of BSG and CSG to lower the interfacial tensions at the interface due to their adsorption at oil-water interfaces in emulsion model systems have been reported previously (Osano, Hosseini-Parvar, Matia-Merino, & Golding, 2014; Timilsena, Adhikari, Kasapis, & Adhikari, 2016).

The effect of DFI on meltdown of ice cream samples in our study was consistent with Muse and Hartel (2004), where increased levels of destabilised fat played a significant role in lowering the meltdown rate.

Table 5.2 Fat globule size distribution, destabilised fat index of ice cream mix and ice cream

Formulation	Fat globule size ($d_{4,3}$) mix (μm)	Fat globule size ($d_{4,3}$) ice cream (μm)	Destabilised fat index (%)
F1	0.55±0.01[a]	5.63±4.83[b]	37.60±5.99[a]
F2	1.00±0.14[a]	40.66±8.87[a]	41.00±5.35[a]
F3	3.23±3.79[a]	71.53±16.00[a]	47.91±3.76[a]
F4	0.73±0.18[a]	1.03±0.18[b]	8.06±4.15[b]
F5	0.56±0.02[a]	56.04±1.41[a]	9.41±0.052[b]

Note: Ice cream formulations: F1 = 0.1% CSG (w/w) +0.15% MDG (w/w); F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F3 = 0.3% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F5 = 0.2% guar gum (w/w) +0.15% MDG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride; $d_{4,3}$= volume average diameter of the mix and melt. ±= standard error of the mean value. Data were based on two replicates. Means with identical superscripts in a column are not significantly different at $P<0.05$.

Texture analysis of ice cream

The ice cream samples were analysed for their firmness which is useful in relating to 'scoopability' using a Texture Analyser (TA- XTplus, UK) (Goh, Nair, & Matia-Merino, 2008). Hardness is influenced by ice phase volume, ice crystal size, overrun, freezing point depression of the mix, fat destabilisation, total solids and the rheological properties of the mix. Figure 5.8 shows the mean force (N) required to penetrate the ice cream samples. Among ice cream samples which contained CSG, the highest level of hardness was obtained for F3 (61.80±39.70 N). The results were

consistent with previous reports where a decrease in overrun (Wilbey, Cooke, & Dimos, 1998) and increase in the concentration of stabilisers (Martinou-voulasiki & Zerfiridis, 1990), viscosity (Muse & Hartell, 2004) and DFI (Tharp, Forrest, Swan, Dunning, & Hilmoe, 1998) enhanced the hardness. In contrast, sample F5 did not follow the trend and showed the highest hardness (71.56±1.03 N) despite having the highest overrun and a relatively low DFI. It may be due to the incapability of guar gum to reduce the rate of ice crystal growth (Javidi, Razavi, Behrouzian, & Alghooneh, 2016).

The hardness of the formulation without stabiliser (F4) with relatively a low overrun and high DFI was 52.90±41 (N). The hardness could be also attributed to the high volume and large size of ice crystals perceived as coarse texture due to the absence of stabilisers in this formulation. In our study, F3 with the highest level of total solids did not show the lowest hardness because the freezing point is primarily affected by low molecular weight sugars, and polysaccharides do not induce significant freezing point depression (Soukoulis, Lebesi, & Tzia, 2009). However, this previous study found that depending on the type of the biopolymer, polysaccharides can substantially affect the freezing point depression. Therefore, further research is required to investigate the effect of CSG on freezing point depression.

Lower DFI and higher overrun of F1 and F2 relative to F3 and F4 contributed to lower level of hardness with F2 showing the lowest hardness. Even though F1 was expected to have the lowest level of hardness due to a higher overrun, lower DFI and stabiliser level, it seemed the latter factor led to the sensation of ice crystals (suggesting larger ice crystals present) which could in turn affect the hardness. It was concluded from our results that the presence and concentration of stabilisers could markedly affect the hardness of ice creams however the statistical analysis showed no significant difference ($p > 0.05$) between the samples. The hardness is also reported to be dependent on polymer network that results from protein-polysaccharide interactions (Goh, Nair, & Matia-Merino, 2008).

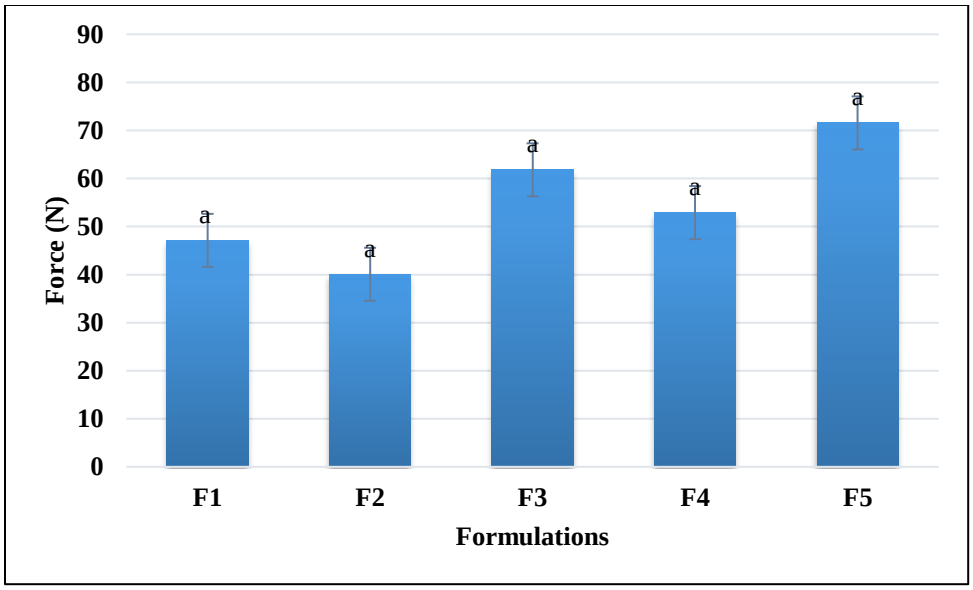


Figure 5.8 Hardness of ice cream samples

Note: Ice cream formulations: F1 = 0.1% CSG (w/w) +0.15% MDG (w/w); F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F3 = 0.3% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F5 = 0.2% guar gum (w/w) +0.15% MDG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates. Means with identical letters are not significantly different at $P<0.05$.

Note: results were recorded in gravitational force (g) and were converted to force (N) by (Hardness (N) = (hardness (g)/1000) × 9.81)).

Sensory evaluation

Sensory properties of the ice cream samples were evaluated by an informal focus group. The perceived melting characteristics supported the experimental results. Based on the collated opinions, the highest meltdown resistance was attributed to F3 which resulted in the perception of ice cream as chewable and undesirable. Contrary, similar to F5, F1 was perceived as slightly gritty and fast-melting with a weaker body and texture. Among ice cream samples, F2 was the most liked formulation in terms of the hardness, creaminess, smoothness, mouth solubility and melting properties.

In a previous study, the microstructure analysis of ice cream containing BSG showed relatively small air cells which was attributed to either the high viscosity of ice cream mix or emulsification properties of BSG. This property imparted positive impacts on the stability of the foam and the

perception of creaminess upon consumption (BahramParvar & Goff, 2013). This ability possibly contributed to the desirable sensory properties of F2.

5.1.4 Summary

The addition of 0.2% CSG (w/w) resulted in ice cream with comparable and even superior characteristics compared to the ice cream stabilised with the same concentration of the commercial stabiliser (guar gum). The ice cream mixes which contained CSG showed typical non-Newtonian pseudoplastic flow behaviour which is largely attributed to the added hydrocolloids. Similar to the guar gum in F5, the addition of chia seed mucilage markedly increased the apparent viscosity of the ice cream mixes compared to the formulation without added CSG (F4). The viscosity of the ice cream mixes increased with increasing the concentration of CSG. Compared to F5 which contained 0.2% guar gum (w/w), F2 with the same concentration of CSG showed a higher viscosity at lower shear rates but the ice cream mixes showed similar behavior at higher shear rates. The higher viscosity at lower shear rate for sample F2 could be related to the viscosity of the melted ice cream in the mouth which could be perceived to be "creamier".

The relative high overrun, meltdown resistance, high DFI and $d_{4,3}$ accompanied by acceptable sensory properties showed the potential of CSG as an alternative stabiliser in ice cream. The sensory evaluation conducted by the focus group stated that the ice cream had desirable hardness, smooth texture and creamy mouthfeel without sensation of ice crystals and off-flavour. Considering the data obtained from the experimental measurements and sensory tests, 0.2% CSG (w/w) appeared to be an optimal concentration for use in ice cream. Therefore, it was used in the subsequent phase.

5.2 Phase II Addition of chia seed gum as stabiliser and emulsifier to ice cream

5.2.1 Introduction

The surface activity and emulsifying properties of CSG were reported to be either superior or comparable to other common gums and industrial polysaccharides (Timilsena, Adhikari, Kasapis, & Adhikari, 2016). The emulsification properties of CSG was presumed to arise from the formation of spatial structures surrounding the oily phase indicating the potential of CSG to provide long-term stability to emulsions (Avila-De La Rosa, Alvarez-Ramirez, Vernon-Carter, Carrillo-Navas, & Pérez-Alonso, 2015). Maestrello et al. (2017) reported the potential of CSG to replace commercial emulsifier and produce ice cream with appropriate chemical composition and sensory acceptance.

Phase I showed that CSG at 0.2% (w/w) was able to stabilise the ice cream in the absence of commercial stabilisers. In this previous phase, the commercial emulsifier (MDG) was used in ice cream formulations. The comparable overrun and higher meltdown resistance obtained for F2 as well as its higher destabilised fat coupled with acceptable sensory properties relative to F5 (which contained commercial stabiliser) could be due to the additional emulsifying properties of CSG. Therefore, in this phase the emulsifying ability of CSG was investigated in the absence of MDG (F7). The results were compared with ice creams which had no CSG. Accordingly, formulations with and without MDG (F4 and F6, respectively) were made in the absence of stabilisers to investigate the emulsifying properties of the CSG.

5.2.2 Materials and methods

The materials and methods used in this phase (phase II) are similar to those described in phase I with some minor differences. In phase II, some ice cream formulations (F6, F7) did not contain MDG (Figure 5.1 and Table 3.1).

5.2.3 Results and discussion

Rheological measurements

As Figure 5.9 shows, F2 and F7 had similar viscosity curves which were different from those of F4 and F6. Similar to F2, F7 had relatively higher apparent viscosity which could be attributed to the presence of CSG in these formulations. It was observed that F7 had the potential to exhibit the same positive attributes as F2 based on the rheological properties.

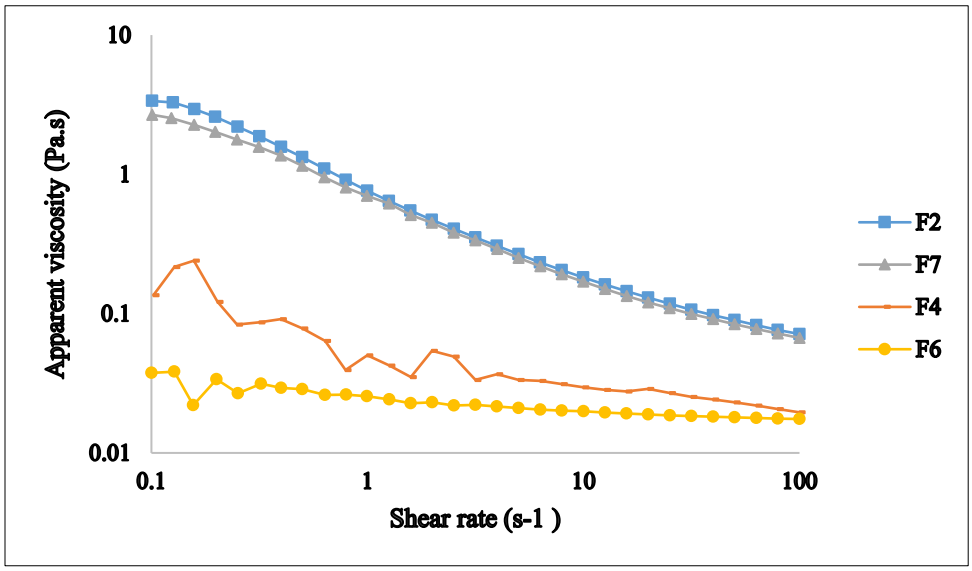


Figure 5.9 Apparent viscosity of ice cream mixes

Note: Ice cream formulations: F2 = 0.2% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F6 = without CSG and MDG; F7 = 0.2% CSG (w/w) with no added MDG. CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates.

Overrun

As shown in Table 5.3, the overrun of F7 (77.86±5.83%) substantially decreased compared to F2 (103.10±0.47%) which was attributed to the absence of MDG. The ice cream mixes which contained MDG had lower surface tension values and tended to produce higher overrun and smaller air bubbles (Goff, Liboff, Jordan, & Kinsella, 1987). The correlation could also be

observed in F4 and F6, where F4 showed higher overrun due to the presence of MDG. As mentioned earlier, the destabilised fat contributes to stabilising the foam. Although F7 exhibited higher DFI and $d_{4,3}$ relative to F4 and F6, which could potentially give higher overrun, the results showed that CSG does not have the ability to enhance the overrun. The high DFI and particle size could be due to aggregation of fat globules caused by CSG instead of the partial coalescence of fats that is necessary for stabilisation of air cells.

The higher overrun obtained for F6 which was devoid of stabiliser and emulsifier compared with F7 may be due to the air cells stabilised by the milk proteins present instead of the partially coalesced fat particles or the lower viscosity of this formulation which allowed the incorporation of air into the mix. Our results for overrun were not consistent with previous studies which could be due to the differences in the extraction of CSG, ice cream processing, equipment, and CSG concentrations. In a previous study, the overrun was higher for the formulations with higher level of CSG (92% and 96% for 1% and 2%, (w/w) respectively) when the gum was used to substitute both stabiliser and emulsifier (Campos, Ruivo, Scapim, Madrona, & Bergamasco, 2016). Maestrello et al. (2017), replaced the emulsifier in a prebiotic ice cream with CSG and found that ice creams with 1.2% and 1.8% CSG had higher overrun (55% and 45% respectively), compared to 0.6% (25%). In another study, the overrun increased from 48.38% to 49.82% when CSG increased from 0.1 to 0.4% (Chavan, Gadhe, Sharma, & Hingade, 2017).

Table 5.3 The overrun, meltdown rate and pH of ice cream formulations

Formulation	Overrun (%)	Meltdown rate ($g.min^{-1}$)	pH
F2	103.10 ± 0.47^{a}	0.96 ± 0.14^{b}	6.55 ± 0.06^{a}
F7	77.86 ± 5.83^{a}	1.61 ± 0.10^{a}	6.55 ± 0.01^{a}
F4	99.33 ± 9.36^{a}	1.15 ± 0.08^{b}	6.57 ± 0.02^{a}
F6	95.93 ± 14.50^{a}	$1.25\pm0.10^{a,b}$	6.52 ± 0.00^{a}

Note: Ice cream formulations: F2 = 0.2% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F6 = without CSG and MDG; F7 = 0.2% CSG (w/w) with no added MDG. CSG = chia seed gum; MDG = mono-/diglyceride. ±= standard error of the mean value. Data were based on two replicates. Means with identical superscripts in a column are not significantly different at $P<0.05$.

Meltdown rate

In this phase, there was an inverse relationship between overrun and meltdown rate. The absence of MDG and lower overrun in F7, led to a significant ($p < 0.05$) increase in meltdown rate from 0.96±0.14 in F2 to 1.61± 0.10 g/min. Sample F7 started melting after 20 min whereas F4 and F6 began to melt after 10 min. However, the two latter formulations showed slower meltdown rates during the 70 min duration (Figure 5.10). Although in comparison with F7, F6 had a lower viscosity and DFI, the higher meltdown rate in F7 could be attributed to an increased rate of heat transfer due to its low overrun. The lower rate of meltdown in F4 was attributed to the presence of MDG which also caused a higher overrun. The results indicate that an emulsifier is required to only improve overrun and meltdown resistance in ice cream.

A previous study showed that the ice creams which contained CSG (as stabiliser and emulsifier), had higher meltdown resistance compared to ice creams which had a blend of commercial stabiliser and emulsifier (Campos, Ruivo, Scapim, Madrona, & Bergamasco, 2016). They also reported that the ice cream which contained 2% CSG (w/w) only started melting after 45 min with lower amounts of melted ice cream at the end of the experiment compared to the ice cream containing 1% CSG (w/w). It was then attributed to the higher overrun obtained for the former formulation. Similarly, Chavan, Gadhe, Sharma, and Hingade (2017), reported lower meltdown resistance for the ice creams with lower concentrations of CSG (1.49 to 1.72 ml/min for ice creams with 0.4% and 0.1% CSG, respectively). On the contrary, Maestrello et al. (2017), found that the higher concentrations of CSG contributed negatively to the melting resistance of the ice cream. In this previous study, the ice creams with higher CSG showed lower meltdown rates during the first 30 min and completely melted after 50 min whereas after that time, the ice cream with lower concentration of CSG showed higher resistance and completely melted after 60 min.

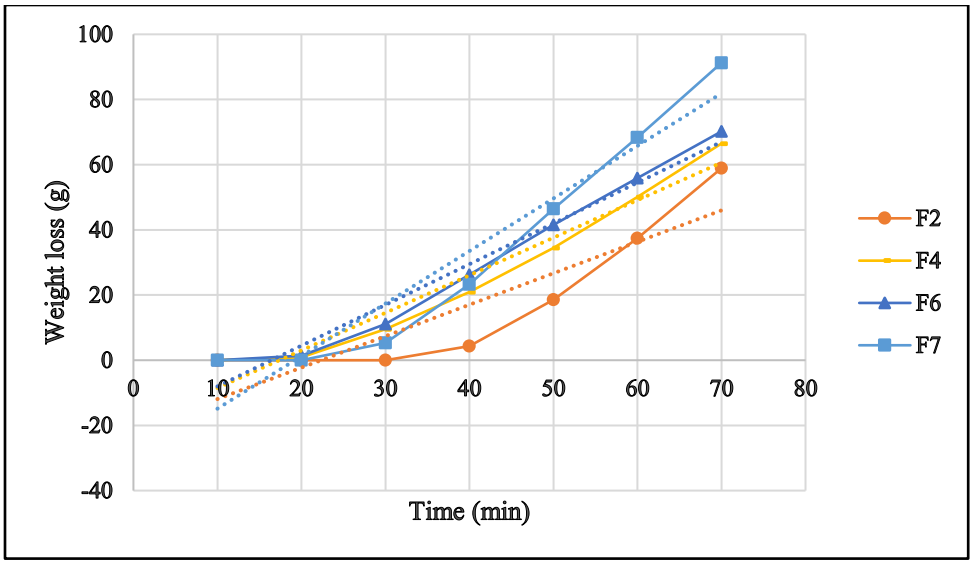


Figure 5.10 Weight loss of ice cream samples during meltdown

Note: Ice cream formulations: F2 = 0.2% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F6 = without CSG and MDG; F7 = 0.2% CSG (w/w) with no added MDG. CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates. Non-continuous lines represent the slope of each meltdown curve.

Destabilised fat index and fat globule size distribution

The presence of MDG can lead to a higher percentage of destabilised fat and large fat aggregates as the freezing progresses (Segall & Goff, 2002). Although the increased fat network is reported to contribute to lowering the meltdown rate, slightly higher DFI and $d_{4,3}$ in F7 did not lead to slower meltdown compared to F4 and F6 with lower DFI and $d_{4,3}$ (Table 5.4).

Table 5.4 Fat globule size distribution, destabilised fat index of ice cream mix and ice cream

Formulation	Fat globule size ($d_{4,3}$) mix (µm)	Fat globule size ($d_{4,3}$) ice cream (µm)	Destabilised fat index (%)
F2	1.00 ± 0.14^{a}	40.66 ± 8.87^{a}	41.00 ± 5.35^{a}
F6	0.57 ± 0.05^{a}	0.91 ± 0.16^{a}	6.37 ± 3.73^{b}
F7	0.65 ± 0.01^{a}	28.15 ± 36.10^{a}	8.43 ± 1.55^{b}
F4	0.73 ± 0.18^{a}	1.03 ± 0.18^{a}	8.06 ± 4.15^{b}

Note: Ice cream formulations: F2 = 0.2% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F6 = without CSG and MDG; F7 = 0.2% CSG (w/w) with no added MDG. CSG = chia seed gum; MDG = mono-/diglyceride; $d_{4,3}$= volume-average mean diameters of the mix and melt. ±= standard error of the mean value. Data were based on two replicates. Means with identical superscripts in a column are not significantly different at $P<0.05$.

Emulsifiers contribute to enhancing the destabilised fat and thereby stabilising the air cells and trapping the aqueous phase of the ice cream which prevents drainage upon meltdown (Goff, 1997). The lower overrun and higher meltdown rate could be due to the absence of emulsifiers in F7 as compared to F2. The arrangement of various components in ice cream and their contribution to the microstructure and increased levels of DFI and $d_{4,3}$ need to be further investigated using scanning electron microscopy. Figure 5.11 shows the average particle size distribution of ice cream mixes.

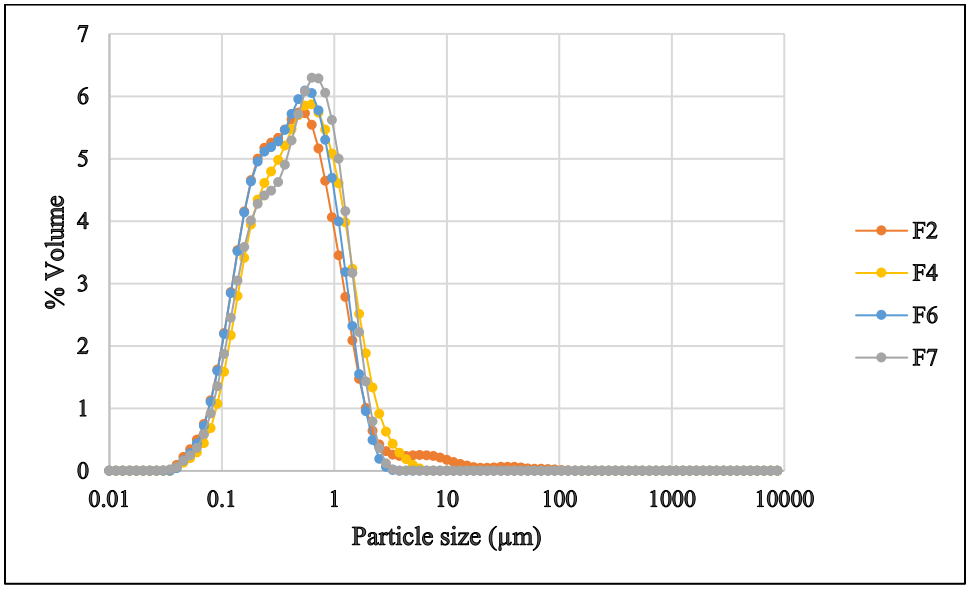


Figure 5.11 Average particle size distribution of ice cream mix

Note: Ice cream formulations: F2 = 0.2% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F6 = without CSG and MDG; F7 = 0.2% CSG (w/w) with no added MDG. CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates.

Similar to the work on incorporating BSG in ice cream (BahramParvar & Goff, 2013), the higher $d_{4,3}$ in F7 could be attributed to the formation of large aggregates by CSG. The polyelectrolyte behavior (negatively charge) of the CSG can cause protein-polysaccharide interactions which may contribute to the formation of aggregates, hence, a higher $d_{4,3}$ value. The lower extent of fat destabilisation in F6 (without emulsifier) was likely due to the high stability of proteins at the fat globules interface, preventing close association (by steric and intermolecular repulsion) and partial coalescence of droplets (Segall & Goff, 2002). Figure 5.12 shows higher distribution of smaller

fat globules for this formulation compared to F2 and F7. F4 showed similar size distribution profiles to F6 although this formulation contained MDG, but when the guar gum was present (as in F5), the distribution profile skewed towards higher values (Figure 5.7).

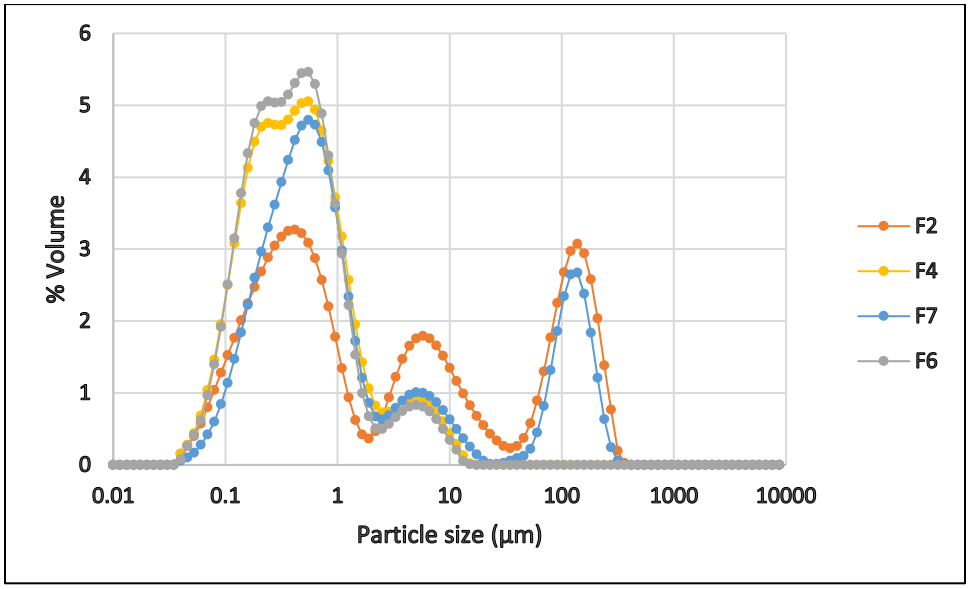


Figure 5.12 Average particle size distribution of ice cream melt

Note: Ice cream formulations: F2 = 0.2% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F6 = without CSG and MDG; F7 = 0.2% CSG (w/w) with no added MDG. CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates.

Texture analysis

Our results showed an inverse relationship between the overrun and hardness for all formulations. F7 with the lowest overrun had the highest value for hardness (93.87±15.5 N) (Figure 5.13). The presence of CSG in F7 compared to F6 and F4, resulted in an increased hardness in this ice cream which could be due to the higher viscosity of F7 (Muse & Hartell, 2004). The presence and absence of emulsifier did not result in a marked difference in hardness of F4 and F6 but we were not able to explain the substantial difference between the hardness of F2 and F7. Although the higher hardness in F4 and F6 relative to F2 could be due to the distribution of large ice crystals reflected by the perceived coarseness, F7 (which was the hardest) was not associated with icy or coarse texture.

Our results did not show a distinct correlation between the DFI and hardness. The results obtained for hardness in our study does not agree with those reported previously which could be due to differences in the formulations and total solids. In a previous study, the hardness of ice cream which contained commercial emulsifier and stabiliser (control sample) was not different ($p > 0.05$) from ice creams substituted with CSG (Campos, Ruivo, Scapim, Madrona, & Bergamasco, 2016). In another study, the reported hardness for the ice creams containing 1.8% CSG was lower than the ice cream which had 1.2% CSG (Maestrello et al., 2017).

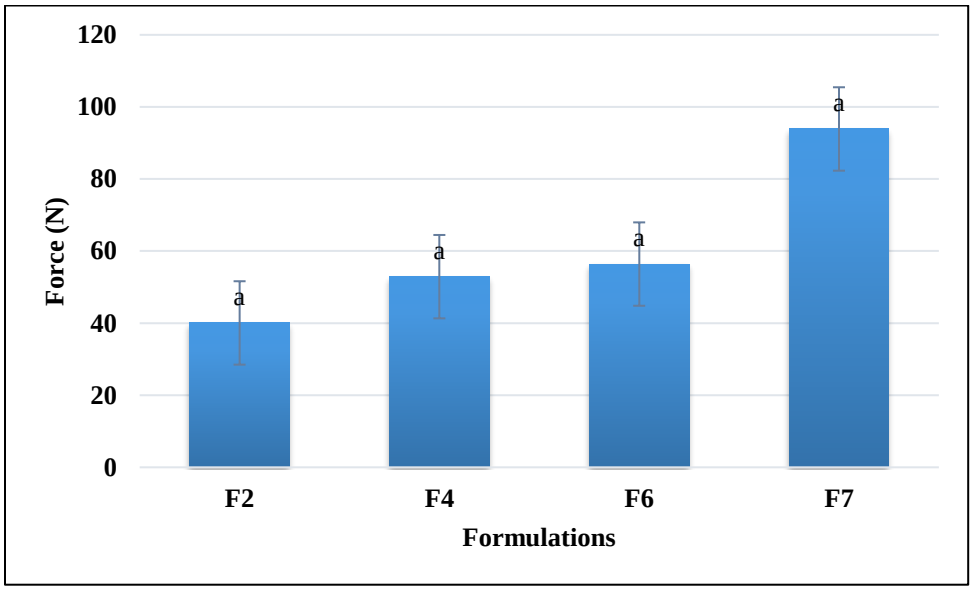


Figure 5.13 Hardness of ice cream samples

Note: Ice cream formulations: F2 = 0.2% CSG (w/w) +0.15% MDG (w/w); F4 = 0.15% MDG (w/w) with no added stabiliser; F6 = without CSG and MDG; F7 = 0.2% CSG (w/w) with no added MDG. CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates. Means with identical letters are not significantly different at $P<0.05$.

Note: results were recorded in gravitational force (g) and were converted to force (N) by (Hardness (N) = (hardness (g)/1000) × 9.81)).

Sensory analysis

The sensory properties of the ice cream samples were evaluated by an informal focus group. The majority of the panelists described that F7 had a slightly harder texture when compared to F2. Some panel members stated that the ice crystals could be slightly perceived in F7 whereas others found the ice cream as smooth as F2. F2 had a better mouthfeel although the overall difference

between the two samples were not pronounced and F7 turned out to be relatively acceptable. Despite having higher overrun and meltdown resistance, F6 and F4 were perceived as being coarse and having short-lasting mouthfeel with weaker body and texture. In a previous study, the addition of 1% CSG (w/w) to ice cream formulation as stabiliser and emulsifier, produced ice cream, with high acceptance in terms of flavour attributes and overall sensory profile (Campos, Ruivo, Scapim, Madrona, & Bergamasco, 2016). In this previous report, the textural properties of the formulation containing 2% CSG (w/w) was acceptable but the taste and colour attributes had the lowest scores because the resultant ice cream had a dark yellow colour which was due to the method used to extract the mucilage. Similar to our findings, Maestrello et al. (2017), reported that the changes in overrun and firmness due to the addition of CS mucilage did not cause negative impact on sensory properties of the product.

5.2.4 Summary

The ice cream formulated with 0.2% CSG (w/w) (F7) in the absence of MDG (commercial emulsifier) had lower overrun and meltdown resistance than the ice cream which contained the commercial emulsifier (F2). F7 had acceptable organoleptic properties compared to F2, F4 and F6. The absence of MDG did not change the viscosity of the ice cream mix but contributed to increasing the hardness of the resultant ice cream. Also the smaller fat aggregates of the ice cream in F7 relative to F2 could be due to the absence of MDG and its partial-destabilising properties under shear. Therefore, it was concluded that there is need to consider addition of a clean label emulsifier to improve the ice cream overrun and melting attributes.

5.3 Phase III Addition of sweet cream buttermilk as emulsifier to ice cream

5.3.1 Introduction

Buttermilk is a by-product released during the churning of cream in the manufacture of butter. Compared to skim milk, buttermilk has higher content of phospholipids (specially lecithin) which are the native emulsifiers in milk (Jinjarak, Olabi, Jimenez-Flores, Sodini, & Walker, 2006). Previous studies have used buttermilk as a source of serum solids in ice cream mix with favorable results. SCB imparts strong emulsifying properties to the ice cream due to the presence of lecithin-protein complex in relatively large amounts similar to egg yolk (Williams, Potter, & Hufnagel, 1950; Schoenfuss & Chandan, 2011). Sweet buttermilk improved whipping ability, overrun and melting resistance and contributed to the production of a creamier ice cream which had drier appearance and smooth body and texture. It decreased the coarseness, sandiness and contributed to retarding the growth of large ice crystals during hardening (Rajor, Patel, Patel, & Gupta, 1901). SCB can be used to replace up to 50% of the SMP (Rothwell, 1974; Hamilton, 1990). Another study reported that the partial replacement of SMP (25%) with SCB could produce ice cream with acceptable organoleptic properties compared to the control ice cream (Shibu, Kumar, Narasımhan, & Pugazhenthi, 2000).

Thus, in order to offset the defects made upon the removal of commercial emulsifiers, SCB was used to confer emulsification attributes, improve the overrun and meltdown resistance. To achieve this goal, two ice cream mixes were formulated in which SCB solids supplied 25% and 50% of the NMS (w/w).

5.3.2 Materials and methods

The materials and methods used in this phase (phase III) are similar to those described in phase II with some minor differences. In phase III, SCB was used to partially substitute SMP and constituted 25% (F8) and 50% (F9) of the NMS (w/w) in the absence of MDG (Table 3.1 and Figure 5.1).

5.3.3 Results and discussion

Rheological measurements

As shown in Figure 5.14, the addition of SCB did not change the viscosity of the ice cream mix over a range of shear rates (0.1 to 100 s^{-1}). Our results were consistent with Yanasugondha (1951), who found that buttermilk powder did not show gelling ability as indicated by the viscosity of the mixes which contained this ingredient as substitute for SMP. Similar results were obtained in another report where replacing the SMP with buttermilk caused a slight but insignificant increase in the mix viscosity (Shibu, Naresh Kumar, Narasimhan & Pugazhenthi, 2000).

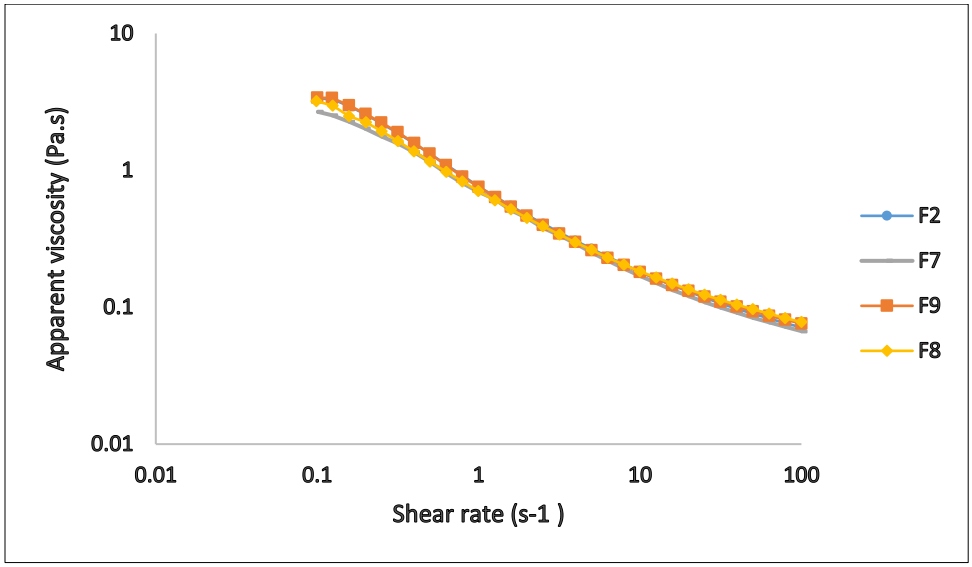


Figure 5.14 Apparent viscosity of ice cream mixes

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w) with no added MDG; F8 = SCB (25% NMS of mix (w/w)) + 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates.

Overrun

In our study, substitution of SMP with SCB increased the overrun (Table 5.5). When SCB solids, provided 25% of NMS (w/w), the overrun increased from 77.86±5.83% (F7) to 82.71±1.61% (F8)

whereas substitution with 50% SCB (w/w) increased the overrun to 88.90±0.10% (F9). When buttermilk substituted the skim milk serum solids, distinct improvement in the whipping ability and emulsifying properties of ice cream mixes were reported which was attributed to the lecithin content of buttermilk (Whitaker, 1930; Walts & Dahle, 1933; Thomas & Combs, 1944; Williams, Potter, & Hufnagel, 1950; Yanasugondha, 1951; Webb & Whittier, 1972; Mahran et al., 1976; Shibu, Naresh Kumar, Narasimhan & Pugazhenthi, 2000). Thomas and Combs (1944), stated that the use of buttermilk powder resulted in an ice cream which was drier in appearance and stiffer in consistency when freshly drawn from the freezer.

Table 5.5 The overrun, meltdown rate and pH of ice cream formulations

Formulation	Overrun (%)	Meltdown rate ($g.min^{-1}$)	pH
F2	103.10 ± 0.47^{a}	0.96 ± 0.14^{b}	6.55 ± 0.06^{a}
F7	77.86 ± 5.83^{b}	1.61 ± 0.10^{a}	6.55 ± 0.01^{a}
F8	82.71 ± 1.61^{b}	1.60 ± 0.08^{a}	6.50 ± 0.01^{a}
F9	88.90 ± 0.10^{b}	1.57 ± 0.05^{a}	6.48 ± 0.01^{a}

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w) with no added MDG; F8 = SCB (25% NMS of mix (w/w)) + 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. ±= standard error of the mean value. Data were based on two replicates. Means with identical superscripts in a column are not significantly different at $P<0.05$.

Meltdown rate

Ice creams which contained SCB (F8 and F9) were slightly more resistant to meltdown relative to F7 but the rates of meltdown in F8 and F9 were still high compared to F2 (Figure 5.15). This could be attributed to the presence of the MDG in F2 which resulted in a higher overrun. Similarly, higher overrun in F9 could contribute to the slightly higher meltdown resistance of this formulation relative to F8 and F7. The results are in agreement with other studies where they reported nearly similar melting resistance for ice creams formulated with and without buttermilk solids (Yanasugondha, 1951; Mahran et al., 1976; Shibu, Naresh Kumar, Narasimhan & Pugazhenthi, 2000). Thomas and Combs (1944), reported that the ice cream containing SCB was characterised by a foamy meltdown with the foam being finer in structure and more stable than that of containing SMP.

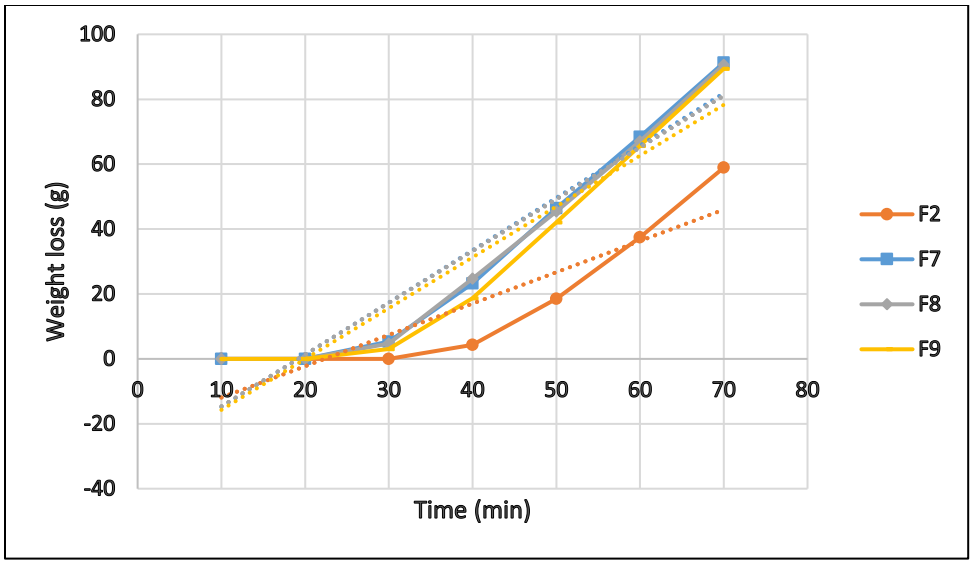


Figure 5.15 Weight loss of ice cream samples during meltdown

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w) with no added MDG; F8 = SCB (25% NMS of mix (w/w)) + 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates. Non-continuous lines represent the slope of each meltdown curve.

Destabilised fat index and fat globule size distribution

Although substitution with SCB improved the overrun and meltdown resistance of ice cream to some extent, the results for DFI and $d_{4,3}$ were lower than those of obtained for F7 (Table 5.6).

Table 5.6 Fat globule size distribution, destabilised fat index of ice cream mix and ice cream

Formulation	Fat globule size ($d_{4,3}$) mix (µm)	Fat globule size ($d_{4,3}$) ice cream (µm)	Destabilised fat index (%)
F2	1.00 ± 0.14^{a}	40.66 ± 8.87^{a}	41.00 ± 5.35^{a}
F7	0.65 ± 0.00^{b}	28.15 ± 36.10^{a}	8.43 ± 1.55^{b}
F8	$0.74\pm0.07^{a,b}$	6.74 ± 0.30^{a}	8.03 ± 1.31^{b}
F9	0.58 ± 0.04^{b}	22.53 ± 2.12^{a}	4.43 ± 3.84^{b}

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w) with no added MDG; F8 = SCB (25% NMS of mix (w/w)) + 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride; $d_{4,3}$= volume-average mean diameters of the mix and melt. ±= standard error of the mean value. Data were based on two replicates. Means with identical superscripts in a column are not significantly different at $P<0.05$.

Compared to F7, it seems that buttermilk did not contribute to increasing the fat destabilisation and partial coalescence (Figure 5.17), showing emulsifying properties. Microstructure studies are required to investigate the contribution of buttermilk solids in the structure of ice cream. There were no published data regarding the effect of buttermilk on DFI and FGSD. Figure 5.16 and Figure 5.17 show the average particle size distribution of the ice cream mixes and ice cream melts, respectively.

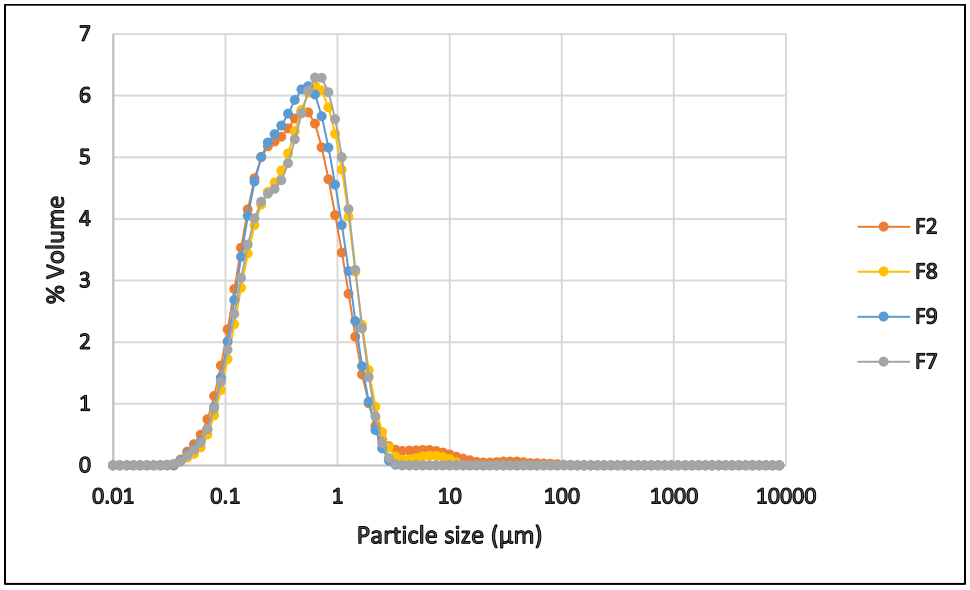


Figure 5.16 Average particle size distribution of ice cream mix

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w) with no added MDG; F8 = SCB (25% NMS of mix (w/w)) + 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates.

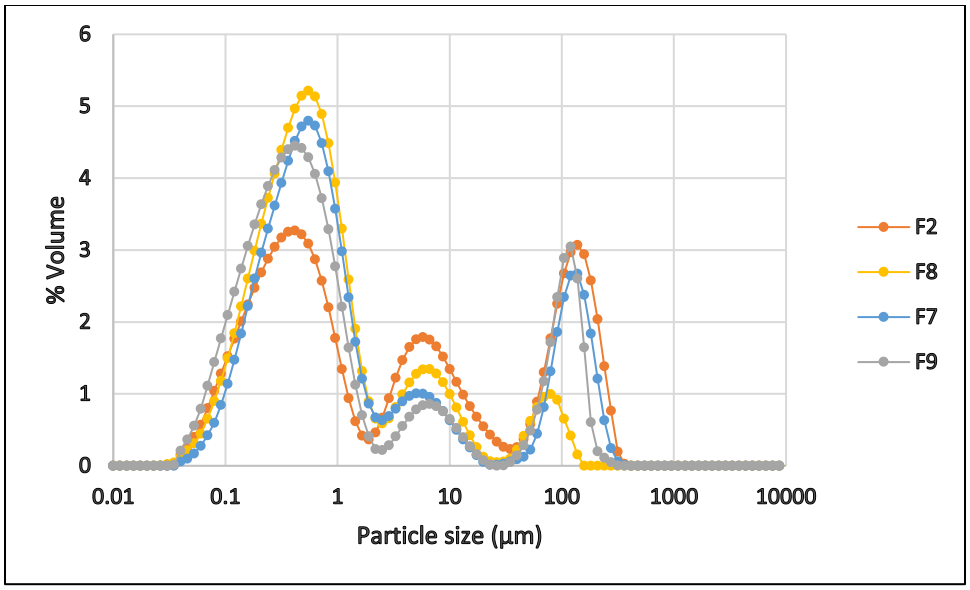


Figure 5.17 Average particle size distribution of ice cream melt

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w) with no added MDG; F8 = SCB (25% NMS of mix (w/w)) + 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates.

Texture analysis

Data analysis showed that there is no significant difference ($p > 0.05$) between the samples in terms of the hardness. However, among the ice cream samples, F8 and F9 had the highest values for hardness (Figure 5.18) even though they had higher overrun than F7 which is reported to have an inverse relationship with hardness. The extent of destabilised fat did not show an obvious impact on hardness. There is no previous report regarding the effect of buttermilk on hardness of ice cream. In a previous study by Yanasugondha (1951), the ice creams containing buttermilk received higher sensory scores for body and texture compared to those containing SMP.

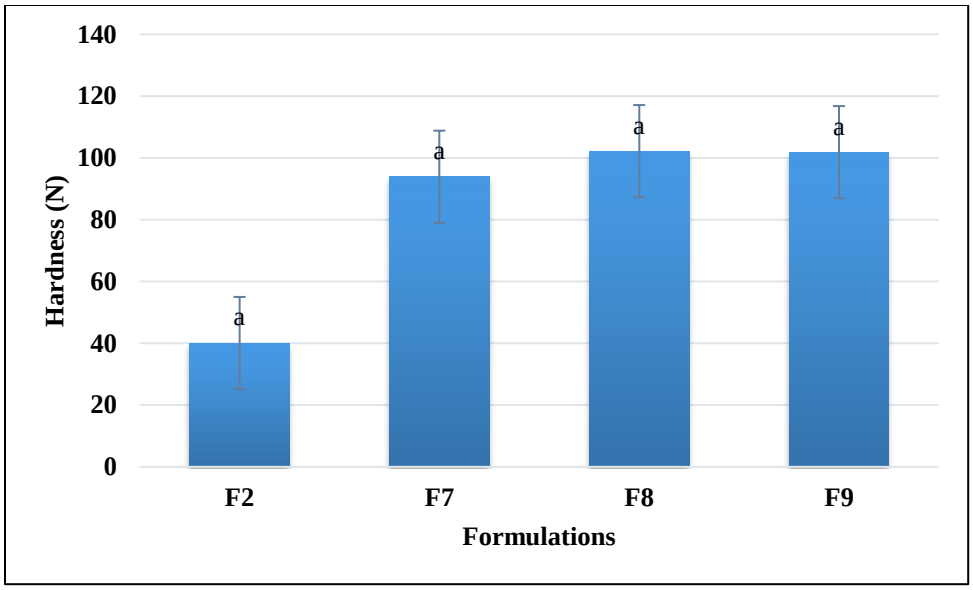


Figure 5.18 Hardness of ice cream samples

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w) with no added MDG; F8 = SCB (25% NMS of mix (w/w)) + 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates. Means with identical letters are not significantly different at $P<0.05$.

Note: results were recorded in gravitational force (g) and were converted to force (N) by (Hardness (N) = (hardness (g)/1000) × 9.81)).

Sensory analysis

Regardless of the experimental data, sensory properties associated with F9 were acceptable by all the panelists in an informal focus group. F8 had a weaker body whereas the body of F9 was perceived to linger longer in the mouth. F8 was also perceived to be colder than F9 with ice crystals which could be slightly perceived. F9 was reported to have desirable hardness, smoothness, mouthfeel and melting quality. The results were in agreement with previous studies where SCB positively affected the sensory properties of ice cream. According to Rajor, Patel, Patel, and Gupta (1901), the addition of sweet buttermilk to soft-serve ice cream mix improved the melting resistance and decreased the coarseness and sandiness of the ice cream. Previous studies reported that the emulsifying properties of buttermilk contributed to retarding the growth of large ice crystals during hardening and developing an ice cream with smooth body and texture (Webb & Whittier, 1972; Mahran et al., 1976). In another study, the ice cream which contained buttermilk had a richer and creamier flavour compared to the ice cream which did not contain this ingredient

(Williams, Potter, & Hufnagel, 1950).) A consumer preference test conducted by Combs (1927) showed that the ice cream which contained SCB was comparable or slightly superior than the ice cream which contained SMP as the source of NMS.

5.3.4 Summary

The addition of SCB at both levels (25% and 50% (w/w)) improved the overrun and meltdown resistance of ice cream compared with F7. SCB did not change the viscosity of the ice cream mix. Generally, the addition of SCB did not increase the FGSD and DFI compared with F7 but the ice cream which had higher level of SCB, had higher FGSD however F8 with lower level of SCB had a higher DFI. Relative to F7, the hardness increased in F8 and F9. F9 was perceived to have favorable sensory attributes over F7, which did not contain SCB, and also F8 with lower amounts of this ingredient. Moreover, the sample F9 could not be distinguished from F2 which contained the commercial emulsifier (MDG). Therefore, the formulation F9 was chosen to be fortified with a probiotic culture in the next phase.

5.4 Phase IV Addition of CSG, SCB and probiotic culture to ice cream

5.4.1 Introduction

As mentioned earlier the functional food market is dominated by products containing probiotic cultures which are perceived to exert health-promoting effects when ingested in adequate amounts (Bourianne, 2018). Ice cream is considered a suitable carrier for the delivery of probiotic bacteria due to the required low temperatures during processing and storage. The consumer acceptance is mainly governed by the organoleptic properties of the food therefore, the functional foods must diverge only minimally from regular food formulations (Soukoulis, Fisk, & Bohn, 2014). This implies that ice cream with a low pH is not acceptable to consumers because ice cream is not an acidic food and the global popularity of the product is mainly due to its sweet flavour beside the smooth texture, refreshing palatability and delightfully coldness (Marshall, Goff, & Hartel, 2003; Cruz, Antunes, Sousa, Faria, & Saad, 2009).

According to previous studies the addition of 10% (v/v) of grown probiotic culture to the ice cream mix before churning resulted in acceptable sensory properties as well as microbial cell counts (Salem, Fathi, & Awad, 2005; Akin, Akin, & Kirmaci, 2007). Therefore, the same method was applied in this Phase.

5.4.2 Materials and methods

Activation and identification of the probiotic culture

The freeze-dried probiotic strain, *Lactobacillus acidophilus* La-5, was activated in MRS broth by incubating at 37°C for 24 h. The grown culture was then streaked on MRS agar and incubated at 37°C for 24 h in CO_2 incubator. In order to obtain a pure culture, another streaking was performed using a single colony from the agar plate and incubated in the same conditions. The cellular morphology of the microorganism was observed by performing Gram staining technique. The short chain Gram positive rods with rounded ends confirmed the cellular characteristics of *Lactobacillus acidophilus* (Kulp & Rettger, 1924).

Preparation of Probiotic culture for use in ice cream processing

The growth medium for the stock culture was prepared by reconstituting 10% (w/v) skim milk powder (SMP) in distilled water and heating in an autoclave at 100°C for 10 min (Goh, Nair, & Matia-Merino, 2008). To prepare the stock culture, the medium was inoculated with the freeze-dried probiotic culture, *Lactobacillus acidophilus* La-5 (1%), and incubated at 37°C for 24 h. The stock culture was used as the inoculum to carry out the second fermentation to ensure the adaptation of the bacterial cultures to the growth conditions. Consequently, to prepare the probiotic culture for use in ice cream, the reconstituted skim milk was inoculated with the stock culture (1% (w/w)) and incubated at 37 °C for 18 h.

Production of probiotic ice cream

Preparation of ice cream

As shown in Figure 5.1, the cultured milk was added to the ice cream mixes (10% (w/w)) prior to freezing. After incorporating the cultured milk, the ice cream mixes consisted of 10% fat (w/w) (derived from 37.3% fat cream and 5.8% fat SCB), 11% NMS (w/w) (derived from cream, SMP and SCB), 12% sugar (w/w), 0.3% vanilla (w/w), 0.2% CSG (w/w) and the balance was with water. The amount of SMP used for preparation of the cultured milk was taken into account when calculating the mix formulation in this phase (Appendix A). The ice cream produced in this phase was denoted as F10. The pH of the fermented milk and the ice cream mix after incorporating the cultured milk was recorded.

Enumeration of viable cells

For testing the viability of probiotics, the cultured milk, ice cream mix and the frozen product samples were serially diluted and pour plated on MRS agar according to Dos Santos Cruxen et al. (2017). The first dilution was prepared by homogenising 10 g of sample in 90 g sterile 0.1 % (w/v) peptone water. One mL of three consecutive dilutions was pour plated and the plates were incubated at 37°C in a CO_2 incubator for 72 h. The grown colonies on each plate were then counted and used to calculate the colony forming units per mL of each sample.

Consumer Sensory Evaluation

The acceptance of ice cream samples (F10 and a commercial vanilla ice cream) were evaluated using a 9-point hedonic scale in a consumer acceptance test (Peryam & Pilgrim, 1957). The sensory attributes of the ice creams such as colour, appearance, texture, meltdown in the mouth, smoothness, sweetness, flavour and overall acceptance were analysed by consumer sensory panelists who attended the sensory evaluation session. The sensory evaluation was carried out in sensory booths in building 26, Massey University, Albany campus. Both samples were scooped in 60-mL coded cups and the consumers rinsed their palates with water between tasting the samples. The collected data were analysed using the univariate of the general linear model (ANOVA) ($p \leq 0.05$).

Stability of the ice cream during storage

Sensory properties of the ice creams were evaluated by consumer sensory acceptance tests after storage for 30 days in freezer (-20°C). Moreover, the viability of the probiotic cells was analysed and pH was measured.

5.4.3 Results and discussion

Rheological measurements

The addition of cultured milk slightly increased the mix viscosity (Figure 5.19) which was consistent with previous reports (Salem, Fathi, & Awad, 2005; Abghari, Sheikh-Zeinoddin, & Soleimanian-Zad, 2011). The slight increase in viscosity could be related to protein precipitation due to the higher acidity and lower pH compared to formulations without the probiotic culture. Turgut and Cakmakci (2009), reported that the probiotic culture affected the viscosity of ice cream mixes and the ice cream mixes which contained *B. bifidum* showed higher viscosity compared to *L. acidophilus* containing mixes. Parameters such as inoculum size, fermentation conditions, pH endpoint and use of ropy (EPS-producing) strains have been reported to have substantial effect on the viscosity of the ice cream mix (Soukoulis, Fisk, & Bohn, 2014).

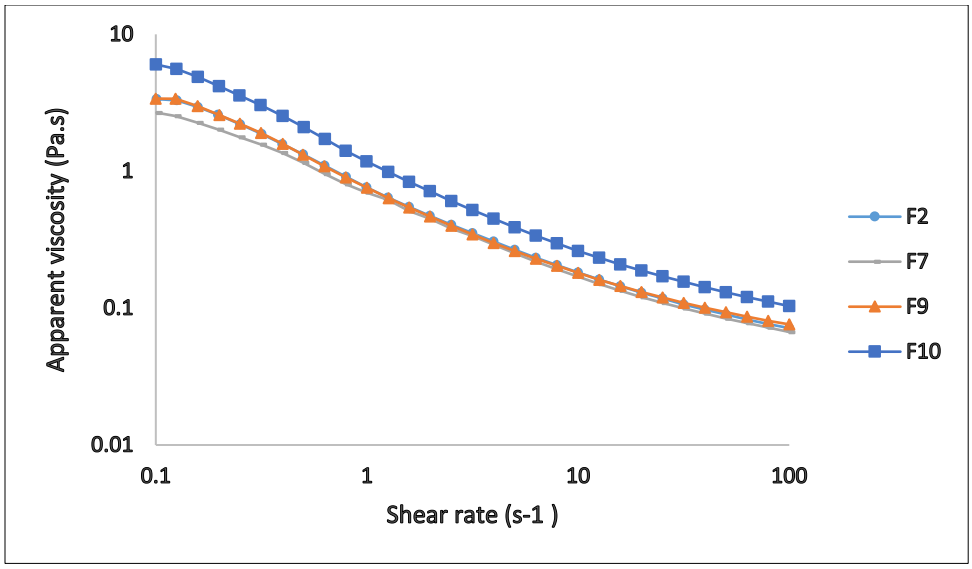


Figure 5.19 Apparent viscosity of ice cream mixes

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w); F10 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w) + 10% probiotic (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates.

Overrun

The overrun slightly decreased by the addition of the probiotic cultured milk (Table 5.7). Similar result reported by Salem, Fathi, and Awad (2005) were attributed to the higher acidity which could affect the proteins and freezing point. Nousia, Androulakis and Fletouris, (2011), found that the addition of probiotic cells in two forms of harvested and freeze-dried, did not affect the pH and overrun of the resultant ice cream.

Table 5.7 The overrun, meltdown rate and pH of ice cream formulations

Formulation	Overrun (%)	Meltdown rate ($g.min^{-1}$)	pH (ice cream mix)
F2	103.10 ± 0.47^{a}	0.96 ± 0.14^{b}	6.55 ± 0.06^{a}
F7	77.86 ± 5.83^{b}	1.61 ± 0.10^{a}	6.55 ± 0.01^{a}
F9	88.90 ± 0.10^{b}	1.57 ± 0.05^{a}	6.48 ± 0.01^{a}
F10	88.49 ± 0.54^{b}	$1.24\pm0.04^{a,b}$	6.27 ± 0.01^{b}

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w); F10 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w) + 10% probiotic (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. CSG = chia seed gum;

MDG = mono-/diglyceride. ±= standard error of the mean value. Data were based on two replicates. Means with identical superscripts in a column are not significantly different at P<0.05.

Meltdown rate

Our results showed that the ice cream which contained the probiotic cultured milk had a slower meltdown rate than F9 which did not contain the probiotic culture (Figure 5.20). In contrast, a previous study reported that the ice cream with added probiotic culture showed faster meltdown rate which was attributed to the alterations in the freezing points and viscosity of the mix (Salem, Fathi, & Awad, 2005). Further, in this previous study, the ice creams which contained *L. rhamnosus* had the fastest meltdown rate whereas *L. acidophilus* La-5 containing ice creams had the slowest rate of meltdown among all ice cream samples which contained various probiotic cultures.

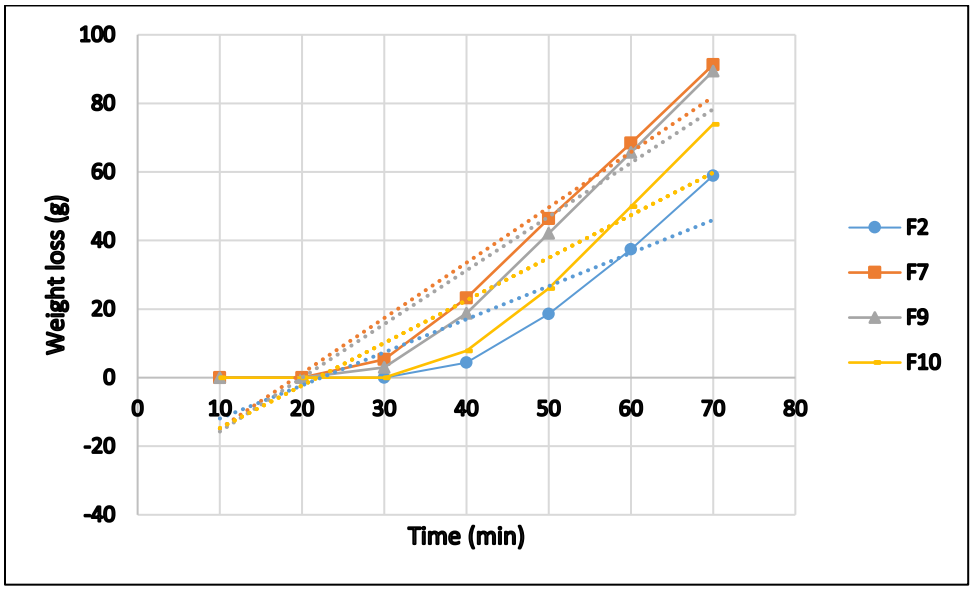


Figure 5.20 Weight loss of ice cream samples during meltdown

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w); F10 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w) + 10% probiotic (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates. Non-continuous lines represent the slope of each meltdown curve.

Destabilised fat index and fat globule size distribution

The higher $d_{4,3}$ obtained for the ice cream mix in F10 compared to F9 (Table 5.8) could be related to the protein precipitation due to the increased acidity resulting from the addition of the probiotic cultured milk.

Table 5.8 Fat globule size distribution, destabilised fat index of ice cream mix and ice cream

Formulation	Fat globule size ($d_{4,3}$) mix (µm)	Fat globule size ($d_{4,3}$) ice cream (µm)	Destabilised fat index (%)
F2	1.00 ± 0.14^{b}	40.66 ± 8.87^{a}	41.00 ± 5.35^{a}
F7	0.65 ± 0.00^{b}	28.15 ± 36.10^{a}	8.43 ± 1.55^{b}
F9	0.58 ± 0.04^{b}	22.53 ± 2.12^{a}	4.43 ± 3.84^{b}
F10	12.90 ± 0.90^{a}	13.83 ± 2.02^{a}	11.96 ± 1.44^{b}

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w); F10 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w) +10% probiotic (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. CSG = chia seed gum; MDG = mono-/diglyceride. $d_{4,3}$ = volume-average mean diameters of the mix and melt ±= standard error of the mean value. Data were based on two replicates. Means with identical superscripts in a column are not significantly different at $P<0.05$.

According to Valdivia-López and Tecante (2015), chia seed mucilage is sensitive to external mono and divalent cations. In acidic conditions, the polysaccharide-protein interactions form larger aggregates (Figure 5.24). Despite the higher $d_{4,3}$ obtained for the mix in this formulation compared to F9, $d_{4,3}$ of the ice cream melt was lower. This could be due to the shearing forces of the dasher together with the ice crystals formed during the churning which may have disrupted the protein aggregates. In order to get a better explanation, confocal laser scanning microscopy (CLSM) could be used which can help visualise the state of fat globules in the melt without disturbing the sample (Cardona, Iriart, & Herrera, 2013). The amount of partial coalescence in F10 was not conclusive due to the discrepancies between the results obtained from FGSD and DFI (Table 5.8).

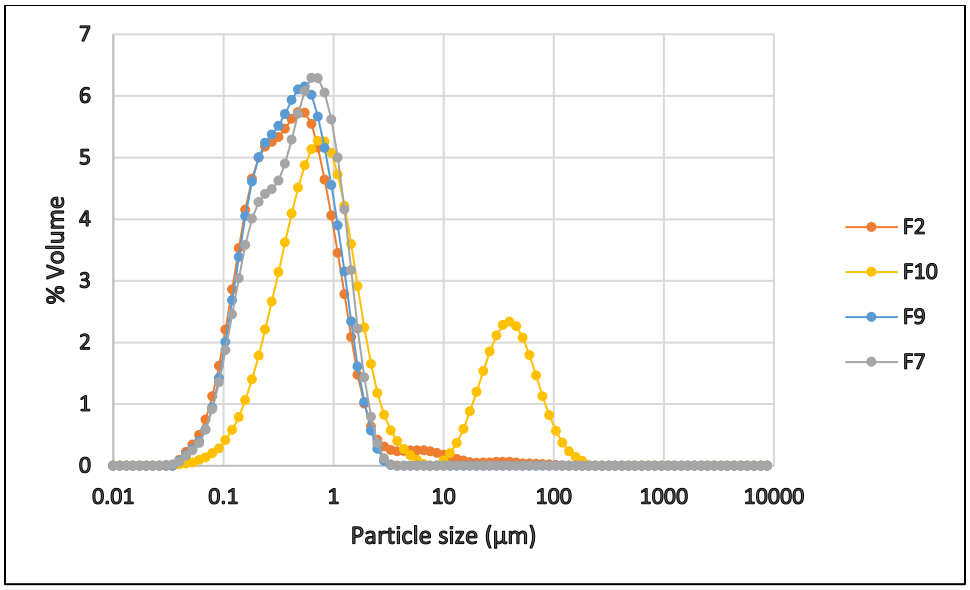


Figure 5.21 Average particle size distribution of ice cream mix

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w); F10 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w) +10% probiotic (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates.

Figure 5.21 and Figure 5.22 showed the average particle size distribution of ice cream mix and melts, respectively. Despite the ice cream mix in F10 which had the largest aggregates, $d_{4,3}$ was the lowest in the melt sample of this formulation compared to other ice cream formulations examined in this Phase.

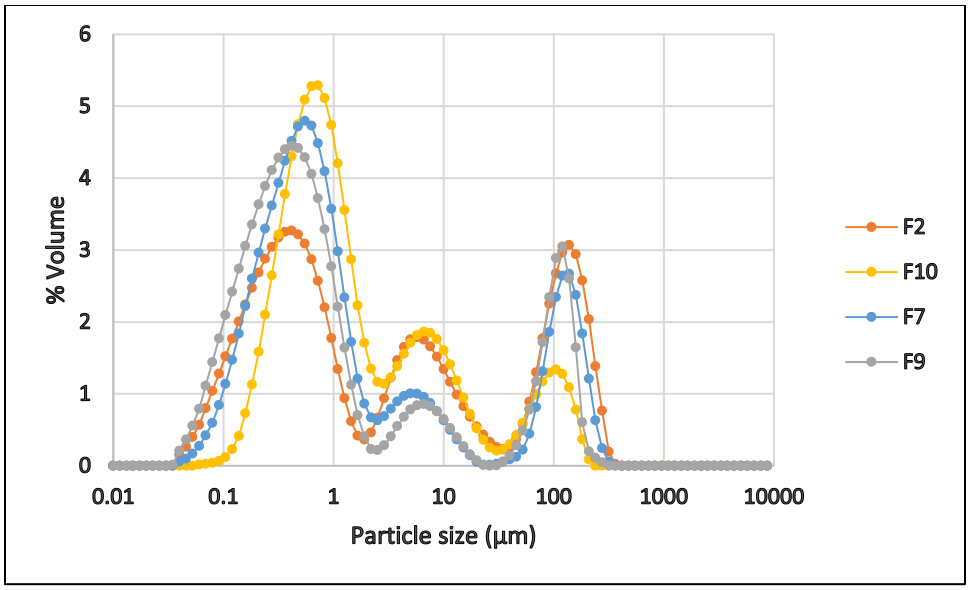


Figure 5.22 Average particle size distribution of ice cream melt

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w); F10 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w) +10% probiotic (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates.

Texture analysis

The ice cream formulation which contained the probiotic culture (F10) had a lower hardness compared to F9 (Figure 5.23). Although F10 was expected to have higher hardness due to slightly higher viscosity, lower overrun and higher DFI compared to F9, the depression in freezing point due to the incorporation of the probiotic cultured milk could contribute to its lower hardness as reported by Salem, Fathi, and Awad (2005).

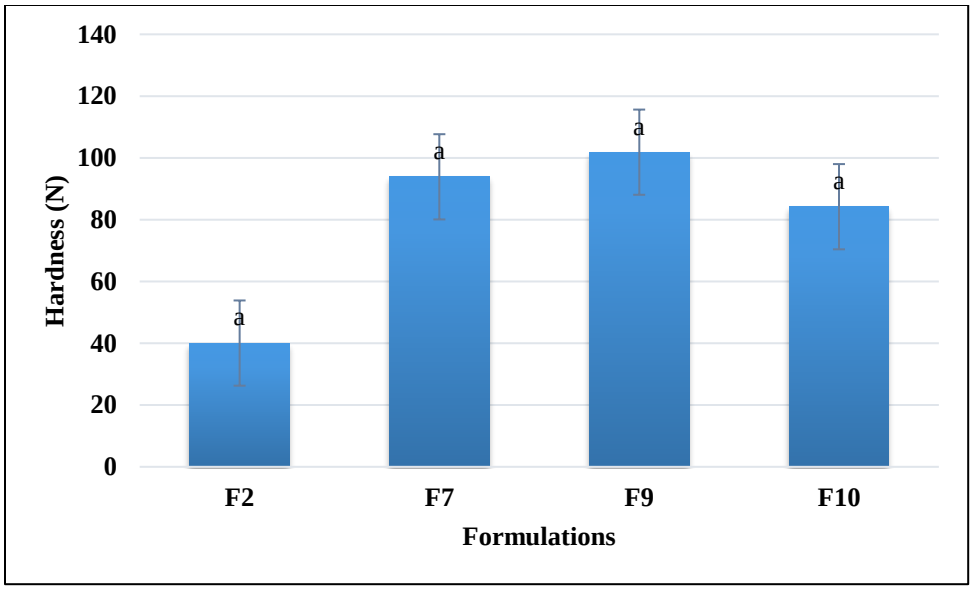


Figure 5.23 Hardness of ice cream samples

Note: Ice cream formulations: F2= 0.2% CSG (w/w) +0.15% MDG (w/w); F7 = 0.2% CSG (w/w); F9 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w); F10 = SCB (50% NMS of mix (w/w)) + 0.2% CSG (w/w) +10% probiotic (w/w). CSG = chia seed gum; MDG = mono-/diglyceride. Data were based on two replicates. Means with identical letters are not significantly different at $P<0.05$.

Note: results were recorded in gravitational force (g) and were converted to force (N) by (Hardness (N) = (hardness (g)/1000) × 9.81)).

Figure 5.24 shows a schematic diagram of the structure of the ice cream in F10. Chia seed is an anionic heteropolysaccharide (xyloglucan) with a high molecular weight (800-2000 kDa) (Timilsena, Adhikari, Kasapis, & Adhikari, 2015). After the incorporation of the probiotic cultured milk, in acidic pH, the interaction of the positively charged casein micelles with the anionic polysaccharide may have contributed to the appearance of large aggregates in ice cream mix. The surface active properties of the gum and its ability to adsorb at the oil and air-water interfaces have been documented (Timilsena, Adhikari, Kasapis, & Adhikari, 2016). The increase in the distribution of larger fat globules could also be likely due to possible bridging of the fat droplets by the polysaccharide (Goh, Nair, & Matia-Merino, 2008). In a study by BahramParvar and Goff (2013), microstructure of the ice cream containing BSG (as stabiliser) showed that the increase in concentration of the gum could have contributed to the penetration of more fat globules at the air interface and producing smaller air cells possibly due to the emulsifying properties of the gum.

The flocculation patterns of CSG-oil emulsions with different ratios were studied by Timilsena, Adhikari, Kasapis, and Adhikari (2016). Measurement of droplet size in the emulsions in this previous study showed that increasing the concentration of the gum resulted in smaller oil droplets covered with the gum which have less tendency to coalesce. Lower concentration of CSG (0.25%) resulted in flocculation whereas this phenomenon was not observed in emulsions with higher ratios of the gum (>0.25%). In order to understand the microstructure of the ice cream illustrated in Figure 5.24, further investigation is required using scanning electron microscopy.

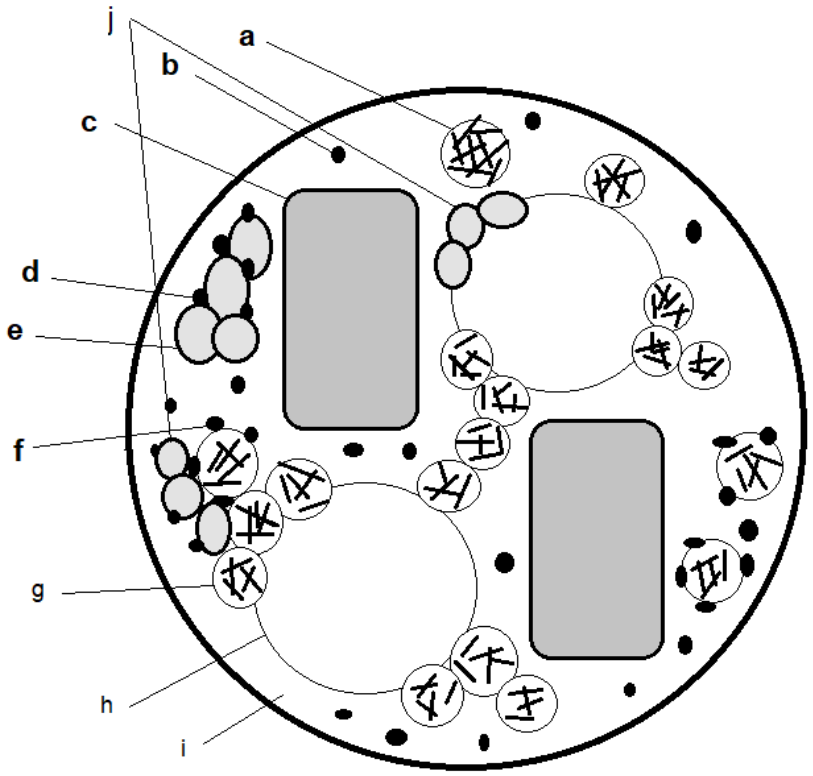


Figure 5.24 A schematic diagram of structure of ice cream, showing the principal structural elements of fat globules and clusters, air bubbles and ice crystals, all embedded within the freeze-concentrated unfrozen phase (Drawn using Microsoft Paint based on Goff, (2011))

Note: a) Fat globules, partially crystalline; b) Casein micelles; c) Ice crystals; d) Mixed membrane of caseins micelles around CSG (in lower pH); e) CSG aggregates; f) Mixed membrane around fat globules of caseins micelles, non-micellar casein, whey protein, and phospholipids from SCB; g) Partially coalesced fat globule network; h) Air bubbles, surrounded by protein membrane and adsorbed fat globules; i) Freeze-concentrated unfrozen phase of sugars, salts, and unadsorbed proteins; j) CSG at the fat globule/air cells interfaces.

Enumeration of viable probiotic bacteria

In our study the initial concentration of *L. acidophilus* La-5 in the cultured milk was 5.17×10^8 CFU/mL. However, Magarinos, Selaive, Costa, Flores and Pizarro (2007), obtained 1.32×10^9 CFU/mL for the same microorganism. The differences could be attributed to the strain, incubation time and temperature and also the growth medium. In the latter report, the number of viable cells decreased from 1.7×10^7 CFU/mL in the mix to 4×10^6 CFU/mL in the ice cream even though in our study we obtained the cell counts of 3.5×10^7 CFU/mL (7.54±0.04 log CFU/mL) and 1.15×10^7 (7.05±0.11 log CFU/mL), respectively. In a report by Salem, Fathi, and Awad (2005), the number of viable cells in the frozen ice cream was 7.48 log CFU/g. Our results were consistent with other studies where freezing of the ice cream mix in the freezer followed by hardening caused a reduction of less than one log viable probiotic cells (Hekmat & MacMahan, 1992; Hagen & Narvhus, 1999; Salem, Fathi, & Awad, 2005).

A higher survival rate for *L. acidophilus* was obtained when the harvested cells were added to the ice cream mix before aging (Abghari, Sheikh-Zeinoddin, & Soleimanian-Zad, 2011). In this case, the number of viable cells after aging was 8.54 CFU/g whereas immediately after freezing (day 0), the number of cells declined to 8.26 CFU/g. The higher survival rate was assumed to be due to the cryotolerance resulting from the exposure of the bacteria to aging temperature (4°C) for 24 hours.

The low temperature has a marked effect on the survival of probiotic bacteria with *Lactobacillus* showing a higher resistance than *Bifidobacterium* (Homayouni, Azizi, Ehsani, Yarmand, & Razavi, 2008). The freezing process resulted in a significant decrease in viable *L. acidophilus* from 7.89 CFU/g to 7.03 CFU/g when the freeze-dried cells were added to the ice cream mix before aging (Nousia, Androulakis & Fletouris, 2011). According to Magarinos et al. (2007), activated cells have the ability to dehydrate themselves and reduce the intracellular ice crystals which may break the cell's cytoplasm membrane during freezing. Therefore, this previous study reported that the addition of activated harvested cells resulted in slight changes in viable cell counts during freezing declining from 7.63 CFU/g to 7.60 CFU/g.

The formation and growth of ice crystals in ice cream mix and increase in the concentration of electrolyte due to the freeze-concentration could negatively affect the viability of the probiotic

microorganisms (Foley & Sheuring, 1966). However, the pH of ice cream mix could also contribute to the survival of probiotic bacteria (Christiansen et al., 1996). Lower pH values (between 5.0 and 5.5) reduced the number of Lactic acid bacteria (LAB) in yogurt ice cream to nearly 13% from its original concentration (Inoue, Shiota, & Ito, 1998). Similarly, the freezing and hardening of the ice cream mix which was fermented with *L. acidophilus* and *B. bifidum* to the pH of 4.9 caused a reduction of less than one log cycle from the initial total colony count (5×10^8 CFU/mL) (Hekmat & McMahan, 1992). Moreover, cryoprotective compounds such as casein, lactose and sucrose can provide good protection against freezing damage whereas milkfat and air bubbles operate as insulator and reduce the rate of heat transfer (Moss & Speck, 1963; Godward & Kailasapathy, 2003).

The decline in bacterial numbers could be due to the physical disintegration of the cells during freezing with agitation and also the incorporation of oxygen into the mix during the freezing-whipping step and ice cream mix processing (Hagen & Narvhus, 1999; Magarinos, Selaive, Costa, Flores & Pizarro, 2007). Depending on the species, probiotic bacteria are regarded as strictly anaerobic (*Bifidobacterium*) or microaerophilic (most *Lactobacillus*), and therefore susceptible to high partial oxygen pressure (Homayouni, Azizi, Ehsani, Yarmand, & Razavi, 2008). The survival could also be affected by osmotic pressure and freeze-concentration of the serum phase associated with the ingredients that elevate the glass transition temperature such as polysaccharides and high-molecular-weight sugars (Pehkonen et al., 2008). *Lactobacillus* bacteria resist better than *Bifidobacterium* in different sucrose concentrations (Homayouni, Azizi, Ehsani, Yarmand, & Razavi, 2008). Several studies have demonstrated that *L. acidoplhilus* is capable of surviving in ice cream (Alamprese, Foschino, Rossi, Pompei, & Savani, 2002; Andriaghetto & Gomes, 2003) and even remaining above the recommended minimum of 10^6 CFU/mL during 12 weeks (Salem, Fathi, & Awad, 2005) and 52 weeks of frozen storage (Hagen & Narvhus, 1999).

Consumer Sensory Evaluation

The average scores for the sensory attributes of the two ice cream samples (F10 and commercial) obtained from the 9-point hedonic scaling acceptance test are shown in Table 5.9. The mean scores showed that the prototype ice cream was comparable to the commercial product and the addition

of CSG, SCB and the probiotic cultured milk had no significant ($p>0.05$) effect on sensory properties of the ice cream such as appearance, colour, sweetness and flavour. However, the two ice cream samples were different in terms of texture, meltdown in the mouth and smoothness ($p<0.05$), the overall acceptability of the two samples were not different ($p>0.05$). In addition, no probiotic flavour was perceived which could be partly due to the high pH of the ice cream (F10). Similarly, Salem, Fathi, and Awad (2005), reported that among ice creams with different probiotic strains, the formulation which contained *L. acidophilus* had the lowest acidity, good body and texture with no perceived cultured milk taste. Turgut and Cakmacki (2009) added the fermented milk to ice cream mix at 10% of the total milk and found that the cultured milk taste described as mildly sour was particularly perceived by consumers.

In previous studies, the probiotic ice creams formulated with cultured milk had lower overall acceptance scores than the control ice creams (Turgut & Cakmacki, 2009; Salem, Fathi, & Awad, 2005). According to Nousia, Androulakis and Fletouris (2011), the sensory acceptance of ice cream manufactured with addition of freeze-dried culture was not markedly different from the control ice cream however the addition of harvested *L. acidophilus* cells resulted in ice cream with significantly higher scores. In contrast, in another study, the probiotic ice cream produced with harvested cells could be significantly distinguished from the control ice cream in terms of sensory properties (Abghari, Sheikh-Zeinoddin, & Soleimanian-Zad, 2011).

Table 5.9 Mean consumer sensory scores for commercial retail ice cream and F10

Ice cream sample	Colour	Appearance	Texture	Meltdown in the mouth	Smoothness	Sweetness	Flavour	Overall acceptance
Commercial	7.33±1.40	7.54±1.25	7.54±1.59	7.38±1.64	7.79±1.10	7.00±1.44	6.92±1.74	7.52±1.32
F10	7.25±1.33	7.25±1.19	6.50±1.77	6.43±1.88	6.54±1.47	7.00±1.35	6.54±1.50	7.10±0.97

Note: data are means ± standard deviations; n = 24 panelists. A 9-point hedonic scale was used.

Stability of the ice cream during frozen storage

The average pH of the cultured milk and the ice cream mix after addition of the cultured milk were 4.6±0.01 and 6.27±0.01, respectively. The results were in agreement with Salem, Fathi, and Awad (2005) who obtained the pH range of 4.30-5.66 and 6.26-6.42 for the cultured milk and the ice cream mix after incorporation of the cultured milk (10% v/v) prior to freezing. In another report, the pH of the probiotic ice cream after the addition of the cultured milk (4%) was 6.51 (Magarinos, Selaive, Costa, Flores & Pizarro, 2007). Generally, the pH of probiotic ice cream which varies from 4.5 to 6.3 for fully fermented and non-fermented formulations, respectively (Saukoulis & Tzia, 2008) can affect the sensory attributes of the ice cream (Favaro-Trindade et al., 2007).

The acceptance test on the ice creams after four weeks of storage (-20°C) showed that the mean consumer sensory scores were not significantly different ($p > 0.05$) compared to the fresh ice cream (Table 5.10). Also the sensory results showed that F10 was not significantly different ($p > 0.05$) from the commercial retail ice cream and after four weeks of storage without any deterioration, it was comparable with the commercial counterpart. Therefore, the storage did not significantly ($p > 0.05$) affect the sensory properties of the ice cream samples. After four weeks of storage, the viable cell counts were above the recommended probiotic counts (1.07×10^7 CFU/mL).

Table 5.10 Mean consumer sensory scores for commercial retail ice cream and F10 after storage (-20°C) for four weeks

Ice cream sample	Colour	Appearance	Texture	Meltdown in the mouth	Smoothness	Sweetness	Flavour	Overall acceptance
Commercial	7.57±1.03	7.61±1.03	7.64±1.25	7.71±0.90	7.68±1.12	7.46±0.84	7.50±1.23	7.54±1.14
F10	7.43±1.17	7.29±1.21	7.32±1.16	7.07±1.33	7.11±1.37	7.00±1.51	7.00±1.44	7.27±1.14

Note: data were means ± standard deviations; n = 28 panelists. A 9-point hedonic scale was used.

5.4.4 Summary

Incorporation of the probiotic culture into the ice cream mix in the form of cultured milk (10% (w/w)) resulted in an ice cream with comparable sensory acceptance to the commercial retail ice cream. The sensory results did not show a significant difference ($p>0.05$) between the overall acceptability of the commercial retail ice cream and F10 (Table 5.9). After four weeks of storage the sensory properties of the ice cream and the viable probiotic cell counts were not negatively affected. The viable cell counts were above the recommended minimum limit of 10^6 CFU/mL for conferring probiotic properties. The pH of the ice cream mix decreased with the incorporation of the probiotic cultured milk from 6.48± 0.01 in F9 to 6.27±0.01 in F10 but it remained stable after four weeks of frozen storage. It is likely that the high pH had positive effects on the sensory attributes of the ice cream and also the survival of the probiotic microorganisms. The incorporation of the probiotic culture enhanced the viscosity and decreased the overrun which could be related to the precipitation of proteins due to the enhanced acidity. Also, the meltdown rate and hardness in F10 decreased compared to F9. The acceptable viability of probiotic microorganism and desirable sensory properties qualify the ice cream for being considered a potential clean label probiotic frozen dessert.

Chapter 6 General discussion

Over the last decade, the sector of functional frozen dairy dessert has experienced a substantial growth, primarily due to the increasing consumer awareness and the desire for functional foods with natural ingredients (Soukoulis, Fisk, & Bohn, 2014). Due to the global popularity of ice cream as well as the low processing and storage temperatures, the frozen dessert is considered a suitable carrier for delivery of bioactive compounds with beneficial functional properties (Turgut & Cakmakci, 2009; Soukoulis, Fisk, & Bohn, 2014).

Stabilisers and emulsifiers are the commonly used additives in ice cream manufacture due to their desirable functional properties. These two additives which are presently used in ice cream are designated with E-numbers, which is unpopular among consumers as they do not provide sufficient information for making informed decisions at purchasing time. Stabilisers are added to ice cream formulations to increase the viscosity and smoothness, improve the body, reduce the ice and lactose crystal growth during storage, maintain shape during melting and enhance air entrapment (Cottrell, Pass, & Phillips, 1980; Goff et al, 1995; BahramParvar & Mazaheri Tehrani, 2011). Another group of additives used in ice cream is emulsifiers which partially destabilise the fat emulsion during the freezing of an ice cream mix. Emulsifiers contribute to desired smooth texture, slightly greasy mouthfeel, dryness upon extrusion, good shape retention and slow meltdown (Berger & White, 1971; Goff & Jordan, 1989; Baer, Wolkow, & Kasperson, 1997). The stabilisation of air cells by partially coalesced fat globules at the interface improves whipping ability, overrun capacity and reduces whipping time (Goff & Jordan, 1989; Baer, Wolkow, & Kasperson, 1997). Therefore, to replace the commercial stabilisers and emulsifiers in ice cream, suitable ingredients with effective functional properties are required to improve the colloidal and structural integrity of the frozen dessert.

In our study CSG and SCB were used to replace stabilisers and emulsifiers respectively. The chia seeds were minimally processed using a modified water-extraction method to produce mucilage (gum) as an ingredient for the production of ice cream. The yield of the CSG from the extraction procedure used in this study was 3.40±0.16% (w/w) which was comparable to a study by Capitani et al. (2015) who used similar conditions.

All the ice cream mixes containing CSG (0.1%, 0.2% and 0.3% (w/w)) exhibited non-Newtonian pseudoplastic flow behaviour of a typical hydrocolloid. The viscosity of the ice cream mix, destabilised fat index and fat globule size distribution ($d_{4,3}$) increased with increasing the concentration of CSG, whereas the overrun and meltdown rate decreased. The sensory evaluation and physico-chemical results showed that CSG desirably substituted the commercial stabiliser at 0.2% (w/w).

At 0.2% CSG (w/w), the absence of MDG (commercial emulsifier) in the formulation affected the sensory and physico-chemical properties of the resultant ice cream. The destabilised fat index, fat globule size distribution, overrun and meltdown resistance of the ice cream decreased while the hardness increased in the absence of MDG. The addition of SCB at two different concentrations (25% and 50% of NMS (w/w)) (Phase III), improved the whipping and emulsifying properties of the ice cream in the absence of MDG. The incorporation of SCB increased the overrun and meltdown resistance of the ice cream especially when it constituted 50% of the NMS (w/w), however it did not increase the fat destabilisation. The sensory properties of the ice cream containing SCB (50% of NMS (w/w)) was acceptable by the focus group which was consistent with previous reports where the SCB contributed to smooth texture, richer and creamier flavour (Williams, Potter, & Hufnagel, 1950; Webb & Whittier, 1972). Addition of probiotic cultured milk (10% (w/w)) (Phase IV) resulted in ice cream with high overall acceptance scores for sensory attributes. Although the overrun was slightly reduced which was probably due to the precipitation of proteins at lower pH (6.27±0.01), the addition of the probiotic culture decreased the meltdown rate and hardness of the final product and increased the destabilised fat index. The sensory acceptance test showed that there were no significant differences ($p>0.05$) between the overall acceptability of the prototype ice cream and a commercial retail product even after four weeks of frozen storage. The ice cream contained more than 10^6 CFU/mL viable probiotic cells, and therefore it may be described as probiotic (FAO/WHO, 2002)

Chapter 7 Conclusion

In this study, the CSG was successfully extracted from organic chia seed using water. Ice cream with added CSG (0.2% (w/w)), SCB (6.08% (w/w) (50% of NMS (w/w))) and probiotic culture (10% (w/w)) received high sensory scores and remained stable after four weeks of frozen storage (-20°C). The performance of CSG and SCB as potential stabiliser and emulsifier, respectively were comparable to the guar gum (commercial stabiliser) and MDG (commercial emulsifier) in ice cream. The ice cream containing CSG and SCB may be described as clean label frozen dessert.

Chapter 8 Recommendations

1. Studying the performance of the ingredients (CSG, SCB and probiotic culture) in ice cream formulations containing low sugar, low fat and high protein with the aim of targeting health conscious consumers. The use of different flavours can also provide variety to consumers
2. Investigating the contribution of the ingredient (CSG, SCB and probiotic culture) in the microstructure of the ice cream using cryo-scanning electron microscopy would provide more information about the function of each ingredient in the ice cream formulation.
3. Large scale consumer tests for the acceptability of the frozen desserts containing CSG, SCB and probiotic culture would provide important information for the potential to upscale and commercialise.
4. Freeze-thaw (heat-shock) cycles are recommended to increase our knowledge about the stability of the product during transportation and temperature fluctuation.
5. Studying the effect of the rheological characteristics of the ice cream mix on the stability of the probiotics in simulated digestion trials.

Chapter 9 References

Abghari, A., Sheikh-Zeinoddin, M., & Soleimanian-Zad, S. (2011). Non-fermented ice cream as a carrier for *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. *International Journal of Food Science and Technology*, 46(1), 84-92.

Adapa, S., Dingeldein, H., Schmidt, K. A., & Herald, T. J. (2000). Rheological properties of ice cream mixes and frozen ice creams containing fat and fat replacers. *Journal of Dairy Science*, 83(10), 2224-2229.

Ahmadi, A., Milani, E., Madadlou, A., Mortazavi, S. A., Mokarram, R. R., & Salarbashi, D. (2014). Synbiotic yogurt ice cream produced via incorporation of microencapsulated *lactobacillus acidophilus* (la-5) and fructooligosaccharide. *Journal of Food Science and Technology*, 51(8), 1568-1574.

Akin, M. B., Akin, M. S., & Kirmaci, Z. (2007). Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice cream. *Food Chemistry*, 104(1), 93-99.

Alamprese, C., Foschino, R., Rossi, M., Pompei, C., & Corti, S. (2005). Effects of *Lactobacillus rhamnosus* GG addition in ice cream. *International Journal of Dairy Technology*, 58(4), 200-206.

Alamprese, C., Foschino, R., Rossi, M., Pompei, C., & Savani, L. (2002). Survival of *Lactobacillus johnsonii* La1 and influence of its addition in retail-manufactured ice cream produced with different sugar and fat concentrations. *International Dairy Journal*, 12(2-3), 201-208.

Alting, A. C., & Van De Velde, F. (2012). Proteins as clean label ingredients in foods and beverages. In *Natural food additives, ingredients and flavourings* (pp. 197-211). Cambridge, UK: Woodhead Publishing Limited.

Altug, T., & Elmaci, Y. (1995). A consumer survey on food additives: food flavors: Generation, analysis and process influence. *Development in Food Science*, 37(1), 1-11.

Alvarez, V. B., Wolters, C. L., Vodovotz, Y., & Ji, T. (2005). Physical properties of ice cream containing milk protein concentrates. *Journal of Dairy Science*, 88(3), 862-871.

Anjaneyalu, Y. V., & Gowda, D. C. (1979). Structural studies of an acidic polysaccharide from *Ocimum basilicum* seeds. *Carbohydrate Research*, 75, 251-256.

Anonymous (2015). *Food additives | Food additive list and their health impact.* (2015). Retrieved from https://www.cbhs.com.au/health-well-being-blog/blog-article/2015/03/12/food-additives-harmful-or-harmless.

Anonymous (2017a). *It's Clear: Transparency is Winning in the U.S. Retail Market. Nielsen Clean Label Report.* August 2017. Retrieved from http:// www.nielsen.com/us/en/insights/reports/2017/transparency-is-winning-the-us-retail-market.html.

Anonymous (2017b). *Transparency and simplicity, The new normal in product development.* Retrieved from https://www.cargill.com/doc/1432106811290/clean-label-white-paper.pdf.

Anonymous (2018a). *2018 DIRA Review: Analysis of industry performance.* Retrieved from https://www.mpi.govt.nz/dmsdocument/31389/send.

Anonymous (2018b). *Consumers' Focus on Health and Wellness Drives Clean-Labeled Product Demand.* Retrieved from https://www.globalfoodforums.com/consumers-increased-focus-on-health-and-wellness-drives-demand-for-clean-labeled-products/

Anonymous (2018c). *The supplier view - 2018 trends: industry remains confident on clean label outlook.* Retrieved from *http://www.foodingredientsfirst.com/news/the-supplier-view-2018-trends-has-clean-label-hit-its-peak.html.*

Anonymous (2019a). *Laser Diffraction (LD).* Retrieved from https://www.malvernpanalytical.com/en/products/technology/light-scattering/laser-diffraction.

Anonymous (2017c). *Consumer principles on the use of food additives and enzymes*. Research report. Kantar public food standards research agency.

Anonymous. (2014). *The clean label guide to Europe*. Retrieved from https://emea.ingredion.com/Campaign/Clean-Label-Guide.html.

Anonymous. (2016). *Ingredients and additives market – global annual review – 2016*. Retrieved from https://store.mintel.com/ingredients-and-additives-market-global-review-2016?_ga=2.176405349.1193762970.1561671321-1468549917.1522979757.

Anonymous. (2019b). *Convert gram-force to newton*. Retrieved from https://www.convertunits.com/from/grams/to/newtons#:~:targetText=How%20many%20grams%20in%201,is%20equal%20to%200.00980665%20newton.

Arbuckle, W. S. (2013). *Ice cream*. New York, USA: Springer.

Arbuckle, W. S., & Frandsen, J. H. (1966). Ice cream. *West Port, Ireland:* AVI Publishing Company.

Aschemann-Witzel, J., Varela, P., & Peschel, A. O. (2019). Consumers' categorization of food ingredients: Do consumers perceive them as 'clean label' producers expect? An exploration with projective mapping. *Food Quality and Preference*, 71, 117-128.

Asioli, D., Aschemann-Witzel, J., Caputo, V., Vecchio, R., Annunziata, A., Næs, T., & Varela, P. (2017). Making sense of the "clean label" trends: A review of consumer food choice behavior and discussion of industry implications. *Food Research International*, 99, 58-71.

Avila-De La Rosa, G., Alvarez-Ramirez, J., Vernon-Carter, E. J., Carrillo-Navas, H., & Pérez-Alonso, C. (2015). Viscoelasticity of chia (*Salvia hispanica L.*) seed mucilage dispersion in the vicinity of an oil-water interface. *Food Hydrocolloids*, 49, 200-207.

Baer, R. J., Wolkow, M. D., & Kasperson, K. M. (1997). Effect of emulsifiers on the body and texture of low fat ice cream. *Journal of Dairy Science*, 80(12), 3123-3132.

BahramParvar, M., & Goff, H. D. (2013). Basil seed gum as a novel stabilizer for structure formation and reduction of ice recrystallization in ice cream. *Dairy Science & Technology*, 93(3), 273-285.

Bahramparvar, M., & Mazaheri Tehrani, M. (2011). Application and functions of stabilizers in ice cream. *Food Reviews International*, 27(4), 389-407.

Bahramparvar, M., Haddad Khodaparast, M. H., & Razavi, S. M. (2009). The effect of Lallemantia royleana (Balangu) seed, palmate-tuber salep and carboxymethylcellulose gums on the physicochemical and sensory properties of typical soft ice cream. *International Journal of Dairy Technology*, 62(4), 571-576.

BahramParvar, M., Razavi, S. M., & Mazaheri Tehrani, M. (2012). Optimising the ice cream formulation using basil seed gum (*Ocimum basilicum L*.) as a novel stabiliser to deliver improved processing quality. *International Journal of Food Science and Technology*, 47(12), 2655-2661.

Bandy, L. (2013). *'Clean Label': An Industry Rather Than a Consumer Term*. Retrieved from http://www.portal.euromonitor.com.ezproxy.massey.ac.nz/portal/Analysis/Tab.

Barford, N. M., Krog, N., Larsen, G., & Buchheim, W. (1991). Effects of emulsifiers on protein-fat interaction in ice cream mix during ageing I: quantitative analyses. *Lipid/Fett*, 93(1), 24-29.

Bearth, A., Cousin, M. E., & Siegrist, M. (2014). The consumer's perception of artificial food additives: Influences on acceptance, risk and benefit perceptions. *Food Quality and Preference*, 38, 14-23.

Berger, K. G., & White, G. W. (1971). An electron microscopical investigation of fat destabilization in ice cream. *International Journal of Food Science and Technology*, 6(3), 285-294.

Berryman, P. (Ed.). (2014). *Advances in Food and Beverage Labelling: Information and Regulations*. Cambridge, UK: Woodhead publishing.

Blanchfield, J. R. (Ed.). (2000). *Food labelling*. Cambridge, England: Woodhead Publishing.

Bochicchio, R., Philips, T. D., Lovelli, S., Labella, R., Galgano, F., Di Marisco, A., Perniola, M., & Amato, M. (2015). Innovative crop productions for healthy food: the case of chia (*Salvia hispanica L*.). In A. Vastolla (Eds.), *The sustainability of agro-food and natural resource systems in the Mediterranean Basin*, (pp. 29-45): SpringerLink.com.

Bolliger, S., Goff, H. D., & Tharp, B. W. (2000). Correlation between colloidal properties of ice cream mix and ice cream. *International Dairy Journal*, 10(4), 303-309.

Bolliger, S., Kornbrust, B., Goff, H. D., Tharp, B. W., & Windhab, E. J. (2000). Influence of emulsifiers on ice cream produced by conventional freezing and low-temperature extrusion processing. *International Dairy Journal*, 10(7), 497-504.

Bolliger, S., Wildmoser, H., Goff, H. D., & Tharp, B. W. (2000). Relationships between ice cream mix viscoelasticity and ice crystal growth in ice cream. *International Dairy Journal*, 10(11), 791-797.

Bourianne, Th. (2018). *Exploring today's top ingredient trends and how they fit into our health-conscious world*. Retrieved from https://www.globalfoodforums.com/store/clean-label-conferences/

Caillet, A., Cogné, C., Andrieu, J., Laurent, P., & Rivoire, A. (2003). Characterization of ice cream structure by direct optical microscopy. Influence of freezing parameters. *LWT-Food Science and Technology*, 36(8), 743-749.

Cakmakci, S., & Dagdemir, E. (2013). A preliminary study on functionality of *Gundelia tournefortii L.* as a new stabiliser in ice cream production. *International Journal of Dairy Technology*, *66*(3), 431-436.

Campos, B. E., Ruivo, T. D., da Silva Scapim, M. R., Madrona, G. S., & Bergamasco, R. D. C. (2016). Optimization of the mucilage extraction process from chia seeds and application in ice cream as a stabilizer and emulsifier. *LWT-Food Science and Technology*, 65, 874-883.

Capitani, M. I., Corzo-Rios, L. J., Chel-Guerrero, L. A., Betancur-Ancona, D. A., Nolasco, S. M., & Tomás, M. C. (2015). Rheological properties of aqueous dispersions of chia (*Salvia hispanica L.*) mucilage. *Journal of Food Engineering*, 149, 70-77.

Capitani, M. I., Nolasco, S. M., & Tomás, M. C. (2013). Effect of mucilage extraction on the functional properties of Chia meals. In I. Muzzalupo (Eds.), *Food Industry*, (pp. 421-437). IntechOpen.

Capitani, M. I., Spotorno, V., Nolasco, S. M., & Tomás, M. C. (2012). Physicochemical and functional characterization of by-products from chia (*Salvia hispanica L.*) seeds of Argentina. *LWT-Food Science and Technology*, 45(1), 94-102.

Cardona, J. A. R., Iriart, C. H., & Herrera, M. L. (2013). Applications of Confocal Laser Scanning Microscopy (CLSM) in Foods. In N. Lagali (Eds.), *Confocal laser microscopy–principles and applications in medicine, biology, and the food sciences*, (pp. 202-234). IntechOpen.

Cerning, J. (1990). Exocellular polysaccharides produced by lactic acid bacteria. *FEMS Microbiology Reviews*, 7(1-2), 113-130.

Chavan, V. R., Gadhe, K. S., Sharma, D., & Hingade, S. T. (2017). Studies on extraction and utilization of chia seed gel in ice cream as a stabilizer. *Journal of Pharmacognosy and Phytochemistry*, 6(5), 1367-1370.

Chaves, M. A., Piati, J., Malacarne, L. T., Gall, R. E., Colla, E., Bittencourt, P. R., de Souza, A. H, Gomes, S. T., & Matsushita, M. (2018). Extraction and application of chia mucilage (*Salvia hispanica L.*) and locust bean gum (*Ceratonia siliqua L.*) in goat milk frozen dessert. *Journal of Food Science and Technology*, 55(10), 4148-4158.

Choo, S. Y., Leong, S. K., & Henna Lu, F. S. (2010). Physicochemical and sensory properties of ice cream formulated with virgin coconut oil. *Food Science and Technology International*, 16(6), 531-541.

Christiansen, P. S., Edelsten, D., Kristiansen, J. R., & Nielsen, E. W. (1996). Some properties of ice cream containing *Bifidobacterium bifidum* and *Lactobacillus acidophilus*. *Milchwissenschaft*, 51(9), 502-504.

Christiansen, P. S., Madeira, A. I. M. R., & Edelsten, D. (1999). The use of ropy milk as stabilizer in the manufacture of ice cream. *Milchwissenschaft*, 54(3), 138-140.

Clarke, C. (2015). *The science of ice cream*. Cambridge, UK: Royal Society of Chemistry.

Cody, T. L., Olabi, A., Pettingell, A. G., Tong, P. S., & Walker, J. H. (2007). Evaluation of rice flour for use in vanilla ice cream. *Journal of Dairy Science*, 90(10), 4575-4585.

Coelho, M. S., & de las Mercedes Salas-Mellado, M. (2015). Effects of substituting chia (*Salvia hispanica L.*) flour or seeds for wheat flour on the quality of the bread. *LWT-Food Science and Technology*, 60(2), 729-736.

Combs, W. B. (1927). Powdered buttermilk in ice cream. *Ice Cream Reviews*, 10(7).

Corredig, M., & Dalgleish, D. G. (1997). Isolates from industrial buttermilk: Emulsifying properties of materials derived from the milk fat globule membrane. *Journal of Agricultural and Food Chemistry*, 45(12), 4595-4600.

Corredig, M., & Dalgleish, D. G. (1998). Buttermilk Properties in Emulsions with Soybean Oil as Affected by Fat Globule Membrane-Derived Proteins. *Journal of Food Science*, 63(3), 476-480.

Costa, M. R., Elias-Argote, X. E., Jiménez-Flores, R., & Gigante, M. L. (2010). Use of ultrafiltration and supercritical fluid extraction to obtain a whey buttermilk powder enriched in milk fat globule membrane phospholipids. *International Dairy Journal*, 20(9), 598-602.

Cottrell, J. I., Pass, G., & Phillips, G. O. (1979). Assessment of polysaccharides as ice cream stabilisers. *Journal of the Science of Food and Agriculture*, 30(11), 1085-1088.

Cottrell, J. I., Pass, G., & Phillips, G. O. (1980). The effect of stabilisers on the viscosity of an ice cream mix. *Journal of the Science of Food and Agriculture*, 31(10), 1066-1070.

Cruz, A. G., Antunes, A. E., Sousa, A. L. O., Faria, J. A., & Saad, S. M. (2009). Ice cream as a probiotic food carrier. *Food Research International*, 42(9), 1233-1239.

Daw, E., & Hartel, R. W. (2015). Fat destabilization and meltdown of ice creams with increased protein content. *International Dairy Journal*, 43, 33-41.

De Falco, B., Amato, M., & Lanzotti, V. (2017). Chia seeds products: an overview. *Phytochemistry Reviews*, 16(4), 745-760.

Dertli, E., Toker, O. S., Durak, M. Z., Yilmaz, M. T., Tatlısu, N. B., Sagdic, O., & Cankurt, H. (2016). Development of a fermented ice cream as influenced by in situ exopolysaccharide production: Rheological, molecular, microstructural and sensory characterization. *Carbohydrate Polymers*, 136, 427-440.

Dervisoglu, M. (2006). Influence of hazelnut flour and skin addition on the physical, chemical and sensory properties of vanilla ice cream. *International Journal of Food Science and Technology*, 41(6), 657-661.

Dervisoglu, M., & Yazici, F. (2006). Note. The effect of citrus fibre on the physical, chemical and sensory properties of ice cream. *Food Science and Technology International*, 12(2), 159-164.

Dewettinck, K., Rombaut, R., Thienpont, N., Le, T. T., Messens, K., & Van Camp, J. (2008). Nutritional and technological aspects of milk fat globule membrane material. *International Dairy Journal*, 18(5), 436-457.

Di Criscio, T., Fratianni, A., Mignogna, R., Cinquanta, L., Coppola, R., Sorrentino, E., & Panfili, G. (2010). Production of functional probiotic, prebiotic, and synbiotic ice creams. *Journal of Dairy Science*, 93(10), 4555-4564.

Dickinson, E., & Wedlock, D. J. (1994). Controlled Particle, Droplet and Bubble Formation, Oxford, England: Butterworth-Heinemann.

Ding, Y., Lin, H. W., Lin, Y. L., Yang, D. J., Yu, Y. S., Chen, J. W., Wang, S. Y., & Chen, Y. C. (2018). Nutritional composition in the chia seed and its processing properties on restructured ham-like products. *Journal of Food and Drug Analysis*, 26(1), 124-134.

Dogan, M., & Kayacier, A. (2007). The effect of ageing at a low temperature on the rheological properties of Kahramanmaras-type ice cream mix. *International Journal of Food Properties*, 10(1), 19-24.

Dogan, M., Kayacier, A., Toker, Ö. S., Yilmaz, M. T., & Karaman, S. (2013). Steady, dynamic, creep, and recovery analysis of ice cream mixes added with different concentrations of xanthan gum. *Food and Bioprocess Technology*, 6(6), 1420-1433.

Duboc, P., & Mollet, B. (2001). Applications of exopolysaccharides in the dairy industry. *International Dairy Journal*, 11(9), 759-768.

Early, R. (Ed.). (1998). *Technology of dairy products*. London, UK: Blackie academic and professional.

Elling, J. L., Duncan, S. E., Keenan, T. W., Eigel, W. N., & Boling, J. (1996). Composition and Microscopy of Reformulated Creams from Reduced-Cholesterol Butteroil. *Journal of Food Science*, 61(1), 48-53.

Emerton, V., & Choi, E. (2008). *Essential guide to food additives*. Cambridge, UK: Royal Society of Chemistry.

Erejuwa, O., Sulaiman, S., & Wahab, M. (2014). Modulation of gut microbiota in the management of metabolic disorders: the prospects and challenges. *International Journal of Molecular Sciences*, 15(3), 4158-4188.

Euromonitor International. (2016). *Clean label revolution: Response of the ingredients industry*. Retrieved from http://www.portal.euromonitor.com.ezproxy.massey.ac.nz/portal/magazine/homemain.

Euromonitor International. (2018). *Ice cream and frozen desserts in New Zealand.* Retrieved from http://www.portal.euromonitor.com.ezproxy.massey.ac.nz/portal/magazine/homemain.

FAO/WHO. (2002). Guidelines for the evaluation of probiotics in food. London, Ontario, Canada, April 30 and May 1. Retrieved from http://www. who. int/foodsafety/publications/fs management/probiotics 2/en/index. html.

Felisberto, M. H. F., Wahanik, A. L., Gomes-Ruffi, C. R., Clerici, M. T. P. S., Chang, Y. K., & Steel, C. J. (2015). Use of chia (*Salvia hispanica L*.) mucilage gel to reduce fat in pound cakes. *LWT-Food Science and Technology*, 63(2), 1049-1055.

Fernandes, S. S., & Mellado, M. D. L. M. S. (2018). Development of mayonnaise with substitution of oil or egg yolk by the addition of chia (*Salvia hispanica L*.) mucilage. *Journal of Food Science*, 83(1), 74-83.

Florencia, F. S. (2013). Rheology of spreadable goat cheese made with autochthonous lactic cultures differing in their ability to produce exopolysaccharides. *Food Science and Technology*, 33(2), 233-238.

Folkenberg, D. M., Dejmek, P., Skriver, A., Guldager, H. S., & Ipsen, R. (2006). Sensory and rheological screening of exopolysaccharide producing strains of bacterial yoghurt cultures. *International Dairy Journal*, 16(2), 111-118.

Freitas, F., Alves, V. D., & Reis, M. A. (2011). Advances in bacterial exopolysaccharides: from production to biotechnological applications. *Trends in Biotechnology*, 29(8), 388-398.

Gelin, J. L., Poyen, L., Courthaudon, J. L., Le Meste, M., & Lorient, D. (1994). Structural changes in oil-in-water emulsions during the manufacture of ice cream. *Food Hydrocolloids*, 8(3-4), 299-308.

Gnanasambandam, R., Torres-Gonzalez, M., Burrington, K.J., & Kapoor, R. (2017). *Technical Report: Milkfat and Related Ingredients Serving Today's Marketplace*. Retrieved from http://www.thinkusadairy.org/resources-and-insights/resources-and-insights/application-and-technical-materials/technical-report-milkfat-and-related-ingredients-serving-todays-marketplace.

Goff, H. D. (1997). Colloidal aspects of ice cream-a review. *International Dairy Journal*, 7(6-7), 363-373.

Goff, H. D. (2008). 65 Years of ice cream science. *International Dairy Journal*, 18(7), 754-758.

Goff, H. D. (2011). Ice cream and frozen desserts: Manufacture. *Encyclopedia of Dairy Sciences*, 899-904.

Goff, H. D. (2016). Ice cream and frozen desserts. *Ullmann's Encyclopedia of Industrial Chemistry*, 1-15.

Goff, H. D. (2016). *Milk Proteins in Ice Cream*. New York, USA: Springer.

Goff, H. D., & Jordan, W. K. (1989). Action of emulsifers in promoting fat destabilization during the manufacture of ice cream. *Journal of Dairy Science*, 72(1), 18-29.

Goff, H. D., Caldwell, K. B., Stanley, D. W., & Maurice, T. J. (1993). The influence of polysaccharides on the glass transition in frozen sucrose solutions and ice cream. *Journal of Dairy Science*, 76(5), 1268-1277.

Goff, H. D., Ferdinando, D., & Schorsch, C. (1999). Fluorescence microscopy to study galactomannan structure in frozen sucrose and milk protein solutions. *Food Hydrocolloids*, 13(4), 353-362.

Goff, H. D., Freslon, B., Sahagian, M. E., Hauber, T. D., Stone, A. P., & Stanley, D. W. (1995). Structural development in ice cream-dynamic rheological measurements. *Journal of Texture Studies*, 26(5), 517-536.

Goff, H. D., Liboff, M., Jordan, W. K., & Kinsella, J. E. (1987). The effects of polysorbate 80 on the fat emulsion in ice cream mix: evidence from transmission electron microscopy studies. *Food Structure*, 6(2), 11.

Goff, H. D., Verespej, E., & Smith, A. K. (1999). A study of fat and air structures in ice cream. *International Dairy Journal*, 9(11), 817-829.

Goh, K. K., Nair, R. S., & Matia-Merino, L. (2008). Exploiting the functionality of lactic acid bacteria in ice cream. *Food Biophysics*, 3(3), 295-304.

Goren, A. C., Kiliç, T., Dirmenci, T., & Bilsel, G. (2006). Chemotaxonomic evaluation of Turkish species of Salvia: Fatty acid compositions of seed oils. *Biochemical Systematics and Ecology*, 34(2), 160-164.

Granato, D., Branco, G. F., Cruz, A. G., Faria, J. D. A. F., & Shah, N. P. (2010). Probiotic dairy products as functional foods. *Comprehensive Reviews in Food Science and Food Safety*, 9(5), 455-470.

Granger, C., Leger, A., Barey, P., Langendorff, V., & Cansell, M. (2005). Influence of formulation on the structural networks in ice cream. *International Dairy Journal*, 15(3), 255-262.

Guinard, J. X., Zoumas-Morse, C., Mori, L., Panyam, D., & Kilara, A. (1996). Effect of sugar and fat on the acceptability of vanilla ice cream. *Journal of Dairy Science*, 79(11), 1922-1927.

Hagiwara, T., & Hartel, R. W. (1996). Effect of sweetener, stabilizer, and storage temperature on ice recrystallization in ice cream. *Journal of Dairy Science*, 79(5), 735-744.

Hamilton, M. P. (1990). Ice cream manufacture. *International Journal of Dairy Technology*, 43(1), 17-20.

Harrigan, W. F. (1998). *Laboratory methods in food microbiology*. London, UK: Academic Press Limited.

Hartel, R. W. (1996). Ice crystallization during the manufacture of ice cream. *Trends in Food Science and Technology*, 7(10), 315-321.

Haynes, I. N., & Playne, M. J. (2002). Survival of probiotic cultures in low-fat ice cream. *Australian Journal of Dairy Technology*, 57(1), 10.

Hekmat, S., & McMahon, D. J. (1992). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food. *Journal of Dairy Science*, 75(6), 1415-1422.

Hickey, C. D., O'Sullivan, M. G., Davis, J., Scholz, D., Kilcawley, K. N., Wilkinson, M. G., & Sheehan, J. J. (2018). The effect of buttermilk or buttermilk powder addition on functionality, textural, sensory and volatile characteristics of Cheddar-style cheese. *Food Research International*, 103, 468-477.

Homayouni, A., Azizi, A., Ehsani, M. R., Yarmand, M. S., & Razavi, S. H. (2008). Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry*, 111(1), 50-55.

Homayouni, A., Azizi, A., Javadi, M., Mahdipour, S., & Ejtahed, H. (2012). Factors influencing probiotic survival in ice cream: a review. *International Journal of Dairy Science*, 7(1), 1-10.

Hosch, A. (2018). The State of the Global Ice Cream Market. Retrieved from https://kerry.com/ph-en/sitecore/content/kerrysite/pages/insights/kerrydigest/2018/the-state-of-the-global-ice-cream-market.

Hosseini-Parvar, S. H., Matia-Merino, L., Goh, K. K. T., Razavi, S. M. A., & Mortazavi, S. A. (2010). Steady shear flow behavior of gum extracted from *Ocimum basilicum L*. seed: Effect of concentration and temperature. *Journal of Food Engineering*, 101(3), 236-243.

Huppertz, T., Smiddy, M. A., Goff, H. D., & Kelly, A. L. (2011). Effects of high pressure treatment of mix on ice cream manufacture. *International Dairy Journal*, 21(9), 718-726.

Huppertz, Th., Smiddy, M., Kelly, A., & Goff, D. (2012). High pressure processing for better ice cream. *Agro-Food Industry Hi-tech*, 23(3), 22-24.

Hyde, K. A., & Rothwell, J. (1973). *Ice cream*. Edinburg, UK: Churchill livingstone.

Innocente, N., Comparin, D., & Corradini, C. (2002). Proteose-peptone whey fraction as emulsifier in ice-cream preparation. *International Dairy Journal*, 12(1), 69-74.

Inoue, K., Shiota, K., & Ito, T. (1998). Preparation and properties of ice cream type frozen yogurt. *International Journal of Dairy Technology*, 51(2), 44-50.

Javidi, F., Razavi, S. M., Behrouzian, F., & Alghooneh, A. (2016). The influence of basil seed gum, guar gum and their blend on the rheological, physical and sensory properties of low fat ice cream. *Food Hydrocolloids*, 52, 625-633.

Jinjarak, S., Olabi, A., Jimenez-Flores, R., Sodini, I., & Walker, J. H. (2006). Sensory evaluation of whey and sweet cream buttermilk. *Journal of Dairy Science*, 89(7), 2441-2450.

Karaca, O. B., Guven, M., Yasar, K., Kaya, S., & Kahyaoglu, T. (2009). The functional, rheological and sensory characteristics of ice creams with various fat replacers. *International Journal of Dairy Technology*, 62(1), 93-99.

Khazaei, N., Esmaiili, M., Djomeh, Z. E., Ghasemlou, M., & Jouki, M. (2014). Characterization of new biodegradable edible film made from basil seed (*Ocimum basilicum L*.) gum. *Carbohydrate Polymers*, 102, 199-206.

Kifah, S. D., Layla, A. A., & Baha, N. A. (2014). Utilization of concentrated buttermilk in functional processed cheese manufacturing and studying some of its physicochemical properties. *Pakistan Journal of Nutrition*, 13(1), 33-37.

Knoshaug, E. P., Ahlgren, J. A., & Trempy, J. E. (2007). Exopolysaccharide expression in *Lactococcus lactis* subsp. *cremoris* Ropy 352: evidence for novel gene organization. *Applied Environmental Microbiology*, 73(3), 897-905.

Koxholt, M. M., Eisenmann, B., & Hinrichs, J. (2001). Effect of the fat globule sizes on the meltdown of ice cream. *Journal of Dairy Science*, 84(1), 31-37.

Kuchta-Noctor, A. M., Murray, B. A., Stanton, C., Devery, R., & Kelly, P. M. (2016). Anticancer activity of buttermilk against SW480 colon cancer cells is associated with caspase-independent cell death and attenuation of Wnt, Akt, and ERK signaling. *Nutrition and Cancer*, 68(7), 1234-1246.

Kulp, W. L., & Rettger, L. F. (1924). Comparative study of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. *Journal of Bacteriology*, 9(4), 357.

Lal, S. N., O'Connor, C. J., & Eyres, L. (2006). Application of emulsifiers/stabilizers in dairy products of high rheology. *Advances in Colloid and Interface Science*, 123, 433-437.

Leon de Pinto, G. L., Rincón, F., & Beltrán, O. (2006). Behaviour of a mixture of Acacia glomerosa, enterolobium cyclocarpum and hymenaea courbaryl gums in ice cream preparation: note. *Food Science and Technology International*, 12(1), 13-17.

Levin, M. A., Burrington, K. J., & Hartel, R. W. (2016). Whey protein phospholipid concentrate and delactosed permeate: Applications in caramel, ice cream, and cake. *Journal of Dairy Science*, 99(9), 6948-6960.

Lin, K. Y., Daniel, J. R., & Whistler, R. L. (1994). Structure of chia seed polysaccharide exudate. *Carbohydrate Polymers*, 23(1), 13-18.

Liong, M. T. (Ed.). (2011). *Probiotics: biology, genetics and health aspects*. Berlin, Germany: Springer-verlag.

Maestrello, C., de Lima Figueiredo, I., Caroline de Souza Takahashi, G., Pimentel, T. C., Visentainer, J. V., de Cassia Bergamasco, R., & Madrona, G. S. (2017). Replacing emulsifier in a prebiotic ice cream: Physical and chemical evaluation and acceptance. *Journal of Culinary Science and Technology*, 16(1), 76-87.

Magariños, H., Selaive, S., Costa, M., Flores, M., & Pizarro, O. (2007). Viability of probiotic microorganisms (*Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* Bb-12) in ice cream. *International Journal of Dairy Technology*, 60(2), 128-134.

Malvern Instruments Ltd. (2013). Mastersizer 300. UK: Malvern Instruments Ltd.

Marshall, R., Goff, H.D., & Hartel, R. (2003). *Ice cream.* New York, USA: Kluwer Academic/Plenum Publishers.

Marshall, R.T., Arbuckle, W.S. (1996). *Ice cream*. New York, USA: Chapman & Hall.

Marshall, V. M., & Rawson, H. L. (1999). Effects of exopolysaccharide-producing strains of thermophilic lactic acid bacteria on the texture of stirred yoghurt. *International Journal of Food Science and Technology*, 34(2), 137-143.

Martinou-voulasiki, I. S., & Zerfiridis, G. K. (1990). Effect of some stabilizers on textural and sensory characteristics of yogurt ice cream from sheep's milk. *Journal of Food Science*, 55(3), 703-707.

Mende, S., Rohm, H., & Jaros, D. (2016). Influence of exopolysaccharides on the structure, texture, stability and sensory properties of yoghurt and related products. *International Dairy Journal*, 52, 57-71.

Méndez-Velasco, C., & Goff, H. D. (2011). Enhancement of fat colloidal interactions for the preparation of ice cream high in unsaturated fat. *International Dairy Journal*, 21(8), 540-547.

Méndez-Velasco, C., & Goff, H. D. (2012). Fat structure in ice cream: A study on the types of fat interactions. *Food Hydrocolloids*, 29(1), 152-159.

Miller-Livney, T., & Hartel, R. W. (1997). Ice recrystallization in ice cream: interactions between sweeteners and stabilizers. *Journal of Dairy Science*, 80(3), 447-456.

Mohammadi, R., Mortazavian, A. M., Khosrokhavar, R., & da Cruz, A. G. (2011). Probiotic ice cream: viability of probiotic bacteria and sensory properties. *Annals of Microbiology*, 61(3), 411-424.

Mohan, A., Quek, S., Gutierrez-Maddox, N., Gao, Y., & Shu, Q. (2017). Effect of honey in improving the gut microbial balance. *Food Quality and Safety*, 1(2), 107-115.

Mohd Ali, N., Yeap, S. K., Ho, W. Y., Beh, B. K., Tan, S. W., & Tan, S. G. (2012). The promising future of chia, *Salvia hispanica L*. *Journal of Biomedicine and biotechnology*, 2012, 1-10.

Moriano, M. E., & Alamprese, C. (2017). Honey, trehalose and erythritol as sucrose-alternative sweeteners for artisanal ice cream. A pilot study. *LWT*, 75, 329-334.

Muñoz, L. A., Cobos, A., Diaz, O., & Aguilera, J. M. (2012). Chia seeds: Microstructure, mucilage extraction and hydration. *Journal of Food Engineering*, 108(1), 216-224.

Muñoz, L. A., Cobos, A., Diaz, O., & Aguilera, J. M. (2013). Chia seed (*Salvia hispanica*): an ancient grain and a new functional food. *Food Reviews International*, 29(4), 394-408.

Murtaza, M. A., Mueenuddin, G., Huma, N., Shabbir, M. A., & Mahmood, S. (2004). Quality evaluation of ice cream prepared with different stabilizers/emulsifier blends. *International Journal of Agriculture and Biology*, 6(1), 65-67.

Muse, M. R., & Hartel, R. W. (2004). Ice cream structural elements that affect melting rate and hardness. *Journal of Dairy Science*, 87(1), 1-10.

Nousia, F. G., Androulakis, P. I., & Fletouris, D. J. (2011). Survival of *Lactobacillus acidophilus* LMGP-21381 in probiotic ice cream and its influence on sensory acceptability. *International Journal of Dairy Technology*, 64(1), 130-136.

O'Connell, J. E., & Fox, P. F. (2000). Heat stability of buttermilk. *Journal of Dairy Science*, 83(8), 1728-1732.

Oortwijn, H., & Walstra, P. (1979). The membranes of recombined fat globules. 2. Composition. *Netherlands Milk and Dairy Journal*, 33, 134-154.

Osano, J. P., Hosseini-Parvar, S. H., Matia-Merino, L., & Golding, M. (2014). Emulsifying properties of a novel polysaccharide extracted from basil seed (*Ocimum basilicum L.*): Effect of polysaccharide and protein content. *Food Hydrocolloids*, 37, 40-48.

Ozdemir, C., Arslaner, A., Ozdemir, S., & Allahyari, M. (2015). The production of ice cream using stevia as a sweetener. *Journal of Food Science and Technology*, 52(11), 7545-7548.

Ozdemir, C., Dagdemir, E., Ozdemir, S., & Sagdic, O. (2008). The effects of using alternative sweeteners to sucrose on ice cream quality. *Journal of Food Quality*, 31(4), 415-428.

Patel, A., & Prajapat, J. B. (2013). Food and health applications of exopolysaccharides produced by lactic acid bacteria. *Advances in Dairy Research*, 1-8.

Pei, Z. J., & Schmidt, K. A. (2010). Ice cream: foam formation and stabilization-a review. *Food Reviews International*, 26(2), 122-137.

Peirce, S. (1997). What Manufacturers Want in Food Ingredients. *Journal of Food Distribution Research*, 39-41.

Peryam, D. R., & Pilgrim, F. J. (1957). Hedonic scale method of measuring food preferences. *Food Technology*. 11, supplementary, 9-14.

Pollan, M. (2008). *In defense of food: An eater's manifesto*. London, England: Penguin Press.

Rajor, R. B. (1980). *Technological studies on the utilization of butter milk and soya bean for the manufacture of 'Softy' Ice cream* (Unpublished doctoral dissertation). National Dairy Research Institute, Karnal, India.

Rajor, R. B., Patel, G. R., Patel, A. A., & Gupta, S. K. (1901). A new" softy" from, buttermilk and soybean. *Indian Dairyman Journal*, 35(1), 29-32.

Razavi, S. M., Mortazavi, S. A., Matia-Merino, L., Hosseini-Parvar, S. H., Motamedzadegan, A., & Khanipour, E. (2009). Optimisation study of gum extraction from Basil seeds (*Ocimum basilicum L.*). *International Journal of Food Science and Technology*, 44(9), 1755-1762.

Regand, A., & Goff, H. D. (2002). Effect of biopolymers on structure and ice recrystallization in dynamically frozen ice cream model systems. *Journal of Dairy Science*, 85(11), 2722-2732.

Regand, A., & Goff, H. D. (2003). Structure and ice recrystallization in frozen stabilized ice cream model systems. *Food Hydrocolloids*, 17(1), 95-102.

Renner, E., Schaafsma, G., & Scott, K. J. (1989). Micronutrients in milk. In E. Renner (Eds.), *Micronutrients in milk and milk based products,* (pp. 1-70). New York, USA: Elsevier.

Rinaldi, M., Dall'Asta, C., Paciulli, M., Guizzetti, S., Barbanti, D., & Chiavaro, E. (2014). Innovation in the Italian ice cream production: effect of different phospholipid emulsifiers. *Dairy Science and Technology*, 94(1), 33-49.

Rothwell, J. (1974). Alternative MSNF ingredients for use in ice cream. *Ice Cream Frozen Confectionery*, 27(3), 178.

Rownan, A. (2017). *Increasing consumer confidence and driving value through clean label claims globally.* Euromonitor International.

Ruger, P. R., Baer, R. J., & Kasperson, K. M. (2002). Effect of double homogenization and whey protein concentrate on the texture of ice cream. *Journal of Dairy Science*, 85(7), 1684-1692.

Sakurai, K. (1996). Effect of production conditions on ice cream melting resistance and hardness. *Milchwissenschaft*, 51, 451-454.

Salem, M. M., Fathi, F. A., & Awad, R. A. (2005). Production of probiotic ice cream. *Polish Journal of Food and Nutrition Sciences*, 14(3), 267.

Saltmarsh, M. (Eds.). (2013). *Essential guide to food additives*. Cambridge, UK: Royal Society of Chemistry.

Schoenfuss, T. C., & Chandan, R. C. (2011). Dairy ingredients in dairy food processing. In R. C. Chandan & A. Kilara (Eds.), *Dairy Ingredients for Food Processing*, USA: Wiley-Blackwell.

Segall, K. I., & Goff, H. D. (1999). Influence of adsorbed milk protein type and surface concentration on the quiescent and shear stability of butteroil emulsions. *International Dairy Journal*, 9(10), 683-691.

Segall, K. I., & Goff, H. D. (2002a). A modified ice cream processing routine that promotes fat destabilization in the absence of added emulsifier. *International Dairy Journal*, 12(12), 1013-1018.

Segall, K. I., & Goff, H. D. (2002b). Secondary adsorption of milk proteins from the continuous phase to the oil–water interface in dairy emulsions. *International Dairy Journal*, 12(11), 889-897.

Shibu, A. V., Kumar, C. N., Narasımhan, R., & Pugazhenthi, T. R. (2000). Substitution of Buttermilk Powder in Ice Cream. *Journal of Veterinary and Animal Sciences*, 31, 25-27.

Shim, S. M., Seo, S. H., Lee, Y., Moon, G. I., Kim, M. S., & Park, J. H. (2011). Consumers' knowledge and safety perceptions of food additives: Evaluation on the effectiveness of transmitting information on preservatives. *Food Control*, 22(7), 1054-1060.

Singh, H., & Tokley, R. P. (1990). Effects of preheat treatments and buttermilk addition on the seasonal variations in the heat stability of recombined evaporated milk and reconstituted concentrated milk. *Australian Journal of Dairy Technology*, 45(1), 10-16.

Siro, I., Kápolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance-A review. *Appetite*, 51(3), 456-467.

Sloan, E., & Adams Hutt, C. (2015). *Coming Clean: What Clean Label Means for Consumers and Industry.* Retrieved from https://www.globalfoodforums.com/2019-clean-label-conference/

Sodini, I., Morin, P., Olabi, A., & Jiménez-Flores, R. (2006). Compositional and functional properties of buttermilk: a comparison between sweet, sour, and whey buttermilk. *Journal of Dairy Science*, 89(2), 525-536.

Sofjan, R. P., & Hartel, R. W. (2004). Effects of overrun on structural and physical characteristics of ice cream. *International Dairy Journal*, 14(3), 255-262.

Sommer, H. H. (1951). *The theory and practice of ice cream making*. USA: The Olsen publishing.

Soukoulis, C., & Fisk, I. (2016). Innovative ingredients and emerging technologies for controlling ice recrystallization, texture, and structure stability in frozen dairy desserts: A review. *Critical Reviews in Food Science and Nutrition*, 56(15), 2543-2559.

Soukoulis, C., Fisk, I. D., & Bohn, T. (2014). Ice cream as a vehicle for incorporating health-promoting ingredients: Conceptualization and overview of quality and storage stability. *Comprehensive Reviews in Food Science and Food Safety*, 13(4), 627-655.

Soukoulis, C., Lebesi, D., & Tzia, C. (2009). Enrichment of ice cream with dietary fibre: Effects on rheological properties, ice crystallisation and glass transition phenomena. *Food Chemistry*, 115(2), 665-671.

Soukoulis, C., Lyroni, E., & Tzia, C. (2010). Sensory profiling and hedonic judgement of probiotic ice cream as a function of hydrocolloids, yogurt and milk fat content. *LWT-Food Science and Technology*, 43(9), 1351-1358.

Tabatabaee Amid, B., & Mirhosseini, H. (2012). Influence of different purification and drying methods on rheological properties and viscoelastic behaviour of durian seed gum. *Carbohydrate Polymers*, 90(1), 452-461.

Tanaka, M., Pearson, A. M., & Deman, J. M. (1972). Measurement of ice cream texture with the constant speed penetrometer. *Canadian Institute of Food Science and Technology Journal*, 5(2), 105-110.

Tarnavolgyi, G. (2003). Analysis of consumer's attitudes towards food additives using focus group survey. *Agriculturae Conspectus Scientificus*, 68(3), 193-196.

Tavares, L. S., Junqueira, L. A., de Oliveira Guimarães, Í. C., & de Resende, J. V. (2018). Cold extraction method of chia seed mucilage (*Salvia hispanica L.*): effect on yield and rheological behavior. *Journal of Food Science and Technology*, 55(2), 457-466.

Tharp, B. W., Forrest, B., Swan, C., Dunning, L., & Hilmoe, M. (1998). Basic factors affecting ice cream meltdown. *International Dairy Federation Special Issue*, (3), 54-64.

Thomas, E. L., & Combs, W. B. (1944). Observations on the use of roller process sweet cream buttermilk powder in ice cream. *Journal of Dairy Science*, 27(6), 419-432.

Timilsena, Y. P., Adhikari, R., Kasapis, S., & Adhikari, B. (2015). Rheological and microstructural properties of the chia seed polysaccharide. *International Journal of Biological Macromolecules*, 81, 991-999.

Timilsena, Y. P., Adhikari, R., Kasapis, S., & Adhikari, B. (2016). Molecular and functional characteristics of purified gum from Australian chia seeds. *Carbohydrate Polymers*, 136, 128-136.

Tomas, A., Paquet, D., Courthaudon, J. L., & Lorient, D. (1994). Effect of fat and protein contents on droplet size and surface protein coverage in dairy emulsions. *Journal of Dairy Science*, 77(2), 413-417.

Tosaki, M., Kitamura, Y., Satake, T., & Tsurutani, T. (2009). Effects of homogenisation conditions on the physical properties of high-fat ice cream. *International Journal of Dairy Technology*, 62(4), 577-583.

Tuormaa, T. E. (1994). The adverse effects of food additives on health: a review of the literature with a special emphasis on childhood hyperactivity. *Journal of Orthomolecular Medicine*, 9, 225-225.

Turgut, T., & Cakmakci, S. (2009). Investigation of the possible use of probiotics in ice cream manufacture. *International Journal of Dairy Technology*, 62(3), 444-451.

Ulbricht, C., Chao, W., Nummy, K., Rusie, E., Tanguay-Colucci, S., Iannuzzi, C. M., Plammootti, J. B., Varghese, M., & Weissner, W. (2009). Chia (*Salvia hispanica*): a systematic review by the natural standard research collaboration. *Reviews on Recent Clinical Trials*, 4(3), 168-174.

Uzomah, A., & Ahiligwo, R. N. (1999). Studies on the rheological properties and functional potentials of achi (*Brachystegea eurycoma*) and ogbono (*Irvingia gabonesis*) seed gums. *Food Chemistry*, 67(3), 217-222.

Valdivia-López, M. Á., & Tecante, A. (2015). Chapter Two - Chia (*Salvia hispanica*): a review of native mexican seed and its nutritional and functional properties. In J. Henry (Eds.), *Advances in food and nutrition research*, (pp. 53-75): Academic Press.

Varga, L. (2007). *Microbiological quality of commercial dairy products.* In: Communicating Current Research and Educational Topics and Trends in Applied Microbiology. Formatex Microbiology Series (1). Formatex, Badajoz, pp. 487-494.

Vivek, B. (2001). *Ice cream: manufacture and technology.* New Delhi, India: Tata McGraw-Hill Publishing Company Limited.

Walts, C. C., & Dahle, C. D. (1933). The effect of egg products on the whipping properties of ice cream mixes made from butter and dried skim milk. *Journal of Agricultural Research*, 47, 967.

Wansink, B., Tal, A., & Brumberg, A. (2014). Ingredient-based food fears and avoidance: Antecedents and antidotes. *Food Quality and Preference*, 38, 40-48.

Whitaker, R. (1930). The influence of the use of butter on the freezing properties of ice cream mix. *Journal of Dairy Science*, 13(1), 1-7.

Whitehurst, R. J. (2004). *Emulsifiers in food technology.* Ames, USA: Blackwell Publishing Ltd.

Wilbey, R. A., Cooke, T., & Dimos, G. (1998). Effects of solute concentration, overrun and storage on the hardness of ice cream. In *Ice cream. IDF Symposium, Athens (Greece),* 18-19 Sep 1997. Brussels, Belgium, International Dairy Federation.

Wildmoser, H., Scheiwiller, J., & Windhab, E. J. (2004). Impact of disperse microstructure on rheology and quality aspects of ice cream. *LWT-Food Science and Technology*, 37(8), 881-891.

Williams, D. H., Potter, F. E., & Hufnagel, C. F. (1950). Concentrated buttermilk in ice cream. *Journal of Dairy Science*, 33(8), 593-98.

Williams, P. G., Stirling, E., & Keynes, N. (2004). Food fears: a national survey on the attitudes of Australian adults about the safety and quality of food. *Asia Pacific Journal of Clinical Nutrition*, 13 (1), 9-32.

Windhab, E. J., & Wildmoser, H. (2002). Extrusion: A novel technology for the manufacture of ice cream. Brussels, Belgium, *Bulletin of the International Dairy Federation*.

Wittinger, S. A., & Smith, D. E. (1986). Effect of sweeteners and stabilizers on selected sensory attributes and shelf life of ice cream. *Journal of Food Science*, 51(6), 1463-1466.

Wong, P. Y. Y., & Kitts, D. D. (2003). A comparison of the buttermilk solids functional properties to nonfat dried milk, soy protein isolate, dried egg white, and egg yolk powders. *Journal of Dairy Science*, 86(3), 746-754.

Yanasugondha, D. (1951). *A Comparison of Sweet Cream Buttermilk Powder with Nonfat Dried Milk Solids in the Manufacture of Ice Cream* (Unpublished master's thesis). Utah State University, Logan, USA.

Yilmaz, M. T., Dertli, E., Toker, O. S., Tatlisu, N. B., Sagdic, O., & Arici, M. (2015). Effect of in situ exopolysaccharide production on physicochemical, rheological, sensory, and microstructural properties of the yogurt drink ayran: an optimization study based on fermentation kinetics. *Journal of Dairy Science*, 98(3), 1604-1624.

Zameni, A., Kashaninejad, M., Aalami, M., & Salehi, F. (2015). Effect of thermal and freezing treatments on rheological, textural and color properties of basil seed gum. *Journal of Food Science and Technology*, 52(9), 5914-5921.

Zanabria Eyzaguirre, R., & Corredig, M. (2011). Milk Lipids | Buttermilk and Milk Fat Globule Membrane Fractions. In J. W. Fuquay (Eds.), *Encyclopedia of Dairy Sciences (Second Edition)* (pp. 691-697). San Diego, USA: Academic Press.

Zhang, J., Zhao, W., Guo, X., Guo, T., Zheng, Y., Wang, Y., Hao, Y., & Yang, Z. (2017). Survival and Effect of Exopolysaccharide-Producing *Lactobacillus plantarum* YW11 on the Physicochemical Properties of Ice Cream. *Polish Journal of Food and Nutrition Sciences,* 67(3), 191-200.

Zulim Botega, D. C., Marangoni, A. G., Smith, A. K., & Goff, H. D. (2013). The potential application of rice bran wax oleogel to replace solid fat and enhance unsaturated fat content in ice cream. *Journal of Food Science,* 78(9), 1334-1339.

Chapter 10 Appendices

10.1 Appendix A

10.1.1 Raw data

Table 10.1 Mean value of hardness (N)

Formulations	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Hardness (N)	47.12± 2.69	40.06± 7.23	61.80± 39.7	52.90± 41	71.56± 1.032	56.39± 8.68	93.88± 15.5	102.17 ±41	101.85 ±33	84.20± 18.7

Note: Each formulation was duplicated.

Table 10.2 Mean weight loss (g) of ice cream during meltdown rate test at 20°C

Time (min)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
10	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0.91±0. 495	0.06±0. 085	1.35±1. 103	0	0	0	0
30	3735±4 .250	0	0	9.58±0. 396	5.01±0. 806	11.125 ±3.132	5.33±1. 527	4.745± 1.365	3.005± 0.007	0
40	18.44± 8.782	4.335± 6.131	0.485± 0.686	20.97± 0.424	15.385 ±2.157	26.265 ±4.716	23.24± 5.685	24.75± 3.677	18.76± 1.471	7.84±0. 792
50	35.615 ±11.08 0	18.545 ±4.490	8.645± 11.901	34.44± 2.913	35.865 ±1.534	41.545 ±6.838	46.41± 5.600	45.465 ±4.009	42.13± 1.655	26.035 ±0.530
60	55.845 ±11.03 8	37.435 ±9.072	28.37± 16.433	50.085 ±7.205	53.54± 5.459	55.905 ±5.537	68.37± 5.558	67.05± 2.687	65.67± 1.881	49.93± 1.047
70	76.855 ±12.93 3	58.93± 5.756	47.455 ±14.91 3	66.5±2. 121	71.84± 5.459	70.21± 4.879	91.265 ±4.306	90.64± 4.002	89.41± 2.786	73.94± 3.620

Note: Each formulation was duplicated.

Table 10.3 Regression equations obtained from meltdown rate curves

Formulation	Regression equation
F1	y = 1.3362x – 26.235
F2	y = 0.965x – 21.566
F3	y = 0.742x – 17.542
F4	y = 1.1525x – 20.032
F5	y = 1.2619x – 24.519
F6	y = 1.2506x – 20.537
F7	y = 1.6129x - 31
F8	y = 1.5955x – 30.584
F9	y = 1.5668x – 31.389
F10	y = 1.2418x – 27.139

Note: The mean meltdown rate (g/min) for each formulation equals to the slope of the respective equation, each formulation was duplicated.

Table 10.4 Overrun (%) of each formulation

Formulation	First replication			Second replication			Mean
	Weight of 250 mL mix (g)	Weight of 250 mL foam (g)	Overrun (%)	Weight of 250 mL mix (g)	Weight of 250 mL foam (g)	Overrun (%)	Overrun (%)
F1	269	132.04	103.73	269.62	126.84	112.57	108.15
F2	264.38	129.96	103.43	257.40	126.94	102.77	103.10
F3	259.57	132.20	96.35	262.43	134	95.84	96.10
F4	270.01	140.11	92.71	272	132.08	105.94	99.33
F5	272.09	123.26	120.74	269.98	124.52	116.82	118.78
F6	270.92	131.38	106.21	269.98	124.52	116.82	95.93
F7	265.12	145.69	81.98	259.18	149.18	73.74	77.86
F8	265	145.95	81.57	259.83	141.34	83.84	82.71
F9	215.24	113.90	88.97	260.41	137.91	88.83	88.90
F10	259.59	138	88.11	257.51	136.34	88.87	88.49

Note: mix = ice cream mix; Foam = ice cream after whipping

Table 10.5 Destabilised fat index (DFI)

Formulation	First replication			Second replication			Mean
	Absorbance of mix (Mean)	Absorbance of foam (Mean)	DFI (%)	Absorbance of mix (Mean)	Absorbance of foam (Mean)	DFI (%)	DFI (%)
F1	1.081	0.629	41.83	1.083	0.72	33.36	37.60
F2	0.964	0.532	44.78	0.964	0.605	37.21	41
F3	1.523	0.753	50.575	1.4	0.7665	45.25	47.91
F4	1.124	1.066	5.12	1.134	1.009	10.99	8.06
F5	1.179	1.068	9.376	1.2065	1.0925	9.45	9.41
F6	1.110	1.069	3.739	1.215	1.1055	9.01	6.37
F7	1.139	1.0555	7.331	1.2795	1.1575	9.53	8.43
F8	1.122	1.022	8.957	1.0845	1.0075	7.10	8.03
F9	1.141	1.06	7.143	1.2	1.1795	1.71	4.43
F10	1.248	1.112	10.938	1.263	1.0995	12.95	11.96

Note: Each absorbance was read twice.

Table 10.6 Apparent viscosity of the ice cream mixes over the shear rate range of 0.1-100 s^{-1}

F1

First replication		Second replication	
shear rate	viscosity	shear rate	viscosity
1.s	Pa.s	1.s	Pa.s
0.0995	0.2591	0.F09844	0.3209
0.1248	0.2827	0.1246	0.3038
0.1599	0.2448	0.1594	0.2831
0.2023	0.2285	0.1974	0.2864
0.2507	0.2222	0.2502	0.2686
0.323	0.2085	0.3216	0.2415
0.3977	0.1875	0.3971	0.226
0.4997	0.1741	0.5014	0.2035
0.6321	0.161	0.6304	0.1843
0.7947	0.1474	0.7913	0.1718
0.9919	0.1357	0.9975	0.1565
1.263	0.1218	1.263	0.1458
1.59	0.1131	1.588	0.1314
1.987	0.1033	1.995	0.121
2.515	0.09527	2.513	0.1108
3.163	0.08746	3.167	0.1014
3.973	0.08065	3.977	0.09333
5.013	0.07405	5.017	0.08546
6.305	0.06823	6.305	0.07847
7.936	0.06301	7.944	0.07227
9.994	0.05836	9.996	0.06688
12.59	0.05407	12.6	0.06194
15.85	0.05023	15.85	0.05764
19.95	0.04689	19.95	0.05375
25.12	0.04376	25.11	0.05044
31.61	0.0411	31.62	0.04749
39.8	0.03875	39.8	0.04495
50.12	0.03674	50.13	0.0426
63.09	0.03489	63.09	0.04062
79.43	0.03331	79.43	0.03883
100	0.03189	99.99	0.03722

F2

First replication		Second replication	
shear rate	viscosity	shear rate	viscosity
1.s	Pa.s	1.s	Pa.s
0.1009	3.363	0.1005	3.2
0.1258	3.285	0.1256	3.153
0.1583	2.941	0.158	2.842
0.1974	2.587	0.2002	2.467
0.2511	2.198	0.2505	2.11
0.3168	1.871	0.3187	1.796
0.3986	1.575	0.3974	1.52
0.4994	1.325	0.5003	1.273
0.6313	1.097	0.6308	1.063
0.7959	0.911	0.7951	0.8886
0.9979	0.7626	1.002	0.7508
1.261	0.6424	1.258	0.6381
1.583	0.5494	1.589	0.5461
1.99	0.4719	1.994	0.4725
2.514	0.4068	2.512	0.408
3.164	0.3522	3.16	0.3548
3.977	0.3061	3.973	0.3102
5.009	0.2671	5.015	0.2714
6.306	0.2335	6.304	0.2393
7.945	0.2055	7.941	0.2116
9.999	0.1818	9.995	0.1873
12.59	0.1617	12.59	0.1672
15.85	0.1447	15.85	0.15
19.95	0.1299	19.96	0.135
25.12	0.1175	25.11	0.1226
31.62	0.1065	31.63	0.1116
39.81	0.09752	39.81	0.1022
50.13	0.08969	50.11	0.09419
63.09	0.08258	63.09	0.08679
79.43	0.07651	79.42	0.08055
100	0.07138	100	0.07516

F3

First replication		Second replication	
shear rate	viscosity	shear rate	viscosity
1.s	Pa.s	1.s	Pa.s
0.09995	10.31	0.1001	11.64
0.1257	9.061	0.1255	10.99
0.1587	7.673	0.1587	9.452
0.1997	6.478	0.1998	8.06
0.2513	5.478	0.2512	6.831
0.3164	4.627	0.3164	5.777
0.3979	3.904	0.3981	4.898
0.5014	3.255	0.501	4.14
0.6278	2.697	0.6322	3.467
0.7962	2.208	0.7934	2.902
0.9975	1.81	1.001	2.399
1.262	1.482	1.258	1.979
1.588	1.232	1.587	1.61
1.993	1.035	1.997	1.299
2.512	0.8752	2.514	1.042
3.162	0.7458	3.165	0.857
3.982	0.6391	3.978	0.7237
5.01	0.551	5.015	0.6165
6.31	0.477	6.306	0.5302
7.94	0.4148	7.946	0.4574
10.01	0.3636	9.996	0.3961
12.6	0.3209	12.58	0.3444
15.85	0.2852	15.85	0.3016
19.95	0.2539	19.95	0.2646
25.11	0.2278	25.12	0.2341
31.63	0.2047	31.63	0.2086
39.81	0.1856	39.81	0.1871
50.12	0.1686	50.13	0.1685
63.1	0.1546	63.09	0.1533
79.43	0.142	79.44	0.1398
99.99	0.1305	100	0.1282

F4

First replication		Second replication	
shear rate	viscosity	shear rate	viscosity
1.s	Pa.s	1.s	Pa.s
0.1022	0.136	0.09994	0.05119
0.1277	0.2168	0.1246	0.02469
0.1571	0.24	0.1584	0.01877
0.2023	0.1214	0.2004	0.02774
0.2489	0.08362	0.2492	0.0265
0.3162	0.08683	0.3236	0.02361
0.3977	0.09154	0.3973	0.02028
0.4999	0.07815	0.5018	0.02164
0.6366	0.06377	0.6355	0.02193
0.7916	0.0394	0.7913	0.02185
0.9972	0.05017	0.9975	0.02076
1.264	0.0425	1.269	0.02089
1.588	0.03513	1.592	0.02017
1.988	0.05419	1.99	0.01991
2.512	0.04914	2.516	0.0197
3.16	0.03347	3.161	0.01977
3.984	0.03682	3.972	0.01951
5.009	0.03344	5.011	0.01907
6.313	0.03292	6.307	0.01879
7.942	0.03117	7.944	0.01858
9.999	0.02962	9.998	0.0185
12.59	0.02839	12.59	0.01829
15.84	0.02758	15.85	0.01813
19.95	0.02876	19.95	0.01797
25.12	0.02694	25.13	0.01786
31.62	0.02522	31.62	0.01774
39.81	0.02421	39.8	0.01765
50.12	0.02312	50.12	0.01758
63.09	0.02187	63.1	0.01752
79.44	0.02066	79.43	0.01744
100	0.01956	100	0.01736

F5

First replication		Second replication	
shear rate	viscosity	shear rate	viscosity
1.s	Pa.s	1.s	Pa.s
0.09992	1.374	0.1011	1.455
0.1233	1.215	0.1242	1.252
0.1594	1.018	0.16	1.059
0.2002	0.8793	0.2019	0.8969
0.2514	0.7728	0.2515	0.7707
0.316	0.6728	0.3189	0.6755
0.3982	0.5999	0.3989	0.5845
0.5006	0.5349	0.5032	0.5208
0.6303	0.4797	0.6338	0.4667
0.7947	0.4295	0.7946	0.4255
0.9979	0.386	0.9988	0.3899
1.262	0.3502	1.264	0.3462
1.586	0.3214	1.588	0.3183
1.994	0.2895	1.991	0.2906
2.51	0.2672	2.509	0.2657
3.163	0.2433	3.16	0.2409
3.984	0.2213	3.975	0.2192
5.008	0.2025	5.01	0.2006
6.306	0.1847	6.311	0.1845
7.938	0.1701	7.943	0.1697
10	0.1576	10	0.1563
12.59	0.1464	12.59	0.1452
15.85	0.1362	15.85	0.1352
19.96	0.1275	19.95	0.1265
25.12	0.12	25.12	0.1188
31.62	0.1128	31.62	0.1118
39.81	0.1063	39.81	0.1053
50.12	0.1005	50.11	0.09951
63.1	0.0949	63.09	0.09396
79.43	0.09008	79.43	0.08908
100	0.08531	100	0.08444

F6

First replication		Second replication	
shear rate	viscosity	shear rate	viscosity
1.s	Pa.s	1.s	Pa.s
0.1004	0.03751	0.1019	0.06012
0.1276	0.0383	0.1276	0.03707
0.156	0.02205	0.1587	0.04434
0.2003	0.03386	0.2009	0.05042
0.2521	0.02675	0.2485	0.04803
0.3216	0.03145	0.3199	0.04907
0.3988	0.02933	0.3933	0.05262
0.5002	0.0287	0.4987	0.05349
0.6375	0.02602	0.6394	0.05134
0.7962	0.02625	0.7962	0.04529
0.995	0.02549	0.9965	0.04405
1.264	0.02417	1.261	0.04446
1.588	0.02278	1.592	0.03839
1.995	0.02304	1.991	0.03945
2.516	0.02189	2.512	0.03668
3.161	0.02214	3.163	0.03316
3.98	0.02152	3.978	0.03018
5.006	0.02097	5.011	0.02781
6.31	0.02045	6.311	0.02722
7.941	0.02012	7.948	0.0256
10	0.01992	10	0.02388
12.59	0.01953	12.59	0.0228
15.84	0.0192	15.85	0.02182
19.95	0.01891	19.95	0.02079
25.12	0.01863	25.12	0.01996
31.62	0.01838	31.63	0.01931
39.81	0.01818	39.81	0.01872
50.12	0.01799	50.12	0.01823
63.09	0.01784	63.1	0.01781
79.43	0.01767	79.43	0.01746
99.99	0.01752	99.99	0.01716

F7

First replication		Second replication	
shear rate	viscosity	shear rate	viscosity
1.s	Pa.s	1.s	Pa.s
0.1009	2.677	0.09955	3.285
0.1239	2.526	0.1249	3.121
0.1578	2.263	0.1531	2.827
0.1974	2.021	0.1975	2.437
0.2493	1.774	0.2537	2.073
0.3147	1.571	0.3154	1.77
0.3973	1.361	0.3995	1.492
0.4998	1.151	0.4993	1.245
0.6314	0.9542	0.6311	1.022
0.7934	0.8052	0.7942	0.841
0.9945	0.6985	0.9969	0.7021
1.26	0.6161	1.263	0.5948
1.588	0.5106	1.583	0.5081
1.991	0.4494	1.988	0.4362
2.509	0.3807	2.508	0.3771
3.164	0.3354	3.17	0.3282
3.983	0.2911	3.989	0.2866
5.01	0.2507	5.006	0.2511
6.311	0.219	6.304	0.2208
7.94	0.1917	7.936	0.1954
9.995	0.1698	9.999	0.1741
12.59	0.1503	12.59	0.1557
15.86	0.134	15.84	0.1401
19.95	0.1205	19.94	0.1268
25.11	0.1091	25.12	0.1155
31.63	0.09956	31.63	0.1054
39.81	0.09141	39.81	0.09713
50.12	0.08389	50.12	0.08977
63.09	0.07767	63.09	0.08318
79.42	0.0721	79.42	0.07753
100	0.06691	100	0.07264

F8

First replication		Second replication	
shear rate	viscosity	shear rate	viscosity
1.s	Pa.s	1.s	Pa.s
0.09892	3.205	0.09831	3.035
0.1246	2.973	0.1261	2.851
0.1575	2.484	0.1558	2.594
0.2006	2.248	0.1981	2.259
0.2514	1.926	0.2507	1.938
0.3171	1.637	0.3173	1.659
0.3977	1.374	0.3974	1.409
0.5	1.159	0.5033	1.185
0.6322	0.9766	0.6321	0.9933
0.7937	0.8301	0.7918	0.839
0.9971	0.7055	1.001	0.7097
1.262	0.6027	1.264	0.6023
1.588	0.5191	1.588	0.5147
1.994	0.4494	1.997	0.4348
2.51	0.3906	2.516	0.3748
3.17	0.3393	3.161	0.3248
3.974	0.2974	3.979	0.2837
5.014	0.2613	5.019	0.2487
6.308	0.2303	6.309	0.2185
7.938	0.2047	7.941	0.1926
10.01	0.1831	9.998	0.1717
12.59	0.1647	12.59	0.1535
15.84	0.1491	15.85	0.1383
19.96	0.1352	19.95	0.1254
25.12	0.1236	25.12	0.1144
31.63	0.1132	31.62	0.1048
39.81	0.1042	39.8	0.09661
50.12	0.09656	50.12	0.08949
63.1	0.0896	63.1	0.08307
79.44	0.08324	79.43	0.07758
100	0.07793	100	0.07268

F9

First replication		Second replication	
shear rate	viscosity	shear rate	viscosity
1.s	Pa.s	1.s	Pa.s
0.1001	3.392	0.1003	4.56
0.1239	3.375	0.1256	4.296
0.1577	2.988	0.1593	3.819
0.2	2.578	0.1989	3.325
0.2503	2.227	0.253	2.857
0.3155	1.897	0.3156	2.444
0.3989	1.585	0.3983	2.062
0.4982	1.322	0.5008	1.731
0.6294	1.089	0.6304	1.434
0.7942	0.901	0.7936	1.185
0.9996	0.7561	1.002	0.9769
1.267	0.6352	1.258	0.8086
1.588	0.5413	1.588	0.6767
1.986	0.4662	1.989	0.5771
2.509	0.3991	2.513	0.4921
3.166	0.3445	3.158	0.4234
3.978	0.299	3.982	0.3662
5.014	0.2606	5.01	0.3188
6.306	0.2295	6.313	0.279
7.944	0.2034	7.942	0.2456
9.999	0.1805	9.999	0.2167
12.6	0.161	12.58	0.1929
15.85	0.1451	15.84	0.1722
19.95	0.1312	19.96	0.1551
25.11	0.1196	25.12	0.1405
31.63	0.1093	31.63	0.128
39.8	0.1008	39.82	0.1175
50.12	0.09336	50.12	0.108
63.09	0.08661	63.1	0.1001
79.43	0.08091	79.43	0.09334
100	0.07603	99.99	0.08703

F10

First replication		Second replication	
shear rate	viscosity	shear rate	viscosity
1.s	Pa.s	1.s	Pa.s
0.1003	6.017	0.1003	5.469
0.1254	5.578	0.1256	5.075
0.1583	4.868	0.1585	4.444
0.1998	4.178	0.1965	3.859
0.2495	3.567	0.2505	3.271
0.3139	3.041	0.3171	2.747
0.3996	2.527	0.3986	2.312
0.5029	2.091	0.5031	1.898
0.6329	1.717	0.6304	1.556
0.7936	1.408	0.7947	1.269
0.9977	1.177	0.9976	1.05
1.263	0.9887	1.26	0.8779
1.584	0.8348	1.591	0.7378
1.99	0.7144	1.997	0.6292
2.515	0.6057	2.511	0.5361
3.167	0.5205	3.161	0.4624
3.983	0.4493	3.996	0.3993
5.009	0.3882	5.015	0.3476
6.305	0.3383	6.309	0.3041
7.942	0.2967	7.943	0.267
10	0.2613	9.998	0.237
12.59	0.2326	12.59	0.2107
15.84	0.2085	15.85	0.1894
19.96	0.1881	19.96	0.1708
25.12	0.1702	25.12	0.1555
31.62	0.1555	31.63	0.1422
39.81	0.1423	39.81	0.1306
50.12	0.1304	50.11	0.1206
63.1	0.1204	63.1	0.1119
79.43	0.1114	79.43	0.1043
100	0.1034	100	0.09781

Table 10.7 The mean pH of each ice cream mix

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
pH	6.54±0.06	6.55±0.06	6.59±0.00	6.57±0.02	6.50±0.00	6.52±0.00	6.55±0.01	6.50±0.01	6.48±0.01	6.27±0.01

Note: Each formulation was duplicated.

Table 10.8 The mean pH of the cultured milk and the ice cream after storage for four weeks at -20°C

Formulation	Cultured milk	Ice cream after storage
pH	4.6±0.01	6.27±0.06

Note: Each formulation was duplicated.

Table 10.9 Viable cell counts

Formulation (F10)	Viable cell counts (CFU/ ml)		
	First replication (mean*)	Second replication (mean*)	Mean (log CFU/ ml)
Fermented milk	4.95×10^8 (8.69 log CFU/ml)	5.40×10^8 (8.73 log CFU/ ml)	8.71±0.03
Ice cream mix	3.75×10^7 (7.57 log CFU/ ml)	3.25×10^7 (7.51 log CFU/ ml)	7.54±0.04
Ice cream after 2 days freezing	9.40×10^6 (6.97 log CFU/ ml)	1.36×10^7 (7.13 log CFU/ ml)	7.05±0.11
Ice cream after 4 weeks of storage at -20°C	1.16×10^7 (7.06 log CFU/ ml)	9.80×10^6 (6.99 log CFU/ ml)	7.02±0.05

Note: Each formulation was duplicated. *Each replication was duplicated.

Table 10.10 Weight of the chia seed gum (g) obtained from 240 g chia seed

Weight of the chia seed gum obtained from 240 g chia seed (g)		
First replication	**Second replication**	**Third replication**
8.58	7.82	8.00
Yield (%)		
3.57	3.26	3.33

Mean yield=3.40±0.16%

Note: The experiment was triplicated.

Table 10.11 Consumer sensory scores for commercial retail ice cream

Sample 614 (commercial retail ice cream)								
Number of panelists	**Colour**	**Appearance**	**Texture**	**Meltdown in the mouth**	**Smoothness**	**Sweetness**	**Flavour**	**Overall acceptance**
1	7	7	7	7	7	6	7	7
2	9	9	9	9	7	7	8	9
3	5	5	5	5	7	4	5	7
4	6	7	7	3	7	4	4	6
5	9	9	9	9	9	8	5	9
6	8	8	8	8	9	8	8	8
7	8	8	9	9	9	8	8	8
8	8	8	8	8	8	8	8	8
9	6	6	7	4	7	6	6	7
10	7	8	9	8	9	9	7	8
11	7	7	8	7	6	7	7	7
12	8	8	9	8	8	7	5	7
13	9	9	9	9	9	7	7	8
14	8	8	7	8	8	8	8	8
15	8	8	7	9	9	9	9	9
16	6	6	6	7	7	7	6	7
17	8	8	9	9	8	4	7	7
18	5	9	5	7	8	7	9	9
19	7	7	7	7	7	7	7	7
20	9	9	9	7	9	7	9	8
21	4	5	3	6	5	7	2	3
22	9	9	9	9	9	9	9	9
23	7	6	7	6	7	6	7	6
24	8	7	8	8	8	8	8	8
Sum of scores	176	181	181	177	187	168	166	173
Mean score	**7.33±1.40**	**7.54±1.25**	**7.54±1.59**	**7.38±1.64**	**7.79±1.10**	**7.00±1.44**	**6.92±1.74**	**7.52±1.32**

Note: A 9-point hedonic scale was used; n = 24 panellists

Table 10.12 Consumer sensory scores for clean label probiotic ice cream (F10)

Sample 902 (clean label probiotic ice cream (F10))								
Number of panelists	**colour**	**appearance**	**texture**	**meltdown in the mouth**	**smoothness**	**sweetness**	**flavour**	**overall acceptance**
1	7	7	6	5	6	7	6	6
2	9	9	4	4	4	6	7	6
3	5	5	6	6	6	7	6	6
4	6	6	7	7	7	7	5	7
5	8	9	9	4	8	5	5	7
6	8	8	7	7	7	8	7	7
7	8	6	4	4	4	7	7	6
8	8	8	4	6	6	3	3	7
9	6	6	5	4	5	7	6	7
10	7	7	9	9	8	7	7	7
11	6	6	4	3	4	6	5	5
12	8	7	8	9	8	8	8	8
13	6	6	6	6	6	8	8	8
14	9	8	6	7	7	7	7	7
15	9	8	7	7	6	6	6	7
16	5	6	7	8	6	6	5	7
17	7	7	6	*	5	7	7	6
18	5	7	8	8	7	8	8	8
19	7	8	8	8	9	9	9	9
20	9	8	8	8	8	6	8	8
21	7	6	3	4	6	7	4	7
22	9	9	9	9	9	9	9	9
23	8	9	8	8	8	9	7	8
24	7	8	7	7	7	8	7	7
Sum of scores	174	174	156	148	157	168	157	149
Mean score	**7.25±1.33**	**7.25±1.19**	**6.50±1.77**	**6.43±1.88**	**6.54±1.47**	**7.00±1.35**	**6.54±1.50**	**7.10±0.97**

Note: A 9-point hedonic scale was used; n = 24 panellists

Table 10.13 Consumer sensory scores for commercial retail ice cream after four weeks of storage at -20°C

Sample 756 (commercial retail ice cream)								
Number of panelists	**colour**	**appearance**	**texture**	**meltdown in the mouth**	**smoothness**	**sweetness**	**flavour**	**overall acceptance**
1	7	7	7	8	7	8	8	7
2	7	6	7	7	6	6	7	7
3	7	7	7	8	8	8	8	7
4	5	7	9	7	8	8	7	8
5	6	6	7	7	7	6	6	6
6	9	9	8	6	9	8	9	8
7	7	7	8	8	8	7	8	8
8	9	9	9	9	9	9	9	9
9	7	8	9	9	8	7	8	8
10	7	7	8	8	8	8	8	8
11	8	8	9	7	8	8	8	8
12	7	8	8	8	8	7	7	8
13	7	7	5	6	7	7	7	6
14	8	8	9	9	9	9	9	9
15	8	8	8	8	8	8	8	8
16	8	8	8	8	8	8	8	8
17	8	8	8	8	8	8	8	8
18	8	8	8	8	8	7	8	8
19	8	8	8	8	8	8	8	8
20	8	8	7	8	8	7	7	7
21	8	8	7	7	6	8	7	8
22	9	9	9	9	9	8	8	9
23	9	9	6	6	8	6	5	5
24	8	8	8	8	8	8	8	8
25	6	6	8	7	7	7	8	8
26	9	9	9	9	9	7	8	8
27	8	7	6	7	6	6	7	7
28	6	5	4	8	4	7	3	4
Sum of scores	212	213	214	216	215	209	210	211
Mean score	**7.57±1.03**	**7.61±1.03**	**7.64±1.25**	**7.71±0.90**	**7.68±1.12**	**7.46±0.84**	**7.50±1.23**	**7.54±1.14**

Note: A 9-point hedonic scale was used; n = 28 panellists

Table 10.14 Consumer sensory scores for clean label probiotic ice cream (F10) after four weeks of storage at -20°C

285 (clean label probiotic ice cream (F10))								
Number of panelists	**colour**	**appearance**	**texture**	**meltdown in the mouth**	**smoothness**	**sweetness**	**flavour**	**overall acceptance**
1	7	6	6	5	6	5	6	6
2	7	6	6	6	6	7	6	6
3	7	7	8	8	8	7	8	8
4	5	7	8	8	8	7	8	7
5	6	7	8	8	8	9	7	7
6	9	9	9	9	9	6	7	8
7	7	8	7	8	7	7	8	8
8	9	9	9	9	9	9	8	9
9	7	7	6	5	6	8	7	6
10	7	7	7	6	7	6	6	7
11	8	8	7	8	7	8	8	7/5
12	7	8	8	7	8	9	9	9
13	5	5	4	5	4	5	4	4
14	8	8	8	8	8	9	9	8
15	8	7	7	7	7	6	6	7
16	6	6	6	5	5	5	6	6
17	8	8	8	7	7	8	7	8
18	8	7	8	8	8	6	6	7
19	8	7	7	7	7	8	7	7
20	8	8	8	6	8	7	8	8
21	8	7	8	6	7	6	6	7
22	9	9	6	6	7	4	4	6
23	9	9	8	8	6	8	8	8
24	8	8	8	8	8	8	8	8
25	6	5	7	6	6	7	8	8
26	9	9	9	9	9	9	9	9
27	8	7	6	6	4	4	4	6
28	6	5	8	9	9	8	8	8
Sum of scores	208	204	205	198	199	196	196	203/5
Mean score	**7.43±1.17**	**7.29±1.21**	**7.32±1.16**	**7.07±1.33**	**7.11±1.37**	**7.00±1.51**	**7.00±1.44**	**7.27±1.14**

Note: A 9-point hedonic scale was used; n = 28 panellists

Table 10.15 pH of the extracted mucilage from chia seed before drying

pH of the extracted chia seed mucilage before drying			
First replication	Second replication	Third replication	Mean
6.15	6.07	6.20	6.14±0.07

10.1.2 Calculation of ice cream mix formulations

The requirements for making 100 g ice cream mix (F1, F2, F3, F4, F5, F6 and F7):

In these formulations the fat (10%) is derived from the cream (37.3% fat).

(10*100)/37.3 = **26.81 g cream**

The amount of NMS obtained from 26.81 g cream: ((100-37.3)*0.09*26.81)/100 = 1.513 g NMS

The required NMS is 11%.

11-1.513=9.487 g NMS is required which is obtained from the SMP.

The SMP contains 0.9% fat and 3.8% moisture: (100-(0.9+3.8))/100=0.953 (the conversion index for calculating the amount of required SMP for obtaining a certain amount of NMS)

9.487/0.953 =**9.95 g SMP**

The sugar is obtained from sucrose (**12 g sucrose)**

0.15 g MDG was used in formulations F1, F2, F3 and F4.

In **F1, F2, F3 and F7**, respectively **0.1, 0.2, 0.3, 0.2 g CSG** and in **F5, 0.2 g guar gum** were added.

In all formulations, **0.3% vanilla** was added.

F1: 100-(26.81+12+9.95+0.15+0.1+0.3) = **50.69 g water**

F2: 100-(26.81+12+9.95+0.15+0.2+0.3) = **50.59 g water**

F3: 100-(26.81+12+9.95+0.15+0.3+0.3) = **50.49 g water**

F4: 100-(26.81+12+9.95+0.15+0.3) = **50.79 g water**

F5: 100-(26.81+12+9.95+0.15+0.2+0.3) = **50.59 g water**

F6: 100-(26.81+12+9.95+0.3) = **50.94 g water**

F7: 100-(26.81+12+9.95+0.2+0.3) = **50.74 g water**

The requirements for making 100 g ice cream mixes (F8 and F9):

In these formulations the fat (10%) is derived from the cream (37.3% fat) and SCB (5.8% fat).

In F8, SCB constitutes 25% of the NMS of the mix. 11*25%= 2.75 g NMS

The SCB contains 5.8% fat and 3.8% moisture: (100-(5.8+3.8))/100=0.904 (the conversion index for calculating the amount of required SCB for obtaining a certain amount of NMS)

2.75/0.904 =**3.04 g SCB**

The amount of fat obtained from 3.04 g SCB: (3.04*5.8)/100=0.18 g fat from SCB

10 – 0.176=9.82 g fat is required to be provided by the cream (37.3% fat)

(100*9.82)/37.3= **26.33 g cream**

The amount of NMS obtained from 26.33 g cream: ((100-37.3)*0.09*26.33)/100 = 1.48 g NMS

11-(2.75+1.48) = 6.77 g NMS is required to be provided by the SMP

6.77/0.953 =**7.10 g SMP**

0.2g CSG and **0.3% vanilla** are added to the formulation F8.

F8: 100-(26.33+12+7.10+0.2+0.3+3.04) = **51.03 g water**

In F9, SCB constitutes 50% of the NMS of the mix. 11*50%= 5.50 g NMS

5.50 g NMS/0.904 =**6.08 g SCB**

The amount of fat obtained from 6.08 g SCB: (6.08*5.8)/100=0.35 g fat from SCB

10 – 0.35=9.65 g fat is required to be provided by the cream (37.3% fat)

(100*9.65)/37.3= **25.87 g cream**

The amount of NMS obtained from 25.87 g cream: ((100-37.3)*0.09*25.87)/100 = 1.46 g NMS

11-(5.50+1.46) = 4.04 g NMS is required to be provided by the SMP

4.04/0.953 =**4.24 g SMP**

0.2g CSG and **0.3% vanilla** are added to the formulation F9.

F9: 100-(25.87+12+4.24+0.2+0.3+6.08) = **51.31 g water**

The requirements for making 100 g ice cream mixes (F10):

The fat content of this formulation is similar to F9 but lower amount of SMP is required due to the addition of 10% fermented reconstituted skim milk to the mix.

The amount of NMS obtained from 10 g skim milk: ((100-0)*0.09*10)/100 = 0.9 g NMS

11-(5.50+1.46+0.9) = 3.14 g NMS is required to be provided by the SMP

3.14/0.953 = **3.30 g SMP**

0.2g CSG and **0.3% vanilla** are added to the formulation F10.

F10: 100-(25.87+12+3.30+0.2+0.3+10+6.08) = **42.25 g water**

Table 10.16 Ice cream mix formulations (Ingredients (g/100 g ice cream mix))

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
Cream	26.81	26.81	26.81	26.81	26.81	26.81	26.81	26.33	25.87	25.87
Sugar	12	12	12	12	12	12	12	12	12	12
SMP	9.95	9.95	9.95	9.95	9.95	9.95	9.95	7.10	4.24	3.30

MDG	0.15	0.15	0.15	0.15	0.15	0	0	0	0	0
Water	50.69	50.59	50.49	50.79	50.59	50.94	50.74	51.03	51.31	42.25
CSG	0.1	0.2	0.3	0	0	0	0.2	0.2	0.2	0.2
Guar gum	0	0	0	0	0.2	0	0	0	0	0
Vanilla	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Fermented milk	0	0	0	0	0	0	0	0	0	10
SCB	0	0	0	0	0	0	0	3.04	6.08	6.08

Note: SCB = sweet cream buttermilk; CSG = chia seed gum; SMP = skim milk powder; MDG = mono-/diglyceride

10.2 Appendix B: Data analysis

Phase I

WORKSHEET 1

One-way ANOVA: Overrun versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	4	629.8	157.44	5.85	0.040
Error	5	134.6	26.92		
Total	9	764.4			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
5.18884	82.39%	68.30%	29.55%

Means

Formulation	N	Mean	StDev	95% CI
1	2	108.15	6.25	(98.72, 117.58)
2	2	103.100	0.467	(93.668, 112.532)
3	2	96.095	0.361	(86.663, 105.527)
4	2	99.32	9.36	(89.89, 108.76)
5	2	118.78	2.77	(109.35, 128.21)

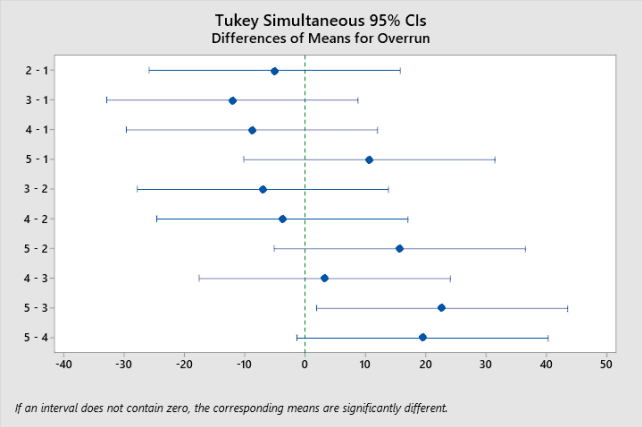
Pooled StDev = 5.18884

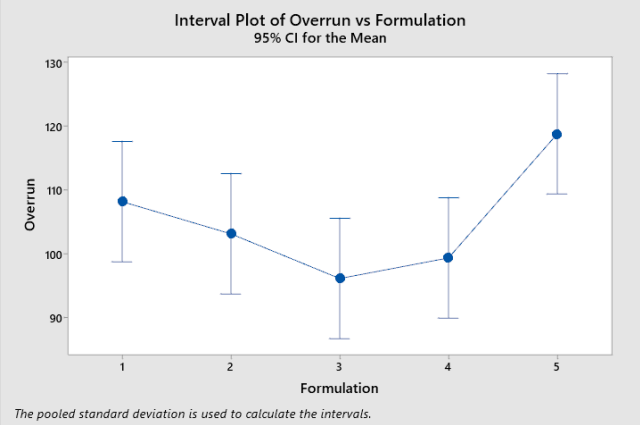
Tukey Pairwise Comparisons

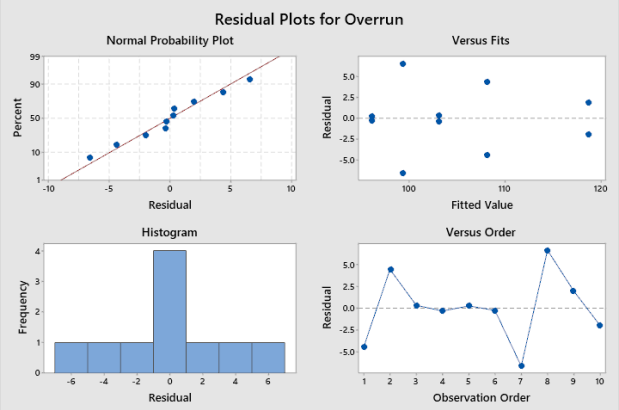
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping	
5	2	118.78	A	
1	2	108.15	A	B
2	2	103.100	A	B
4	2	99.32	A	B
3	2	96.095		B

Means that do not share a letter are significantly different.







WORKSHEET 1

One-way ANOVA: Meltdown rate versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	4	0.4638	0.11596	2.84	0.141
Error	5	0.2040	0.04081		
Total	9	0.6679			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.202015	69.45%	45.01%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
1	2	1.335	0.247	(0.968, 1.702)
2	2	0.960	0.141	(0.593, 1.327)
3	2	0.740	0.325	(0.373, 1.107)
4	2	1.1500	0.0849	(0.7828, 1.5172)
5	2	1.2600	0.0990	(0.8928, 1.6272)

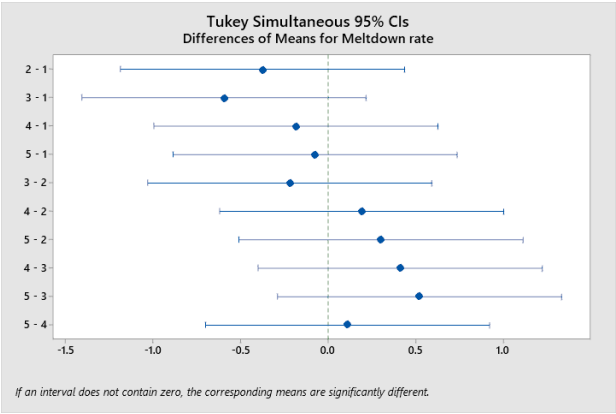
Pooled StDev = 0.202015

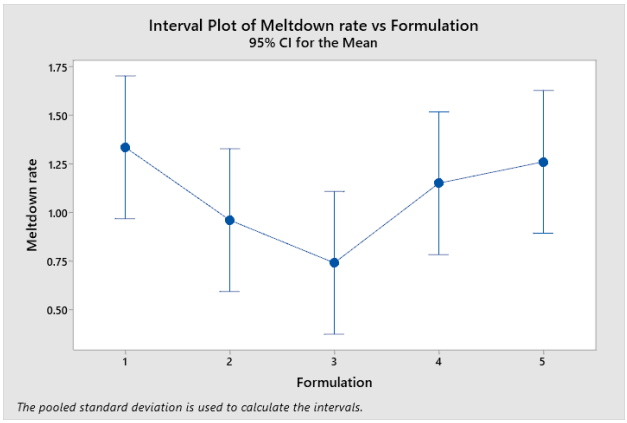
Tukey Pairwise Comparisons

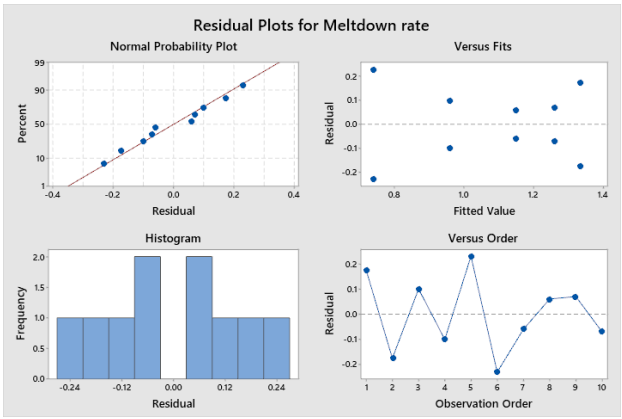
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
1	2	1.335	A
5	2	1.2600	A
4	2	1.1500	A
2	2	0.960	A
3	2	0.740	A

Means that do not share a letter are significantly different.







WORKSHEET 1

One-way ANOVA: DFI versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	4	2794.95	698.74	36.43	0.001
Error	5	95.91	19.18		
Total	9	2890.85			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.37962	96.68%	94.03%	86.73%

Means

Formulation	N	Mean	StDev	95% CI

1	2	37.59	5.99	(29.63, 45.56)
2	2	41.00	5.35	(33.03, 48.96)
3	2	47.91	3.76	(39.95, 55.87)
4	2	8.05	4.15	(0.09, 16.02)
5	2	9.4130	0.0523	(1.4523, 17.3737)

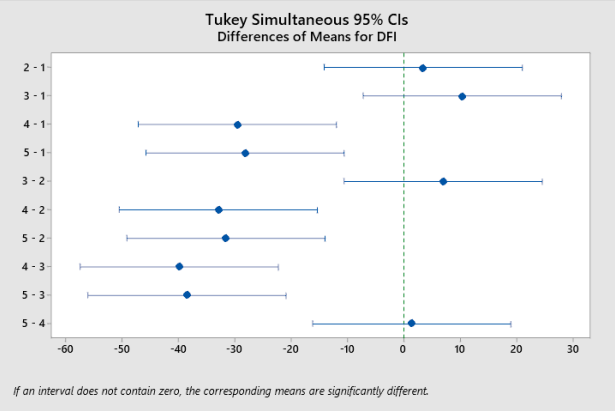
Pooled StDev = 4.37962

Tukey Pairwise Comparisons

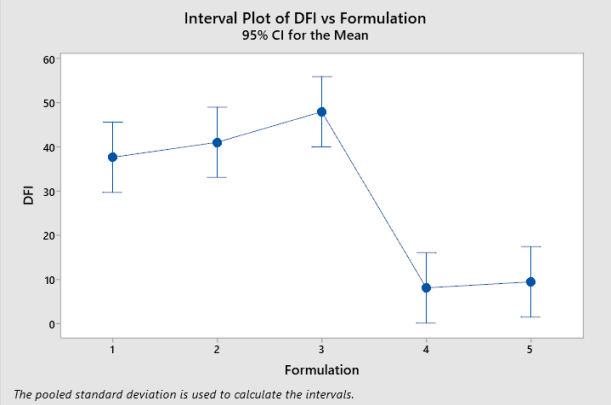
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
3	2	47.91	A
2	2	41.00	A
1	2	37.59	A
5	2	9.4130	B
4	2	8.05	B

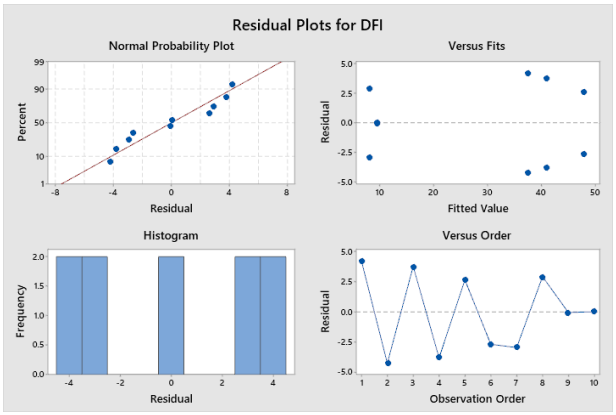
Means that do not share a letter are significantly different.



If an interval does not contain zero, the corresponding means are significantly different.



The pooled standard deviation is used to calculate the intervals.



WORKSHEET 1

One-way ANOVA: Fat globule size distribution (Mix) versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	4	10.46	2.614	0.91	0.524
Error	5	14.39	2.879		
Total	9	24.85			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.69669	42.08%	0.00%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
1	2	0.55350	0.01202	(-2.53052, 3.63752)
2	2	1.000	0.145	(-2.084, 4.085)
3	2	3.23	3.79	(0.15, 6.32)
4	2	0.730	0.185	(-2.355, 3.814)
5	2	0.5555	0.0219	(-2.5285, 3.6395)

Pooled StDev = 1.69669

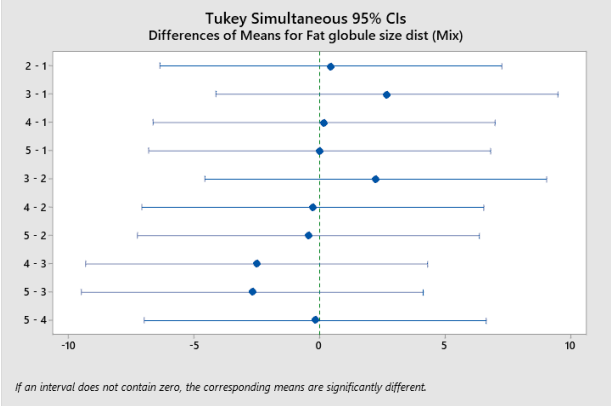
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

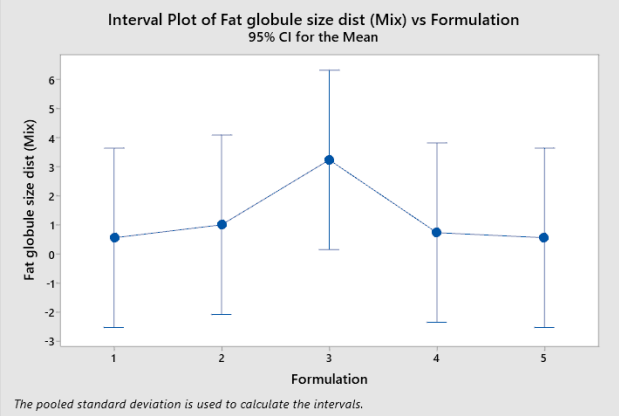
Formulation	N	Mean	Grouping
3	2	3.23	A
2	2	1.000	A
4	2	0.730	A

5	2	0.5555	A
1	2	0.55350	A

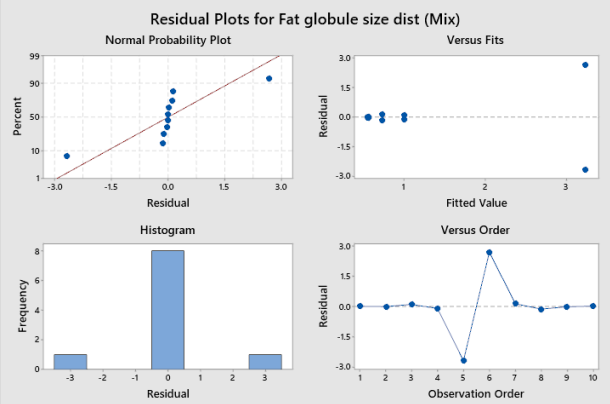
Means that do not share a letter are significantly different.



If an interval does not contain zero, the corresponding means are significantly different.



The pooled standard deviation is used to calculate the intervals.



WORKSHEET 1

One-way ANOVA: Fat globule size distribution (Foam) versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	4	7651.2	1912.79	26.64	0.001
Error	5	359.0	71.81		
Total	9	8010.2			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
8.47401	95.52%	91.93%	82.07%

Means

Formulation	N	Mean	StDev	95% CI
1	2	5.63	4.83	(-9.77, 21.03)
2	2	40.66	8.87	(25.25, 56.06)
3	2	71.5	16.0	(56.1, 86.9)
4	2	1.031	0.178	(-14.372, 16.434)
5	2	56.04	1.41	(40.63, 71.44)

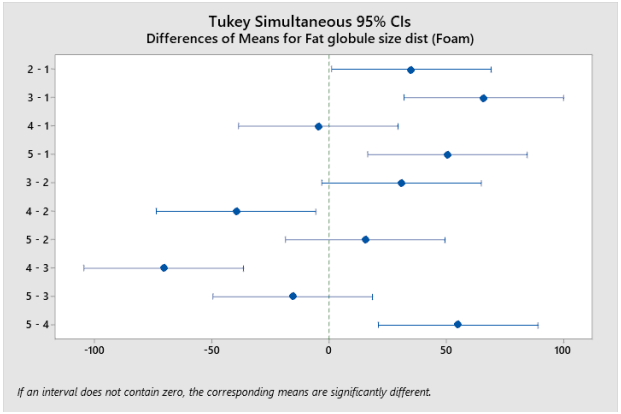
Pooled StDev = 8.47401

Tukey Pairwise Comparisons

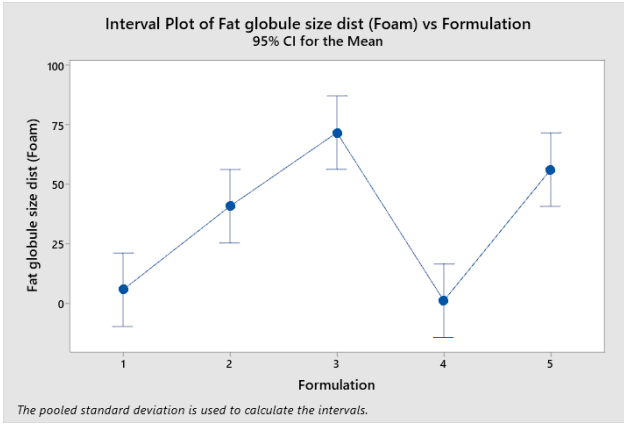
Grouping Information Using the Tukey Method and 95% Confidence

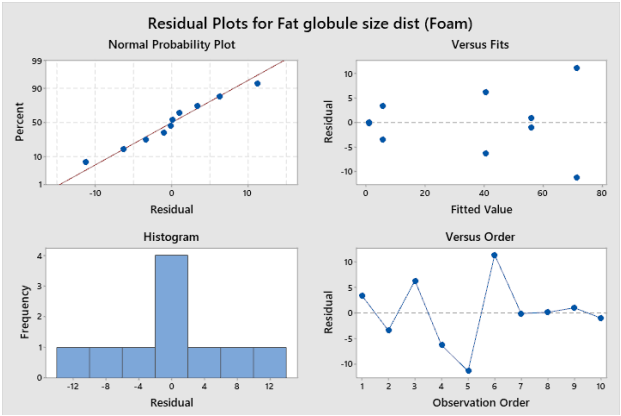
Formulation	N	Mean	Grouping	
3	2	71.5	A	
5	2	56.04	A	
2	2	40.66	A	
1	2	5.63		B
4	2	1.031		B

Means that do not share a letter are significantly different.



If an interval does not contain zero, the corresponding means are significantly different.





WORKSHEET 1

One-way ANOVA: pH versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	4	0.008840	0.002210	1.61	0.303
Error	5	0.006850	0.001370		
Total	9	0.015690			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0370135	56.34%	21.41%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
1	2	6.5400	0.0566	(6.4727, 6.6073)
2	2	6.5500	0.0566	(6.4827, 6.6173)
3	2	6.590	0.000	(6.523, 6.657)
4	2	6.5650	0.0212	(6.4977, 6.6323)
5	2	6.500	0.000	(6.433, 6.567)

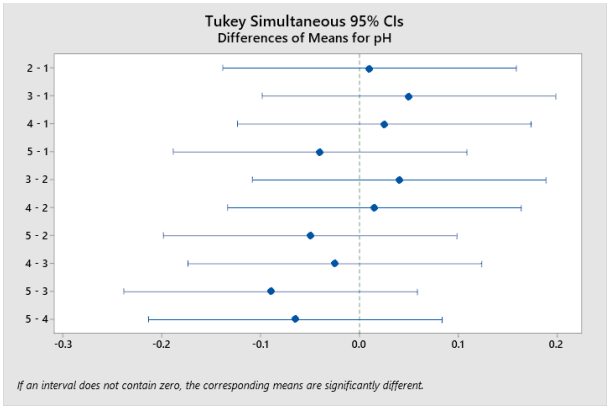
Pooled StDev = 0.0370135

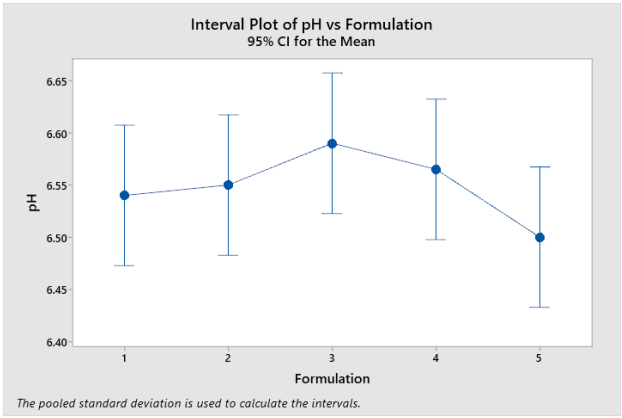
Tukey Pairwise Comparisons

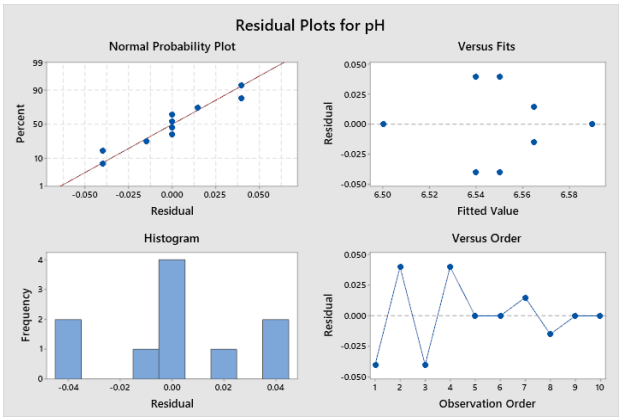
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
3	2	6.590	A
4	2	6.5650	A
2	2	6.5500	A
1	2	6.5400	A
5	2	6.500	A

Means that do not share a letter are significantly different.







WORKSHEET 1

One-way ANOVA: Hardness versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	4	1219	304.8	0.46	0.764
Error	5	3314	662.9		
Total	9	4534			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
25.7462	26.90%	0.00%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
1	2	47.12	2.69	(0.32, 93.91)
2	2	40.06	7.23	(-6.74, 86.86)
3	2	61.8	39.7	(15.0, 108.6)
4	2	52.9	41.0	(6.1, 99.7)
5	2	71.560	1.032	(24.762, 118.358)

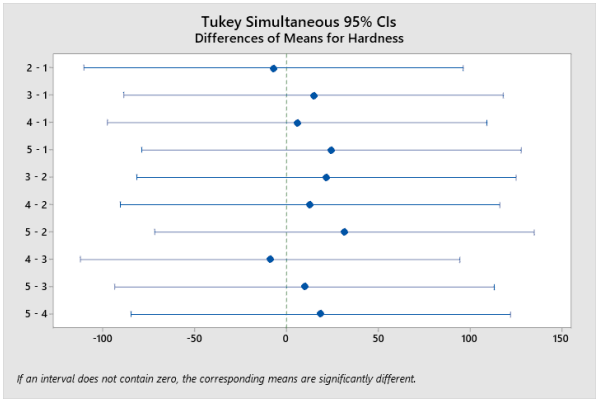
Pooled StDev = 25.7462

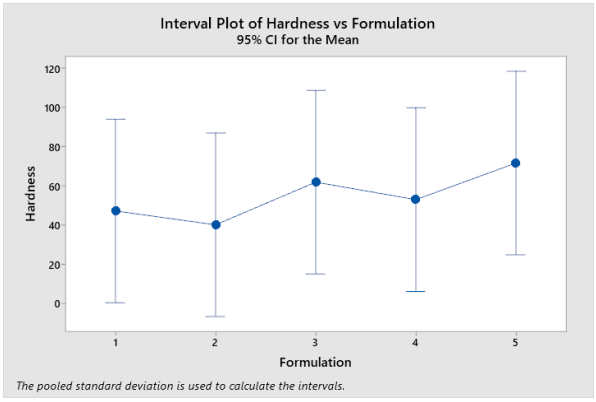
Tukey Pairwise Comparisons

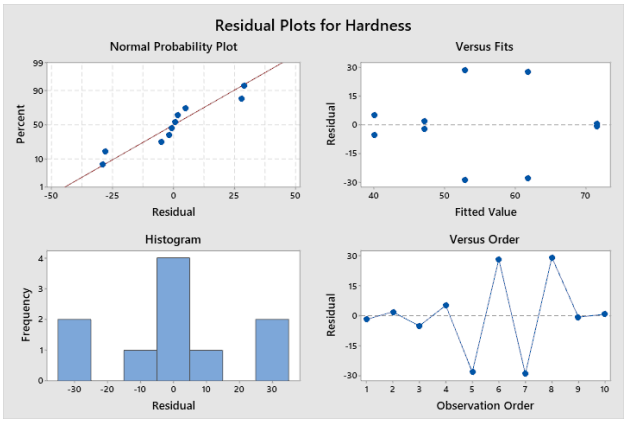
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
5	2	71.560	A
3	2	61.8	A
4	2	52.9	A
1	2	47.12	A
2	2	40.06	A

Means that do not share a letter are significantly different.







Phase II

WORKSHEET 1

One-way ANOVA: Overrun versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 4, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	750.8	250.25	3.01	0.158
Error	4	333.0	83.26		
Total	7	1083.8			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
9.12469	69.27%	46.22%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
2	2	103.100	0.467	(85.186, 121.014)
4	2	99.32	9.36	(81.41, 117.24)
6	2	95.9	14.5	(78.0, 113.8)
7	2	77.86	5.83	(59.95, 95.77)

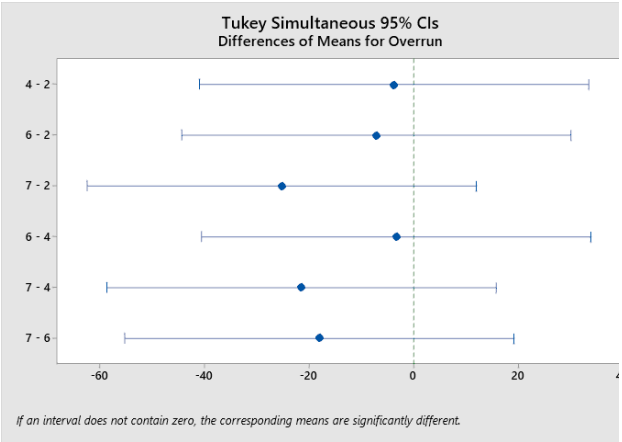
Pooled StDev = 9.12469

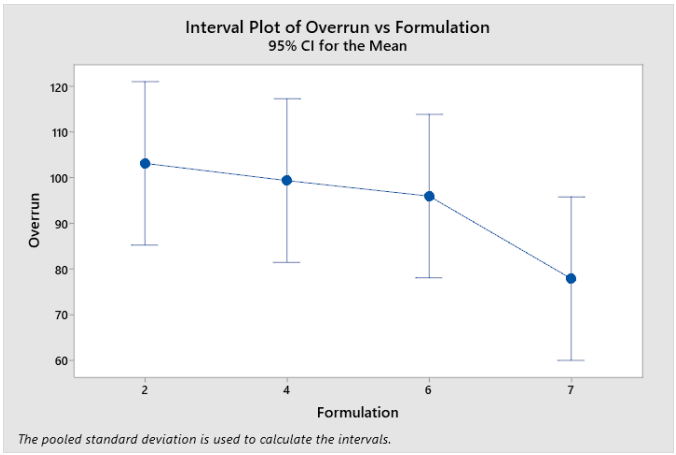
Tukey Pairwise Comparisons

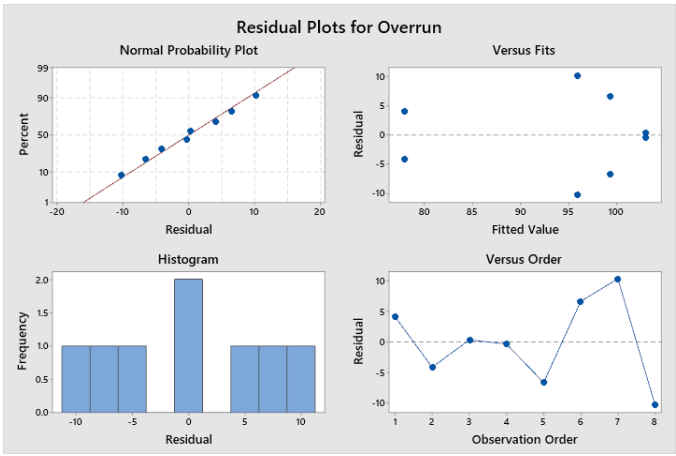
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
2	2	103.100	A
4	2	99.32	A
6	2	95.9	A
7	2	77.86	A

Means that do not share a letter are significantly different.







WORKSHEET 1

One-way ANOVA: Meltdown rate versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 4, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	0.44695	0.14898	12.73	0.016
Error	4	0.04680	0.01170		
Total	7	0.49375			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.108167	90.52%	83.41%	62.09%

Means

Formulation	N	Mean	StDev	95% CI
2	2	0.960	0.141	(0.748, 1.172)
4	2	1.1500	0.0849	(0.9376, 1.3624)
6	2	1.2500	0.0990	(1.0376, 1.4624)
7	2	1.6100	0.0990	(1.3976, 1.8224)

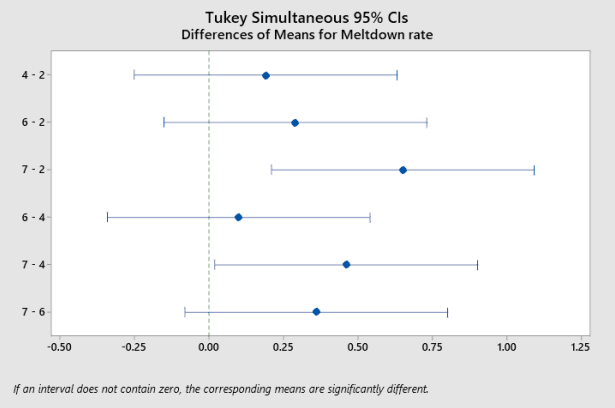
Pooled StDev = 0.108167

Tukey Pairwise Comparisons

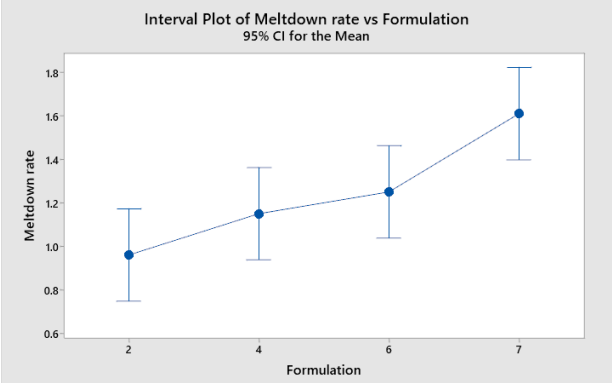
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
7	2	1.6100	A
6	2	1.2500	A B
4	2	1.1500	B
2	2	0.960	B

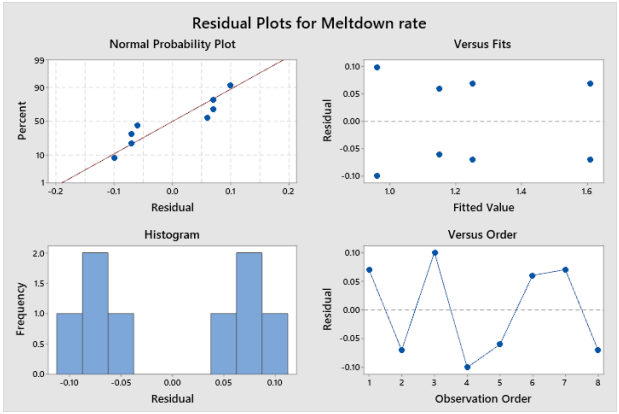
Means that do not share a letter are significantly different.



If an interval does not contain zero, the corresponding means are significantly different.



The pooled standard deviation is used to calculate the intervals.



WORKSHEET 1

One-way ANOVA: DFI versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 4, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	1675.63	558.54	35.92	0.002
Error	4	62.19	15.55		
Total	7	1737.82			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.94305	96.42%	93.74%	85.69%

Means

Formulation	N	Mean	StDev	95% CI
2	2	41.00	5.35	(33.25, 48.74)
4	2	8.05	4.15	(0.31, 15.80)
6	2	6.37	3.73	(-1.37, 14.12)
7	2	8.43	1.55	(0.69, 16.17)

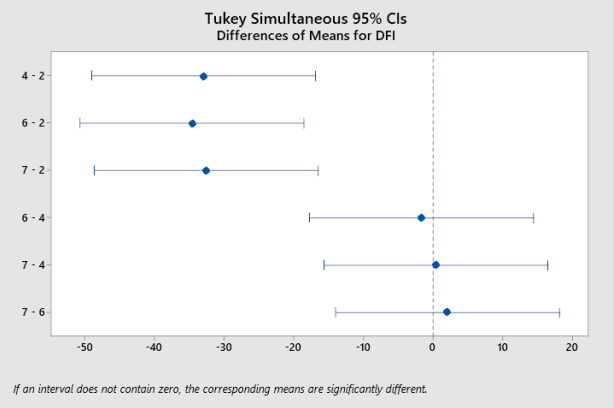
Pooled StDev = 3.94305

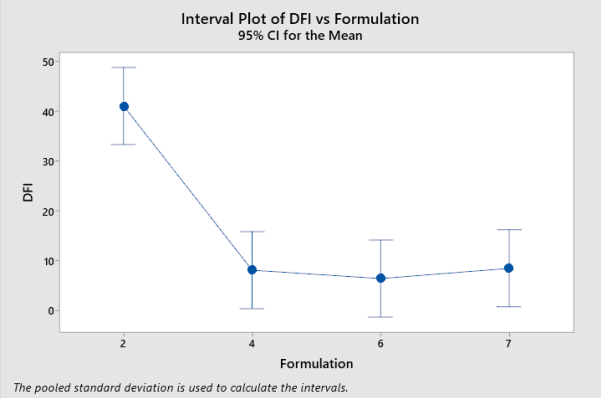
Tukey Pairwise Comparisons

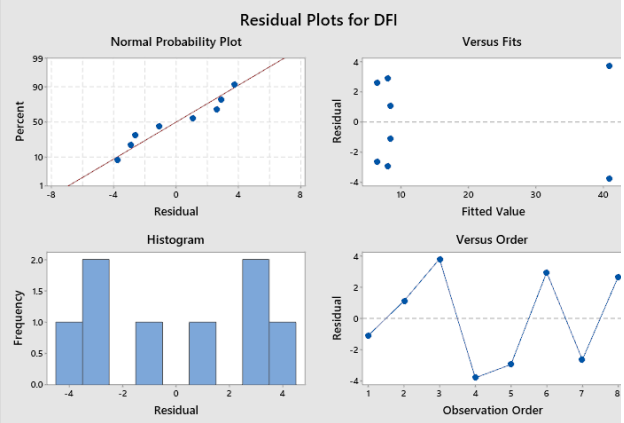
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
2	2	41.00	A
7	2	8.43	B
4	2	8.05	B
6	2	6.37	B

Means that do not share a letter are significantly different.







Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 4, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	0.20603	0.06868	4.73	0.084
Error	4	0.05804	0.01451		
Total	7	0.26407			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.120460	78.02%	61.54%	12.08%

Means

Formulation	N	Mean	StDev	95% CI
2	2	1.000	0.145	(0.764, 1.237)
4	2	0.730	0.185	(0.493, 0.966)
6	2	0.5745	0.0544	(0.3380, 0.8110)
7	2	0.65250	0.00212	(0.41601, 0.88899)

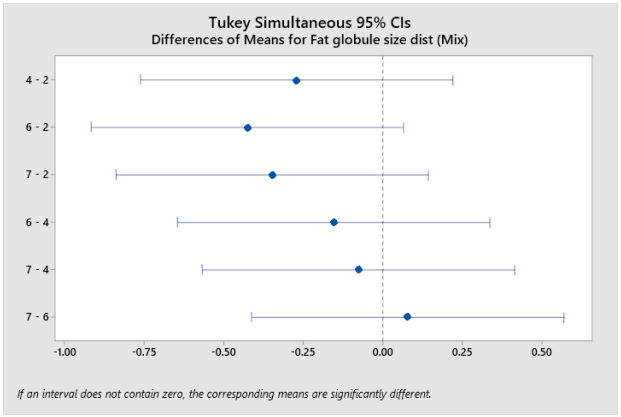
Pooled StDev = 0.120460

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
2	2	1.000	A
4	2	0.730	A
7	2	0.65250	A
6	2	0.5745	A

Means that do not share a letter are significantly different.

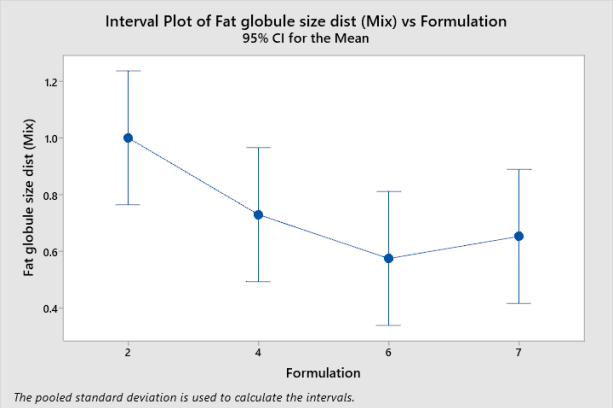


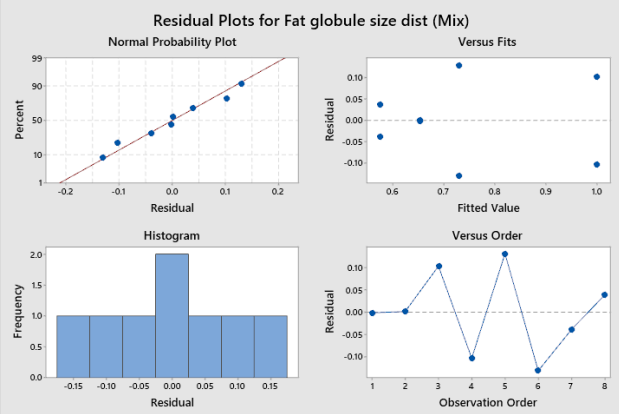
WORKSHEET 1

One-way ANOVA: Fat globule size distribution (Mix) versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05





WORKSHEET 1

One-way ANOVA: Fat globule size distribution (Foam) versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 4, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	2392	797.3	2.31	0.218
Error	4	1379	344.8		
Total	7	3771			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
18.5689	63.43%	35.99%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
2	2	40.66	8.87	(4.20, 77.11)
4	2	1.031	0.178	(-35.424, 37.486)
6	2	0.912	0.160	(-35.543, 37.367)
7	2	28.1	36.1	(-8.3, 64.6)

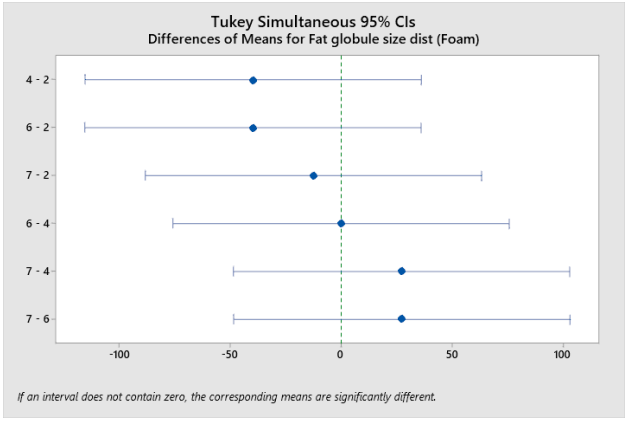
Pooled StDev = 18.5689

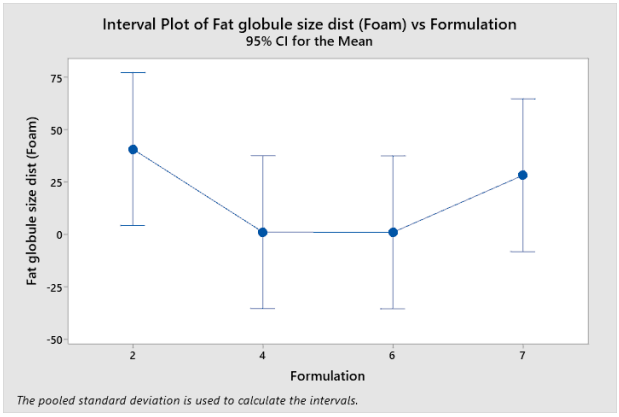
Tukey Pairwise Comparisons

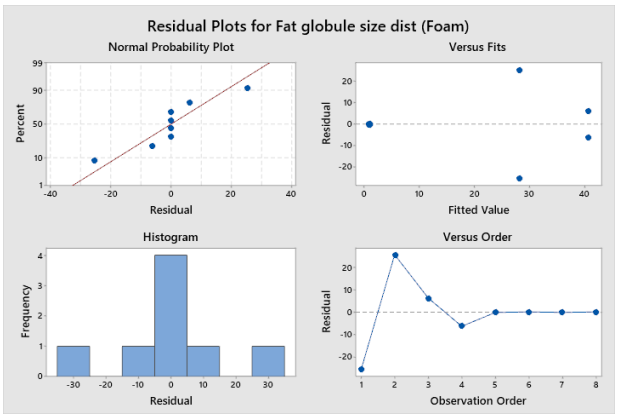
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
2	2	40.66	A
7	2	28.1	A
4	2	1.031	A
6	2	0.912	A

Means that do not share a letter are significantly different.







WORKSHEET 1

One-way ANOVA: pH versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 4, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	0.002250	0.000750	0.81	0.551
Error	4	0.003700	0.000925		
Total	7	0.005950			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0304138	37.82%	0.00%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
2	2	6.5500	0.0566	(6.4903, 6.6097)
4	2	6.5650	0.0212	(6.5053, 6.6247)
6	2	6.520	0.000	(6.460, 6.580)
7	2	6.55500	0.00707	(6.49529, 6.61471)

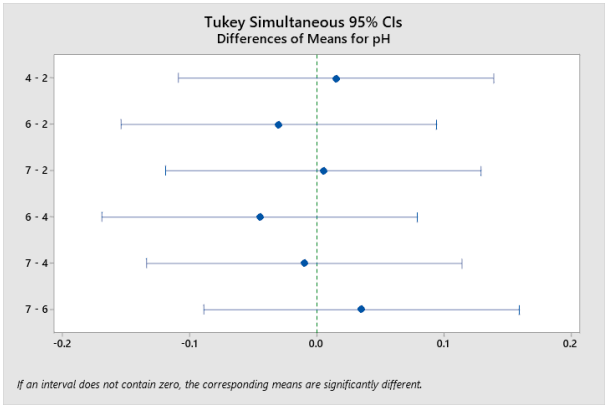
Pooled StDev = 0.0304138

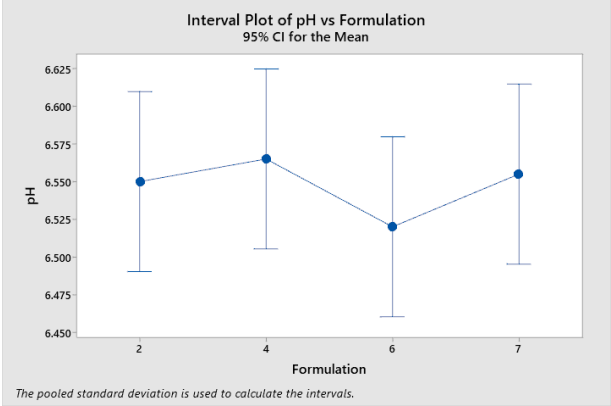
Tukey Pairwise Comparisons

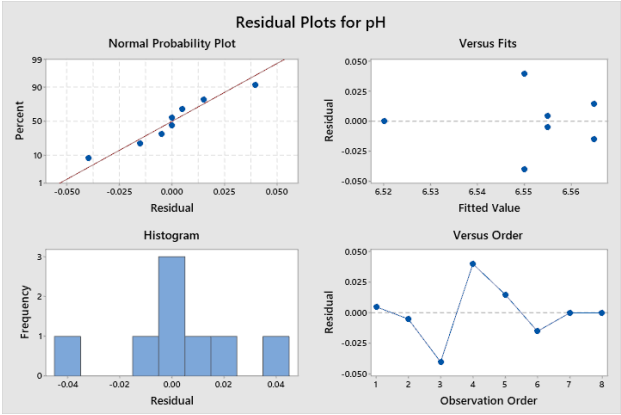
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
4	2	6.5650	A
7	2	6.55500	A
2	2	6.5500	A
6	2	6.520	A

Means that do not share a letter are significantly different.







WORKSHEET 1

One-way ANOVA: Hardness versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 4, 6, 7

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	3212	1070.7	2.09	0.244
Error	4	2046	511.4		
Total	7	5258			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
22.6151	61.09%	31.91%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
2	2	40.06	7.23	(-4.34, 84.46)
4	2	52.9	41.0	(8.5, 97.3)
6	2	56.39	8.68	(11.99, 100.78)
7	2	93.9	15.5	(49.5, 138.3)

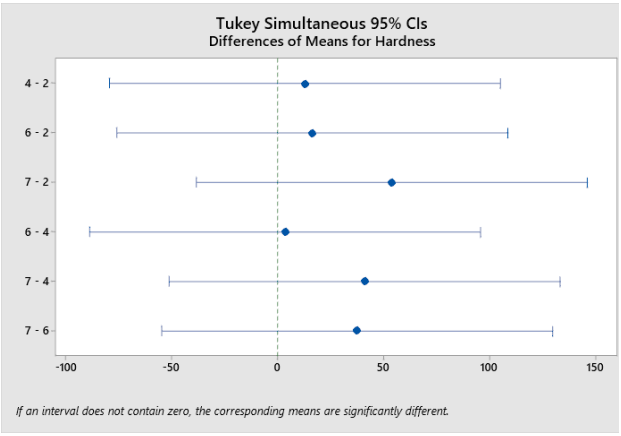
Pooled StDev = 22.6151

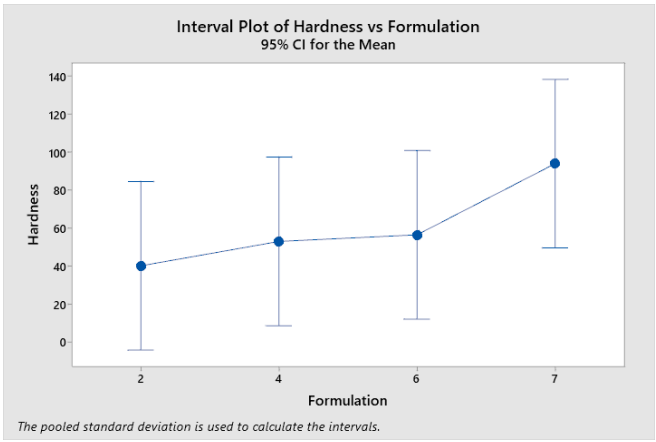
Tukey Pairwise Comparisons

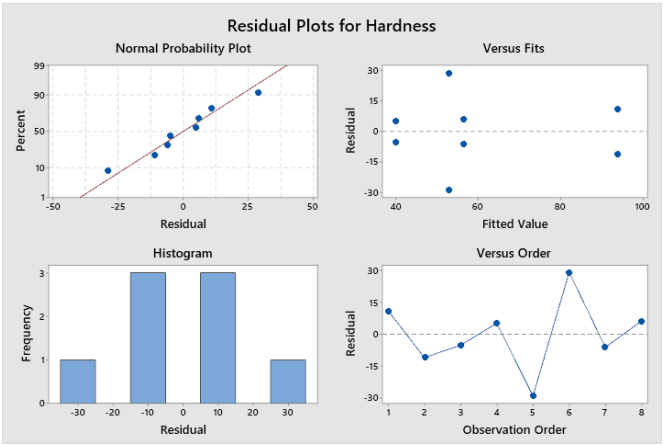
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
7	2	93.9	A
6	2	56.39	A
4	2	52.9	A
2	2	40.06	A

Means that do not share a letter are significantly different.







Phase III

WORKSHEET 1

One-way ANOVA: Overrun versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 8, 9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	719.19	239.731	26.09	0.004
Error	4	36.75	9.188		
Total	7	755.95			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.03121	95.14%	91.49%	80.55%

Means

Formulation	N	Mean	StDev	95% CI
2	2	103.100	0.467	(97.149, 109.051)
7	2	77.86	5.83	(71.91, 83.81)
8	2	82.70	1.61	(76.75, 88.66)
9	2	88.9000	0.0990	(82.9490, 94.8510)

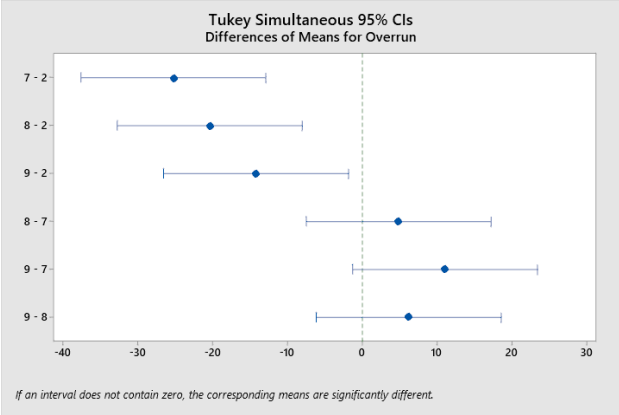
Pooled StDev = 3.03121

Tukey Pairwise Comparisons

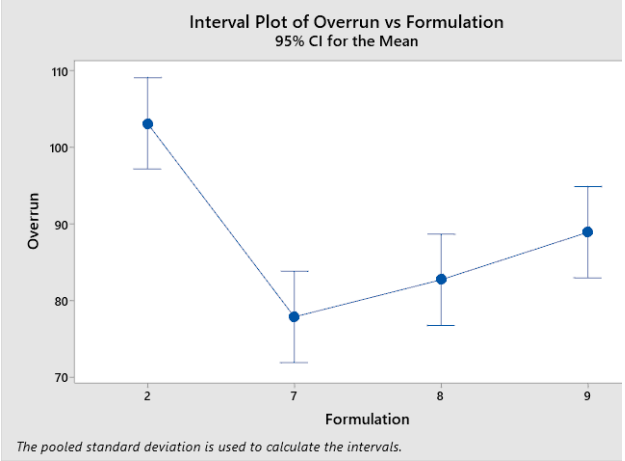
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
2	2	103.100	A
9	2	88.9000	B
8	2	82.70	B
7	2	77.86	B

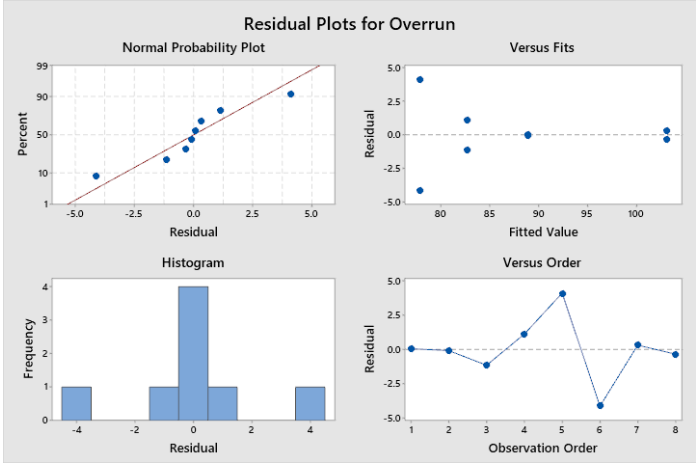
Means that do not share a letter are significantly different.



If an interval does not contain zero, the corresponding means are significantly different.



The pooled standard deviation is used to calculate the intervals.



WORKSHEET 1

One-way ANOVA: Meltdown rate versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 8, 9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	0.59745	0.199150	20.80	0.007
Error	4	0.03830	0.009575		
Total	7	0.63575			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0978519	93.98%	89.46%	75.90%

Means

Formulation	N	Mean	StDev	95% CI
2	2	0.960	0.141	(0.768, 1.152)
7	2	1.6100	0.0990	(1.4179, 1.8021)
8	2	1.5950	0.0778	(1.4029, 1.7871)
9	2	1.5650	0.0495	(1.3729, 1.7571)

Pooled StDev = 0.0978519

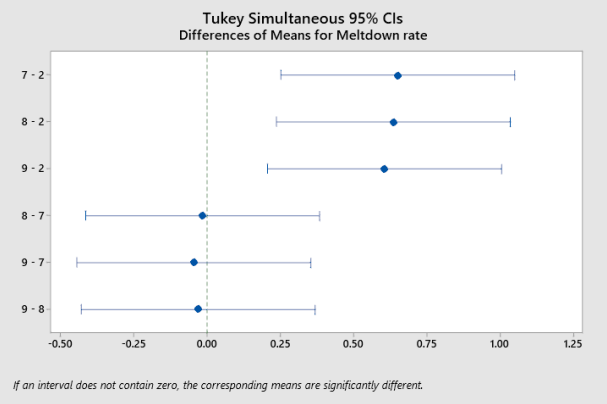
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

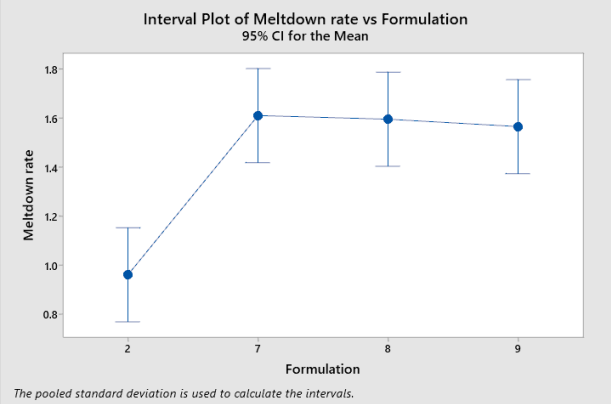
Formulation	N	Mean	Grouping
7	2	1.6100	A
8	2	1.5950	A

9	2	1.5650	A
2	2	0.960	B

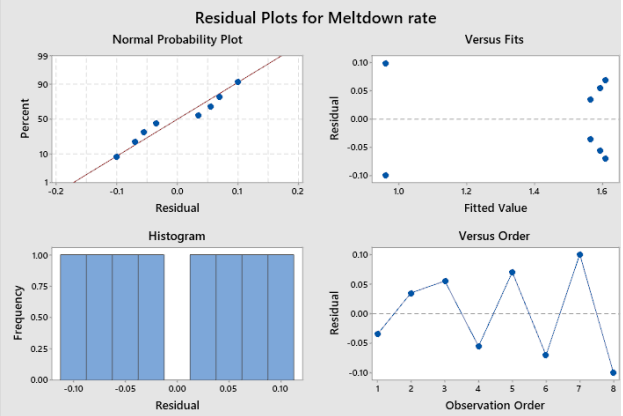
Means that do not share a letter are significantly different.



If an interval does not contain zero, the corresponding means are significantly different.



The pooled standard deviation is used to calculate the intervals.



Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 8, 9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	1756.79	585.60	49.25	0.001
Error	4	47.56	11.89		
Total	7	1804.34			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.44814	97.36%	95.39%	89.46%

Means

Formulation	N	Mean	StDev	95% CI
2	2	41.00	5.35	(34.23, 47.76)
7	2	8.43	1.55	(1.66, 15.20)
8	2	8.030	1.315	(1.260, 14.800)
9	2	4.43	3.84	(-2.34, 11.20)

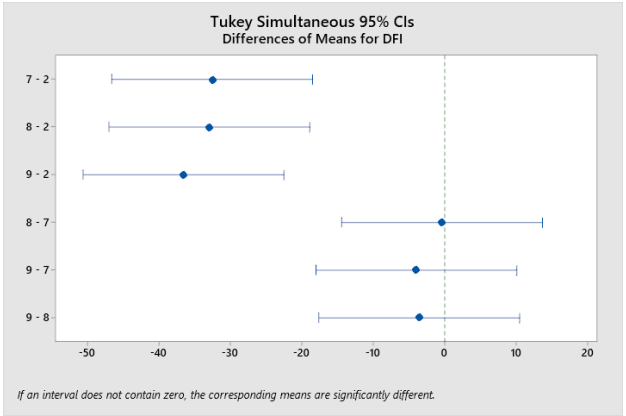
Pooled StDev = 3.44814

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
2	2	41.00	A
7	2	8.43	B
8	2	8.030	B
9	2	4.43	B

Means that do not share a letter are significantly different.

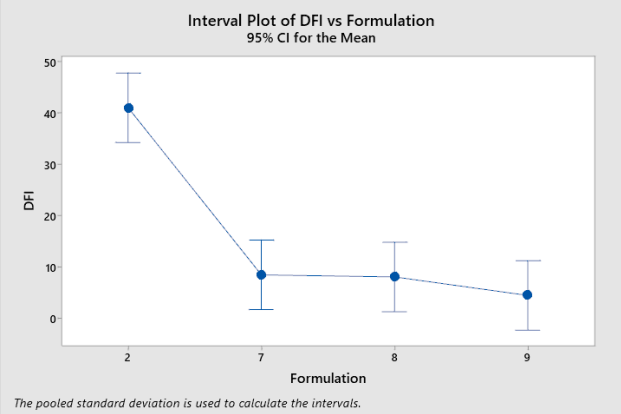


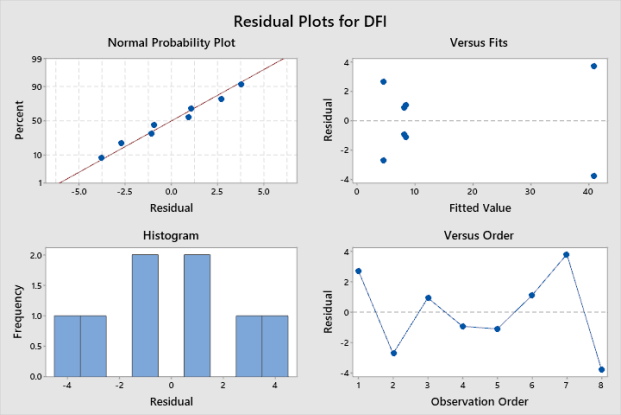
If an interval does not contain zero, the corresponding means are significantly different.

WORKSHEET 1

One-way ANOVA: DFI versus Formulation

Method





Formulation	N	Mean	StDev	95% CI
2	2	1.000	0.145	(0.839, 1.162)
7	2	0.65250	0.00212	(0.49051, 0.81449)
8	2	0.7395	0.0686	(0.5775, 0.9015)
9	2	0.5805	0.0389	(0.4185, 0.7425)

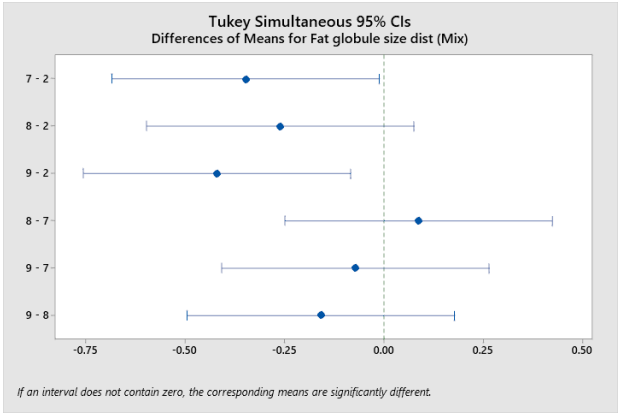
Pooled StDev = 0.0825136

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping	
2	2	1.000	A	
8	2	0.7395	A	B
7	2	0.65250		B
9	2	0.5805		B

Means that do not share a letter are significantly different.



WORKSHEET 1

One-way ANOVA: Fat globule size distribution (Mix) versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 8, 9

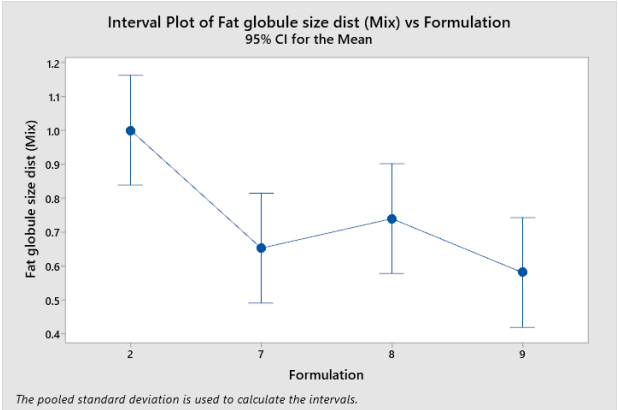
Analysis of Variance

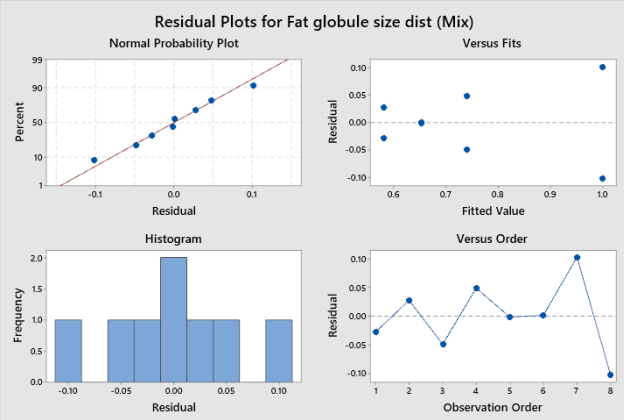
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	0.20183	0.067276	9.88	0.025
Error	4	0.02723	0.006809		
Total	7	0.22906			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0825136	88.11%	79.19%	52.44%

Means





WORKSHEET 1

One-way ANOVA: Fat globule size distribution (Foam) versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 8, 9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	1187	395.7	1.14	0.433
Error	4	1384	345.9		
Total	7	2571			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
18.5994	46.18%	5.81%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
2	2	40.66	8.87	(4.14, 77.17)
7	2	28.1	36.1	(-8.4, 64.7)
8	2	6.743	0.304	(-29.772, 43.258)
9	2	22.53	2.12	(-13.99, 59.04)

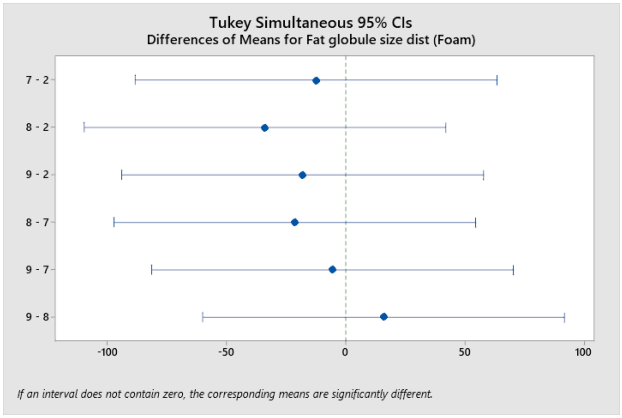
Pooled StDev = 18.5994

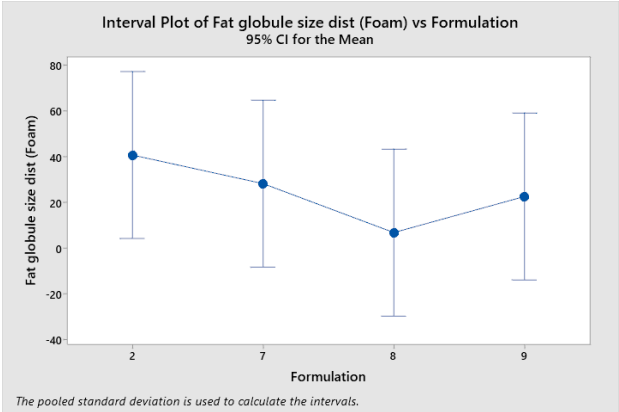
Tukey Pairwise Comparisons

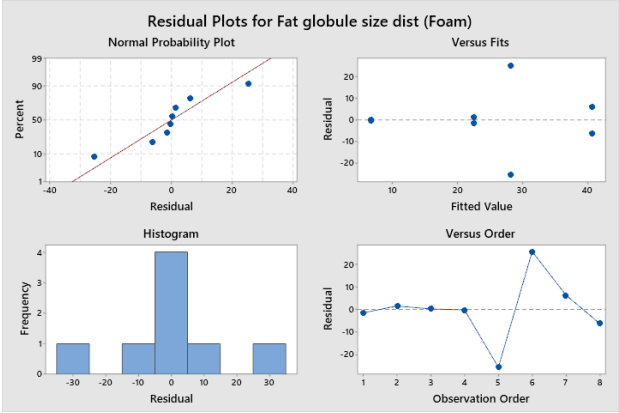
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
2	2	40.66	A
7	2	28.1	A
9	2	22.53	A
8	2	6.743	A

Means that do not share a letter are significantly different.







WORKSHEET 1

One-way ANOVA: pH versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 8, 9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	0.009100	0.003033	3.47	0.130
Error	4	0.003500	0.000875		
Total	7	0.012600			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0295804	72.22%	51.39%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
2	2	6.5500	0.0566	(6.4919, 6.6081)
7	2	6.55500	0.00707	(6.49693, 6.61307)
8	2	6.5000	0.0141	(6.4419, 6.5581)
9	2	6.47500	0.00707	(6.41693, 6.53307)

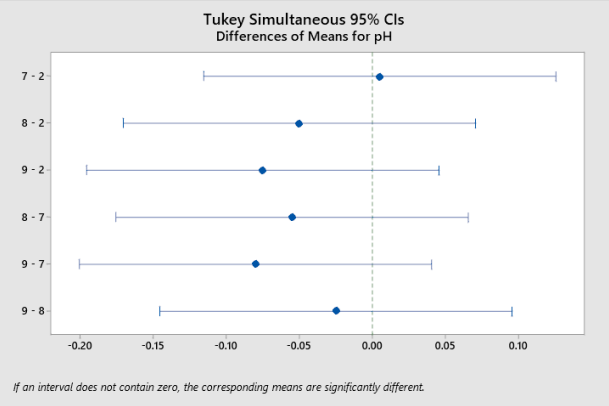
Pooled StDev = 0.0295804

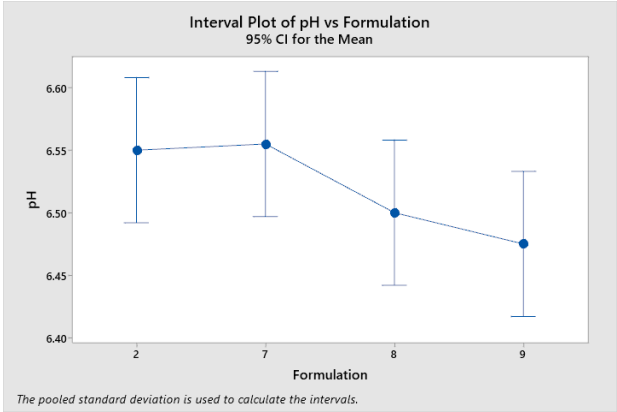
Tukey Pairwise Comparisons

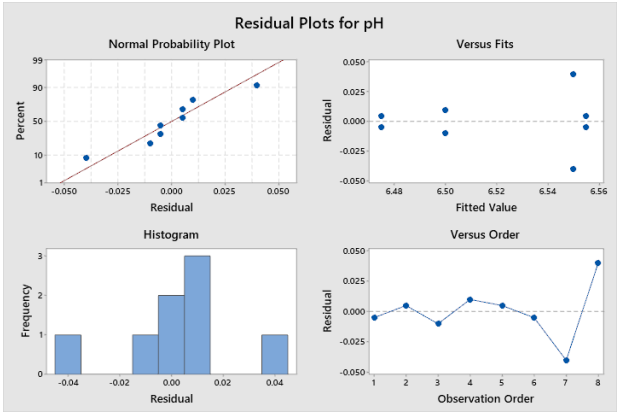
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
7	2	6.55500	A
2	2	6.5500	A
8	2	6.5000	A
9	2	6.47500	A

Means that do not share a letter are significantly different.







WORKSHEET 1

One-way ANOVA: Hardness versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 8, 9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	5352	1784.0	2.33	0.216
Error	4	3061	765.2		
Total	7	8413			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
27.6627	63.62%	36.33%	0.00%

Means

Formulation	N	Mean	StDev	95% CI

2	2	40.06	7.23	(-14.25, 94.37)
7	2	93.9	15.5	(39.6, 148.2)
8	2	102.2	41.0	(47.9, 156.5)
9	2	101.8	33.0	(47.5, 156.2)

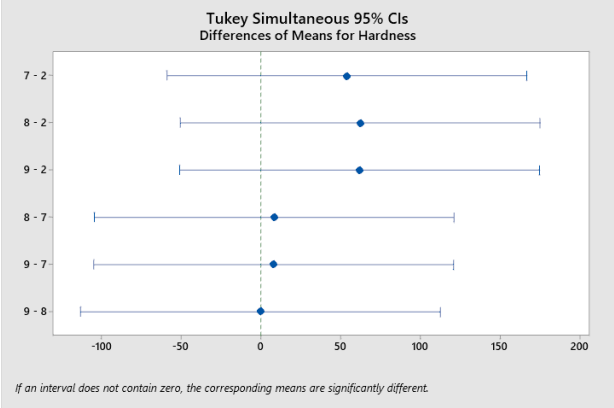
Pooled StDev = 27.6627

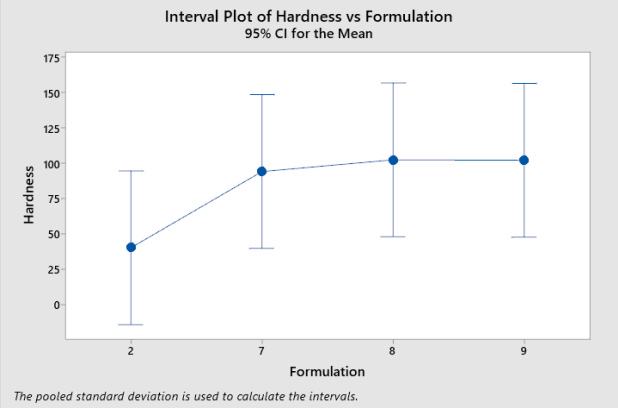
Tukey Pairwise Comparisons

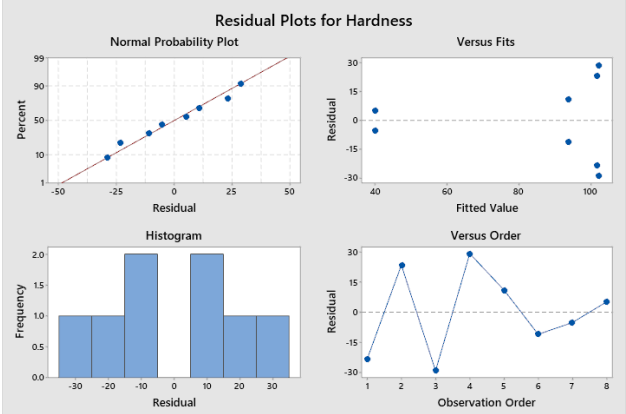
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
8	2	102.2	A
9	2	101.8	A
7	2	93.9	A
2	2	40.06	A

Means that do not share a letter are significantly different.







Phase IV

WORKSHEET 1

One-way ANOVA: Overrun versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 9, 10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	643.60	214.533	24.90	0.005
Error	4	34.47	8.616		
Total	7	678.06			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.93535	94.92%	91.10%	79.67%

Means

Formulation	N	Mean	StDev	95% CI
2	2	103.100	0.467	(97.337, 108.863)
7	2	77.86	5.83	(72.10, 83.62)
9	2	88.9000	0.0990	(83.1372, 94.6628)
10	2	88.490	0.537	(82.727, 94.253)

Pooled StDev = 2.93535

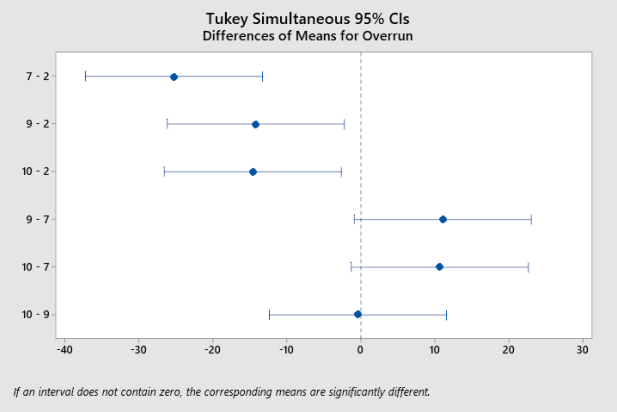
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

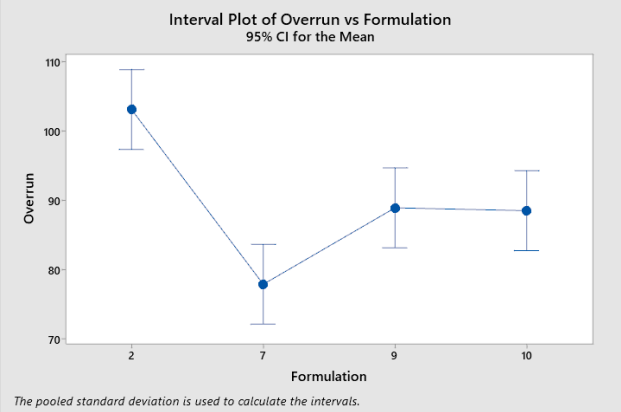
Formulation	N	Mean	Grouping

2	2	103.100	A
9	2	88.9000	B
10	2	88.490	B
7	2	77.86	B

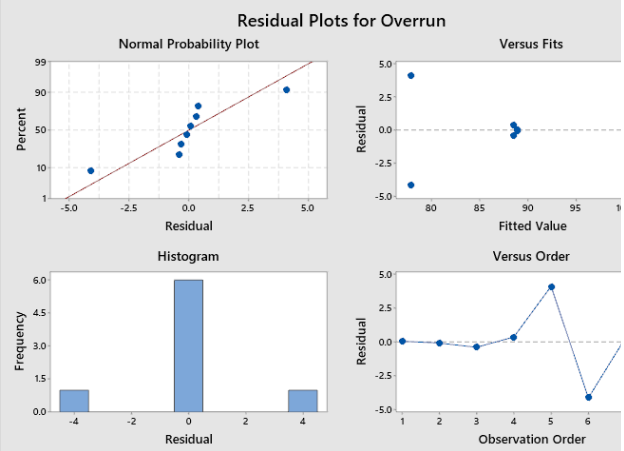
Means that do not share a letter are significantly different.



If an interval does not contain zero, the corresponding means are significantly different.



The pooled standard deviation is used to calculate the intervals.



One-way ANOVA: Meltdown rate versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 9, 10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	0.55574	0.185246	21.76	0.006
Error	4	0.03405	0.008512		
Total	7	0.58979			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0922632	94.23%	89.90%	76.91%

Means

Formulation	N	Mean	StDev	95% CI
2	2	0.960	0.141	(0.779, 1.141)
7	2	1.6100	0.0990	(1.4289, 1.7911)
9	2	1.5650	0.0495	(1.3839, 1.7461)
10	2	1.2400	0.0424	(1.0589, 1.4211)

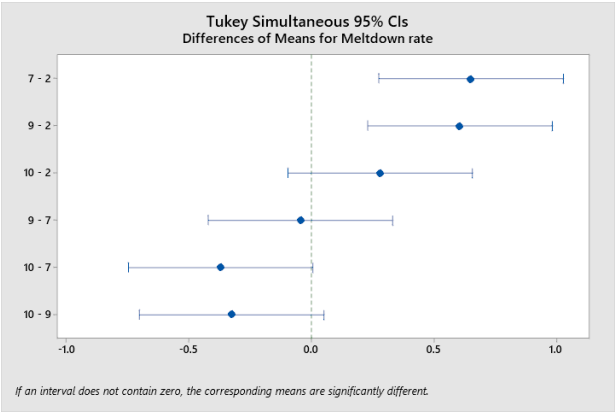
Pooled StDev = 0.0922632

Tukey Pairwise Comparisons

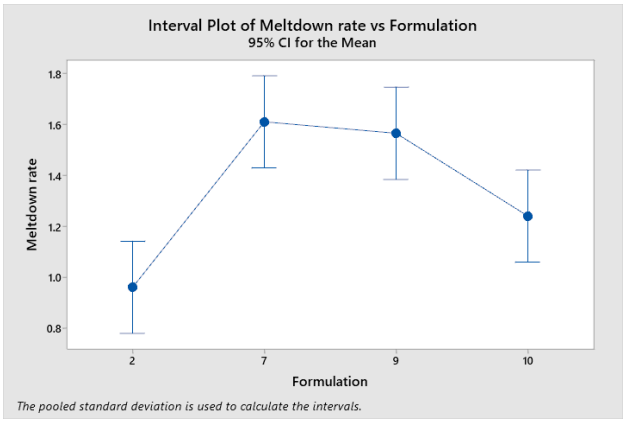
Grouping Information Using the Tukey Method and 95% Confidence

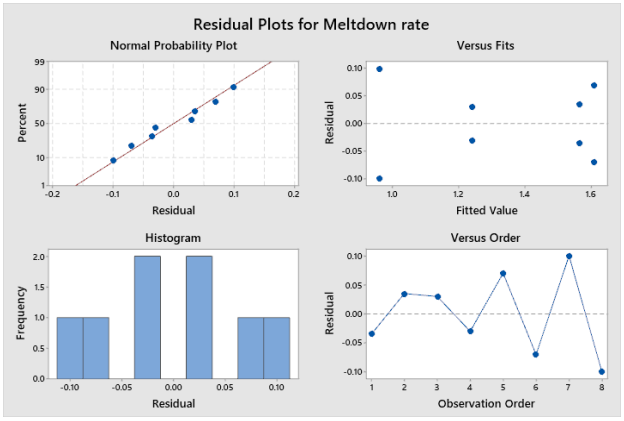
Formulation	N	Mean	Grouping	
7	2	1.6100	A	
9	2	1.5650	A	
10	2	1.2400	A	B
2	2	0.960		B

Means that do not share a letter are significantly different.



If an interval does not contain zero, the corresponding means are significantly different.





2	2	41.00	5.35	(34.20, 47.79)
7	2	8.43	1.55	(1.64, 15.23)
9	2	4.43	3.84	(-2.37, 11.22)
10	2	11.96	1.44	(5.16, 18.75)

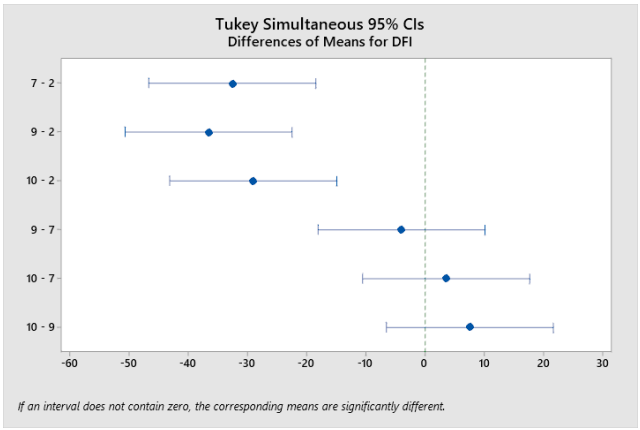
Pooled StDev = 3.46099

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
2	2	41.00	A
10	2	11.96	B
7	2	8.43	B
9	2	4.43	B

Means that do not share a letter are significantly different.



WORKSHEET 1

One-way ANOVA: DFI versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 9, 10

Analysis of Variance

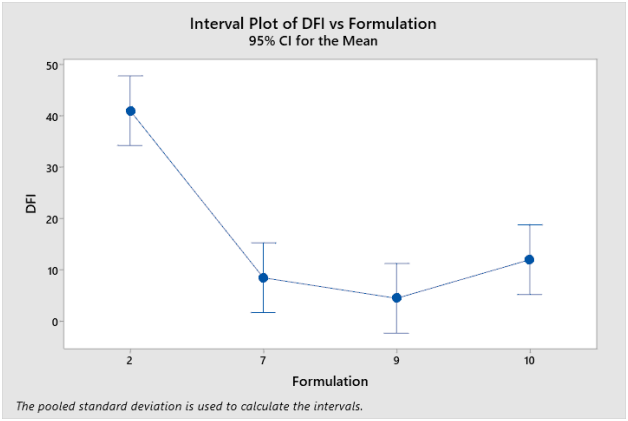
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	1663.01	554.34	46.28	0.001
Error	4	47.91	11.98		
Total	7	1710.92			

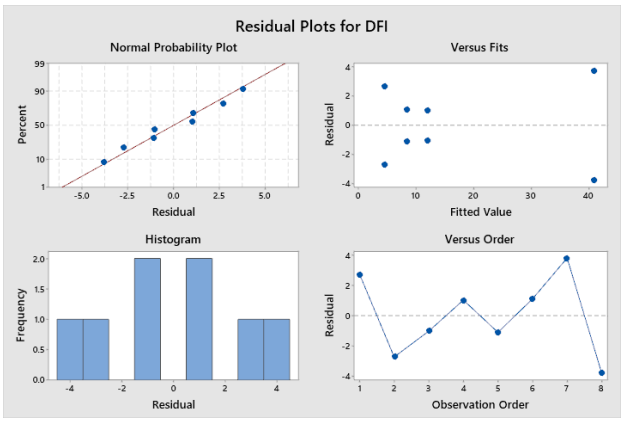
Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.46099	97.20%	95.10%	88.80%

Means

Formulation	N	Mean	StDev	95% CI





WORKSHEET 1

One-way ANOVA: Fat globule size distribution (Mix) versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 9, 10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	221.873	73.9575	354.68	0.000
Error	4	0.834	0.2085		
Total	7	222.707			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.456636	99.63%	99.34%	98.50%

Means

Formulation	N	Mean	StDev	95% CI
2	2	1.000	0.145	(0.104, 1.897)
7	2	0.65250	0.00212	(-0.24399, 1.54899)
9	2	0.5805	0.0389	(-0.3160, 1.4770)
10	2	12.901	0.901	(12.005, 13.797)

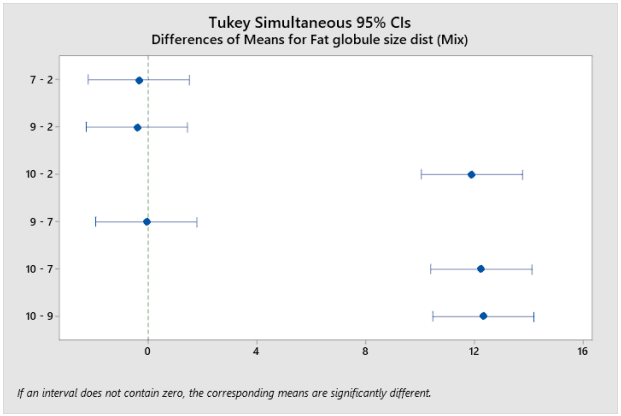
Pooled StDev = 0.456636

Tukey Pairwise Comparisons

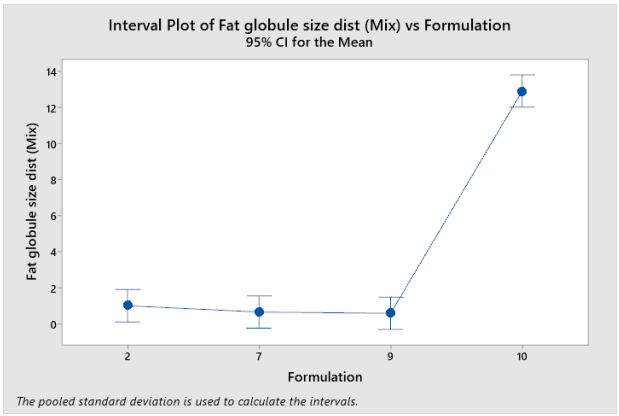
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
10	2	12.901	A
2	2	1.000	B
7	2	0.65250	B
9	2	0.5805	B

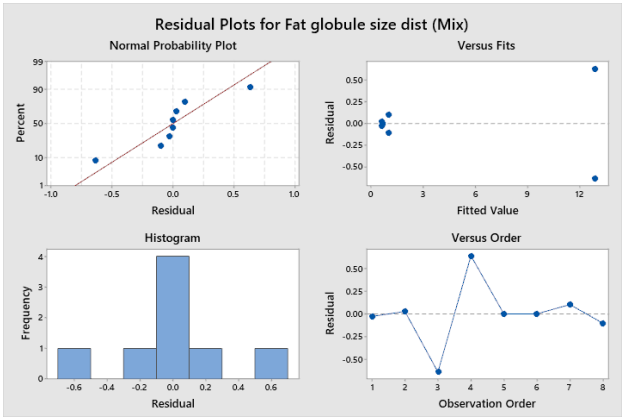
Means that do not share a letter are significantly different.



If an interval does not contain zero, the corresponding means are significantly different.



The pooled standard deviation is used to calculate the intervals.



WORKSHEET 1

One-way ANOVA: Fat globule size distribution (Foam) versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal

Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 9, 10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	758.6	252.9	0.73	0.586
Error	4	1387.7	346.9		
Total	7	2146.4			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
18.6262	35.35%	0.00%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
2	2	40.66	8.87	(4.09, 77.23)
7	2	28.1	36.1	(-8.4, 64.7)
9	2	22.53	2.12	(-14.04, 59.09)
10	2	13.83	2.02	(-22.74, 50.40)

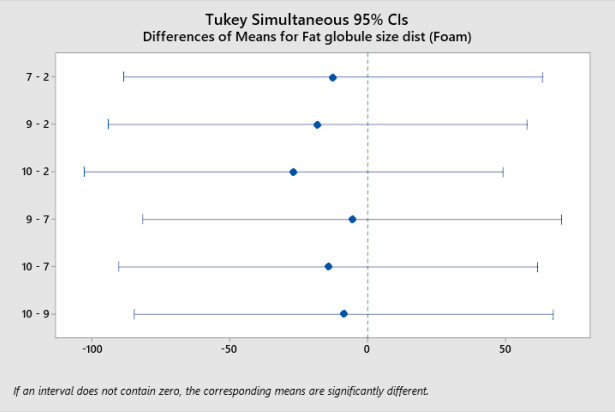
Pooled StDev = 18.6262

Tukey Pairwise Comparisons

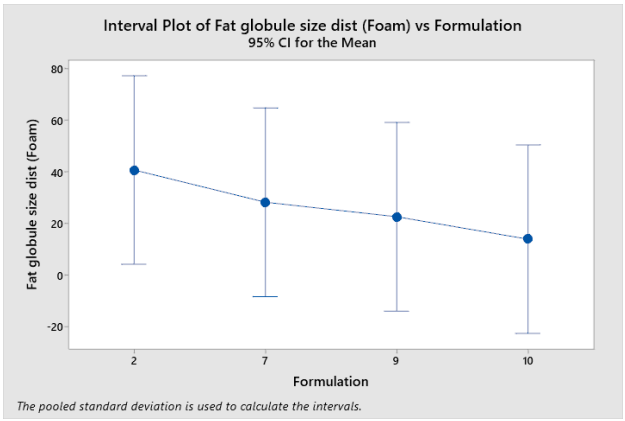
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
2	2	40.66	A
7	2	28.1	A
9	2	22.53	A
10	2	13.83	A

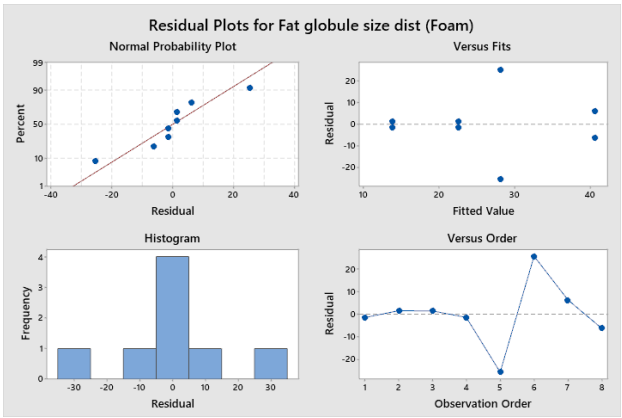
Means that do not share a letter are significantly different.



If an interval does not contain zero, the corresponding means are significantly different.



The pooled standard deviation is used to calculate the intervals.



WORKSHEET 1

One-way ANOVA: pH versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 9, 10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	0.110738	0.036913	44.07	0.002
Error	4	0.003350	0.000838		
Total	7	0.114088			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0289396	97.06%	94.86%	88.25%

Means

Formulation	N	Mean	StDev	95% CI

2	2	6.5500	0.0566	(6.4932, 6.6068)
7	2	6.55500	0.00707	(6.49818, 6.61182)
9	2	6.47500	0.00707	(6.41818, 6.53182)
10	2	6.26500	0.00707	(6.20818, 6.32182)

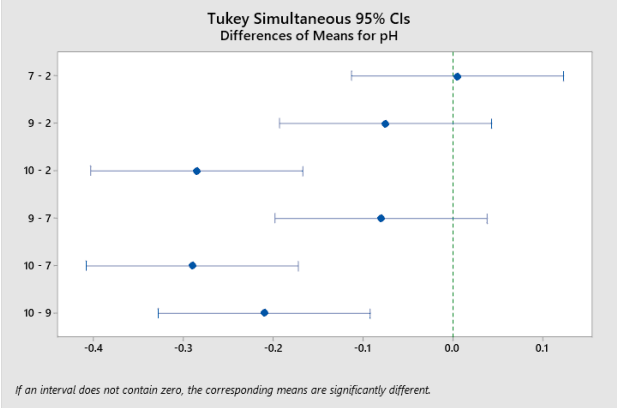
Pooled StDev = 0.0289396

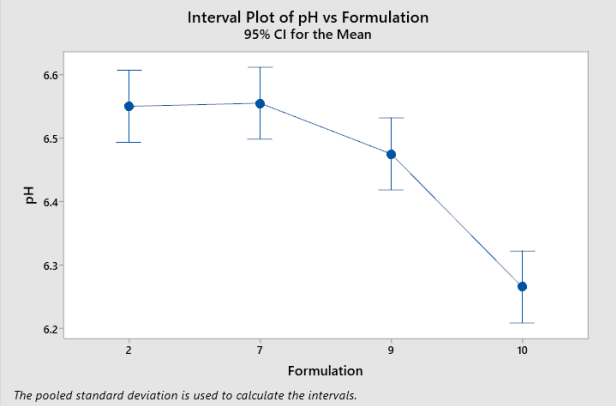
Tukey Pairwise Comparisons

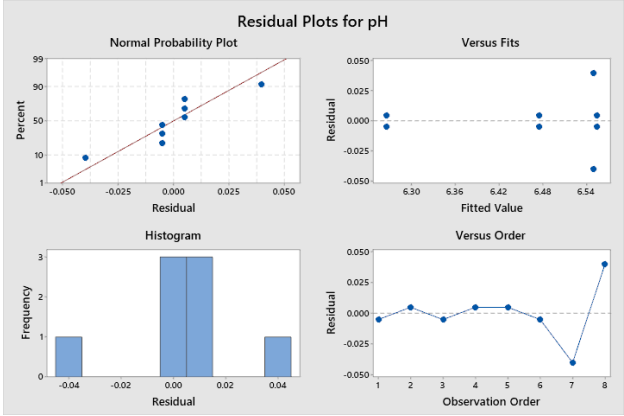
Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
7	2	6.55500	A
2	2	6.5500	A
9	2	6.47500	A
10	2	6.26500	B

Means that do not share a letter are significantly different.







WORKSHEET 1

One-way ANOVA: Hardness versus Formulation

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Formulation	4	2, 7, 9, 10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	4566	1521.9	3.53	0.128
Error	4	1727	431.7		
Total	7	6292			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
20.7777	72.56%	51.97%	0.00%

Means

Formulation	N	Mean	StDev	95% CI
2	2	40.06	7.23	(-0.73, 80.85)
7	2	93.9	15.5	(53.1, 134.7)
9	2	101.8	33.0	(61.1, 142.6)
10	2	84.2	18.7	(43.4, 125.0)

Pooled StDev = 20.7777

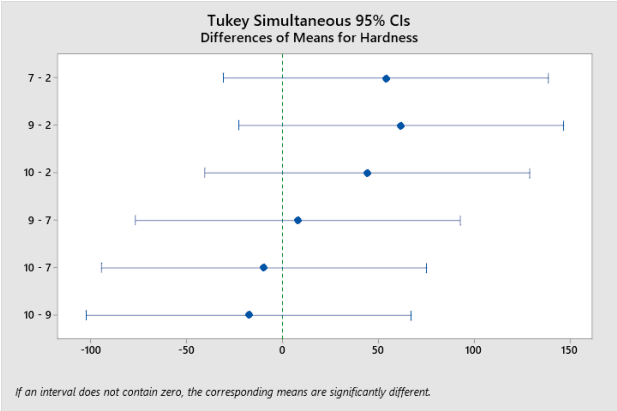
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

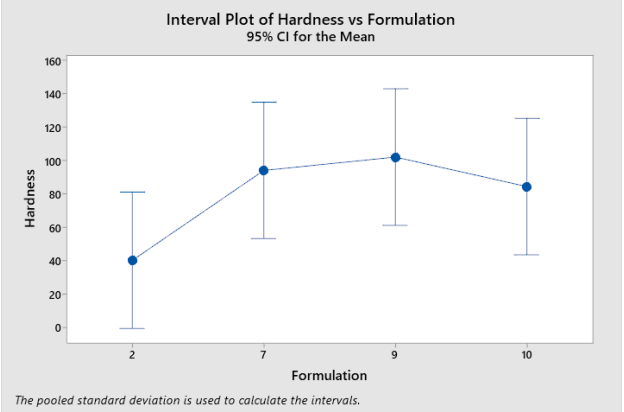
Formulation	N	Mean	Grouping
9	2	101.8	A
7	2	93.9	A
10	2	84.2	A

2		2	40.06 A

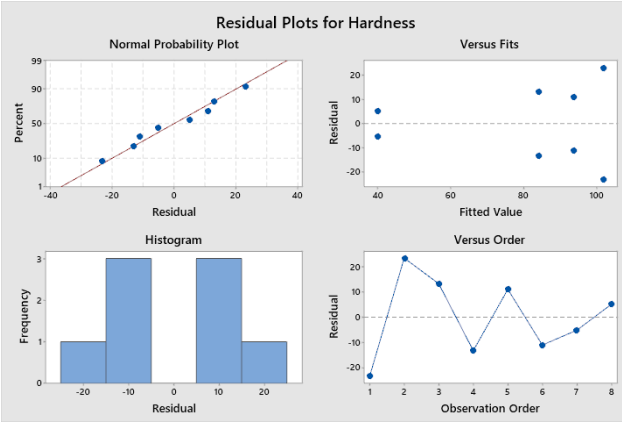
Means that do not share a letter are significantly different.



If an interval does not contain zero, the corresponding means are significantly different.



The pooled standard deviation is used to calculate the intervals.



10.3 Appendix C: Data analysis of sensory evaluation results

WORKSHEET 1

General Linear Model: colour versus formulation, day

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
formulation	Fixed	2	1, 2
day	Fixed	2	2, 30

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
formulation	1	0.346	0.34615	0.23	0.632
day	1	1.122	1.12179	0.75	0.389
Error	101	151.571	1.50070		
Lack-of-Fit	1	0.023	0.02289	0.02	0.902
Pure Error	100	151.548	1.51548		
Total	103	153.038			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.22503	0.96%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.396	0.120	61.39	0.000	
formulation					
1	0.058	0.120	0.48	0.632	1.00
day					
2	-0.104	0.120	-0.86	0.389	1.00

Regression Equation

colour = 7.396 + 0.058 formulation_1 - 0.058 formulation_2
- 0.104 day_2 + 0.104 day_30

Fits and Diagnostics for Unusual Observations

Obs	colour	Fit	Resid	Std Resid	
21	4.000	7.349	-3.349	-2.78	R
52	5.000	7.558	-2.558	-2.12	R
80	5.000	7.442	-2.442	-2.02	R
89	5.000	7.442	-2.442	-2.02	R

R Large residual

WORKSHEET 1

General Linear Model: appearance versus formulation, day

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
formulation	Fixed	2	1, 2
day	Fixed	2	2, 30

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
formulation	1	2.462	2.46154	1.82	0.181
day	1	0.066	0.06616	0.05	0.826
Error	101	136.857	1.35502		
Lack-of-Fit	1	0.006	0.00572	0.00	0.949
Pure Error	100	136.851	1.36851		
Total	103	139.385			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.16405	1.81%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.421	0.114	64.82	0.000	
formulation					
1	0.154	0.114	1.35	0.181	1.00
day					
2	-0.025	0.114	-0.22	0.826	1.00

Regression Equation

appearance = 7.421 + 0.154 formulation_1 - 0.154 formulation_2 - 0.025 day_2 + 0.025 day_30

Fits and Diagnostics for Unusual Observations

Obs	appearance	Fit	Resid	Std Resid	
3	5.000	7.550	-2.550	-2.22	R
21	5.000	7.550	-2.550	-2.22	R
76	5.000	7.600	-2.600	-2.27	R

R Large residual

WORKSHEET 1

General Linear Model: texture versus formulation, day

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
formulation	Fixed	2	1, 2
day	Fixed	2	2, 30

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
formulation	1	11.115	11.115	5.30	0.023
day	1	5.500	5.500	2.62	0.108
Error	101	211.846	2.097		
Lack-of-Fit	1	3.352	3.352	1.61	0.208
Pure Error	100	208.494	2.085		
Total	103	228.462			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.44827	7.27%	5.44%	1.59%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.251	0.142	50.91	0.000	
formulation					
1	0.327	0.142	2.30	0.023	1.00
day					
2	-0.231	0.142	-1.62	0.108	1.00

Regression Equation

texture = 7.251 + 0.327 formulation_1 - 0.327 formulation_2 - 0.231 day_2 + 0.231 day_30

Fits and Diagnostics for Unusual Observations

Obs	texture	Fit	Resid	Std Resid	
21	3.000	7.348	-4.348	-3.05	R
45	3.000	6.694	-3.694	-2.59	R
76	4.000	7.809	-3.809	-2.67	R
89	4.000	7.155	-3.155	-2.21	R

R Large residual

WORKSHEET 1

General Linear Model: meltdown in the mouth versus formulation, day

Method

Factor coding (-1, 0, +1)
Rows unused 1

Factor Information

Factor	Type	Levels	Values
formulation	Fixed	2	1, 2
day	Fixed	2	2, 30

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
formulation	1	15.603	15.6034	7.45	0.007
day	1	6.041	6.0410	2.88	0.093
Error	100	209.413	2.0941		
Lack-of-Fit	1	0.565	0.5647	0.27	0.606
Pure Error	99	208.849	2.1096		
Total	102	230.854			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.44711	9.29%	7.47%	3.61%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.150	0.143	49.95	0.000	
formulation					
1	0.389	0.143	2.73	0.007	1.00
day					
2	-0.243	0.143	-1.70	0.093	1.00

Regression Equation

meltdown in the mouth = 7.150 + 0.389 formulation_1 - 0.389 formulation_2 - 0.243 day_2 + 0.243 day_30

Fits and Diagnostics for Unusual Observations

Obs	meltdown in the mouth	Fit	Resid	Std Resid
4	3.000	7.296	-4.296	-3.02 R
9	4.000	7.296	-3.296	-2.31 R
35	3.000	6.517	-3.517	-2.47 R

R Large residual

WORKSHEET 1

General Linear Model: smoothness versus formulation, day

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
formulation	Fixed	2	1, 2
day	Fixed	2	2, 30

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
formulation	1	20.346	20.346	12.40	0.001
day	1	1.322	1.322	0.81	0.371
Error	101	165.678	1.640		
Lack-of-Fit	1	2.975	2.975	1.83	0.179
Pure Error	100	162.702	1.627		
Total	103	187.346			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.28077	11.57%	9.81%	6.22%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.280	0.126	57.79	0.000	
formulation					
1	0.442	0.126	3.52	0.001	1.00
day					
2	-0.113	0.126	-0.90	0.371	1.00

Regression Equation

smoothness = 7.280 + 0.442 formulation_1 - 0.442 formulation_2 - 0.113 day_2 + 0.113 day_30

Fits and Diagnostics for Unusual Observations

Obs	smoothness	Fit	Resid	Std Resid
21	5.000	7.609	-2.609	-2.07 R
26	4.000	6.724	-2.724	-2.16 R
31	4.000	6.724	-2.724	-2.16 R
35	4.000	6.724	-2.724	-2.16 R
76	4.000	7.835	-3.835	-3.04 R
89	4.000	6.951	-2.951	-2.34 R
103	4.000	6.951	-2.951	-2.34 R

R Large residual

WORKSHEET 1

General Linear Model: sweetness versus formulation, day

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
formulation	Fixed	2	1, 2
day	Fixed	2	2, 30

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
formulation	1	1.625	1.625	0.95	0.331
day	1	1.393	1.393	0.82	0.368
Error	101	172.357	1.707		
Lack-of-Fit	1	1.393	1.393	0.81	0.369
Pure Error	100	170.964	1.710		
Total	103	175.375			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.30633	1.72%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.116	0.128	55.39	0.000	
formulation					
1	0.125	0.128	0.98	0.331	1.00
day					
2	-0.116	0.128	-0.90	0.368	1.00

Regression Equation

sweetness = 7.116 + 0.125 formulation_1 - 0.125 formulation_2 - 0.116 day_2 + 0.116 day_30

Fits and Diagnostics for Unusual Observations

Obs	sweetness	Fit	Resid	Std Resid
3	4.000	7.125	-3.125	-2.43 R
4	4.000	7.125	-3.125	-2.43 R
17	4.000	7.125	-3.125	-2.43 R
32	3.000	6.875	-3.875	-3.01 R
98	4.000	7.107	-3.107	-2.41 R
103	4.000	7.107	-3.107	-2.41 R

R Large residual

WORKSHEET 1

General Linear Model: flavour versus formulation, day

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
formulation	Fixed	2	1, 2
day	Fixed	2	2, 30

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
formulation	1	5.087	5.0865	2.35	0.129

day	1	7.011	7.0112	3.24	0.075
Error	101	218.893	2.1673		
Lack-of-Fit	1	0.101	0.1010	0.05	0.830
Pure Error	100	218.792	2.1879		
Total	103	230.990			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.47216	5.24%	3.36%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.990	0.145	48.28	0.000	
formulation					
1	0.221	0.144	1.53	0.129	1.00
day					
2	-0.260	0.145	-1.80	0.075	1.00

Regression Equation

flavor = 6.990 + 0.221 formulation_1 - 0.221 formulation_2
- 0.260 day_2 + 0.260 day_30

Fits and Diagnostics for Unusual Observations

Obs	flavor	Fit	Resid	Std Resid	
4	4.000	6.950	-2.950	-2.04	R
21	2.000	6.950	-4.950	-3.42	R
32	3.000	6.508	-3.508	-2.42	R
76	3.000	7.471	-4.471	-3.08	R
89	4.000	7.029	-3.029	-2.09	R
98	4.000	7.029	-3.029	-2.09	R
103	4.000	7.029	-3.029	-2.09	R

R Large residual

WORKSHEET 1

General Linear Model: overall acceptance versus formulation, day

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
formulation	Fixed	2	1, 2
day	Fixed	2	2, 30

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
formulation	1	3.291	3.2909	2.51	0.116
day	1	0.206	0.2060	0.16	0.692
Error	101	132.231	1.3092		
Lack-of-Fit	1	0.234	0.2344	0.18	0.674
Pure Error	100	131.997	1.3200		
Total	103	135.728			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.14421	2.58%	0.65%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.357	0.113	65.38	0.000	
formulation					
1	0.178	0.112	1.59	0.116	1.00
day					
2	-0.045	0.113	-0.40	0.692	1.00

Regression Equation

overall acceptance = 7.357 + 0.178 formulation_1
- 0.178 formulation_2 - 0.045 day_2
+ 0.045 day_30

Fits and Diagnostics for Unusual Observations

Obs	overall acceptance	Fit	Resid	Std Resid	
21	3.000	7.490	-4.490	-3.99	R
71	5.000	7.580	-2.580	-2.29	R
76	4.000	7.580	-3.580	-3.17	R
89	4.000	7.224	-3.224	-2.86	R

R Large residual