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**Native Milk Fat Globules and Buttermilk Powder: Novel
Emulsifiers for making Ice cream**

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Abstract

Monoglycerides have been used in ice cream formulations for over seven decades. Monoglyceride addition is known to promote the dual mechanisms of (i) fat agglomeration and (ii) the adsorption of fat droplets to bubble surfaces during freezing which in turn improves the melt stability and sensory attributes of ice cream. The synthetic nature of commercial emulsifiers, coupled with trends in clean label manufacture, provides the motivation for exploring formulation/processing pathways that can generate equivalent product structures without these additives. This thesis represents a research study to replicate the whipping functionality of non-homogenized cream in an ice cream formulation and the use of milk phospholipids as an alternative to monoglycerides in ice cream production.

In the first part of the study, ice cream mixes were prepared with the partial replacement of homogenized anhydrous milk fat with different % of non – homogenized cream. The resultant ice creams were analysed for microstructure, melt stability, material properties, and size distribution of melted ice cream to measure the extent of fat agglomeration. The differences in degree of fat destabilization, structural and material properties indicated that the replacement of homogenized fat with non-homogenized cream resulted in a positive effect on melt stability and a softer texture. Overall, findings suggest that replacing 40% of total fat in ice cream with native milk fat globules provides optimum results and can potentially be used as an alternative to monoglycerides.

In the second part of the study, ice creams were prepared using a phospholipid enriched buttermilk powder (BMP @ 1-4%) as part of a conventional formulation (~35% total solids). Mix processing was adapted to allow the BMP to be either the sole emulsifier during homogenization, or a part of the combined milk solids fraction. Melt stability and material properties of the ice creams were evaluated, along with confocal and cryo-scanning electron microscopy analysis, and particle sizing of ice cream melts to determine the extent of fat agglomeration. All ice creams containing BMP were seen to have a positive effect on melt stability. Measured differences in the extent of fat agglomeration, ice cream structural and material properties indicated that structural assembly during freezing varied according to both BMP content and mix processing pathway. Overall, findings suggest that milk phospholipids can be used to replicate functional aspects of monoglyceride properties in ice cream.

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Contents

Abstract.....	iii
Acknowledgements.....	iv
Symbols and abbreviations	viii
List of figures.....	x
List of tables.....	xiii
1. Introduction.....	1
1.1.1 Objective (i): To conduct a study to find the potential for native milk fat globules as natural emulsifiers for ice cream.....	1
1.1.2 Objective (ii): Extrapolation of the effect of native milk fat globules to analyse the impact of buttermilk powder as a replacement for synthetic emulsifiers in ice cream	2
2. Literature Review.....	4
2.1 Introduction.....	4
2.2 Ingredients of ice cream.....	5
2.2.1 Fat	5
2.2.2 Milk Solid not fat.....	7
2.2.3 Sweeteners	8
2.2.4 Stabilizers.....	10
2.2.5 Emulsifiers	10
2.2.6 Other ingredients.....	11
2.3 Processing of ice cream.....	12
2.4 Structure of Ice cream.....	14
2.4.1 Air cells.....	15
2.4.2 Ice crystals	16
2.4.3 Serum phase	18
2.5 Formation of fat structure in ice cream.....	20
2.5.1 Partial coalescence	20
2.5.2 Factors affecting the degree of fat destabilization	27
2.5.3 Conclusion	37
3. Materials and Methodology	38
3.1 Raw Materials	38
3.2 Equipment.....	38
3.3 Formulations	38
3.3.1 Fat ratios for native milk fat globules formulations.....	39
3.3.2 Levels of buttermilk powder	40
3.4 Process flow.....	40
3.4.1 Preparation of ice cream with non – homogenized cream	40
3.4.2 Preparation of ice cream with buttermilk powder using traditional processing.....	43

3.4.3	Preparation of ice cream with butter milk powder using selective adsorption processing	44
3.5	Methods.....	45
3.5.1	Overrun	45
3.5.2	Particle Size distribution	45
3.5.3	Hardness.....	45
3.5.4	Meltdown Stability.....	46
3.5.5	Confocal Microscopy.....	46
3.5.6	Cryo – Scanning Electron Microscopy	46
4.	Effects of non-homogenized cream addition on ice cream.....	48
4.1	Introduction.....	48
4.2	Overrun	48
4.3	Microstructure.....	50
4.3.1	Confocal microscopy	50
4.3.2	Scanning electron microscopy	53
4.4	Particle size distribution.....	56
4.5	Hardness.....	64
4.6	Melting stability	67
4.7	Final discussion.....	72
5.	Effect of buttermilk powder addition on ice cream	74
5.1	Introduction.....	74
5.2	Traditional processing.....	75
5.2.1	Overrun	75
5.2.2	Microstructure.....	76
5.2.3	Particle size distribution.....	81
5.2.4	Hardness.....	83
5.2.5	Melting stability	85
5.2.6	Final discussion.....	87
5.3	Selective adsorption	89
5.3.1	Overrun	89
5.3.2	Microstructure.....	90
5.3.3	Particle size distribution.....	95
5.3.4	Hardness.....	99
5.3.5	Melting stability	100
5.3.6	Final discussion.....	102
6.	Conclusion and Future prospective.....	104
7.	References.....	105
8.	Appendices.....	110

Appendix A: Composition of raw materials	110
Appendix B: Formulations with non-homogenized cream	111
Appendix C: Formulations with buttermilk powder	114
Appendix D: Confocal images.....	116
Appendix E: SEM Images	121

Symbols and abbreviations

SFC	Solid fat content
AMF	Anhydrous milk fat
MFGM	Milk fat globule membrane
ANZFS	Australia New Zealand Food Standard
MSNF	Milk solid not fat
TS	Total solids
WPC	Whey protein concentrate
SMP	Skim milk powder
CS	Corn starch
DE	Dextrose equivalent
LBG	Locust bean gum
SMS	Small molecule surfactant
MDG	Mono- diglycerides
PS	Polysorbates
PGMS	Propylene glycol monostearates
HTST	High-temperature short time
TM	Mechanical glass transition temperature
TG	Glass transition temperature
PL	Phospholipids
θ_o	Contact angle of fat crystals in the oil phase
θ_w	Contact angle of fat crystals in the water phase
α	Encounter efficiency
T_{max}	Tempering temperature
t_{max}	Tempering time
NZMP	New Zealand Milk Powder
UHT	Ultra-high temperature
PC	Positive control

NC	Negative control
C: AMF	Ratio of non-homogenized cream to anhydrous milk fat
BMP	Buttermilk powder
SEM	Scanning electron microscopy
D[3,2]	The surface weighted mean diameter
D[4,3]	Volume weighted mean diameter
N	Newton

List of figures

Figure 2.1 Various products obtained from hydrolysis of corn starch (Goff & Hartel, 2013b).....	9
Figure 2.2 Industrial schematic of ice cream production (Bylund, 2003).....	13
Figure 2.3 Structural components of ice cream (Walstra et al., 2005).....	14
Figure 2.4 Contribution of different ingredients to structural phases of ice cream (Clarke, 2005)	15
Figure 2.5 Formation of ice crystals in ice cream (Cook & Hartel, 2010).....	17
Figure 2.6 Phase diagram of ice cream mix (Hartel, 1996)	19
Figure 2.7 Various destabilizations mechanism in an emulsion (Darling, 1982)	21
Figure 2.8 Contrast between Coalescence and Partial coalescence(Walstra & Boode, 1993).....	22
Figure 2.9 Fat aggregates produced during partial coalescence (Goff, 1997b)	22
Figure 2.10 Formation of butter grains (Walstra et al., 2005)	24
Figure 2.11 Air-water interface in whipped cream (Rybak, 2016)	25
Figure 2.12 Comparing fat interface for ice cream with and without emulsifiers (Rybak, 2016)	27
Figure 2.13 Arrangement of different components in MFGM (Jukkola & Rojas, 2017)	31
Figure 2.14 Contact angles of a crystal at oil – water interface (Fredrick et al., 2010)	34
Figure 2.15 Different positions of a crystal at oil- water interface (Fredrick et al., 2010)	35
Figure 2.16 Types of fat globules based on type of fat crystal network (Walstra & Boode, 1993).....	35
Figure 3.1 Process flow of ice cream manufacturing with non-homogenized cream	41
Figure 3.2 Process flow of ice cream manufacturing with buttermilk powder using traditional processing	43
Figure 3.3 Process flow for different phases prepared for selective adsorption	44
Figure 4.1 Confocal laser scanning microscopic images of melted ice cream for standard fat ice cream controls.....	50
Figure 4.2 Confocal microscopy images for melted standard ice cream with different ratios cream ($C_{AMF}^{20:80}$, $C_{AMF}^{40:60}$, $C_{AMF}^{45:55}$, and $C_{AMF}^{50:50}$).....	51
Figure 4.3 Confocal microscopy images for melted standard ice cream with different ratios cream ($C_{AMF}^{55:45}$, $C_{AMF}^{60:40}$, $C_{AMF}^{80:20}$, and $C_{AMF}^{100:00}$).....	52
Figure 4.4 The micrographs for PC, NC, $C_{AMF}^{40:60}$ and $C_{AMF}^{60:40}$ at 250 X magnification	54
Figure 4.5 The microscopic images for PC, NC and $C_{AMF}^{40:60}$ and $C_{AMF}^{60:40}$ formulations at 3500 x magnification (s – serum phase, I – ice phase, a – air cells and f – fat particles)	55
Figure 4.6 Particle size distribution of standard fat ice cream mix for positive control, negative control, $C_{AMF}^{20:80}$ and $C_{AMF}^{40:60}$	57
Figure 4.7 Particle size distribution of standard fat ice cream mix of positive control, $C_{AMF}^{45:55}$, $C_{AMF}^{50:50}$, $C_{AMF}^{55:45}$, $C_{AMF}^{60:40}$, $C_{AMF}^{80:20}$ and $C_{AMF}^{100:00}$	58
Figure 4.8 Particle size distribution of standard fat melted ice cream for positive control, negative control, $C_{AMF}^{20:80}$, and $C_{AMF}^{40:60}$ formulations.....	59

Figure 4.9 Particle size distribution of standard fat melted ice cream for positive control, $C_{AMF}^{45:55}$, $C_{AMF}^{50:50}$, $C_{AMF}^{55:45}$, $C_{AMF}^{60:40}$, $C_{AMF}^{80:20}$ and $C_{AMF}^{100:00}$ formulations.	60
Figure 4.10: Particle size distribution of low-fat melted ice cream for positive control, negative control, and $C_{AMF}^{20:80}$ formulations.	62
Figure 4.11 Particle size distribution of low-fat melted ice cream for positive control, $C_{AMF}^{60:40}$, $C_{AMF}^{80:20}$, and $C_{AMF}^{100:00}$ formulations.....	62
Figure 4.12 Dripping curves for different formulations of standard fat ice creams with various % of non-homogenized cream at 20°C	69
Figure 4.13 Dripping curves for different formulations of low-fat ice creams with cream.	70
Figure 4.14 The mechanism of agglomeration in ice cream with native milk fat globules	72
Figure 5.1 Confocal laser scanning microscopic images of melted ice cream for standard fat ice cream controls.....	76
Figure 5.2 Confocal laser scanning microscopic images of melted ice cream with variable % of BMP	77
Figure 5.3 SEM images for controls and ice cream with 1 % and 3 % BMP at 250 x magnification ..	79
Figure 5.4 SEM images for controls and ice cream with 1 % and 3 % BMP at 3500 x magnification (s – serum phase, I – ice phase, a – air cells, and f – fat particles)	80
Figure 5.5 Model size distribution for melted controls and BMP Trad 1% ice creams.....	81
Figure 5.6 Model size distribution for melted positive control, BMP Trad 2%, 3 %, and 4% ice creams	82
Figure 5.7 Dripping rate curves for controls and ice cream with variable % of buttermilk powder produce using traditional processing at 20°C.....	86
Figure 5.8 Fat globules interface with the incorporation of BMP using traditional processing	87
Figure 5.9 The confocal microscopic images for control ice creams.....	90
Figure 5.10 The confocal microscopic images for ice cream formulations with variable level of buttermilk powder incorporated by selective adsorption method	91
Figure 5.11 Scanning electron microscopic images for controls and formulation with 1 & 3 % buttermilk powder at 250x magnification	93
Figure 5.12 Scanning electron microscopic images for controls and formulation with 1 & 3 % buttermilk powder at 3500x magnification ((s – serum phase, I – ice phase, a – air cells and f – fat particles).....	94
Figure 5.13 The model size distribution for ice cream mix for different formulations with BMP added using selective adsorption	95
Figure 5.14 Model size distribution for melted positive control, negative control and $BMP_{Selective}^{1\%}$..	96
Figure 5.15 Particle size distribution for positive control, $BMP_{Selective}^{2\%}$, $BMP_{Selective}^{3\%}$, and $BMP_{Selective}^{4\%}$	97

Figure 5.16 The melting rate curves for controls and ice cream formulations with different % of BMP added using selective adsorption at 20°C.....	101
Figure 5.17 Aged fat globule interface with BMP incorporated using selective adsorption	102
Figure 8.1 Confocal images for postive and negative controls.....	116
Figure 8.2 Confocal images for $C_{AMF}^{20:80}$ (left) and $C_{AMF}^{40:60}$ (right).....	117
Figure 8.3 Confocal images for $C_{AMF}^{45:55}$ (left) and $C_{AMF}^{50:50}$ (right).....	118
Figure 8.4 Confocal images for $C_{AMF}^{55:45}$ (left) and $C_{AMF}^{60:40}$ (right).....	119
Figure 8.5 Confocal images for $C_{AMF}^{80:20}$ (left) and $C_{AMF}^{100:00}$ (right).....	120
Figure 8.6 SEM images of negative control at different magnifications (area 1).....	121
Figure 8.7 SEM images of negative control at different magnifications (area 2).....	121
Figure 8.8 SEM images of positive control at different magnifications (area 1).....	122
Figure 8.9 SEM images of $C_{AMF}^{40:60}$ at different magnifications (area 1).....	122
Figure 8.10 SEM images of $C_{AMF}^{60:40}$ at different magnifications (area 1).....	123
Figure 8.11 SEM images of $C_{AMF}^{60:40}$ at different magnifications (area 2).....	123
Figure 8.12 SEM images of BMP Trad 1% ice cream at different magnifications (area 1)	124
Figure 8.13 SEM images of BMP Trad 1% ice cream at different magnifications (area 2)	124
Figure 8.14 SEM images of BMP Trad 3% ice cream at different magnifications (area 1)	125
Figure 8.15 SEM images of BMP Trad 3% ice cream at different magnifications (area 2)	125
Figure 8.16 SEM images of BMP Selective adsorption 1% ice cream at different magnifications (area 1)	126
Figure 8.17 SEM images of BMP Selective adsorption 1% ice cream at different magnifications (area 2)	126
Figure 8.18 SEM images of BMP Selective adsorption 3% ice cream at different magnifications (area 1)	127
Figure 8.19 SEM images of BMP Selective adsorption 3% ice cream at different magnifications (area 2)	127

List of tables

Table 2.1 Consumption of ice cream in different countries (Atlas, 2020).....	4
Table 2.2 Different compositions for ice cream (Goff & Hartel, 2013b)	5
Table 4.1 The overrun measurements for full fat controls and full fat ice cream made with variable proportion of non-homogenized cream.....	48
Table 4.2 The overrun measurements for low fat controls and low-fat ice cream made with variable proportion of non-homogenized cream.....	49
Table 4.3 Value of D[4,3] and D [3,2] for various standard fat ice cream formulations.	61
Table 4.4 Hardness values for standard fat ice creams with various ratios of non-homogenized cream.	65
Table 4.5 Hardness values for low-fat ice creams with various % of cream.	65
Table 4.6 % mass of melted ice cream (at the end of 2hrs) for standard fat ice cream formulations ..	70
Table 4.7 % mass of melted ice cream (at the end of 2hrs) for low-fat ice cream formulations	70
Table 5.1 Overrun % for ice cream formulations made with traditional processing with different % of buttermilk powder	75
Table 5.2 Hardness values (in N) for controls and ice cream with variable % of BMP made by using traditional processing	84
Table 5.3 % the final mass of melted ice cream for controls and formulations with different % of BMP	85
Table 5.4 Overrun % for controls and ice cream manufactured using selective adsorption with different level of buttermilk powder	89
Table 5.5 Hardness values for controls and formulations with different % of buttermilk incorporated using selective adsorption technique.....	99
Table 5.6 % the final mass of melted ice cream for controls and formulations with different % of BMP	100
Table 8.1 Composition of SMP.....	110
Table 8.2 Composition of Glucose syrup.....	110
Table 8.3 Composition of AMF	110
Table 8.4 Composition of Non - homogenized cream	110
Table 8.5 Composition of buttermilk powder	111
Table 8.6 Calculations for standard fat ice cream mix with non-homogenized cream	112
Table 8.7 Calculations for low fat ice cream mix with non-homogenized cream	113
Table 8.8 Calculations for ice cream mix with buttermilk powder.....	115

1. Introduction

With changing food consumption habits, there is increasing demand for more natural, environmentally friendly, clean labelled and sustainable food products. It has led the food industry to focus on replacing synthetic emulsifiers with natural alternatives in food products. Natural emulsifiers are amphiphilic proteins, polysaccharides, lipids, phospholipids, and biopolymers (McClements & Gumus, 2016). Following the present trend of replacing synthetic ingredients, studies have also been conducted on finding replacement for synthetic chemically derived emulsifiers in ice cream. It is also worth acknowledging that some other ingredients such as sweeteners and stabilizers which are also important to make a clean label ice cream. As these ingredients are derived from natural substances, thereby focus of this study is to specifically replace the chemically synthesized emulsifiers in ice creams.

Segall and Goff (2002) attempted to prepare ice cream without emulsifiers by modifying the process of ice cream making. In place of the traditional processing technique in which all the ingredients are mixed and then homogenized after blending, they suggested a two-phase processing technique. The quality of ice cream in terms of the degree of fat destabilization and melting properties was comparable with ice cream containing emulsifiers. Due to increased processing requirements, the technique was suggested to be less economical.

Another study used different phospholipids (soya, rice, and milk) to replace synthetic emulsifiers in Italian ice cream (gelato). It was reported that gelato made with soya and rice phospholipids was comparable to control in terms of its degree of fat destabilization but had less overrun. Whereas gelato with bovine phospholipids did not foam well, and a low-quality product in terms of overrun hardness and melting stability was obtained (Rinaldi et al., 2014). Continuing the search for natural emulsifiers for ice cream a master study was suggested with following objectives and hypotheses.

1.1.1 Objective (i): To conduct a study to find the potential for native milk fat globules as natural emulsifiers for ice cream

The first objective of this research work was to explore the possibility of using native milk fat globules as a naturally emulsifier in ice cream. In this context, the definition of native milk fat globule is the milk fat globule, which is interracially covered with a milk fat globule membrane.

The hypothesis suggested for this objective comes from the traditional making of whipped cream or butter. It has been well established that native milk globules present in non-

homogenized cream are capable of undergoing partial coalescence when the appropriate solid fat content (SFC) content is maintained by controlling the temperature (as described in the literature). This property of native milk globules is already being used to produce whipped cream by non-homogenized cream at the industrial level. It also implies that the milk fat globule membrane's tri-layer structure can provide the required emulsifying properties. With this information, we hypothesise that partly replacing the fat source (anhydrous milk fat) in ice cream with non-homogenized cream will produce ice cream comparable with conventional emulsifiers. Partial replacement is key to this approach, on the basis that we would expect complete utilisation of non-homogenized fat droplets in the formulation to result in over-churning and destabilization of the ice cream. Partial replacement is anticipated to result in more limited churning, as only a fraction of the total fat droplets will be susceptible to this effect. Accordingly, we partly replaced anhydrous milk fat(AMF) with non-homogenized in different ratios and analysed the resultant ice creams. The critical processing change we pursued was adding non-homogenized cream after mix homogenization as homogenization of native fat globules would have disrupted the milk fat globule membrane(MFGM) and disown their purpose in the formulation.

1.1.2 Objective (ii): Extrapolation of the effect of native milk fat globules to analyse the impact of buttermilk powder as a replacement for synthetic emulsifiers in ice cream

After investigating our first objective, findings indicated that native milk fat globules can be a possible alternative natural emulsifier for ice cream. The results of this study provided the rationale for second objective of the research, which was to investigate the possibility of using phospholipid-enriched buttermilk powder as a natural emulsifier for ice cream.

Buttermilk is an aqueous remnant of the butter production process. It is rich in water-soluble components (proteins, lactose) and fractions of milk fat globule membrane. Natural milk fat globule membrane, composed of proteins, phospholipids, and triglycerides, is known to be surface-active in nature(Roesch et al., 2004). In our study in objective (i), we found that the natural milk fat globules can produce a good quality ice cream in terms of texture and melting stability. As buttermilk powder is enriched with milk fat phospholipids, we hypothesize that it can be a potential natural emulsifier for ice cream. Although, it is known that phospholipids are the main surface-active components in milk globule membrane fractions, in our research; we have not focused on explicitly extracting the phospholipids from the buttermilk. This is

because extracting phospholipids from butter milk powder and then using it as a natural emulsifier would not be an economically viable option. For this objective, we used different % of buttermilk powder in ice cream and analysed its effect on the properties of ice cream. We used two processing techniques, traditional and selective adsorption method.

In traditional processing techniques, all the ingredients are blended, and homogenization is performed after combining. Whereas in the selective adsorption method, ice cream mix blending is composed of two phases. One phase having buttermilk powder, sugar and stabilizers dissolved in water (Phase A), and the other phase is having skim milk powder dissolved in water (Phase B). Phase A is homogenized at conventional homogenization pressure. Phase B is mixed into phase A after homogenization.

2. Literature Review

2.1 Introduction

Ice cream is one of the most iconic dairy products consumed worldwide. As per World atlas 2020, New Zealand is the highest per capita consumer of ice cream, followed by the USA. It has been reported that on average a person consumed 28.4 L of ice cream in the year 2019 in New Zealand. Table 1.1 outlines the top 10 consumers of ice cream worldwide (Atlas, 2020).

Table 2.1 Consumption of ice cream in different countries (Atlas, 2020)

Rank	Country	Per capita ice-cream consumption (litres/year)
1	New Zealand	28.4
2	United States	20.8
3	Australia	18.0
4	Finland	14.2
5	Sweden	12.0
6	Canada	10.6
7	Denmark	9.8
8	Ireland	8.4
9	Italy	8.0
10	United Kingdom	7.0

The term “ice cream” includes a wide range of frozen desserts. These different types of frozen desserts are dairy ice cream, non-dairy ice cream, frozen yoghurt, gelato, milk ice, fruit ice, and many more (Clarke, 2015). The legal definition of the term “ice cream” and standards are variable across in different nations. In North America and New Zealand, it is essential to have milk fat in frozen products to be defined as ice cream, whereas it is not required in the EU unless the product is specified as “dairy ice cream” (Goff & Hartel, 2013b). The New Zealand ice cream industry follows the standards set by Australia New Zealand Food Standard Code(ANZFS). As per the definition of ANZFS, “ice cream means a sweet frozen food that is made from cream or milk products or both, and other foods, and is generally aerated. It should have milk fat not less than 100g/kg and food solids not less than 168g/L of ice cream” (Code, 2015).

Generally, ice cream is classified into regular, low fat, and premium based on its fat content. All these types of ice cream are available in different flavours worldwide. Compositionally, ice cream is made up of milk fat, milk solids not fat (milk protein, lactose, and minerals), sucrose, sweeteners (glucose syrup, high fructose corn syrups, etc.), stabilizers, and emulsifiers. Other than these ingredients, ice cream may also contain different flavours, colours, and fruits. These ingredients can be derived from various sources and perform a specific ice cream role (Arbuckle, 2013). Table 1.1 outlines different generic compositions of ice cream. The composition selection depends on the choice of the manufacturer as local market requirements. (Goff & Hartel, 2013b)

Table 2.2 Different compositions for ice cream (Goff & Hartel, 2013b)

Compositions (%)			
Milk fat	10.0	11.0	12.0
Milk solids-not-fat	11	11	10.5
Sucrose	10	10	12
Corn syrup solids	5	5	4
Stabilizer	0.35	0.35	0.3
Emulsifier	0.15	0.15	0.15
Total solids	36.5	37.5	38.95

2.2 Ingredients of ice cream

Ingredients used for ice cream manufacturing can be grouped as major ingredients, minor ingredients, and inclusions. Major ingredients are fat, milk proteins, sugar, and water, which form the bulk of ice cream. The air in ice cream also forms a significant proportion. Emulsifiers, stabilizers, and flavouring compounds are classified as minor ingredients. Inclusions are speciality ingredients such as chocolate, wafer, fruits, which are used to give particularity to ice cream (Clarke, 2015).

2.2.1 Fat

Fat is one of the critical ingredients in ice cream. It provides rich flavour, smooth texture and contributes to bodybuilding in ice cream. It contributes significantly to the melting properties

of ice cream. It also helps in processing by lubricating the barrel during freezing (Clarke, 2005). Additionally, the formation of the weak interfacial membrane by additives during aging and, incorporation of air with continuous shearing during freezing causes fat in the ice cream mix to undergo partial coalescence. This partial coalescence of fat during freezing results in the structure formation of ice cream. Therefore, the selection of fat for ice cream is of critical importance (Goff, 1997b). According to their source, the fat used in ice cream can be grouped as dairy or non-dairy fat (Goff, 1997a).

Milk fat

Milk fat is the most common type of fat used in ice cream. It is responsible for the richness of dairy flavours associated with ice cream (Goff & Hartel, 2013b). Naturally, milk fat is present as fat globules dispersed in the serum part of milk. These fat globules have a size range of 0.2 to 15 μm . Nearly, 98% of milk fat is triglycerides which are composed of a wide range of fatty acids with a melting range from $-40\text{ }^{\circ}\text{C}$ to $40\text{ }^{\circ}\text{C}$ (Walstra et al., 2005).

Different sources of milk fat used in mix preparation are cream, butter, plastic cream, anhydrous milk fat, and ghee (Arbuckle, 2013). Choice of the source depends on the ease of availability and processing. Fresh cream separated from fresh milk is the foremost source of fat for mix preparation in New Zealand. Fresh cream, without microbial and chemical defects, is the premier source of richness. Being a liquid, cream provides ease of processing. Other dry ingredients i.e., skim milk powder, sugar, stabilizers can be easily dissolved into the cream. Despite being a potential source of milk fat, use of fresh cream is not very common outside New Zealand as it is an expensive source, has a low shelf life, and requires extra storage care to prevent any deterioration. Butter is another source of milk fat. As fat in butter is present as water in oil emulsion an inversion of emulsion needs to be done before use. This adds an extra processing requirement in ice cream making. Anhydrous milk fat (AMF) is arguably the most common source of milk fat used industrially, especially where the supply of fresh cream is low. Low moisture content and ease of transportation make it an appropriate source of milk fat. It does require to be melted prior to mixing. Other milk fat sources can be plastic fat and ghee which are only used occasionally (Goff & Hartel, 2013a).

Non-dairy fats

With the shift in food habits to a vegan lifestyle along with issues of lactose intolerance and allergies focus has been shifting to non-dairy ice cream (2019). Vegetable fats have been

extensively used in European countries as they are less costly than milk fat. The important parameters for the selection of non-dairy fat are their melting profile, crystallization rate, crystals type triglyceride content (high content leads to waxy product), and solid fat content at aging and freezing temperatures (Goff & Hartel, 2013b). Solid fat content at freezing temperature is one of the important factors determining the susceptibility of fat towards partial coalescence which is a major structuring mechanism. Palm kernel, palm oil, and coconut oil are the main non-dairy fats used in ice cream. (Clarke, 2005).

Fat replacers

With the increasing risk of coronary heart disease due to fat consumption, there is rising demand for low-fat ice creams (Akalın & Karagöz, 2008). The function of fat replacers is to mimic the structuring mechanism provided by fat and to limit the undesirable effects of replacing fat in ice cream. Based on their composition fat replacers are of three types- carbohydrates-based, protein-based, and lipid-based. A typical example of fat replacers is modified starch, maltodextrins, whey protein concentrates, soya protein, structure lipids, etc. They can be used alone or in combination with others (Akbari et al., 2019).

2.2.2 Milk Solid not fat

Milk solids not fat (MSNF) is the fraction of milk other than fat. It is comprised of milk proteins, lactose, and minerals (Walstra et al., 2005). Milk proteins perform a range of functions in ice cream. They help in the emulsification of fat during homogenization by adsorbing to the interface of newly formed fat globules. They contribute to the whippability of ice cream mix by stabilizing newly formed air bubbles during freezing. They also contribute to serum phase viscosity in ice cream thereby limiting the mobility of water and thus prevent water recrystallization. Other than these functions, they are a source of nutrition, contribute to the perceived whiteness of ice cream, and provides a unique dairy flavour to ice cream (Tharp & Young, 2012). Milk proteins are made of caseins and whey proteins. The ratio of casein to whey proteins is 80:20 in cow milk (McSweeney & O'Mahony, 2015). Ice cream mix contains 3-4 % milk proteins (Goff & Hartel, 2013b).

Lactose is milk sugar. It contributes to the freezing point depression of the ice cream mix and to a small extent adds to the sweetness of ice cream (around ~ 1% of sweetness) (Tharp & Young, 2012). There is around 5% lactose in the ice cream mix. A high quantity of lactose in the mix leads to “sandiness”, a defect that arises due to the crystallization of lactose in ice

cream (Clarke, 2005). Milk salts provide a typical mineral taste to ice cream and to a certain degree contribute to freezing point depression (Tharp & Young, 2012).

Concentrated milk, dried milk powders, whey protein concentrates, and other powder blends are commonly used sources of milk solid not fat in ice cream formulations. Concentrated skim milk, with 25-30 % TS, has been one of the conventional sources of MSNF. It is produced by evaporating skim milk under a vacuum. As concentrated milk is in a liquid state so this is easier to handle production-wise. Low shelf life and regular supply are some of the limitations of using concentrated milk. Milk powder is a popular source which has been used extensively. Dried milk is produced by spray-drying concentrated milk. Ease of storage and long shelf life are encouraging benefits provided by powders to the producer (Goff & Hartel, 2013b).

Whey protein concentrates (WPCs) are an economical source of MSNF. They are cheaper than skim milk powder. A high content of lactose in WPC, around 72-73 % as compared to 52-54 % in SMP, can lead to the issue of lactose crystallization in ice cream (Goff & Hartel, 2013a). Another limitation of using WPC is the heat instability of whey proteins which can affect the functionality of proteins in ice cream and their strong flavour (Clarke, 2005).

2.2.3 Sweeteners

“Sweetener” is the term used to describe the substances contributing to the sweetness of ice cream. Sweeteners are also important to deliver flavour (Tharp & Young, 2012). They provide a sweet taste to ice cream which complement the various flavour used in ice cream and improve consumer acceptance. Also sweeteners such as polyhydric alcohols lower freezing point of the ice cream mix, controlling the % of the ice in the final product, which in turn, controls the softness of ice cream (Clarke, 2005). The typical concentration of sweeteners used is around 12-15 % of ice cream mix (Arbuckle, 2013). A range of sweeteners such as sucrose, corn starch derivatives, high fructose corn syrup, honey, glucose syrup, maple syrup, sugar alcohols, etc. are used in ice cream manufacturing (Asghar et al., 2013). The selection of sweeteners depends upon their relative sweetness, energy contribution, and degree of freeze point depression. A proper mixture of sweeteners is required to achieve the desired softness in ice cream (Goff & Hartel, 2013b)

Sucrose, also known as table sugar, is derived from sugarcane or sugar beet. It is the most common sweetener used in ice cream. Chemically, it is a disaccharide having glucose and fructose as monomeric units. It is readily soluble in water and can form a supersaturated solution at a concentration of more than 67%. At the storage temperature of ice cream (-18 °C),

sucrose is present in a supersaturated solution. As a rule, 1% of sucrose causes freeze point depression by 0.1 °C (Clarke, 2005). A high proportion of sucrose leads to the formation of too soft ice cream and a low proportion results in less sweet and icy ice cream. To resolve this issue, a part of the sucrose can be replaced with an alternative sweetener that is less sweet and has a high colligative effect of freeze point depression (Walstra et al., 2005).

Corn syrups are common alternative sweeteners used to replace a part of the sucrose. These are derivatives of corn starch with different degrees of hydrolysis (Clarke, 2005). Corn starch (CS) is hydrolysed using enzymes and by controlling the hydrolysis different products can be obtained. Figure 2.1 depicts different sweeteners obtained by varying degrees of corn starch hydrolysis.

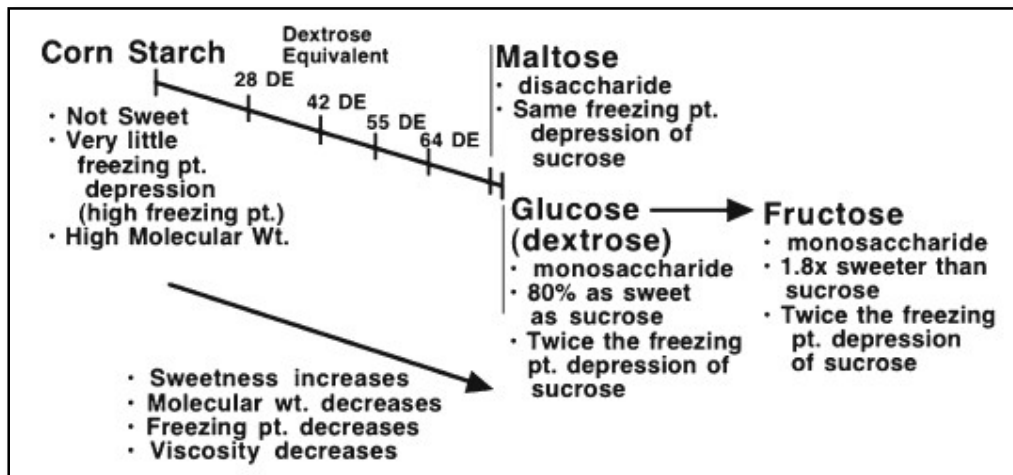


Figure 2.1 Various products obtained from hydrolysis of corn starch (Goff & Hartel, 2013b)

CS derivatives are relatively less sweet than sugar and those with high dextrose equivalent (DE) value have a high freeze point depression as compared to sugar. A high DE value derivative produces softer ice cream whereas incorporation of low DE value derivatives results in firmer ice cream. They also improve the melting properties of ice cream. In general, a mixture of sucrose and CS derivatives are used, with sucrose concentration 12-13 % and CS derivatives concentration 3-4 %. Maple syrup, honey, sugar alcohols are some other examples of sweeteners in ice cream (Goff & Hartel, 2013b).

With changing lifestyles, the demand for low sugar products is on the rise. It has shifted the focus to using non-nutritive sweeteners in ice cream. Saccharin, aspartame, and sucralose are typical non-nutritive sweeteners. More information on the use of non-nutritive sweeteners in ice cream and their effect can be found in the paper by Asghar et al. (2013).

2.2.4 Stabilizers

The primary purposes of stabilizers in ice cream are to enhance mix viscosity, produce smooth texture, prevent shrinkage, prevent ice and lactose crystal growth, and controlling migration of moisture from ice cream to air. They also interact with milk proteins to prevent wheying off (Bahramparvar & Mazaheri Tehrani, 2011). Most of these functions are outcomes of the increased viscosity of the unfrozen portion of ice cream caused by the binding of water to these macromolecules (Goff & Hartel, 2013b).

Most stabilizers used in ice cream are polysaccharides originated from plants. They are long-chain biopolymers with hydroxy groups. Being large molecules, they are not readily hydratable in water and often require shearing or heating to assist with mixing in water (Clarke, 2005). The nominal usage of stabilizers is around 0.1 – 0.5%. Locust bean gum (LBG), guar gum, and carrageenan are the primary stabilizers used in ice cream. Some stabilizers are not compatible with milk proteins and compete to bind water. This results in wheying off in ice cream. Therefore, κ -carrageenan, which can bind to caseins, is often used as a secondary stabilizer in ice cream formulation to prevent phase separation (Bahramparvar & Mazaheri Tehrani, 2011). More detailed information about the type and properties of stabilizers in ice cream can be found in the review by Bahramparvar and Mazaheri Tehrani (2011).

2.2.5 Emulsifiers

For a long time, emulsifiers have been a common ingredient for ice cream manufacturing. They are food additives that lower the interfacial tension between two immiscible phases and result in the formation of stable emulsions. They may also offer other technical benefits such as controlling crystallization, reducing viscosity, and forming a complex with starch in some food systems. Emulsifiers are amphiphilic molecules that have a hydrophilic head and lipophilic tail. (Hasenhuettl & Hartel, 2008).

In contrast to their name emulsifiers are used in ice cream for the destabilization of fat. As an adequate amount of milk proteins is present in the ice cream mix to form a stable emulsion, emulsifiers are not required in the general sense of stabilizing the emulsion (Clarke, 2005). Being a highly surface-active compound, synthetic emulsifiers have a stronger emulsifying capacity as compared to proteins. During aging, an emulsifier displaces a portion of proteins from the surface of fat droplets which results in the weakening of the membrane surrounding the fat droplets. This weak membrane makes fat droplets susceptible to partial coalescence during dynamic freezing and results in the formation of fat structure in ice cream. Formation of fat structure provides stiffness to ice cream which further helps in the extrusion of ice cream.

Emulsifiers also promote the adsorption of fat on air bubbles which provides melting stability to ice cream (Goff & Jordan, 1989).

Traditionally, egg yolk was a commonly used emulsifier in ice cream. In modern times, common emulsifiers used in ice cream include a blend of mono-diglycerides (MDG), polysorbates (PS), and propylene glycol monostearate (PGMS). Mono – diglycerides are produced by the esterification of fatty acids with glycerol (which is usually present in excess). MDG contains more than 40% monoglycerides, which are predominantly responsible for surface-active properties. Diglycerides have two fatty acids esterified to glycerol. Similar to triglycerides, they are lipophilic and possess low emulsifying capacity as compared to monoglycerides. Mixtures containing more than 90% of monoglycerides are also available commercially. The nominal concentration of MDG used is around 0.2 – 0.3 % of the mix. Polysorbates on the other hand are esters of fatty acid of sorbitol with polyoxyethylene groups added to give hydrophilic nature. Polysorbates are regarded as an active drying agent as they promote partial coalescence of fat whereas MDG promotes uniform distribution of air cells. Typical concentration of PS used is around 0.02 to 0.04 % of mix(Goff & Hartel, 2013b; Hasenhuettl & Hartel, 2008).

With the rising demand for eco-friendly, natural, and sustainable products, the requirement for natural additives is on the rise. This has also encouraged the use of natural emulsifiers in ice cream. Egg yolk, lecithin, phospholipids, proteins provide an alternative to synthetic emulsifiers in ice cream. But the use of these emulsifiers is limited due to their efficacy in ice cream systems. The more information of about the emulsifiers can be found in literature by Goff & Hartel, 2013b; Hasenhuettl & Hartel, 2008.

2.2.6 Other ingredients

The use of flavours, inclusions, colours, essences, and other novelty ingredients is on the rise in ice cream. The usage of ice cream along with cakes and puddings has greatly extended the variety in ice cream. Sandwiches, cups, bars are some popular novelty products. In terms of flavour, there is a wide range including cookie dough, buttered pecan, moose tracks, and many more available in the market (Association, 2021).

2.3 Processing of ice cream

Figure 2.2 summarises the manufacturing process of ice cream. The processing of ice cream can be divided into two sub-processes: Mix manufacturing and freezing operation. (Goff & Hartel, 2013a)

Mix preparation comprises the blending of raw materials, homogenization, pasteurization, cooling, and aging. Commonly, a mixing tank with heating capability and a high shear mixer is used for mixing the ingredients. Dry ingredients are added through a high shear venturi mixture to ensure proper mixing. It is common practice to pre-blend sugar and stabilizers to avoid lump formation. All the liquid ingredients are added directly into the mixing tank. Nowadays, a vacuum blending system is used to avoid air incorporation and foam formation during blending (Goff & Hartel, 2013b; Tharp & Young, 2012).

After blending of ingredients, the mix is homogenized and pasteurized. The primary goal of pasteurization is to inactivate any pathogenic microorganisms. Heat treatment also results in the deactivation of lipolytic and proteolytic enzymes which can cause off-flavours in the final product. Depending upon volume, the mix can be batch pasteurized or HTST (high-temperature short time) pasteurized.

In batch operation, the mix is first heated to pasteurization temperature (around 70 °C) in a tank, and it is kept there for a specific holding time (30 min). After holding, the hot mix is homogenized and cooled to 4° C. In continuous operation, the mix is preheated to 60-65 °C and homogenized. Homogenization of mix is done in two stages to avoid clumping of fat globules. Normally, a homogenizing pressure of 10-14 MPa is used. After homogenization, the mix is heated to 83° C using a plate heat exchanger and pass-through holding tube of 15s. Immediately to holding mix at pasteurization temperature the mix is cooled to 4 °C and stored in aging tanks (Bylund, 2003; Goff & Hartel, 2013b).

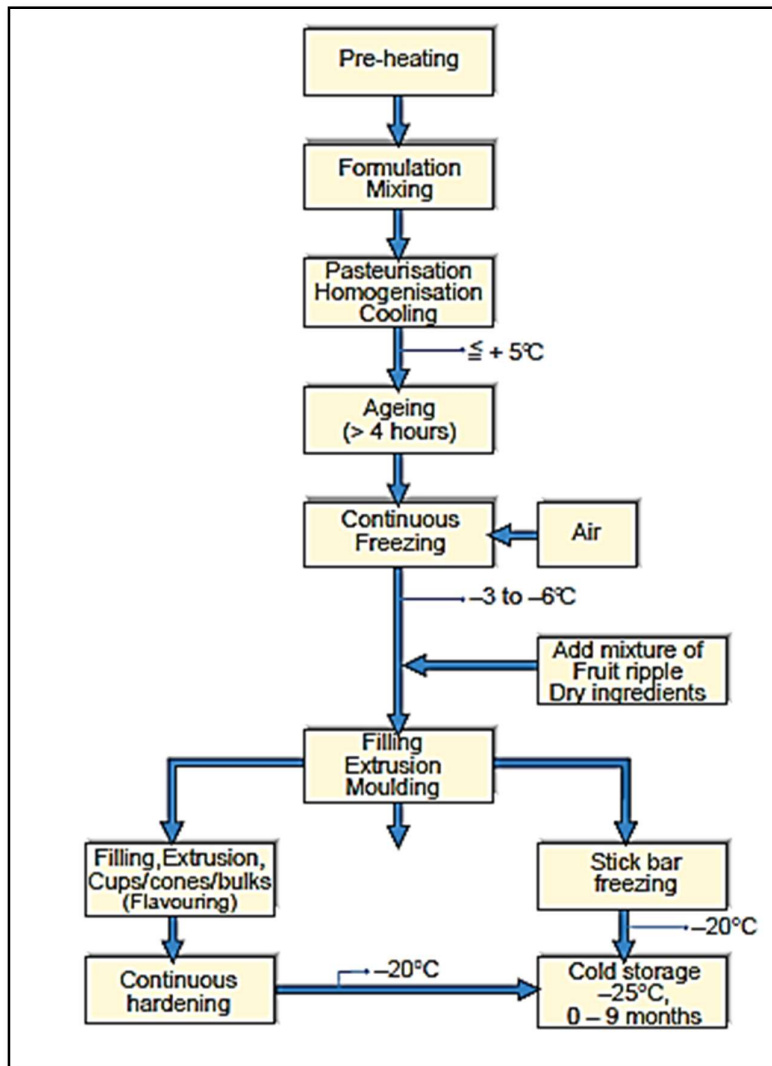


Figure 2.2 Industrial schematic of ice cream production (Bylund, 2003)

The mix is then stored at 4°C in aging tanks. The purpose of aging is to provide sufficient time for the crystallization of fat and displacement of proteins by emulsifiers from fat globules. It also helps in the hydration of protein and development of mix viscosity. The mix is aged at 4°C for a minimum of 4 hrs (Clarke, 2005).

Followed aging, the mix is transferred to the ice cream freezer. Air is whipped into a continuously freezing mix. The formation of air cells, ice crystals, and partial coalescence of fat are changes that happen during freezing. Freezing can be done in a batch or continuous freezer. As 35-65 % of water is frozen, ice cream is still pumpable and transferred to filling or extrusion machines. After filling, ice cream is hardened in a tunnel freezer at -32°C and transferred to storage (Arbuckle, 2013; Goff, 1997b)

2.4 Structure of Ice cream

Arguably, ice cream is one of the most complex food colloids, having multiple phases. The structure of ice cream can be broken down into four phases. The dispersed phases are air bubbles, fat droplets (partially coalesced fat), and ice crystals with serum or matrix continuous phase (Goff & Hartel, 2013b). In phase volume terms, air bubbles are 50 % of total volume, ice crystals are around 30%, fat globules are around 5 % and the rest is serum phase. Figure 2.3 shows different phases of ice cream at -5 °C (Walstra et al., 2005).

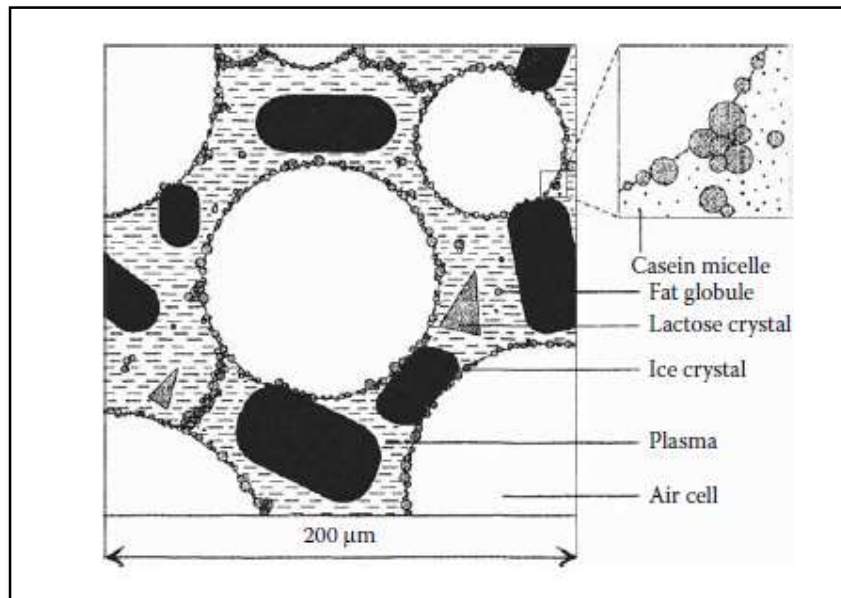


Figure 2.3 Structural components of ice cream (Walstra et al., 2005)

Being a composite material ice cream is made up of a range of ingredients i.e., cream, SMP, stabilizers, emulsifiers, sweeteners, flavours, and air incorporated during freezing. These ingredients and their interaction contribute to the structural make-up of ice cream. Figure 2.4 shows the relationship of ingredients to structural elements of ice cream (Clarke, 2005).

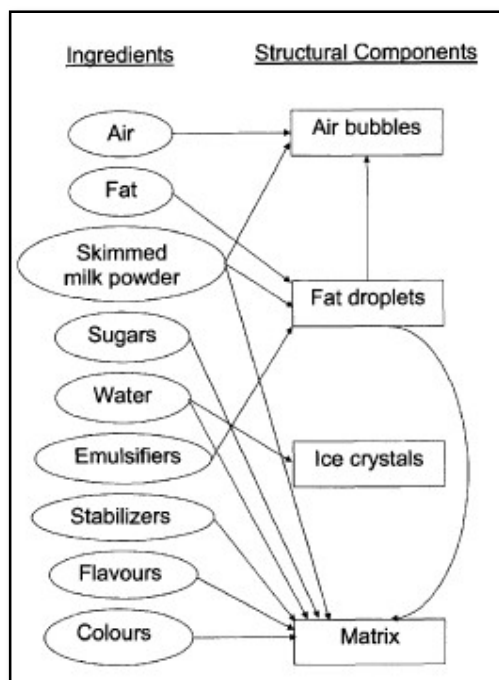


Figure 2.4 Contribution of different ingredients to structural phases of ice cream (Clarke, 2005)

The following sub-sections will discuss the properties of these phases in ice cream and briefly describe the formation of them in ice cream during processing. The fat droplet phase will be discussed separately in section 2.5.

2.4.1 Air cells

Air plays important role in ice cream. It is present in the form of small bubbles in ice cream. The size of air bubbles can be from few micrometres to over 100 μm with 20-25 μm average nominal size. It is desirable to have smaller evenly distributed air cells that provide smoothness to ice cream. The amount of air in ice cream is measured by overrun. Overrun is “% increase in the volume of ice cream greater than the volume of mix used to produce that ice cream” Overrun of ice cream can vary widely depending upon the type of ice cream and legal requirements. Overrun can be as low as 25% or it can as high as 150% but it is most commonly set at 100% (Clarke, 2005; Goff & Hartel, 2013b).

Air is incorporated during dynamic freezing of the mix. Different freezers use different methods of incorporating air into ice cream which impacts the final size and distribution of air cells. A batch freezer incorporates air at atmospheric pressure by folding and mixing it into an ice cream mix. In continuous freezer, freezing is done under pressure which results in the expansion of air cells as ice cream gets extruded from the freezer. (Koxholt et al., 2000).

Irrespective of the type of freezer, the incorporated air is comminuted into smaller bubbles by the shearing forces in the barrel. The breakdown of bulk air into cells and distribution in ice cream depends upon shear forces produced by both ice crystals and the dasher (Goff & Science, 2002). Chang and Hartel (2002a) reported that the formation of ice crystals is necessary for the formation of small air cells as whipping alone (without freezing) does not yield the required size of air cells. Essentially, a longer residence time in the freezer is required to form uniform and small air bubbles, contrary to the short residence time required for the formation of small ice crystals (Chang & Hartel, 2002a).

The new air cells formed during dynamic freezing are stabilized by two mechanisms: adsorption of proteins and adsorption of fat droplets. Proteins along with surface-active emulsifiers get adsorbed into the air matrix interface and lower the surface tension. Being large molecules, proteins provide steric stabilization against coalescence. Adsorption of partially coalesced fat on protein-coated air serum interface gives extended stability to air cells by pickering stabilization. As ice cream has less fat % than whipping cream, the air serum interface in ice cream is not completely covered with fat (Goff et al., 1999b).

Following the dynamic freezing, the size of air cells in ice cream tends to change during static freezing. Air bubbles can potentially undergo disproportionation, coalescence, drainage, and distortion depending upon processing conditions. The rate of these mechanisms defines the distribution of air cell sizes in the final product and the storage conditions of ice cream. Shrinkage of ice cream is one of issue encountered during the storage of ice cream. Shrinkage is defined as when the ice cream starts to pull away from the walls of the container (Chang & Hartel, 2002a, 2002b; Clarke, 2005; Goff & Hartel, 2013b)

The actual mechanisms behind the shrinkage of ice cream are not fully understood yet. Drastic changes in pressure over air cells are the main reason for the shrinkage of ice cream. Some of contributing factors to shrinkage are high overrun and delicate air lamella. Fluctuation in pressure can even happen during transporting from plain area to mountains. It can also be due to the melting, and recrystallization of ice crystals during temperature cycling, which results in pressure changes (Chang & Hartel, 2002b; Goff & Hartel, 2013a).

2.4.2 Ice crystals

Ice is a discrete phase in ice cream. It is present in form of ice crystals. Ice crystals are a crucial structural element of ice cream as their amount and size distribution affect various product properties i.e. hardness, smoothness, cooling effect, and rate of melting (Clarke, 2005).

The formation of ice crystals occurs during dynamic freezing. Approximately half of the volume of water is frozen during dynamic freezing (with about 75-80% of total water present as frozen after hardening). Understanding the actual mechanism behind ice crystals formed in the freezer has been an area of interest for researchers, but our knowledge of the actual mechanism remains limited due to technical issues in visualizing the actual process inside the scraped surface heat exchanger(Goff & Hartel, 2013b). Cook and Hartel (2010) have summarized the process of ice crystallization. Mix at 1-4°C is pumped into the freezer barrel. The mix is cooled by evaporating refrigerant till the temperature at the wall is low enough to cause supercooling which is required to start ice nucleation. At supercooled conditions, an ice layer rapidly forms on the wall of the freezer. Ice is formed as a slushy layer in place of the fully crystallized layer. This slush layer comprises small ice crystals along with sugar and other solutes. The dasher in the freezer scrapes off the slush layer and this layer gets mixed into a warm bulk mix. The slush layer melts and cools the bulk mix. As the bulk mix is cooled enough to reach the freezing point of the mix, ice crystals in the slush layer will not melt and break down into small crystals. Breakdown and mixing of slush layer into mix depend on the type of freezer and design of dasher(Cook & Hartel, 2010; Hartel, 1996).

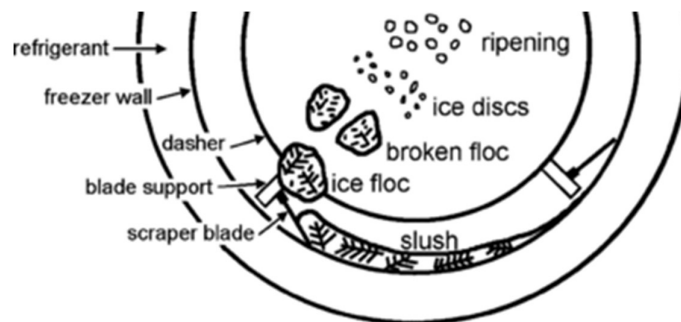


Figure 2.5 Formation of ice crystals in ice cream (Cook & Hartel, 2010)

As freezing proceeds, these ice crystals from the slush layer will further ripen and grow into disc-shaped ice crystals, which can be seen in ice cream after extrusion. Figure 2.5 depicts the mechanism of ice crystals formation in ice cream(Cook & Hartel, 2010).

As the amount of ice further increases during hardening, ice will form on already present ice crystals rather than forming new small ice crystals. This increases the size of ice crystals. Depending upon the rate of cooling during hardening, ice crystals can undergo ripening which results in the loss of smaller ice crystals. Temperature fluctuations during storage and

distribution of ice cream is another important aspect that may lead to the growth of ice crystals due to recrystallization of melted ice (Donhowe & Hartel, 1996).

It is important to have the right amount of ice as it affects the smoothness of ice cream. The amount of ice in ice cream primarily depends upon freeze point depression caused by solute concentration. Sweeteners are the major solutes that affect the freezing point of ice cream. This will in turn affect the supercooling temperature. Therefore, the selection of sweeteners is crucial to control the quality of ice cream. Along with the amount of ice in ice cream, the size distribution of ice crystals is also important for a good quality of ice cream. The nominal size of ice crystals is around 15-30 μm in freshly extruded ice cream and 35-40 μm in hardened. Large ice crystals (greater than 50 μm) can be easily detected in the mouth and leads to grainy texture in ice cream whereas a high amount of ice results in hard ice cream. Therefore, it is essential to control the freezing of mix for even dispersion of ice crystals (Goff & Hartel, 2013b; Regand & Goff, 2003).

Ice recrystallization is one of the major defects encountered in ice cream. Ice cream becomes “icy” or “coarse” with uneven coldness. It is due to large ice crystal formation due to recrystallization of ice during distribution and storage (Arbuckle, 1960). Temperature fluctuations during the storage of ice cream are the prime reason for ice crystallization. The other contributing factors are low total solids, lower freezing temperature, and high extrusion temperature. Controlling the viscosity of the serum phase is crucial to control the extent of ice recrystallization. Therefore, stabilizers are often added to avoid this particular defect in ice cream (Adapa et al., 2000).

2.4.3 Serum phase

The serum is the unfrozen part of ice cream which holds all the structural elements of ice cream together. In colloidal aspect, the serum is the continuous phase and all other phases (ice crystals, air cells, fat globules) are dispersed into it. It consists of unfrozen water (at a given temperature of the product), proteins (which do not adsorb on-air – matrix interface), stabilizers, sweeteners, and other dissolved solutes (Arbuckle, 1960).

The composition of the serum phase is temperature dependent. During dynamic freezing, depending upon the drawing temperature of ice cream 33-60 % of water is frozen. The remaining 23- 57 % of water is frozen during the hardening of ice cream. As more and more water in serum is converted to ice crystals during hardening the concentration of solutes tends to increase relatively. This increase in the concentration of solutes leads to further freeze point

depression and tends to increase the viscosity of the serum phase (Clarke, 2005; Cook & Hartel, 2010; Goff & Hartel, 2013b).

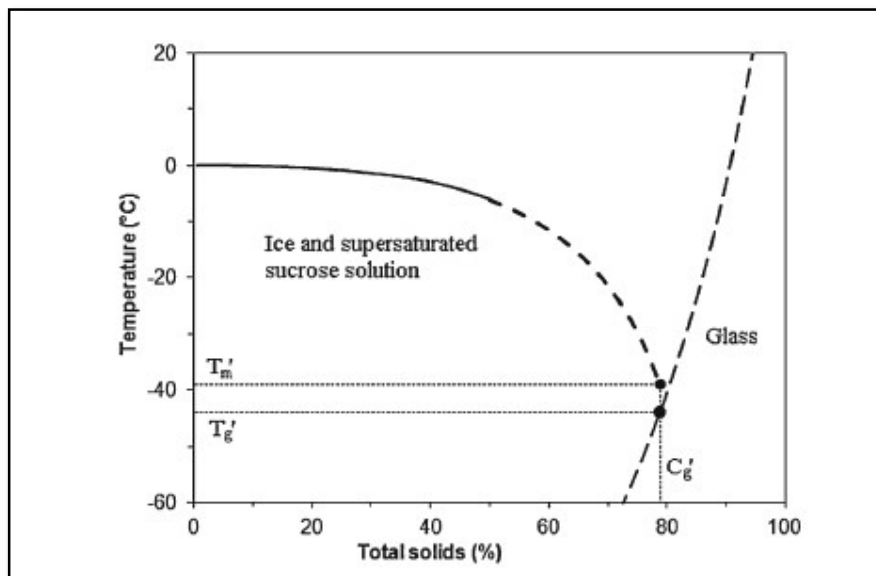


Figure 2.6 Phase diagram of ice cream mix (Hartel, 1996)

As indicated in figure 2.6, at a certain time point the temperature of ice cream reaches to mechanical glass transition temperature ($T_m \sim -32$ °C). Below T_m the molecules in a serum are immobile and there will be no further ice formation in ice cream. T_g (~ -40 °C) is glass transition temperature at maximum freeze concentration at which ice cream becomes sugar glass. From the manufacturer's point of view, it is crucial to store ice cream near T_m as it will remain more stable. However, ice cream is usually stored at -18 °C which is higher than T_m . Thereby, concentration kinetics of the serum phase are important to determine the stability of ice cream (Cook & Hartel, 2010; Goff & Hartel, 2013b; D. W. Stanley et al., 1996).

Milk proteins also form a significant portion of the serum phase. They interact with water and determine the rheological properties of the aqueous phase. Freeze concentration of proteins results in a significant increase in viscosity which largely controls the formation of ice crystals during the hardening of ice cream and the recrystallization of ice during the storage of ice cream. Another important role protein performs in the serum phase is that they interact with stabilizers, particularly with κ -carrageenan. Being polymeric molecules, proteins and hydrocolloids are not compatible with each other. This leads to phase separation in unfrozen ice cream. The phase separation may result in leakage of serum in ice cream and soft-serve ice cream. During storage it leads to separation of ice cream into two phases. Thereby, this phase separation is not desirable in ice cream. To counter this issue, a second stabilizer i.e., κ -

carrageenan is often added to ice cream. κ -carrageenan interacts with caseins and prevents this phase separation (Bahramparvar & Mazaheri Tehrani, 2011; Clarke, 2005; Goff & Hartel, 2013b).

Lactose is present in a supersaturated form in the serum phase. It is a disaccharide, with relatively low solubility in water (around 16 %). The normal % of the lactose in ice cream mix is around 6%. It crystallizes in two forms: α monohydrate and β anhydride. The formation of α form leads to a sandiness defect in ice cream. During freezing, the concentration of lactose is increased multiple times (~ 40 %) and this may lead to the formation of lactose crystals. But increased solution viscosity by stabilizers and proteins helps to keep lactose in a supersaturated state. It is worth noting the increasing the level of lactose in ice cream (by using whey protein concentrate) will lead to freezing point depression, producing hard ice cream with sandiness defect (Arbuckle, 2013; Goff & Hartel, 2013b; Reid & Powell, 1933).

2.5 Formation of fat structure in ice cream

Fat is an important structural element of ice cream. Fat in ice cream is ideally present as “partially coalesced aggregates” and “fat droplets”. It is present in both serum and at the interface of air cells in ice cream (Clarke, 2005). These fat aggregates are responsible for the stabilization of the newly formed air cells. They also determine the quality of ice cream in terms of its melting properties, dryness, and stiffness. Therefore, it is essential to control the agglomeration of fat to produce a good quality ice cream (Goff & Hartel, 2013b). Agglomeration of fat is also referred to as “destabilization of fat” or “partial coalescence” (Tharp & Young, 2012).

The scope of this research is mainly focused on the manipulation of the formation of fat structures and controlling the extent of fat agglomeration in ice cream. Thereby, it is essential to understand the basics of partial coalescence of fat and how different factors affect it. This section will first focus on understanding the mechanism of partial coalescence. It will elaborate how partial coalescence of fat is the mechanism behind the structure formation in butter, whipped cream, and ice cream. It will also discuss various factors that can be used to manipulate the fat structuring mechanism in ice cream.

2.5.1 Partial coalescence

In terms of thermodynamic behaviour, an emulsion is a highly unstable system. An emulsion tends to undergo phase separation because of interfacial forces acting between the two phases. Therefore, an emulsifier is often added to reduce the interfacial tension between phases and

make a stable emulsion. Though emulsifiers tend to stabilize the emulsions to a certain degree, they cannot completely prevent destabilization mechanisms. These different destabilizations which can occur in an emulsion are creaming, aggregation such as flocculation, coalescence, and Ostwald ripening (Darling, 1982; Goff, 1997b; McClements, 2015). Figure 2.7 depicts various destabilization mechanism to which an emulsion may be susceptible.

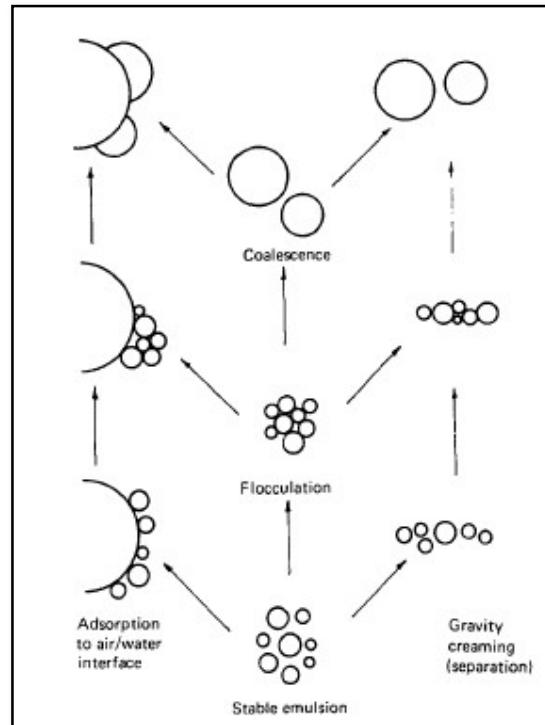


Figure 2.7 Various destabilizations mechanism in an emulsion (Darling, 1982)

Partial coalescence is also a destabilization mechanism in emulsions which is a special manifestation of coalescence. It happens when two or more partly crystalline oil droplets come into contact and form an irregularly shaped aggregate (McClements, 2015).

An emulsion in which all the fat in droplets present in a liquid state does not undergo partial coalescence. Upon cooling the emulsion, crystallization of fat initiates. This crystallization can be primary (formation of homogenous small fat crystals) or secondary (formation of fat crystals on existing impurities i.e. SMS). With time, fat crystals grow and become larger. At a certain stage of growth, these fat crystals protrude outside of the fat globules. During this stage, on the application of shear stress, the fat droplets will collide with each other and the fat membrane between droplets will rupture and oil from one droplet will be transferred to the other. The presence of fat crystals in oil droplets in these emulsions inhibits the complete flow of fat from one globule to another and each globule will partially retain its original shape. This leads to the

formation of irregular clumps of fat as shown in figure 2.8. This mechanism is known as partial coalescence (Walstra & Boode, 1993).

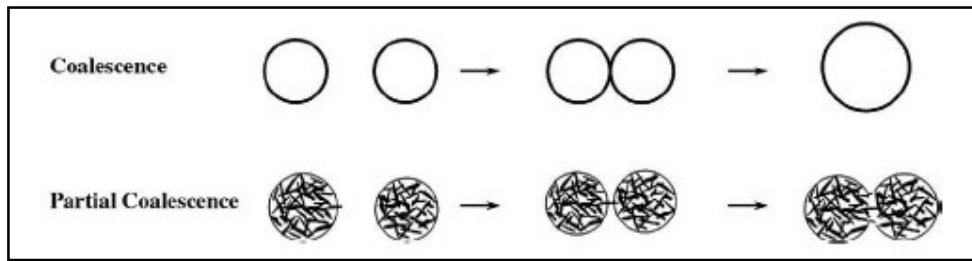


Figure 2.8 Contrast between Coalescence and Partial coalescence(Walstra & Boode, 1993)

Partial coalescence results in the formation of fat aggregates as indicated in figure 2.9. Aggregates produced by partial coalescence can result in a significant structural difference in emulsions as compared to full coalescence. Aggregates formed can lead to an increase in viscosity. A higher degree of aggregation can lead to the formation of a continuous network as occurs in whipped cream and give the product solid-like characteristics (through yield stress)(Fredrick et al., 2010; Goff, 1997b).

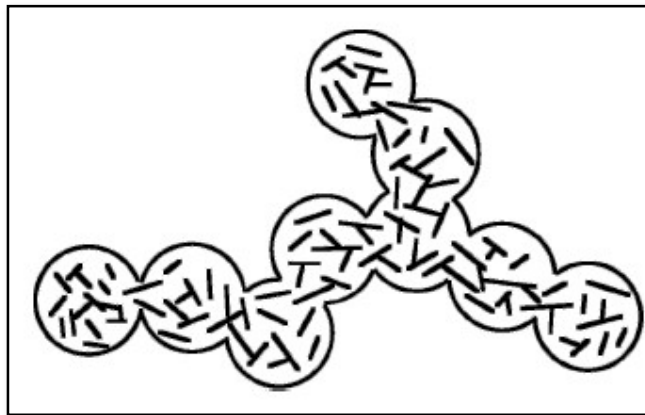


Figure 2.9 Fat aggregates produced during partial coalescence (Goff, 1997b)

The susceptibility of an emulsion to undergo partial coalescence determines the final physical state of the emulsion. In general, destabilization mechanisms are undesirable in emulsions, but partial coalescence is one such phenomenon that essential to produce fat structure in dairy emulsions (Fredrick et al., 2010; Goff, 1997b).

Note: For this document, the terms “fat agglomeration”, “fat aggregation”, “partially coalesced fat” are used interchangeably with “partial coalescence”.

There are pre-requisite conditions for partial coalescence to happen in an emulsion. As outlined by Fredrick et al. (2010) these conditions are:

- Presence of partial crystalline fat phase - which is dependent on the melting profile of the fat present in the emulsion
- Fat crystals should have a tendency to network formation and some fat crystals should be protruding, which will assist in piercing the membrane of aggregating globules
- The gap between the two aggregating fat globules should be less than the protruding length of fat crystals
- Formation of aggregate in emulsion should not result in immobilization of emulsified phase

2.5.1.1 Partial coalescence: a structuring mechanism in different dairy emulsions

As mentioned, partial coalescence is one of the key mechanisms in the butter processing, and formation of structure in whipped cream and ice cream. The difference between butter and whipped cream lies with the fat content in the final product. Fat is around 80-82 % in the case of butter and 35-40 % for whipped cream. In terms of raw material, butter and whipped cream are both made from cream with 38- 40% fat and the difference lies with the extent of partial coalescence. In ice cream the total fat content is around 10%. The difference from whipped cream or butter lies with the level of fat available for partial coalescence and the processing conditions during shearing (Rybak, 2016).

Butter

The formation of butter grains during the churning of cream relies on the beating of air into cream. Generally, a non-homogenized cream (in which fat globules are present in their native state) is used for the production of butter. During churning, the air is beaten as small bubbles in the cream. Fat globules (present as partial crystals) come in contact with these air bubbles. During this contact, the content of the globule membrane and some liquid fat get spread over the air-water interface. This results in the sticking of fat globules to air bubbles. This sticking of fat globules is known as flotation. Air bubbles will have multiple fat globules attached. As shown in figure 2.10, these globules get attached with the help of liquid fat. Continuous agitation cause air bubbles to coalesce with each other, thereby decreasing the surface area and this releases small fat clumps (Rybak, 2016; Walstra et al., 2005).

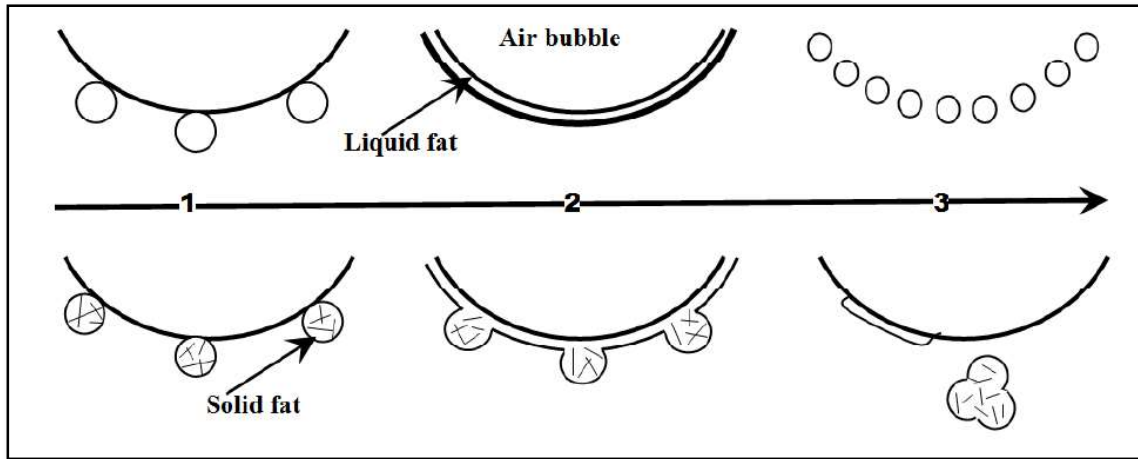


Figure 2.10 Formation of butter grains (Walstra et al., 2005)

These fat clumps formed during the initial stage undergo partial coalescence with other fat globules and grow larger. With time, clumps will form big aggregates and air bubbles will begin to destabilize. At end of churning, there is a little foam is present as there are few fat globules are available to stabilize. It can be inferred that floatation is required to form initial aggregates of fat in churning and partial coalescence by shearing dominates in the latter part of butter making process (Fox & McSweeney, 2007; Rybak, 2016; D. Stanley et al., 1996).

Whipped cream

Partial coalescence is a major phenomenon responsible for the conversion of viscous cream into the solid-like viscoelastic structure of whipped cream during whipping. The whipping process involves the beating of air into cream. Air bubbles formed during the initial stage of whipping are stabilized by milk proteins including casein and whey proteins. As whipping proceeds, mechanical treatment results in disruption of fat globule membrane, and the hydrophobic portion of fat globules get exposed to the air- plasma interface. Due to interfacial tension difference, fat globules penetrate the existing protein layer on the air bubbles and get attached to the air-plasma interface. During contact with the air-water interface, liquid fat gets partially spread over the air-serum interface. This liquid fat helps to form aggregates with other fat globules and results in the formation of a fat network throughout the cream. This network entraps air cells and produces a stiff whipped cream (Anderson & Brooker, 1988; Brooker et al., 1986; Fox & McSweeney, 2007).

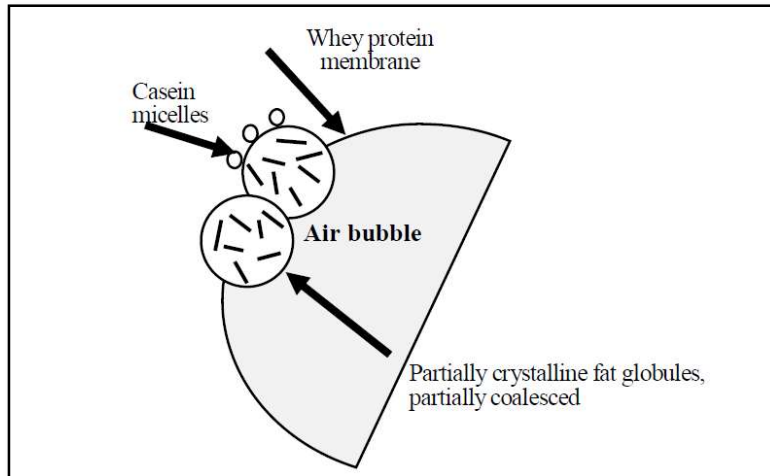


Figure 2.11 Air-water interface in whipped cream (Rybak, 2016)

Figure 2.11 shows the typical air-water interface in whipped cream. The nature of the membrane surrounding fat globules is critical for the whipping capacity of cream. It is difficult to whip a homogenized cream. Fat globules in homogenized cream have a protein membrane which makes them stable against partial coalescence. Therefore, small-molecule surfactants are used in homogenized cream to displace a portion of protein from the surface of fat globules and make cream whippable. Another approach is to use low homogenization pressure (1 to 4 MPa), which results in the formation of fat clumps which can be whipped. Like butter, where flotation mechanism is an initial requirement for butter grains to form, formation of structure in whipping cream is mainly due to flotation mechanism (Brooker et al., 1986; Goff, 1997b; D. Stanley et al., 1996).

Ice cream

The formation of fat structure in ice cream by partial coalescence is quite similar to heavy whipped cream. The difference lies in the amount of fat present in the ice cream mix and its interaction with ingredients during homogenization. Homogenization of ice cream is a crucial step in determining the properties of the fat interface. Homogenization of the mix produces smaller fat globules (0.5 to 2 μm). The new fat globules formed during homogenization are devoid of any membrane. The surface-active components (casein, whey proteins, phospholipids, and synthetic emulsifiers) present during homogenization will start to get adsorbed on newly formed fat globules and stabilize. These compounds will form a membrane around fat globules in the ice cream mix. The membrane around fat globules keeps developing during aging and undergoes rearrangement (Goff & Jordan, 1989; Goff, 1997b; Goff & Science, 2002).

During the initial stage of adsorption, caseins and whey proteins get adsorbed. During aging the synthetic emulsifier having higher surface tension lowering capacity than proteins, displace a portion of proteins from the surface of fat globules and renders them more susceptible to partial coalescence. These small surfactant molecules form patches on the surface of fat globules, sufficient to produce a stable emulsion but weak enough to be disturbed by shearing action during freezing. Barford et al. (1991) reported that 10-12 mg of protein adsorbed per m² of fat in the absence of emulsifier in contrast to 5-6 mg of protein with emulsifiers. The ratios of casein and whey protein are also one of the important aspects in terms of SMS displacing the proteins. It has been reported that adsorbed casein micelles on the fat interface are easier to displace by SMS as they do not provide full coverage of fat globules and SMS can easily adsorb between two casein micelles. Adsorbed whey protein layers are more difficult to displace, as they provide full coverage. Therefore, the ratio of casein and whey proteins present in the mix is also crucial for determining the stability of fat globules against partial coalescence (Barford et al., 1991; Bolliger et al., 2000; Goff, 1997b; Segall & Goff, 1999).

Aging for 4-24 hrs provides enough time for the above-mentioned re-arrangement and crystallization of fat. The next stage of structure formation in ice cream occurs during concomitant whipping and freezing in the ice cream freezer. During freezing, air is incorporated into the mix and small air cells start to form in the mix. Newly formed air cells are stabilized by milk proteins. As well incorporating air, whipping also applies shear stress, which increases with the formation of ice crystals. Upon continuous whipping and application of shear stress (due to dasher movement and frozen phase) the fat present in the ice cream mix undergoes partial coalescence. The partially coalesced fat entraps the air cells and interacts with the protein present at air cells. This leads to the formation of a semi-continuous network of fat throughout the ice cream. This network contributes to the dryness of ice cream upon extrusion, smooth texture, and melting resistance in ice cream (Goff et al., 1999b; Goff, 1997a, 1997b; Goff & Hartel, 2013b).

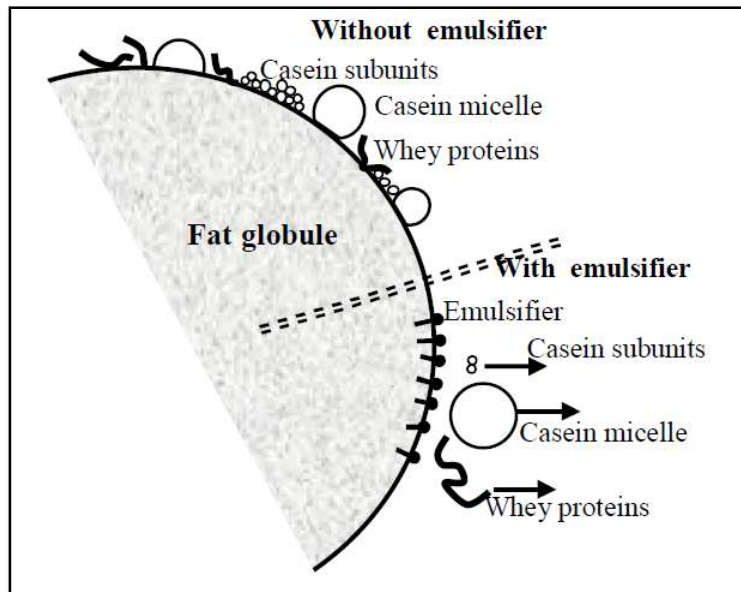


Figure 2.12 Comparing fat interface for ice cream with and without emulsifiers (Rybak, 2016)

Comparing the fat interface between two ice cream mixes with and without emulsifier. As shown in figure 2.12, it can be visualized that fat globules without emulsifiers have a thick interfacial layer. This thick layer will limit the ability of fat globules to undergo partial coalescence and air cells will not be stabilized by partially coalesced fat. The resultant ice cream will also lack dryness, smooth texture, and melting resistance (Goff, 1997a, 1997b; Rybak, 2016).

As outlined before, in lieu of finding novel emulsifiers for ice cream the objectives of this research are to use natural emulsifiers along with adapted processing where necessary. The basis of using these alternative means revolves around controlling the degree of partial coalescence in ice cream. Therefore, it is essential to investigate factors that influence the extent of partial coalescence. It is also worth noting that factors discussed in this text are general factors and it is rare that all these factors can be manipulated to get desired attributes in a product. Subsequent sections will discuss factors that control the kinetics of partial coalescence which in turn affect the degree of fat destabilization.

2.5.2 Factors affecting the degree of fat destabilization

Walstra (2003) defined the rate of partial coalescence as “ the product of encounter frequency and capture efficiency (α)”, where encounter frequency/collision frequency is the measure of how frequent two fat globules are colliding with each other and capture efficiency is the probability of two fat globules partially coalescing during their collision (Walstra, 2003).

Multiple factors affect collision frequency and capture probability and resulting rate of aggregation. These factors can be categorized into two regimes: the process parameters, and the emulsion composition and formulation (Fredrick et al., 2010).

2.5.2.1 Emulsion composition and formulation

Structurally an emulsion can be divided into three different phases: the dispersed oil phase, interfacial layer between the oil and continuous phase, and the continuous phase. The composition of these phases is crucial to determine the tendency of an emulsion to undergo partial coalescence (Fredrick et al., 2010).

The type and composition of the fat largely determines the oil phase's composition. The composition of continuous or serum phase mainly depends upon water-soluble compounds and their behaviour. Other than composition, concentration of different components in their respective phases is crucial to predict the stability of emulsion against partial coalescence.

2.5.2.1.1 Composition of the oil-water interfacial layer

The composition of the interfacial layer is a prime factor in determining the stability of an emulsion (McClements, 2015). It is difficult to assess the stability of an emulsion in which the composition of the interfacial layer is dynamic. Changes in the type of emulsifiers or their concentration during homogenization affect the surface load, behaviour of fat crystallization, and thickness of the surface film. While these dynamic alterations make it hard to predict the stability of emulsion there are some experimentally demonstrated trends (Fredrick et al., 2010).

During homogenization, fat in the emulsion is converted into small globules and the surface-active compounds present during homogenization will adsorb onto these newly formed fat globules. Under quiescent conditions, repulsion forces are acting between emulsion droplets, providing stability against partial aggregation. The colloidal repulsion forces can be steric repulsion or electrostatic depending upon the type of surfactants and their physical properties i.e., pH of the continuous phase. The application of large shear forces can help to overcome these forces (Boode, 1992).

In terms of the type of surfactants, different types of emulsifiers can be found in food emulsions. These emulsifiers are proteins, small molecule surfactants (SMS), lipids (phospholipids), hydrocolloids, and many more. The scope of the present discussion is limited to protein, SMS, and phospholipids. In general, it has been demonstrated that emulsions stabilized by proteins have lower rate of partial coalescence than small molecule surfactants.

This is because protein adsorbed on the interface provides higher steric stabilization than small molecule surfactants. The greater stability of protein stabilized emulsions is also attributed to the formation of the rigid interfacial layer formed by proteins, which limits crystal protrusion thereby reducing the capture efficiency. Whereas, small-molecule- surfactant forms a weak interfacial layer that is easy for protruding fat crystals to penetrate (Van Boekel & Walstra, 1981).

In terms of concentration of emulsifiers, increasing surface load or fuller coverage of fat globules in the emulsion provides better stability. Therefore, increasing protein or SMS concentration in the emulsion increases its stability against partial coalescence. This increase in stability is limited to the extent of interfacial coverage (Anderson & Brooker, 1988).

Proteins

In the case of protein stabilized emulsions, stability of the emulsion is positively correlated to an increase in thickness and viscoelasticity of the protein layer at the interface and steric repulsion with increasing protein concentration on the interface. The charge on proteins at the nominal pH of the emulsion and their structure are other factors crucial to determining the efficiency of proteins to produce a stable emulsion. Differential surface-active behavior is exhibited by different proteins owing to their differing thickness and packing at the interfacial layer (Fredrick et al., 2010).

For milk proteins, it has been illustrated that casein stabilized emulsions are more stable against partial coalescence than whey protein stabilized emulsions (Boode, 1992). One important consideration in the case of milk protein-based emulsion is changes in protein with processing i.e. heat treatment. Heating before or after emulsification can induce structural changes in proteins which can alter their interfacial behaviour (Das & Kinsella, 1990). Das and Kinsella (1990) reported that pre heated whey protein stabilized emulsions are less stable than emulsion stabilized by native whey protein. Whereas heat treatment does not have much impact on casein-stabilized emulsions, owing to the high heat stability of casein.

Small molecule surfactants (SMS)

Small molecule surfactants are typically fatty acids esterified with hydrophilic groups (glycerol, sorbitols). Some examples of SMS are mono diglycerides (MDG), sorbitans esters of fatty acids, and sucrose esters. Due to their efficient packing at the interface, small molecule surfactants are more surface active than proteins. Therefore they undergo competitive adsorption with proteins in emulsions and displace them (Hasenhuettl & Hartel, 2008; McClements, 2015).

In terms of interfacial protein displacement, it has been illustrated that adsorbed interfacial casein micelles are more easily displaced than whey proteins by SMS. Mackie et al. (1999) described how displacement of interfacial protein occurs in three stages. First, SMS form nuclei at protein defect sites. Second, with time these nuclei grow larger causing compression of the protein layer. Lastly, the protein network collapses, and proteins get displaced into the aqueous phase. Water-soluble SMS is more effective in displacing interfacial proteins than oil-soluble SMS (Mackie et al., 1999).

Dickinson et al. (2001) stated that displacement of proteins by SMS weakens the interfacial layer and decreases emulsion stability. As described earlier, displacement of proteins by SMS is the main structuring mechanism for structure formation in ice cream. Other than reducing protein surface load, SMS can increase the wettability of crystals by aqueous phase and increase the protrusion length of crystals across the interface. Thereby, the emulsion becomes susceptible to partial coalescence. It can be concluded that SMS can be used to control the capture efficiency of fat globules thereby controlling fat destabilization in emulsions (Dickinson et al., 2001).

Zhang and Goff (2005) studied the ability of two different emulsifiers, glycerol monostearate (GMS, saturated) and glycerol monooleate (GMO, unsaturated) to displace different milk proteins from the oil-water interface in ice cream. The two emulsifiers were tested in two ice cream formulations (one having SMP as a protein source and the other having whey protein isolate (WPI) as a protein source). It was reported that the unsaturated emulsifier (GMO) was more effective in displacing interfacial caseins than the saturated emulsifier (GMS). For ice cream made only with WPI, neither emulsifier was found to be fully effective against displacing interfacial whey proteins (Zhang & Goff, 2005).

Phospholipids: interfacial layer in milk and milk products

Phospholipids are polar lipids, derivatives of triglycerides with phosphatidic acid and that have various organic groups attached to them. Phospholipids, being amphiphilic molecules, are surface-active compounds (Walstra et al., 2005). They are the main components of the interfacial layer of native milk fat globules, known as the milk fat globule membrane (MFGM). In general, the milk fat globule membrane constitutes phospholipids, a mixture of proteins, neutral lipids, polar lipids, cholesterol, and others. As shown in figure 2.13, glycerophospholipids, phosphosphingolipids, and cholesterol are distributed in a tri-layer system in an MFGM in milk.

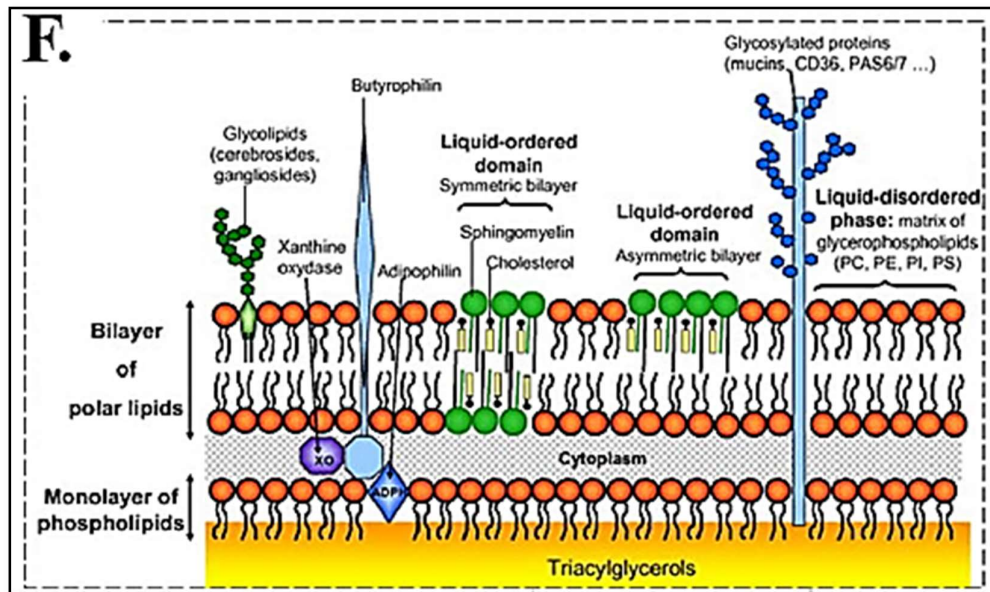


Figure 2.13 Arrangement of different components in MFGM (Jukkola & Rojas, 2017)

Triacylglycerols in fat globules are surrounded by a monolayer of phospholipids followed by a protein coating. Further to this protein space is a bilayer of polar lipids. “This bilayer is separated into liquid-ordered domains, rich in sphingolipids and cholesterol, and liquid-disordered domains of glycerophospholipids” (Jukkola & Rojas, 2017). The role of MFGM is to provide stability to fat globules against physical instabilities i.e., coalescence and enzymatic degradation due to lipase enzyme. It also determines the colloidal interaction of fat globules in milk (Jukkola & Rojas, 2017; Walstra et al., 2005). It is also worth noting that MFGM structure in native milk can be altered used different techniques. Mechanical treatments of milk or milk products such as homogenization, shearing, whipping and others result in deformation of the native MFGM. The extent of deformation is proportional to the magnitude of the forces applied during processing.

As discussed, phospholipids (PLs) play important role in stabilizing the native milk globules in milk. With the recent trend of claiming “all-natural” labels on food products, there has an upward trend in exploring the use of natural phospholipids as potential emulsifiers in food applications. In dairy emulsions, the use of phospholipids has been very limited due to the unavailability of trial volumes.

2.5.2.1.2 Fat volume fraction

The proportion of fat in an emulsion is also an important attribute in deciding whether an emulsion will undergo partial coalescence or not. Generally, increasing the fat proportion in

emulsion decreases its stability. Increasing the fat percentage in an emulsion tends to increase the encounter frequency of partially solidified fat droplets. It is also worth noting that in the case of dairy emulsion such as whipped cream, a minimum % of fat is required for partial coalescence to happen during whipping (Fredrick et al., 2010; Vanapalli et al., 2002).

2.5.2.1.3 Composition of Continuous phase

The composition of the continuous phase is another attribute that plays a role in determining the stability of the emulsion. The pH and ionic of an emulsion are crucial in determining the extent of repulsive forces acting between the droplets, which influences the stability of an emulsion. Other than pH and ionic strength, the solute components such as sugar, biopolymers, and surface-active compounds can affect the stability of emulsions by affecting the viscosity or competitive adsorption with protein or other mechanisms (Fredrick et al., 2010; Thanasukarn et al., 2004).

2.5.2.2 Process parameters

Process parameters are the variables of unit operations used for the processing of an emulsion. They may or may not be constant during processing. Flow conditions (which determine the application of different mechanical shear forces) and time-temperature profile during processing are the main process parameters that affect the kinetics of partial coalescence (Fredrick et al., 2010).

2.5.2.2.1 Flow conditions

The motion of droplets in an emulsion determines how often they may come in contact with each other (in other words the collision frequency). In stationary conditions, the motion of oil droplets in the emulsion is a result of Brownian motion, thus a low collision frequency. Application of hydrodynamic mechanical forces (“proportional to shear rate in shear forces”) during processing will ramp up the motion among the droplets and will result in an increased encounter frequency of droplets, thus increased rate of partial coalescence. Therefore creating high turbulent flows in emulsions positively contribute to the partial coalescence of emulsions (Boode, 1992).

In the case of shear forces, the application of a velocity gradient across the flow layers will also alter the encounter probability. This is due to fact that an applied velocity gradient will result in the rolling of droplets over each other and increase the probability of film penetration between two droplets by protruding crystals. Moreover, the applied force will overcome the repulsive forces acting between the droplets, thereby reducing the distance between droplets to

less than the length of protruding crystals and resulting in increased capture efficiency. It has been demonstrated that an emulsion has a threshold shear rate above which it undergoes partial coalescence. Below this threshold value, there is no or minimal partial coalescence (Boode & Walstra, 1993; Fredrick et al., 2010).

2.5.2.2.2 Time-temperature profile

The processing temperature and prior applied time-temperature conditions largely determine the physical state of the fat in an emulsion which is one of the important factors in determining the stability of emulsion against partial coalescence (Fredrick et al., 2010).

As the temperature of the emulsion reaches undercooled conditions, the oil fraction in the emulsion starts to undergo crystallization. This results in partial solidification of the fat present in droplets dispersed in the continuous phase of an emulsion. The solid fat content (SFC), size and morphology of crystals, and their distribution/arrangement in these partly crystallized droplets influence the ability of fat to undergo partial aggregation. In addition, the freezing of water present in the continuous phase (below 0° C) results in the formation of ice crystals. This further enhances the rate of partial coalescence. This section will discuss the outlined factors (Boode et al., 1993; Darling, 1982).

Solid Fat Content (SFC)

Solid fat content is the amount of solid fat present in emulsion droplets, and it greatly influences the rate of partial coalescence. Upon crystallization, a bundle of fat crystals starts to form inside the globules. The extent of this network is determined by SFC at a given temperature. Below a certain threshold value of solid fat content, crystals do not form a network and do not protrude outside the fat globules. Thus, below a certain threshold value of SFC emulsions remain stable against partial coalescence. While a minimum SFC is required for partial coalescence to occur, but that requirement of some liquid fat is indispensable. Oil is required for wetting the protruding crystals of another oil droplet into a fat globule. In absence of oil, there is no wettability among crystals, which is required to form junctions between two colliding droplets. Therefore, it is the balance of SFC and oil content in fat globule that is a prerequisite for partial coalescence (Boode, 1992; Boode & Walstra, 1993; Boode et al., 1993; Fredrick et al., 2010).

At a given temperature, the SFC of fat largely depends upon the fatty acid composition of triglycerides, morphology, and location of crystals. In terms of SFC required for partial coalescence, Davies et al. (2000) reported that 10-50 % SFC is the optimum range for

maximizing the rate of partial coalescence. In the case of ice cream, the presence of an optimal amount of SFC is required to build a fat structure during freezing. The high amount of oil in fat globules leads to the spreading of oil over air bubbles and results in collapsing of air cells. As it is difficult to determine the optimum content of SFC, it is suggested that SFC content in the range of 1/3 to 2/3 of total fat is optimum for partial coalescence in ice cream. Optimal partial coalescence leads to a the dense aggregate structure (Boode & Walstra, 1993; Davies et al., 2000; Goff & Hartel, 2013b; Hasenhuettl & Hartel, 2008).

Arrangement of Crystals

Arrangement of crystals in fat globules is during the crystallization of fat is another critical aspect for determining the tendency of globules to undergo partial coalescence. Even at an optimum amount of solid fat content fat droplets can be stable against partial coalescence if crystals are not protruded outwards from the interface. The contact angle of the crystal in the oil phase (θ_o) and water phase (θ_w) determines its protrusion tendency. Thermodynamically, the fat crystal formed in globules tends to move at the oil-water interface. As shown in figure 2.14, a typical crystal at interface experiences three different interfacial forces: forces acting between water-oil interface, water- solid interface, and oil -solid interface. These forces acting on the crystal determine its contact angles (θ_w and θ_o) (Boode et al., 1993; Fredrick et al., 2010; Walstra & Boode, 1993).

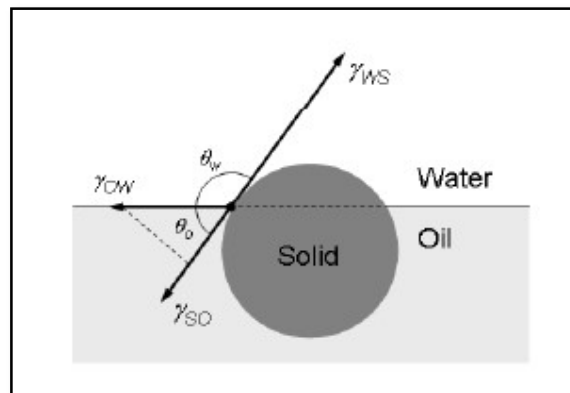


Figure 2.14 Contact angles of a crystal at oil – water interface (Fredrick et al., 2010)

For the majority of emulsions, the contact angle with water (θ_w) is between 120 -160 degrees and in this range, the crystal is wetted by the oil phase and has the lowest free interfacial energy. At smaller θ_w , the crystal will have high interfacial energy and will be primarily

wetted by the aqueous phase. Irrespective of the wetting scenario and free energy state, the free moving crystal tends to be at a tangent to the oil-water interface as shown in figure 1.16 (Boode et al., 1993; Fredrick et al., 2010; Walstra & Boode, 1993).

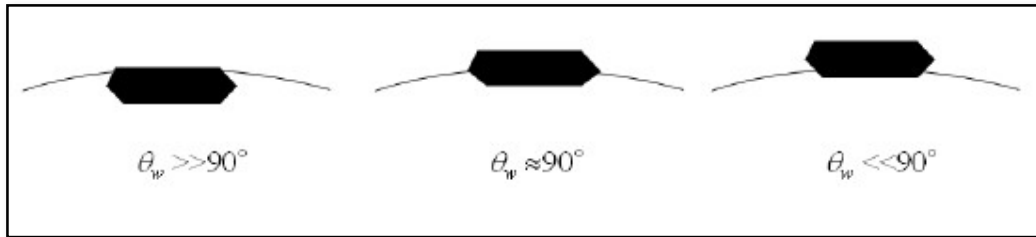


Figure 2.15 Different positions of a crystal at oil- water interface (Fredrick et al., 2010)

Based on crystal arrangement in fat globules, Walstra and Boode (1993) defined six classes of fat globules. These classes are O, N1, N2, L, M, and K type fat globules as shown in figure 1.17.

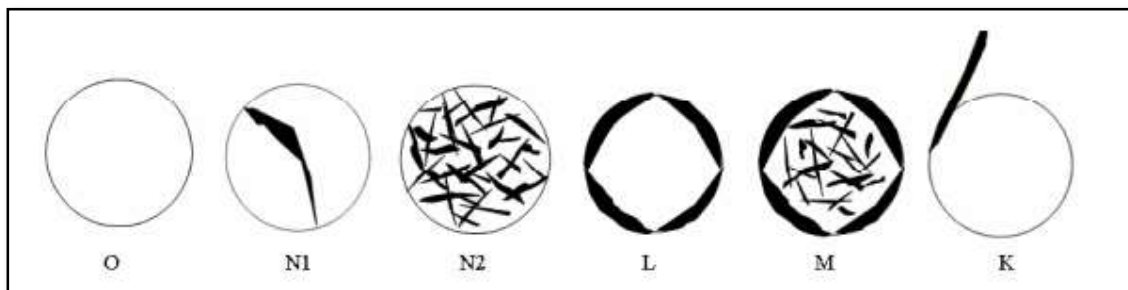


Figure 2.16 Types of fat globules based on type of fat crystal network (Walstra & Boode, 1993)

In the case of layered (L type) fat globule the encounter efficiency (α) is dependent on θ_w . At $\theta_w \gg 90^\circ$, the crystals are wetted by oil phase and will rarely undergo partial coalescence. At $\theta_w = 90^\circ$, the crystals are wetted by both water and oil phases. At $\theta_w \ll 90^\circ$, the aqueous phase will be mainly wetting the crystal, and aggregation between fat globules will occur only by bridging so it will rarely undergo partial coalescence. In the case of N-type crystals, the capture efficiency is predominantly high at $\theta_w \ll 90^\circ$ as a large number of crystals will be protruded. In the case of N2 type fat globules, the capture efficiency will be highest as α reaches 1 (Boode et al., 1993; Fredrick et al., 2010; Walstra & Boode, 1993).

It is difficult to predict and control the type of network developed during the crystallization of fat. Thereby, not much research has been conducted on specifically altering the crystal network in dairy emulsions such as whipped cream and ice cream.

Tempering

Tempering or rebodilying is known to be a mechanism for increasing the viscosity of cream. Tempering is the phenomenon where a cooled emulsion is warmed to a temperature (T_{\max}) at which a small fraction of solid fat is left in the fat globule. The emulsion is left at a tempering temperature for some time (t_{\max}) and then slowly cooled. Tempering has an adverse effect on the stability of emulsions. In concentrated emulsions, tempering leads to “body formation” or “solidification of emulsion” due to extensive partial coalescence. The emulsion becomes more viscous and does not flow. In dilute emulsions, tempering can make emulsions more susceptible against partial coalescence (Fox & McSweeney, 2007; Walstra et al., 2005).

Boode et al. (1991) explained the mechanism behind the increase in partial coalescence susceptibility after tempering. At cooled conditions, before tempering, the fat crystals remain embedded in the crystal network and cannot move to the oil-water interface, which is a thermodynamically favoured position. After heating to tempering temperature (T_{\max}), the majority of crystals are lost and only a few small crystals remain in oil droplets. These crystals are now free to move at the interface. Eventually, they will move to the oil-water interface. After crystals rearrangement are at the interface, upon slow cooling, crystals will start to grow and form big crystals. As more crystals are on the interface, a large number of crystals will be protruding outside in the aqueous phase which will increase the capture efficiency. It should be noted cooling during tempering should be slow, as fast cooling can cause undercooling which will eventually lead to the formation of new crystals rather than the growth of existing crystals. Another critical parameter is tempering time. Enough time should be available for crystal rearrangement. Tempering can be said to be a phenomenon of altering the arrangement of crystals to make emulsion more prone to partial coalescence by using a time-temperature cycle (Boode, 1992; Boode et al., 1991; Boode & Walstra, 1993; Fredrick et al., 2010).

It is also essential to have a minimum SFC at tempering temperatures for this to occur in emulsions. Boode (1992) reported that emulsions having less than 25% fat are not susceptible to tempering and a minimum amount of 1.5 – 8 % SFC at tempering temperature is required to achieve effects of tempering. Moens et al. (2016) reported the use of tempering in altering whipping properties in cream. Both natural cream and recombined cream (before whipping) were tempered at two different temperatures (T_{\max}), 20° and 30°C. It was observed that tempering resulted in increased susceptibility of creams to undergo shear-induced partial coalescence. In the case of natural cream, shorter whipping time and high overrun were

observed. In contrast, low over run as compared to control and low whipping time was observed in the recombined cream (Boode, 1992; Boode et al., 1991; Fredrick et al., 2010; Moens et al., 2016).

So, far no pieces of evidence outline the effect of tempering or crystals rearrangement on fat destabilization in ice cream.

Morphology, Crystal size, and polymorphism

In general, an increase in the size of fat crystals increase the susceptibility of emulsions to undergo partial coalescence. This is because, as the crystals size increases, more crystals will protrude outside of the fat globule. A high proportion of crystals protruding from the fat globule will increase the collision frequency. The size of fat crystals in the fat globule is largely determined by the time-temperature profile of emulsion. In terms of polymorphism of fat crystals, so far no correlation has been reported between polymorphism and susceptibility of emulsion to undergo partial coalescence (Boode et al., 1991; Fredrick et al., 2010; Johansson, 1996; Moens et al., 2016).

2.5.3 Conclusion

To conclude, it can be inferred that partial coalescence is the main phenomenon in the formation of structure in dairy emulsions such as whipped cream, ice cream, and butter. There are many compositional and process factors that affect the kinetics of partial coalescence in an emulsion. In terms of emulsion compositional factors, the interfacial composition is a major factor affecting the stability of emulsion and has been reported to alter the stability of emulsions. Regarding process factors, flow conditions, SFC and tempering are important factors that can be used to alter the kinetics of partial coalescence in emulsions. In the context of the application of these factors in ice cream, only limited scientific work has been carried out to study their effect on the degree of fat destabilization.

3. Materials and Methodology

A standard vanilla-flavoured ice cream formulation was used for this study. Vanilla flavour was used to avoid any interference arising from use of multiple colours or flavour systems.

3.1 Raw Materials

The table lists the various ingredients used for manufacturing ice cream.

Table 3.1: Various ingredients used for manufacturing ice cream

Ingredients	Manufacturer
Skim milk powder	NZMP, Fonterra Co-operative Group
Anhydrous milk fat	Fonterra Co-operative Group
Locust bean gum	Hawkins Watts Limited
Guar gum	Ingredient Stop Ltd
Non – homogenized cream	Anchor, Fonterra Co-operative Group
Sucrose – Raw sugar	Davis Food Ingredients
Glucose Syrup	Davis Food Ingredients
Mono-diglycerides	Ingredient Stop Ltd
Natural Vanilla Flavour	Pacific Flavours
Buttermilk powder	Fonterra Co-operative Group

The composition of raw materials is listed in appendix A.

3.2 Equipment

All the trials were carried in a food pilot plant at the School of Food and Advanced Technology at Massey University. Table 3.2 lists the various equipment used for ice cream manufacturing.

Table 3.2: Equipment used for ice cream processing

Equipment	Manufacturer Details
Stand Mixing Unit	Kika Labortechnik
Homogeniser	Rannie: Serial # 1.88234
Ultra-high temperature processing (UHT pasteurizer)	Massey University
Laminar Flow Cabinet	Air Care Technology Ltd: AC 1100
Ice cream machine	Tetra Pak Hoyer Denmark

3.3 Formulations

Table 3.3 depicts the formulations for standard and low-fat ice cream used for this study. A standard ice cream formulation was used to study the effect of both native milk fat globules and buttermilk powder. The impact of native milk fat globules was further tested on a low-fat ice cream formulation.

Table 3.3: Composition of standard and low-fat ice creams

Ingredients	Standard ice cream (10% fat)	Low fat ice cream (5% fat)
Fat	10	5
Milk Solid Not Fat (MSNF)	11	14
Sucrose	9	10
Glucose Syrup	4	5
Guar	0.1	0.1
LBG	0.2	0.2
Mono-diglycerides (MDG)	0.3	0.3
Flavour (vanilla)	0.1	0.1
Water	65.3	65.3

3.3.1 Fat ratios for native milk fat globules formulations

In the ice cream made using native milk fat globules, non-homogenized cream was used as a source of membrane material. Mono-diglycerides were only used in the positive control. Total fat in other formulations was supplied using AMF and non-homogenized cream. Different ratios of non-homogenized cream and AMF, as outlined in table 3.4, were used to vary the quantity of native milk fat globules. Non-homogenized cream also has a certain amount of milk solids, not fat (~5%). Therefore, the quantity of skim milk powder in formulations was adjusted accordingly. Appendix outline the mix calculations for these formulations.

Table 3.4: Formulations with different ratios of cream and AMF

Formulation	% of Total Fat by Non-homogenised cream	% of Total Fat by AMF	Remark
Positive Control (PC)	0	100	With Emulsifier (MDG)
Negative Control (NC)	0	100	No emulsifier
$C_{AMF}^{20:80}$	20	80	Without MDG
$C_{AMF}^{40:60}$	40	60	Without MDG
$C_{AMF}^{45:55}$	45	55	Without MDG
$C_{AMF}^{50:50}$	50	50	Without MDG
$C_{AMF}^{55:45}$	55	45	Without MDG
$C_{AMF}^{60:40}$	60	40	Without MDG
$C_{AMF}^{80:20}$	80	20	Without MDG
$C_{AMF}^{100:00}$	100	0	Without MDG

3.3.2 Levels of buttermilk powder

Buttermilk powder with 7.7% phospholipid content was tested to replace synthetic emulsifiers in ice cream. The amount of buttermilk powder was varied to have different levels of phospholipids. Table 3.5 and 3.6 outline the different % of buttermilk powder used during traditional processing and selective adsorption method. As buttermilk powder contains a certain amount of milk proteins, the milk solids not fat the content of ice cream formulations was adjusted to match MSNF content with the standard fat formulation. The appendix outlines the mix calculation with buttermilk powder.

Table 3.5: Different % of buttermilk powder used for traditional processing

Formulation	% Buttermilk powder	Remark
BMP _{Trad} ^{1%}	1	Without MDG
BMP _{Trad} ^{2%}	2	Without MDG
BMP _{Trad} ^{3%}	3	Without MDG
BMP _{Trad} ^{4%}	4	Without MDG

Table 3.6: Different % of buttermilk powder used for selective adsorption method

Formulation	% Buttermilk powder	Remark
BMP _{Selective} ^{1%}	1	Without MDG
BMP _{Selective} ^{2%}	2	Without MDG
BMP _{Selective} ^{3%}	3	Without MDG
BMP _{Selective} ^{4%}	4	Without MDG

3.4 Process flow

3.4.1 Preparation of ice cream with non – homogenized cream

Figure 3.1 illustrates the process flow to make ice cream with non-homogenized cream. The first step in ice cream making was mix preparation. All the ingredients were weighted as per formulation requirements. Hot water, at around 50° C, was weighed in a stainless bucket. A mechanical mixing unit was used for the mixing of ingredients. The mixing speed was kept constant throughout the trials. In the mixing sequence of ingredients, first skim milk powder was dissolved into hot water. The rate of addition of skim milk powder was controlled manually to avoid any lump formation. Pre-weighed frozen anhydrous milk fat was melted by immersing the container in hot water to reach a temperature above 40°C. After melting, mono-diglycerides were added (only for the positive controls) into melted AMF and mixed using a large spoon.

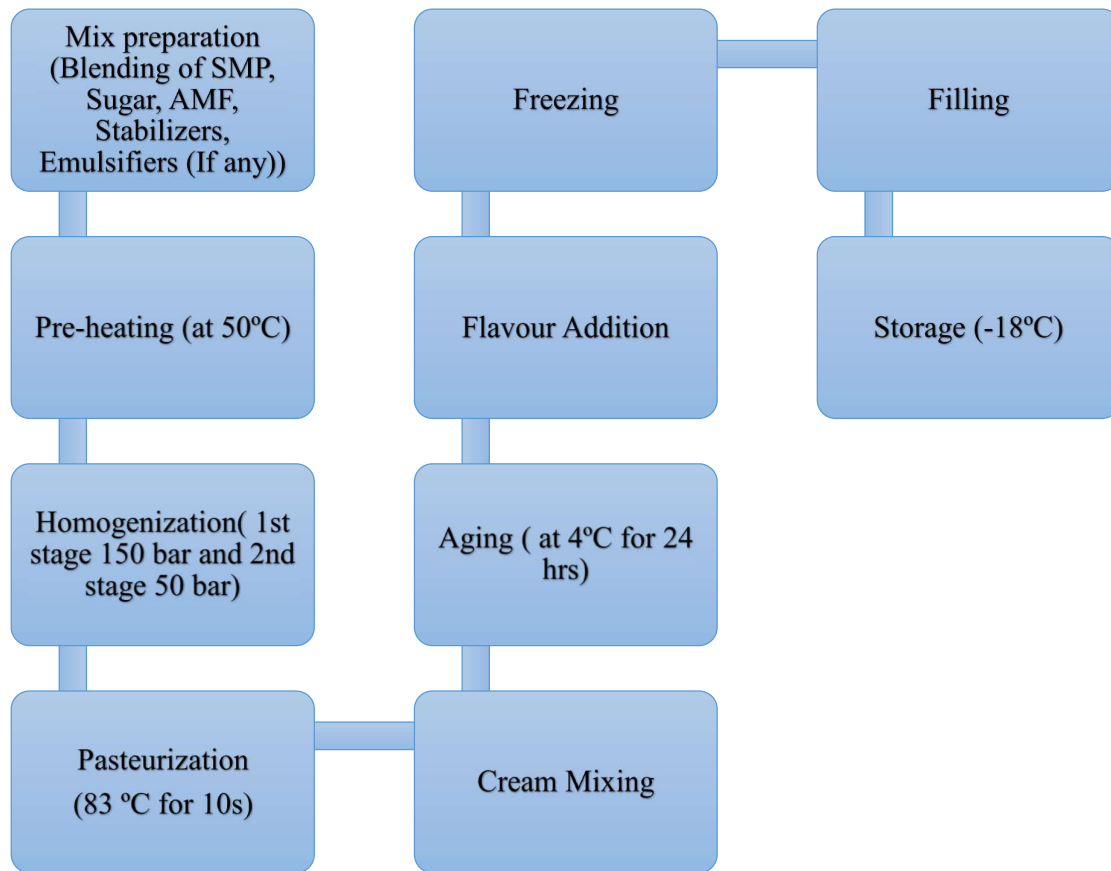


Figure 3.1 Process flow of ice cream manufacturing with non-homogenized cream

A premixing of stabilizers (locust bean gum and guar gum) with sugar was done to avoid lump formation (fisheye effect). This mixture of sugar and stabilizers was added after the dissolution of skim milk powder. After the dissolution of sugar and stabilizer, melted AMF (including MDG in positive control) was added. After mixing all of the ingredients, mixing was continued for the next 5-10 min to produce a uniform ice cream mix.

As the water temperature dropped during the mix preparation process, the mix was preheated again to 50°C by immersing the steel bucket into hot water and agitating with a spoon. This preheating was required to have the fat in a completely liquid state, a prerequisite for homogenization. Mix at 50°C was homogenized in 2 stages using a high-pressure homogenizer. The first and second stage pressure was set at 150 bar and 50 bar, respectively. The second stage of homogenization is usually used in ice cream making to avoid the formation of fat clusters after 1st stage.

Followed homogenization, the ice cream mix was pasteurized using a high-temperature short-time tubular pasteurizer. Mix was heated to temperature 83° C and held for 10s at this temperature in a holding tube. After holding, the ice cream mix was immediately cooled to 15

°C. The mix flow rate was kept around 1.6 L/min. Fill to drain and flush to drain for equipment were 150s and 110s, respectively. These times are based on the transition of the product to the water stream at the product collection point. All the product -water mix phase was drained. Pasteurized product was collected in alcohol sanitized plastic containers with lids.

In ice cream with native fat globules, a commercially pasteurized non-homogenized cream was added into pasteurized mix before aging. A mechanical mixer was used to mix a pre-weighed amount of non-homogenized cream. Mixing of cream and pasteurized ice cream mix was done at low speed to keep the milk fat globule membrane intact. A low-speed mixing (50 rpm) for 2 min was used for all the formulations. After cream addition, the resultant ice cream mix was aged at 4 °C for 24 hrs. After aging, natural vanilla flavour was added to the mix. After flavour additional, the mix was introduced into the ice cream freezing machine.

The overrun was set at 100% during freezing, and mix viscosity was adjusted to 70%. The mix was pumped with a flow rate of 20 L/ hr, and ice cream with a drawing temperature of -6°C was collected in different pottles. Ice cream was collected into plastic pottles. For the melting test, special cardboard boxes were used to have equal volume blocks. All the ice cream pottles and boxes were stored at -18 °C. A food-grade dye was used to differentiate between different batches during ice cream production. It is worth noting that the hardening of ice cream was not done in this study due to the non-availability of a hardening system.

3.4.2 Preparation of ice cream with buttermilk powder using traditional processing

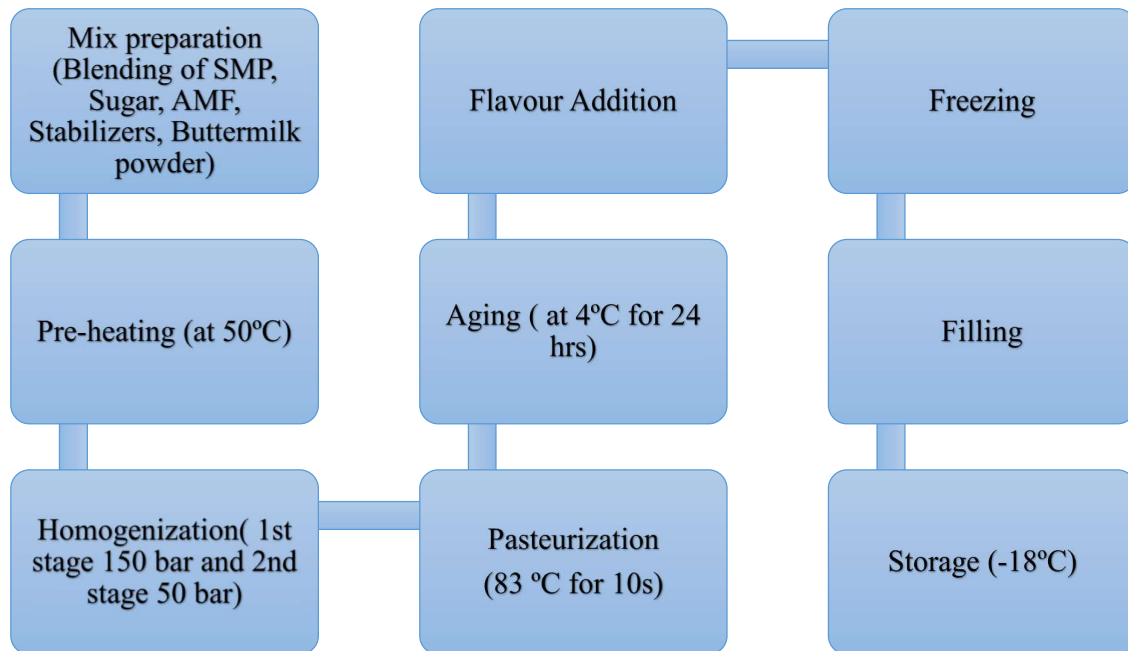


Figure 3.2 Process flow of ice cream manufacturing with buttermilk powder using traditional processing

The process flow of ice cream making with buttermilk powder using traditional processing is shown in figure 3.2. Traditional processing implies the conventional way of manufacturing ice cream with mono-diglycerides. The only difference is in place of MDG; we used buttermilk powder as an emulsifier.

Dry mixing of buttermilk was done with skim milk powder. This dry mixture was dispersed in hot water at 50°C. Next other ingredients (sugar, stabilizers, and AMF) were added. The further processing was similar to the process flow described in section 3.4.1.

3.4.3 Preparation of ice cream with butter milk powder using selective adsorption processing

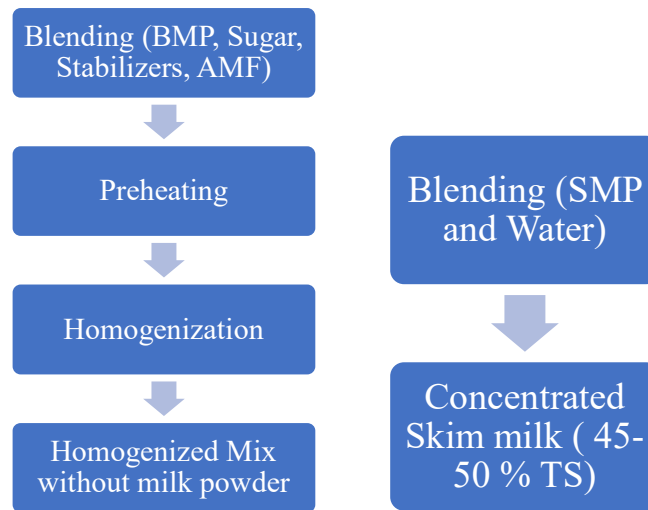


Figure 3.3 Process flow for different phases prepared for selective adsorption

Figure 3.3 shows the process flow of two different phases to make ice cream using the selective adsorption method. Selective adsorption in this context means to allow the buttermilk powder to get selectively adsorbed on the newly created interface of fat globules in the absence of milk proteins. For this, the blending unit operation was split into the preparation of two phases. One phase is a mix without milk powder. For the preparation of this phase, a variable amount of buttermilk powder as outlined in the formulations table was mixed into hot water at 50°C (amount of hot water is about 70% of total water required). Following the buttermilk powder addition, the other ingredients (sugar, stabilizers, and melted anhydrous milk fat) were dispersed using a mixer.

After preparation, the mix was preheated to 50°C. Following heating, the mix was homogenized using high-pressure homogenization. The pressures for the first and second stages were set at 150 bar and 50 bar, respectively. The presence of buttermilk powder during homogenization allowed the protein and phospholipids to adsorb on the interface of newly formed fat globules. The other phase, concentrated skim milk, was prepared by mixing the SMP into hot water at 50°C.

Before pasteurization, both phases (homogenized mix without milk proteins and concentrated skim milk) were mixed using a mechanical agitator. After mixing, the product was pasteurized and aged. The aged mix was processed further, as discussed in section 3.4.1.

3.5 Methods

3.5.1 Overrun

Overrun of ice cream is the measure of air content in ice cream. By definition, overrun is a “% increase in the volume of ice cream greater than the volume of mix used to produce that ice cream”

The formula used for the calculation of overrun is:

$$\text{Over run} = \frac{(\text{Wt. of the mix} - \text{Wt. of ice cream})}{\text{Wt. of ice cream}} \times 100$$

For overrun measurement, we used a steel cup. The weight of mix after aging was measured followed by measurement of weight of ice cream immediate after freezing. All the measurements were done in triplicates.

3.5.2 Particle Size distribution

The particle size distribution of ice cream mix and melted ice cream was determined using laser scattering using Mastersizer 2000 (Malvern Panalytical Ltd, UK). The small volume sample presentation unit was used for uniform dispersion of the sample. The refractive index for the ice cream mix was set at 1.460 with a 0.001 adsorption value. The refractive index of dilutant (RO water) was set at 1.33 with a 0.001 adsorption value. Before the measurements, ice cream was melted by keeping pottles at 4 °C overnight. After melting, the ice cream was diluted with an equal amount of water. The diluted sample was thoroughly mixed. The sample was then dispersed in water using a dispersion unit and an adequate amount of sample was used to get an obscuration range between 8-12 %. All the measurements were done at room temperature. A set of 8-12 replicates were performed for each sample. The average curve for all the measurements was reported as the final result.

3.5.3 Hardness

The hardness of ice cream was measured using Texture Analyzer (TA.XT. Plus, Stable Micro Systems, UK). The measurement of ice cream was done at -15 °C. Samples from the freezer were quickly transported to texture analyser at room temperature using a chilly bin pre-conditioned to freezer temperature (by overnight storing the bin in the freezer). All the measurements were done quickly to avoid any variability due to temperature fluctuation. Flat cylindrical steel rode with 10 mm diameter was used for penetration with the speed of 10mm/s. The depth of penetration was set to 10mm. The force (in N) required for fixed penetration depth

at fixed penetration speed was reported as the hardness value of ice cream. A set of 5 replicates were measured for each sample.

3.5.4 Meltdown Stability

A steel wire mesh having 64 holes / 100 square cm was used to measure the melt stability of ice cream. A constant volume block of ice creams was weighed along with a cardboard box. The ice cream block was removed from the cardboard box and the weight of the cardboard was measured to get the weight of the ice cream. The ice cream box was transferred to a wire mesh at room temperature. A plastic box on weighing balance was used to collect the melted ice cream sipping through a wire mesh.

Another plastic box was used to cover the ice cream block to minimize the effect of variation in air flow on the melting properties of ice cream. The weight of melted ice cream was measured at an interval of 10 min for 2 hrs. The melt stability of ice cream was compared by noting the weight of melted ice cream at the end of 2 hrs. A set of 3 replicates was analysed for each formulation.

3.5.5 Confocal Microscopy

Confocal laser scanning microscopy was performed using a microscope (Zeiss Airyscan, LSM 900). The ice cream pottles were kept at room temperature for 30min. 0.5-1g of ice cream was transferred to a microtube. A drop of each Nile red and fast green dye were added to the microtube. The tube was mixed by shaking it to stain the ice cream with respective dyes. The stained ice cream was transferred to a concave slide using a dropper. After covering with a cover slip, the slide was transferred to the microscope platform. The objective lens (40x) was focused on a sample using fine adjustment. After focusing, the software was started to observe the sample in different lasers.

Two laser lights, one for the Nile red and the other for fast Green were used to visualize stained ice cream. Nile red was excited at 561nm with a collecting frequency set at 570-611 nm. Fast green was excited at 633nm with a collecting frequency set at 643-743 nm. The brightness and contrast of images were adjusted manipulating smart gain and smart offset values. A set of 3 images were captured for each formulation.

3.5.6 Cryo – Scanning Electron Microscopy

The microstructure of ice cream samples was visualized using cryo-scanning electron microscopy (Philips, XL series, X30 SGEFG). A piece of ice cream was cut using a surgical blade (cooled by dipping in liquid nitrogen) from ice cream at -18°C. This small piece was

immediately transferred to liquid nitrogen. The sample was then hooked to the holder by using melted ice cream as a glue. After that, the holder along with the ice cream sample was dipped in liquid nitrogen under vacuum (slushing) to cool the sample to -196 °C. Once cooled, the sample was transferred to the preparation chamber. By using a surgical blade, the cooled ice cream sample was broken to create a new surface. After that heater was switched on to sublime the ice at -96° C. Once all the ice was sublimed the sample was sputter-coated with gold particles. After coating, the specimen was transferred to the main chamber at -140 °C, where the sample was visualized under an electron beam at different areas to get images. Three different areas of the specimen were visualized at different magnification levels. A set of best images from one area is used in the results and other images can be found in appendix.

4. Effects of non-homogenized cream addition on ice cream

4.1 Introduction

In non-homogenized cream, the milk fat globules are present in their native state (with intact milk fat globule membrane). As hypothesized previously that native milk fat globules can act as potential natural fat droplets with emulsifiers in ice cream. Therefore, a varying degree of non-homogenised cream was added into ice cream formulation by partly or wholly replacing the conventional fat source (anhydrous milk fat). The resultant ice cream was tested for physical and microstructural properties. The result and discussion are presented in subsequent sections of this chapter. The hypothesis was further tested on low-fat ice cream formulations, and the resultant ice cream was analysed only for physical properties. The results for low-fat ice cream are presented along with the standard fat ice cream.

Note: The formulation codes have been defined in section 3.3.

4.2 Overrun

Overrun is a measure of the amount of air incorporated in ice cream. A high overrun indicates a large amount of air in ice cream(Chang & Hartel, 2002a). A high overrun result in softer and slightly higher melt stable ice cream.

Table 4.1 The overrun measurements for full fat controls and full fat ice cream made with variable proportion of non-homogenized cream

Ice creams	Overrun (%)
Negative Control	^a 121 ± 0.4
C:AMF - 20:80	^a 117 ± 3.24
C:AMF - 40:60	^a 118 ± 3.24
C:AMF - 45:55	^a 118 ± 3.24
C:AMF - 50:50	^a 118 ± 3.24
C:AMF - 55:45	^a 118 ± 3.24
C:AMF - 60:40	^a 121 ± 1.39
C:AMF - 80:20	^a 118 ± 2.98
C:AMF - 100:0	^a 117 ± 1.96
Positive Control	^a 116 ± 2.48

Note: Mean of overrun values is followed by (±) standard deviation. Within rows, different superscripts in front of mean values indicate significance (p<0.05) The superscript along with values shows the grouping of mean values using Tukey methods and 95% confidence. Mean values followed by different superscripts are significantly different , n = 5.

The results (table 4.1) shows that the overrun values for full fat controls and ice cream is statistically similar. It indicates that partial replacement of fat in ice cream system with non-homogenized cream does not have significant effect on overrun of ice cream.

Table 4.2 *The overrun measurements for low fat controls and low-fat ice cream made with variable proportion of non-homogenized cream*

Ice creams	Overrun (%)
Negative Control	^a 105 ± 3.73
Positive Control	^a 106 ± 1.76
C:AMF - 20:80	^a 102 ± 2.76
C:AMF - 40:60	^a 102 ± 3.47
C:AMF - 60:40	^a 104 ± 0.63
C:AMF - 80:20	^a 105 ± 0.75

Note: Mean of overrun values is followed by (±) standard deviation. Within rows, different superscripts in front of mean values indicate significance (p<0.05) The superscript along with values shows the grouping of mean values using Tukey methods and 95% confidence. Mean values followed by different superscripts are significantly different , n = 5.

Table 4.2 shows that all the low-fat control and formulation with non- homogenized cream has similar overrun value. It indicates that with replacement of fat in ice cream with non – homogenized cream in low fat ice creams does not have any significant effect over run of ice creams.

It can be observed that standard fat ice creams had a high overrun as compared to low-fat ice creams. This can be attributed to the difference in sugar % and milk solid not fat % in both the formulations. Referring to formulations in section 3.3, the standard fat formulation has 13 % sweeteners and 11 % MSNF. In contrast, low-fat ice cream has 15% sweeteners and 13% MSNF. A high level of sweeteners and milk solid not fat content in low-fat ice cream contribute to high mix viscosity. A high concentration of these solids may further affect the ice phase volume, thus affecting the viscosity. It has been reported that overrun tends to decrease with an increase in viscosity of mix as it is challenging to incorporate air in a more viscous ice cream mix (Clarke, 2005).

Overall, the variations in overrun among the formulations of each type of ice creams are not significant. The non – significant variations can be explained by the difficulty in controlling the amount of air admitted by the batch freezer (Warren & Hartel, 2018). Low variation in overrun indicates that all the formulations for the respective type of ice cream could incorporate

approximately the same amount of air. It eliminates the possibility of the impact of overrun on melt stability and other physical properties of ice cream. With this discussion, it can be implied that replacing the AMF with non-homogenized cream in ice cream does not affect the overrun of ice cream.

4.3 Microstructure

4.3.1 Confocal microscopy

The microstructure of melted ice cream formulations was visualized using confocal laser scanning microscopy. As melted ice cream was used for imaging, the resultant images do not fully illustrate the native structure of ice cream. It provides information on the distribution of fat globules and protein on air cells and the matrix of melted ice cream. It is important to note that the fat globules seen in these images are coalesced fat globules, which were present as partially coalesced aggregates in ice cream. Therefore, a direct interpretation of fat aggregates size cannot be made. The images shown in this section are of standard fat ice creams, and no imaging was done for low-fat ice creams.

Before discussing the confocal images, it is vital to establish that the size of fat globules correlates to the size of partially coalesced fat in ice cream. The discussion is based on visual interpretation. There are not quantitative measurements present to support the graphic details.

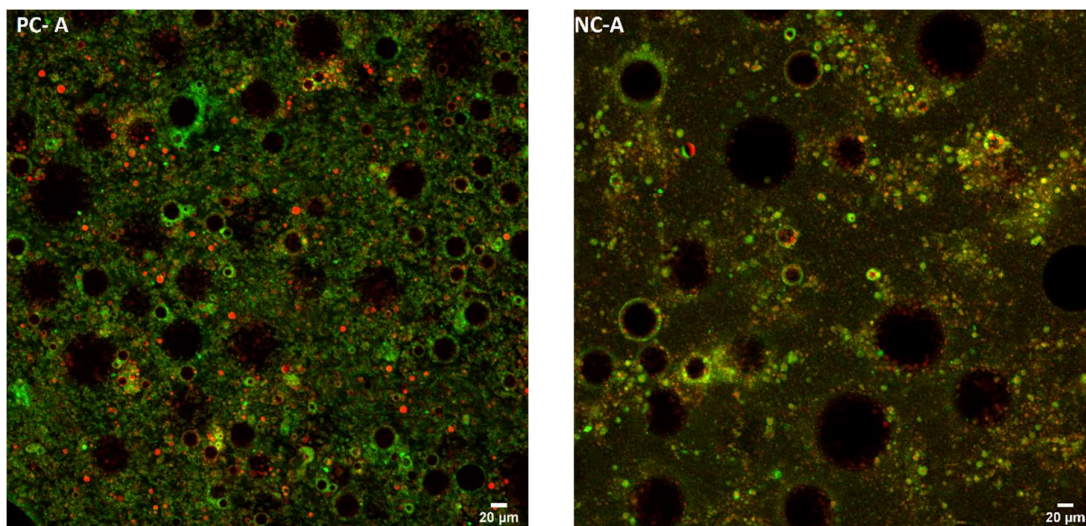
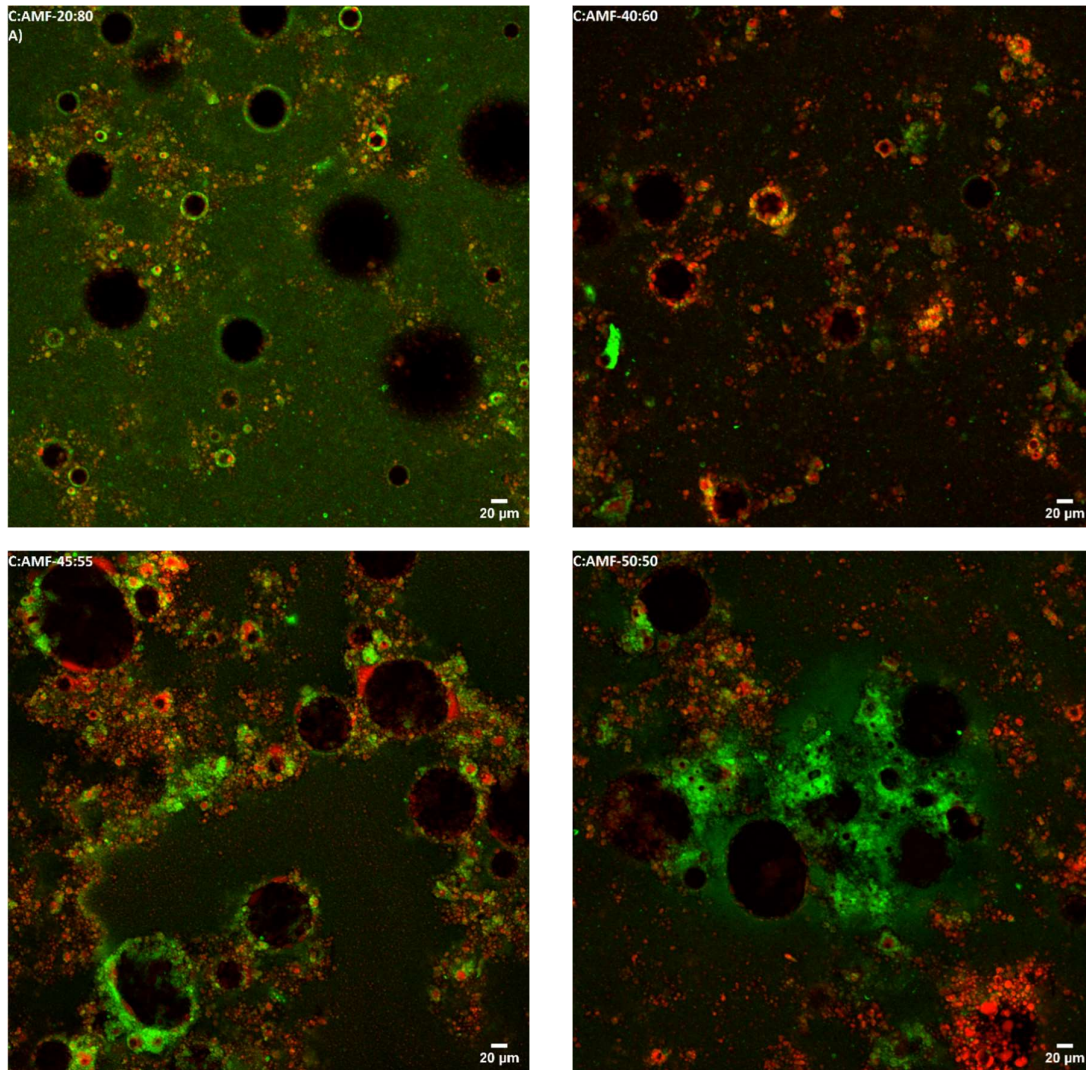


Figure 4.1 Confocal laser scanning microscopic images of melted ice cream for standard fat ice cream controls.

Figure 4.1 depicts the confocal images of positive and negative control formulations. For the positive control, the small fat globules can be seen distributed throughout the matrix. The

macro red dots represent the agglomerated fat present in ice cream. As expected for positive control, the agglomerated fat is uniformly distributed throughout the matrix. The air lamella is covered with smaller size fat globules along with the protein particles. Few agglomerated fat particles are present in the matrix in negative control, illustrates a lower degree of fat destabilization. The air bubbles are primarily covered with milk proteins, and a minimal



amount of fat particles is also present along with proteins on the bubble's interface.

Figure 4.2 Confocal microscopy images for melted standard ice cream with different ratios cream ($C_{AMF}^{20:80}$, $C_{AMF}^{40:60}$, $C_{AMF}^{45:55}$, and $C_{AMF}^{50:50}$)

The confocal images for $C_{AMF}^{20:80}$, $C_{AMF}^{40:60}$, $C_{AMF}^{45:55}$ and $C_{AMF}^{50:50}$ formulations are shown in figure 4.2. A low number of agglomerated fat particles can be seen in $C_{AMF}^{20:80}$, and these aggregated fat particles are present in the cluster-like structure. Occasionally, agglomerated fat is also present on-air lamellar area. In the case of $C_{AMF}^{40:60}$, the air lamella is primarily covered

with agglomerated fat. The larger fat aggregates can be seen nearly uniformly distributed in the matrix of ice cream with occasional cluster formation.

For formulation $C_{AMF}^{45:55}$, a big cluster of agglomerated fat globules can be seen along with a large region devoid of fat. The density of fat particles varies widely among the air cells, with some air bubbles have a high density of agglomerated fat on the interface while others have lower. In formulation $C_{AMF}^{50:50}$, the matrix comprises clusters of agglomerated fat particles and fat depleted regions. The lamella of air bubbles appears to be either covered with big, aggregated fat particles or utterly devoid of fat.

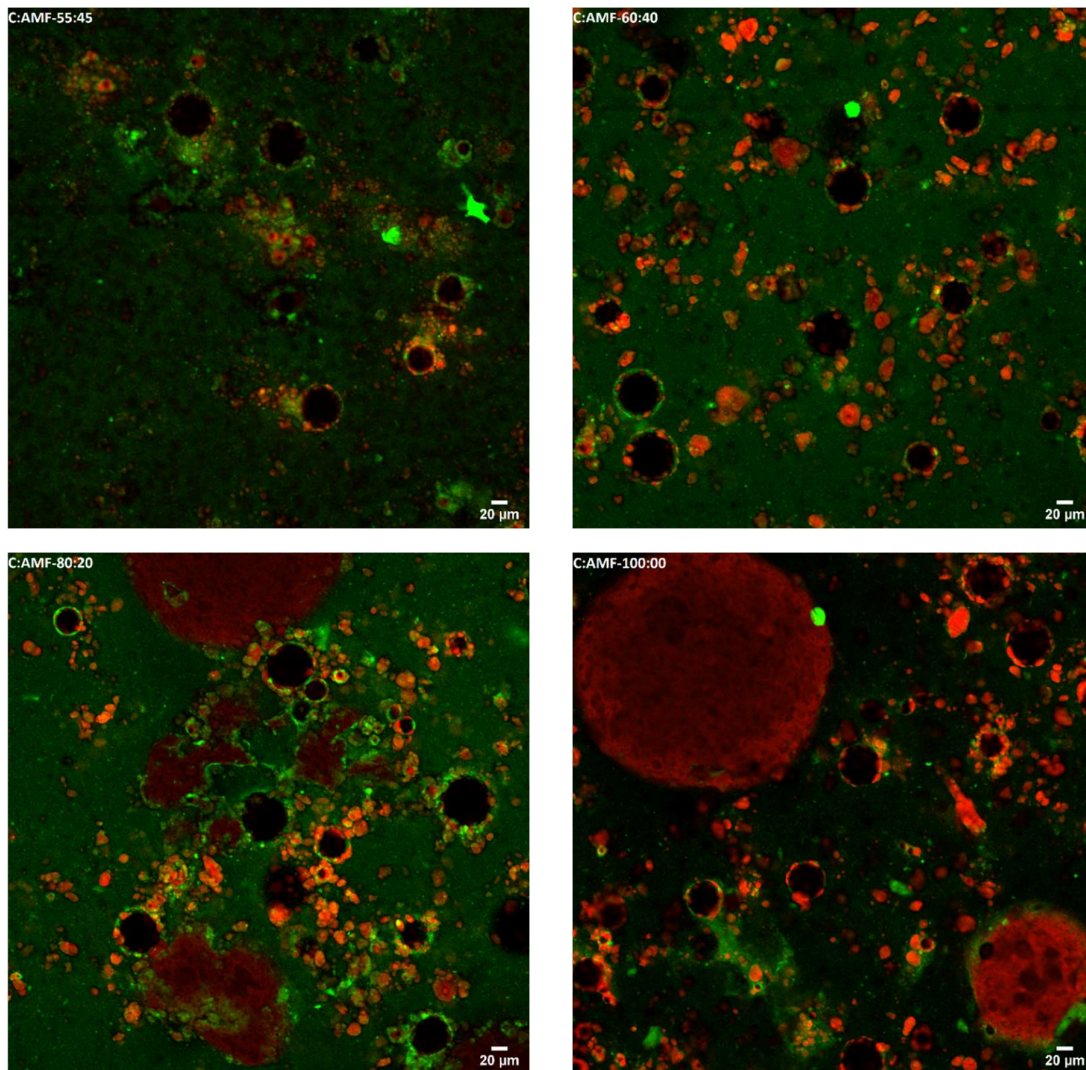


Figure 4.3 Confocal microscopy images for melted standard ice cream with different ratios cream ($C_{AMF}^{55:45}$, $C_{AMF}^{60:40}$, $C_{AMF}^{80:20}$, and $C_{AMF}^{100:00}$)

Figure 4.3 shows the microstructural images for $C_{AMF}^{55:45}$, $C_{AMF}^{60:40}$, $C_{AMF}^{80:20}$ and $C_{AMF}^{100:00}$. For formulation $C_{AMF}^{55:45}$, a smaller number of aggregated fat particles can be visualized. The

fat particles are present in clusters, and in some local sites, the matrix is completely devoid of fat. Some air cells are covered with large fat particles, while others are completely devoid of fat molecules. In $C_{AMF}^{60:40}$, large, aggregated fat particles comparable to the size of air cells can be seen in the matrix. Some of these big aggregates are present in on-air cells, while others have formed a cluster in the matrix. Some matrix areas do not have any fat globules indicating that extensive agglomeration has resulted in the formation of fat-free regions.

For both $C_{AMF}^{80:20}$ and $C_{AMF}^{100:00}$, substantial fat aggregates having a size equivalent to air cells can be easily located in melted ice cream. The clustering of fat aggregates is more prominent in both the formulations, and only some of the air cells are covered with large aggregates.

From the results of all the formulations, it can be interpreted that increasing the content of non-homogenized cream in ice cream results in extensive agglomeration in ice cream. As fat agglomerates tend to grow larger, more localized clustering of these fat particles can be seen in ice cream. The formation of these larger aggregates further impacts the distribution of aggregated fat on air cells. With more extensive aggregates formation, a non-uniform distribution of fat and a lower number density of aggregates can also be observed in the structure of ice cream. This non-uniform distribution results in the variations in melt stability and hardness of the ice cream.

4.3.2 Scanning electron microscopy

Scanning electron microscopy was used for visualizing the structure of ice cream. The controls and two formulations ($C_{AMF}^{40:60}$ and $C_{AMF}^{60:40}$) with non-homogenized cream were analysed to get micrographs at different magnifications. Figure 4.4 represents the microscopic images for both controls, $C_{AMF}^{40:60}$ and $C_{AMF}^{60:40}$, at 250x magnification. It is evident from micrographs that the size of air cells varies widely with the formulation. In ice cream with MDG, air cells' distribution appears to be a combination of very fine and smaller air bubbles. Whereas the air cells in negative control are relatively large, and fine air cells are absent.

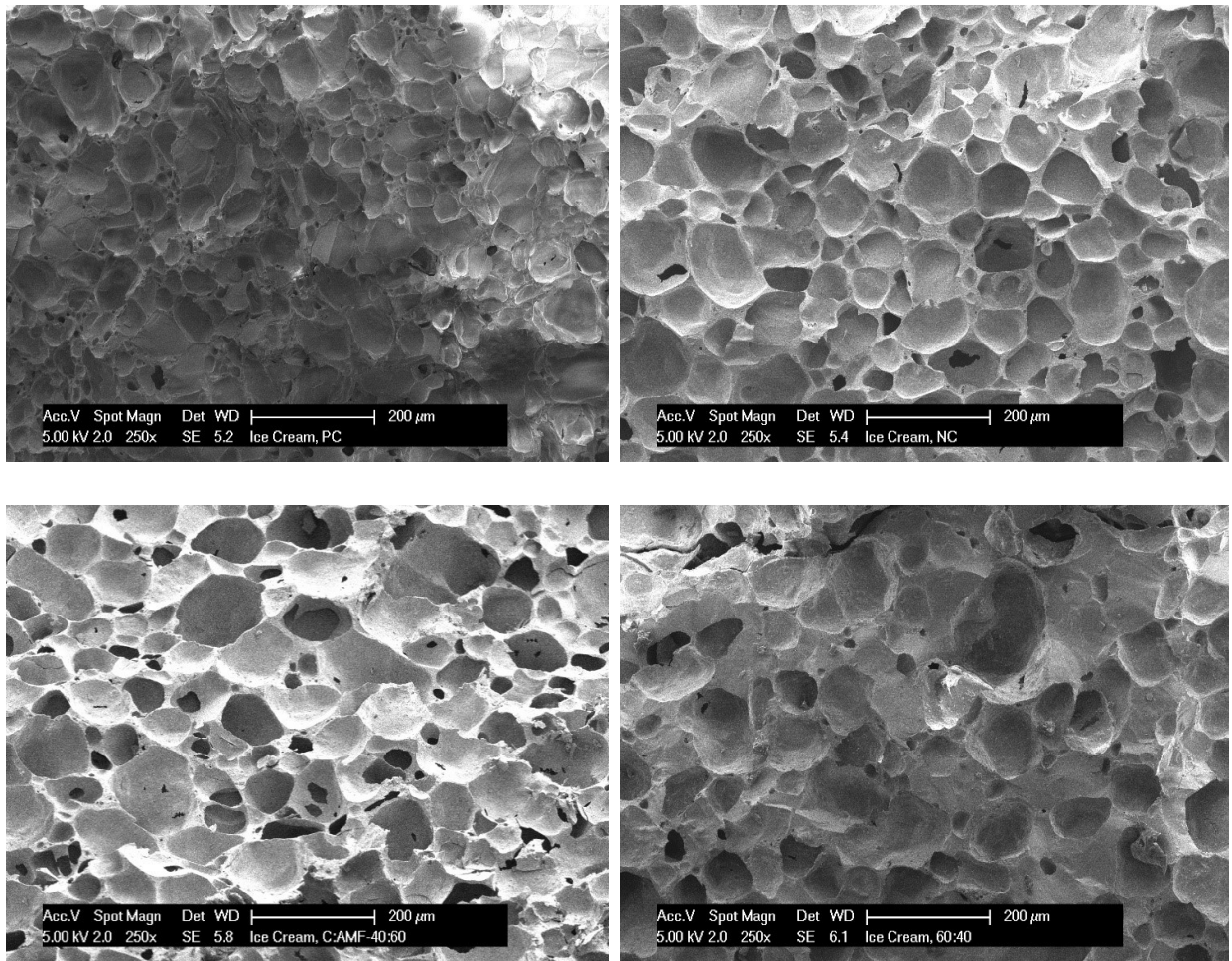


Figure 4.4 The micrographs for PC, NC, $C_{AMF}^{40:60}$ and $C_{AMF}^{60:40}$ at 250 X magnification

The air cells in both $C_{AMF}^{40:60}$ and $C_{AMF}^{60:40}$ is larger than the positive control, which suggests that the fat agglomeration occurring in the formulations with native fat globules is not efficient enough to control the size of air cells. Compared to ice cream with MDG, the number density of fine air cells tends to be lower for formulation with non-homogenized cream. The distribution of the ice phase is another crucial aspect. A uniform distribution of the ice phase can be easily seen in both the control formulations. The ice phase is difficult to locate in $C_{AMF}^{40:60}$, which signifies the fat agglomeration has affected the ice crystallization mechanism in ice cream.

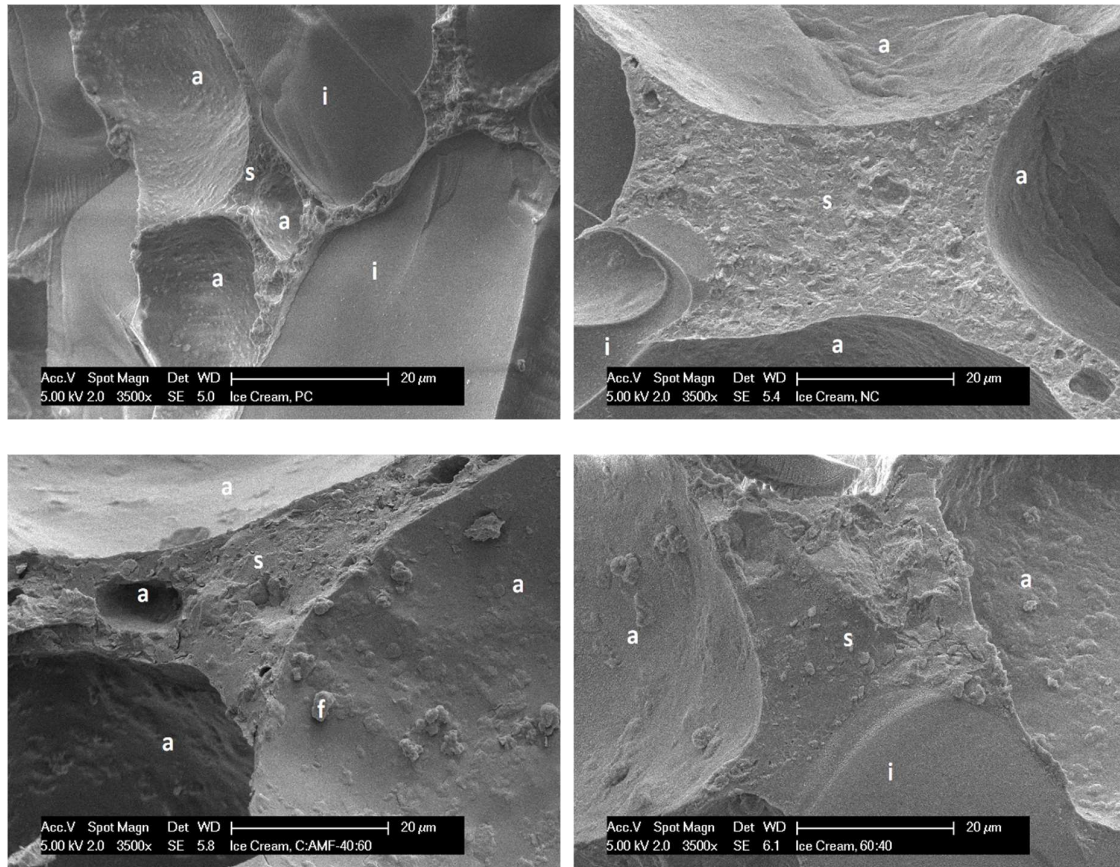


Figure 4.5 The microscopic images for PC, NC and $C_{AMF}^{40:60}$ and $C_{AMF}^{60:40}$ formulations at 3500 x magnification (s – serum phase, I – ice phase, a – air cells and f – fat particles)

Figure 4.5 represents the microscopic images of positive control, negative control, $C_{AMF}^{40:60}$, and $C_{AMF}^{60:40}$ at 3500x magnification. In the case of positive control, it can be seen the size of agglomerated fat molecules is relatively small. The air cells are covered with fat particles, and this coverage is uniform throughout the air bubble. The fat is also distributed uniformly in the matrix. Whereas in ice cream without MDG, the air cells are not covered with fat particles, and no agglomerated fat can be located in the matrix phase.

In the case of $C_{AMF}^{40:60}$ and $C_{AMF}^{60:40}$, the size of fat agglomerates is relatively large compared to positive. The large-size fat agglomerates are clustering together. These partially coalesced fat clusters are non-uniformly distributed on-air lamella. The protein and small fat particles can be located in the serum phase.

In summary, the outcomes from scanning electron microscopy(SEM) suggest that native fat globules are effective replacement to synthetic emulsifiers and are capable of achieving fat

structuring in ice cream. The partial coalescence occurring in the formulation with non-homogenized cream produces large aggregates and relatively large air cells. The big size air cells can be explained by the fact that the number density of fat aggregates required to stabilize air cells decreases with the formation of sizeable aggregates in ice creams having the same amount of fat. Therefore, a relatively smaller number of fat particles are available to stabilize air cells, resulting in formation of large air cells. Another important observation is the approximate absence of sizeable ice crystals in formulation with 40% cream. As suggested by Adapa et al. (2000) it may be due to effect of agglomeration of fat on ice crystals formation. The formation of large aggregates due to the agglomeration of fat may have interfered with the growth of ice crystals. It may have led to the formation of tiny ice crystals which are not visible under microscopy. The presence of sizeable ice crystals in the formulation with 60% cream goes against this explanation. The observation can explain that in the formulation with more than 40% cream, the distribution of fat aggregates is highly non-uniform in nature, as seen in the confocal images. This non-uniform distribution may result in localized hindrance in ice crystals growth; thus, ice cream with 60% cream does have sizeable ice crystals, but as seen in the SEM image, their distribution is non-uniform.

4.4 Particle size distribution

Fat globules in ice cream mix tend to either undergo partial coalescence to form large, destabilized fat clusters or remain intact (in terms of size and distribution) during freezing. The particle size distribution analysis in melted ice cream shows the volume % of fat globules and milk proteins in different size ranges. The volume % or peak height of fat globules for different ice cream formulations provides qualitative information on the extent of fat destabilization in a particular formulation. In general, the size distribution for fat aggregates is mono model for ice cream mix, and bimodal for ice cream. In the bimodal distribution for ice cream, the first peak represents the intact fat globules, and the second peak represents the aggregated fat particles.

Standard Ice cream (10% Fat)

Figure 4.6 and figure 4.7 show the particle size distribution of ice cream mixes for positive control, negative control, and formulations with different ratios of non-homogenized cream. A unimodal fat globules distribution can be seen in the case of the positive and negative control. At the same time, a bimodal distribution has been reported for formulations with non-

homogenized cream (except $C_{AMF}^{100:00}$). The distribution further became mono modal in ice cream mix with 100% non-homogenized cream as a fat source.

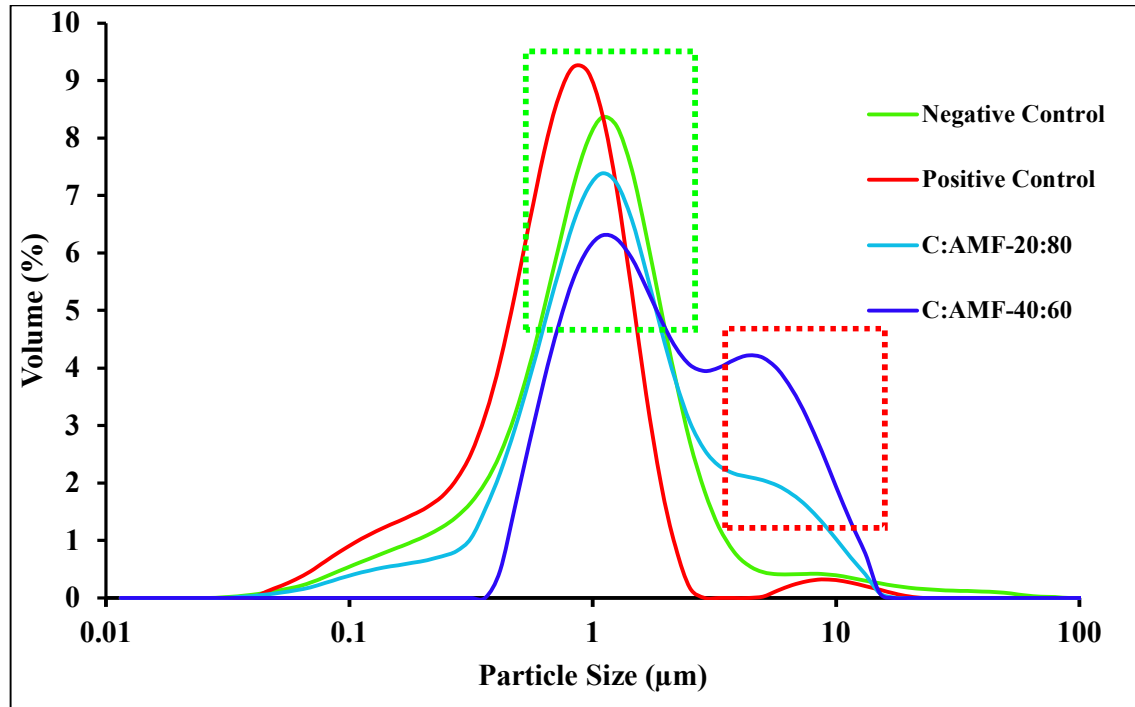


Figure 4.6 Particle size distribution of standard fat ice cream mix for positive control, negative control, $C_{AMF}^{20:80}$ and $C_{AMF}^{40:60}$

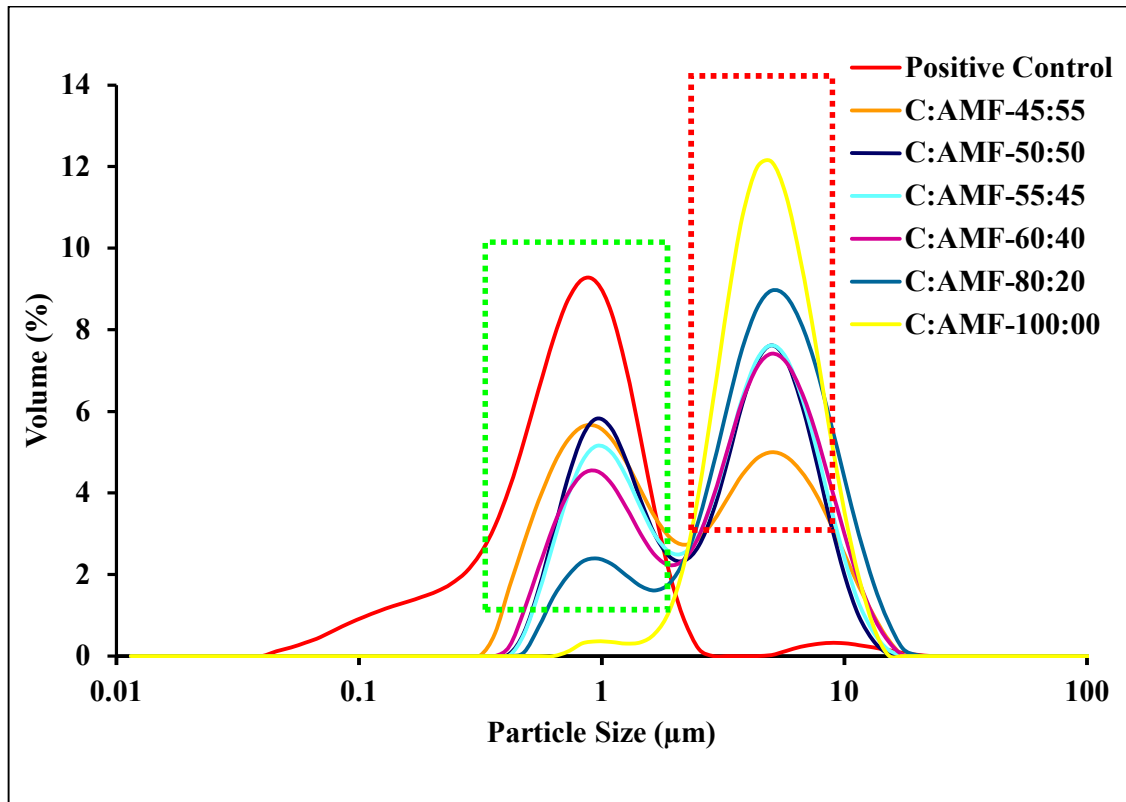


Figure 4.7 Particle size distribution of standard fat ice cream mix of positive control, $C_{AMF}^{45:55}$, $C_{AMF}^{50:50}$, $C_{AMF}^{55:45}$, $C_{AMF}^{60:40}$, $C_{AMF}^{80:20}$ and $C_{AMF}^{100:00}$.

The first peak marked in the green box represents the fat globules formed during the homogenization of the ice cream mix. With the introduction of non-homogenized cream in formulations, the height or volume % globules of the first peak started to decrease, and the curve become bimodal in nature. The first peak is absent in $C_{AMF}^{100:00}$. The second peak marked in the red box represents the native fat globules present in non-homogenized cream. As cream was added after the mix's homogenization, a second peak was expected for these formulations. With the increasing % of the cream, there is an increase in the height of the second peak, which represents the rising amount of native fat globules. Then, for mix with 100% cream, the presence of only the second peak indicates that all the fat present is in the form of native fat globules.

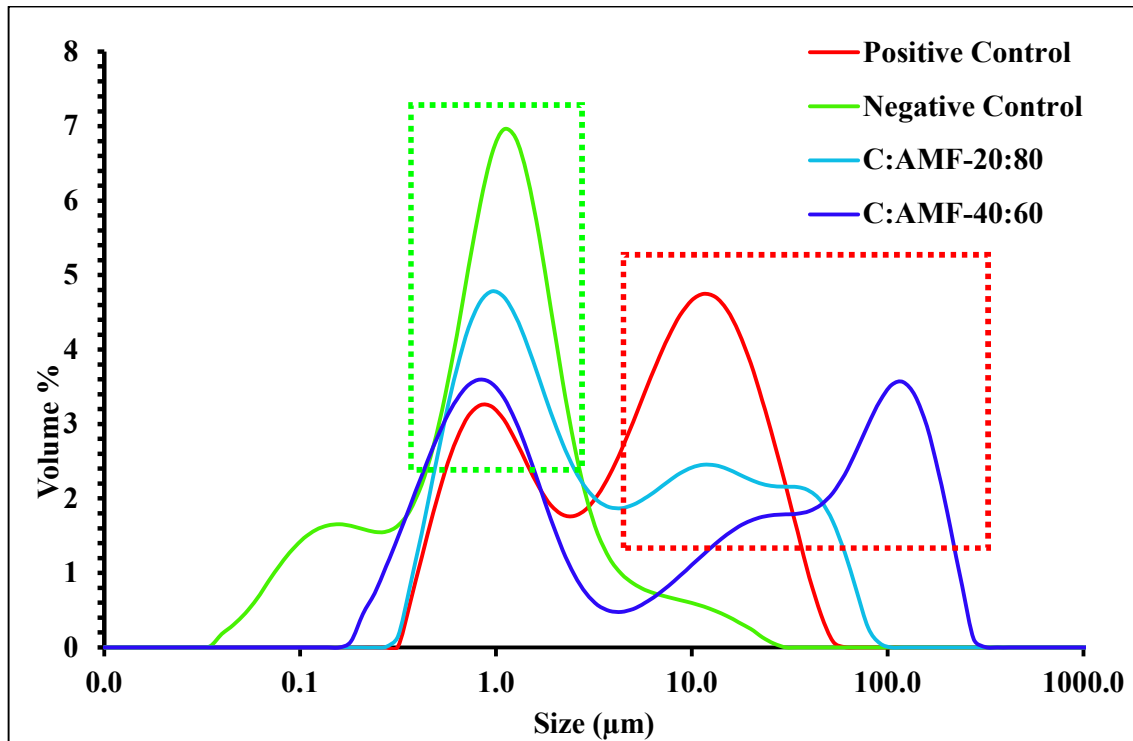


Figure 4.8 Particle size distribution of standard fat melted ice cream for positive control, negative control, $C_{AMF}^{20:80}$, and $C_{AMF}^{40:60}$ formulations.

The size distribution for melted positive control, negative control, $C_{AMF}^{20:80}$, and $C_{AMF}^{40:60}$ ice creams is shown in figure 4.8. Fat globules in the ice cream mix undergo partial coalescence during freezing, which leads to the formation of fat aggregates or partially coalesced fat. The first peak marked in the green box represents the size range of fat globules which have not participated in partial coalescence during freezing. As expected, the negative control has the highest volume % of original fat globules. The volume % of original fat globules is low in $C_{AMF}^{20:80}$ as compared to the negative control. The height of the first peak, representing the original fat globules is similar as ice cream mix, remains comparable for both positive control and $C_{AMF}^{40:60}$. The second peak marked in the red box represents the fat aggregates formed due to agglomeration during freezing. The absence of fat aggregates in the negative control indicates the fat globules in the mix did not undergo partial coalescence during freezing. In positive control, a range (3-60 μm) of fat aggregates can be observed with maximum volume % between 10-13 μm (approximately). The introduction of the cream in formulations results in more diversified size range fat aggregates compared to positive control. The fat aggregates have a size range of 3 -90 μm , with the majority of fat aggregates having a size from 6 to 40 μm in $C_{AMF}^{20:80}$. In contrast, the ice cream formulation with 40% cream has fat aggregates ranging from 4 to 300 μm with a peak value of 90-140 μm .

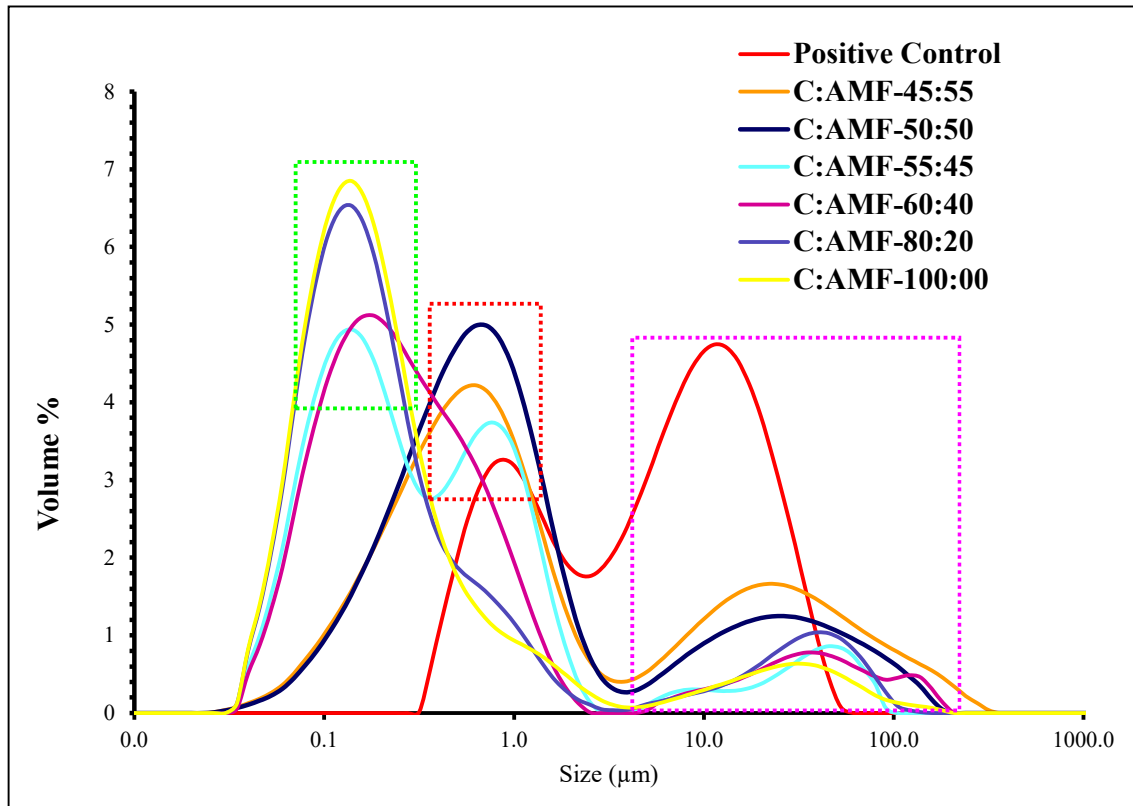


Figure 4.9 Particle size distribution of standard fat melted ice cream for positive control, $C_{AMF}^{45:55}$, $C_{AMF}^{50:50}$, $C_{AMF}^{55:45}$, $C_{AMF}^{60:40}$, $C_{AMF}^{80:20}$ and $C_{AMF}^{100:00}$ formulations.

Figure 4.9 shows the particles distribution curves for $C_{AMF}^{45:55}$, $C_{AMF}^{50:50}$, $C_{AMF}^{55:45}$, $C_{AMF}^{60:40}$, $C_{AMF}^{80:20}$ and $C_{AMF}^{100:00}$. The fat globules distribution for these formulations is not entirely bimodal. Three major peaks characterizing different components can be seen in the graphs. The first peak, marked in the green box, is approximately in size range 100 to 200 nm, and represents the casein micelles. As the amount of cream increases more than 50% in ice cream, the plateau for casein micelles becomes evident in size distribution. The height of the plateau is least for $C_{AMF}^{55:45}$ and tends to rise with an increasing amount of cream.

The second modal distribution marked in the red box represents the original fat globules same as the mix which has not undergone partial coalescence during the freezing process. A wide range of volume % of original fat globules can be observed in $C_{AMF}^{45:55}$, $C_{AMF}^{50:50}$, $C_{AMF}^{55:45}$, and a negligible volume for $C_{AMF}^{60:40}$. The absence of a second peak in formulations with cream > 60 % indicates that all the fat underwent partial coalescence in these formulations and resulting in excessive fat destabilization. The third peak marked in the pink box indicates the fat aggregates. The destabilized fat in all the formulation has a diversified size range. The volume % of fat aggregates for all the formulations show a low amount of fat aggregates,

opposite to interpretation from the second peak. It possibly indicates the limitation of particle size distribution technique for measuring the volume % of large fat aggregates. It is further supported by the fact that fat aggregates in formulations with more than 40% cream were easily visible in melted ice creams. The size of these aggregates was outside of the instrument's measurement range.

D [3,2] and D[4,3]

Table 4.3 Value of D[4,3] and D [3,2] for various standard fat ice cream formulations.

Formulation	D [4, 3] - Volume weighted mean (µm)	D [3, 2] - Surface weighted mean (µm)
Positive Control	9.79	2.29
Negative Control	1.80	0.45
C:AMF-20:80	11.05	1.75
C:AMF-40:60	45.71	1.59
C:AMF-45:55	15.75	0.49
C:AMF-50:50	9.81	0.48
C:AMF-55:45	4.10	0.20
C:AMF-60:40	7.01	0.20
C:AMF-80:20	5.21	0.16
C:AMF-100:00	3.78	0.15

Table 4.3 shows the value for d[4,3] and d[3,2]. As reported by Goff et al. (1999a), these parameters are not sufficient to explain the bimodal distribution, which can be acknowledged with the above data. Further, with the limitation of the laser scattering technique for measuring big fat aggregates (due to excessive fat destabilization), the values d[4,3] and d[3,2] may lead to erroneous interpretation and results should be considered qualitatively at best.

Low fat (5%) ice cream

As reported in the previous section, incorporating native fat globules in ice cream results in the destabilization of fat. This hypothesis was further tested on low-fat ice cream having 5% fat. Figure 4.10 shows the particle distribution for the low-fat positive control, negative control, and C_{AMF}^{20:80} melted ice creams, and figure 4.11 depicts the size distribution for melted positive control, C_{AMF}^{40:60}, C_{AMF}^{60:40}, and C_{AMF}^{80:20} low-fat ice creams.

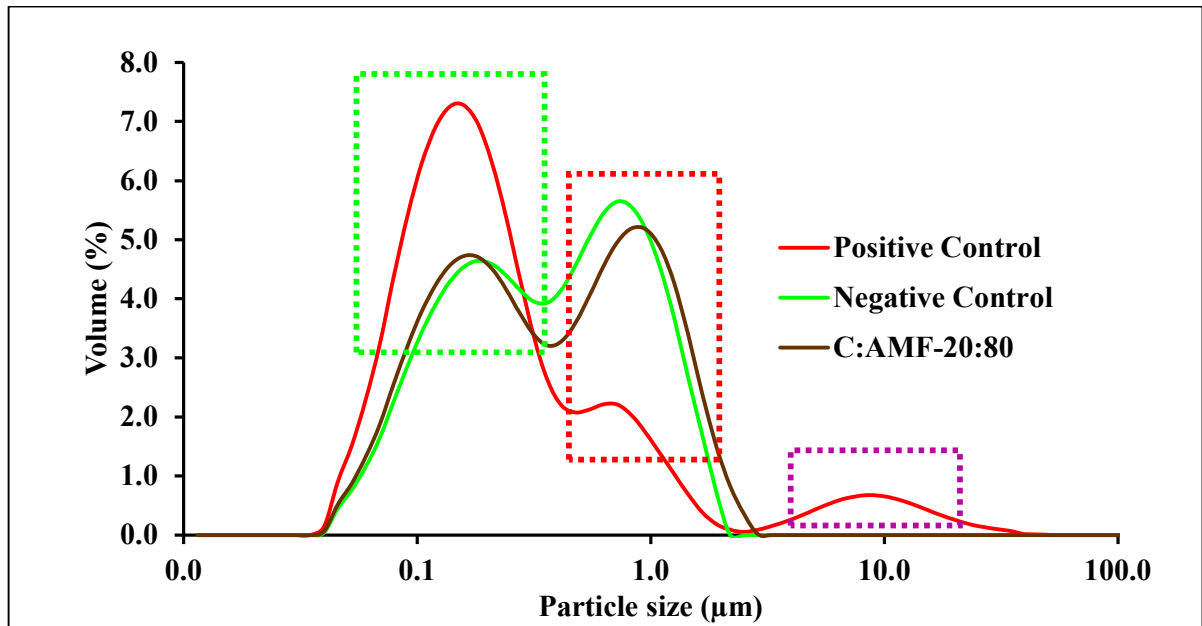


Figure 4.10: Particle size distribution of low-fat melted ice cream for positive control, negative control, and $C_{AMF}^{20:80}$ formulations.

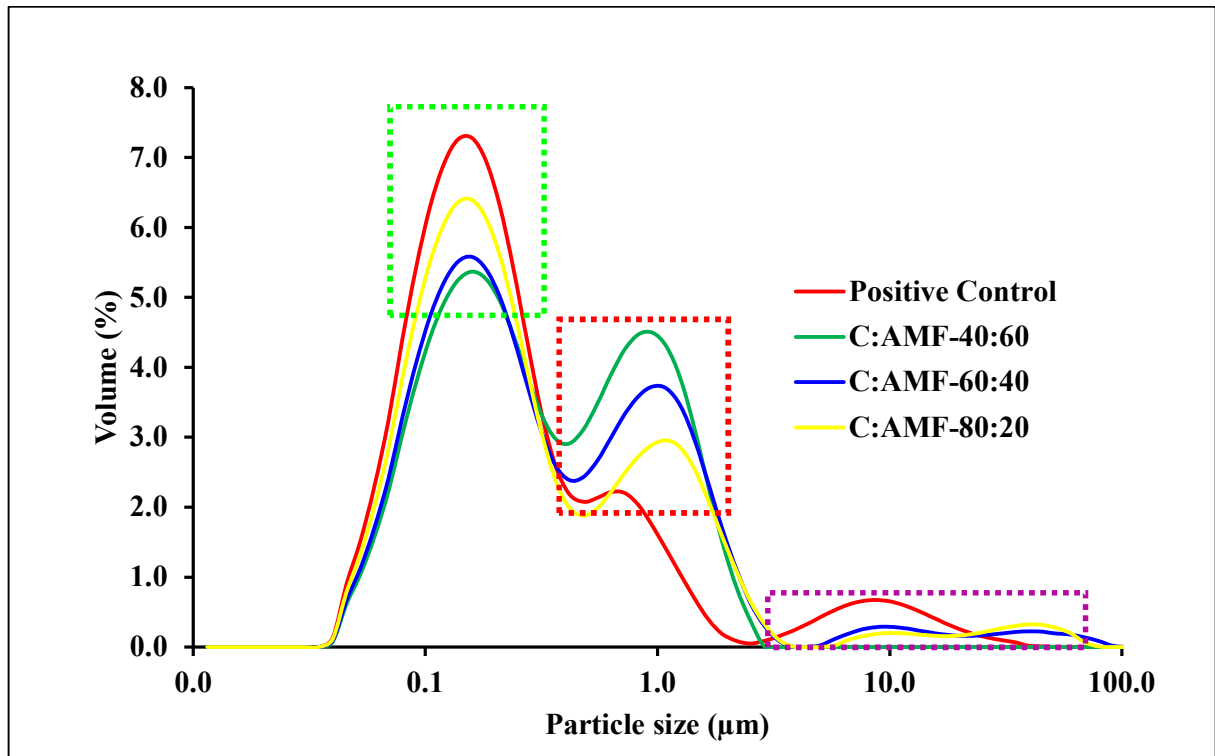


Figure 4.11 Particle size distribution of low-fat melted ice cream for positive control, $C_{AMF}^{60:40}$, $C_{AMF}^{80:20}$, and $C_{AMF}^{100:00}$ formulations

The first peak marked in the green box in Figures 4.10 and 4.11 represents the micellar caseins. The presence of the first peak in all the low-fat formulations is due to a higher amount of milk solid not fat, and lower fat content in these formulations as compared to standard fat ice creams (refer to formulations in section 3.3). The second peak marked in the red box in Figures 4.10 and 4.11 constitutes the size range of original fat globules in ice cream mix, which have not participated in partial coalescence during freezing of mix. As expected, a high volume % of original mix fat globules has been reported for negative control and the lowest volume % for positive control. The incorporation of non-homogenized cream resulted in a decrease in peak height of the original mix fat globules. The reduction in volume % of original fat globules becomes more evident with an increasing % of cream.

The third peak marked in the pink box in Figures 4.10 and 4.11 indicates the distribution of fat aggregates. The absence of the third peak for negative control and $C_{AMF}^{20:80}$ indicates the non-lack of partial coalescence of fat for these formulations. The size of fat aggregates for positive control ranges from 2-35 μm , with most aggregates of 8-9 μm . In contrast, a very low volume % and wide size range (5-91 μm) of fat aggregates have been reported for $C_{AMF}^{40:60}$, $C_{AMF}^{60:40}$, and $C_{AMF}^{80:20}$.

From these results for both standard and low-fat ice creams, it is evident that the fat globules in ice cream without emulsifiers (negative control) underwent minimal destabilization. It is due to the presence of a strong interfacial proteinaceous membrane. In the positive control, a high degree of fat destabilization indicates the displacement of interfacial proteins by monoglycerides. This displacement of proteins results in the weakening of the interfacial membrane, making the fat globules susceptible to partial coalescence during freezing and promoting the adsorption of these aggregates on air bubbles (Goff & Jordan, 1989). It should be emphasized that only a fraction of protein is displaced by mono-diglycerides; therefore, a comparatively lower area of weak interface is present in ice cream with monoglycerides. This area act as a possible site for partial coalescence. With this lower area of the weak interface, the action of shear forces resulted in the formation of 9-12 μm fat aggregates.

The presence of fat aggregates in particle size distribution for ice cream with native fat globules (without any added emulsifiers) signifies that the incorporation of native fat globules has resulted in fat destabilization. The inclusion of native fat globules in the ice cream mix results in the presence of two types of fat globules: 1. Fat globules having milk protein interfacial

membrane and, 2. Fat globules having a native membrane composed of phospholipids, proteins, and triglycerides. As seen in the negative control, fat globules having protein-membrane are stable against the shear forces, so they do not participate in partial coalescence.

The phospholipid interfacial membrane in native fat globules is weak and susceptible to shear forces. Therefore, during freezing, these globules underwent partial coalescence. The presence of a more considerable, more fragile interfacial area than ice cream with MDG resulted in the formation of larger fat aggregates, which is evident from the size of fat aggregated ranging from 3 to 300 μm . The size of these aggregates tends to increase with increasing % of native fat globules in ice cream. This mechanism possibly explains the presence of big aggregates in ice cream with non-homogenized cream. These aggregates can be easily visualized in confocal images, as discussed in previous sections. It is worth noting that non-homogenized fat globules are only expected to interact with each other and not with protein stabilized fat droplets.

4.5 Hardness

The hardness of ice cream is an essential aspect during scooping of ice cream in retail operations. The hardness of ice cream is influenced by overrun, ice phase (volume and size), and to a minor degree by the extent of fat destabilization in ice cream. The ice phase volume and size of ice crystals are majorly contributed to hardness of ice cream. Higher ice phase volume and larger ice crystals result in harder ice cream and vice versa (Roland et al., 1999).

Hardness values for the present study have been reported as force (in N) required to penetrate fixed depth (20mm) in ice cream at $-15\text{ }^{\circ}\text{C}$.

Standard fat ice cream

Table 4.4 shows the hardness values for positive control, negative control, and various standard fat ice cream formulations with non-homogenized cream. The hardness value for positive control was 59 N and for negative control was 69.5 N. A variable trend in hardness values had been reported with the incorporation of non-homogenized cream in the ice creams. Compared to the positive control (59 N), there is no significant decrease in hardness (52.6 N) for formulation with 20% cream. The hardness values drop down to 39.7 N for $C_{\text{AMF}}^{40:60}$, indicating a very soft ice cream compared to positive control. With further increase in cream (more than 40% in ice cream), an upward trend has been reported in hardness value of ice cream until 80 % cream. The hardness value for $C_{\text{AMF}}^{100:00}$ is 49.5 N.

Table 4.4 Hardness values for standard fat ice creams with various ratios of non-homogenized cream.

Ice cream	Hardness
Negative Control	^a 69.5 ± 1.7
Positive Control	^b 59.0 ± 4.9
C:AMF - 20:80	^b 52.6 ± 4.3
C:AMF - 40:60	^c 39.7 ± 3.6
C:AMF - 45:55	^b 52.8 ± 3.0
C:AMF - 50:50	^b 55.8 ± 2.2
C:AMF - 55:45	^b 56.1 ± 1.6
C:AMF - 60:40	^a 70.7 ± 4.8
C:AMF - 80:20	^a 74.8 ± 7.3
C:AMF - 100:0	^b 49.5 ± 4.4

Note: Mean of hardness values is followed by (±) standard deviation. Within rows, different superscripts in front of mean values indicate significance (p<0.05) The superscript along with values shows the grouping of mean values using Tukey methods and 95% confidence. Mean values followed by different superscripts are significantly different, n = 5.

Low fat ice creams

The hardness value for negative control is 34.3 N which is higher than positive control with 29.3 N hardness. The introduction of non-homogenized cream in low-fat recipes resulted in lowering the hardness value until formulations having cream ≤ 40 %. With a further increase in cream % (>40), the ice cream becomes harder. The value of hardness for C_{AMF}^{60:40} is 32.1N, statistically similar to the positive control. A significantly higher value of hardness, 50.4 N, has been reported for C_{AMF}^{80:20}.

Table 4.5 Hardness values for low-fat ice creams with various % of cream.

Ice cream	Hardness
Negative Control	^b 34.3 ± 2.14
Positive Control	^c 29.8 ± 1.11
C:AMF - 20:80	^d 26.1 ± 1.47
C:AMF - 40:60	^d 25.2 ± 1.47
C:AMF - 60:40	^{bc} 32.8 ± 1.92
C:AMF - 80:20	^a 50.4 ± 2.69

Note: Mean of hardness values is followed by (\pm) standard deviation. Within rows, different superscripts in front of mean values indicate significance ($p < 0.05$). The superscript along with values shows the grouping of mean values using Tukey methods and 95% confidence. Mean values followed by different superscripts are significantly different, $n = 5$.

As mentioned, ice phase (volume and size of crystals) and agglomerated fat are major structural elements affecting the hardness of ice cream. It is also important to acknowledge that variations in a structural component of ice cream result in changes in other structures of ice cream. Thus, it is difficult to explain the differences in hardness in ice cream due to variation in one structural element of ice cream, as in the present study (variation in the degree of fat destabilization).

The variation in hardness for different formulations can be explained by the theory of viscosity of ice cream phases other than the air phase. The non-air ice cream phase consists of the ice phase, serum phase, and agglomerated fat. The hypothesis states that the hardness of ice cream increases with an increase in viscosity of the non-air ice cream phase. A higher viscosity non-air ice cream phase can be due to an increased number of ice crystals (high ice phase volume), larger ice crystals, the presence of agglomerated fat clusters, protein-protein aggregates, or other factors.

The results show that the negative control ice cream is harder than ice cream with monodiglycerides (positive control). Both positive and negative control have the same level of sweeteners, thus the same ice phase volume. The difference may lie with the structure of the fat. The fat is present as small, distributed globules in the negative control with only a few globules adsorbed on air cells. In contrast, fat is present in agglomerated form adsorbed on air cells and in the matrix for positive control. The presence of the majority of fat in the matrix phase results in a relatively more viscous non-air ice cream phase in the negative control as compared to positive control. Thus, the positive control ice cream is softer than negative control.

In standard fat ice cream, with the inclusion of cream, a variable trend in hardness has been reported. Up to 40% cream incorporation, ice cream tends to become softer than the positive control. This increase in softness can be explained by the fact that the inclusion of cream resulted in the formation of large fat aggregates, and as is evident from confocal images (for $C_{AMF}^{20:80}$ and $C_{AMF}^{40:60}$), these fat aggregates are evenly adsorbed on air cells. Thus, more fat is present in on-air cells, and the relative amount of fat in the non-air ice cream phase decreases with an increase in cream % (till 40%). Along with a decrease in fat content, as evident in the SEM image for $C_{AMF}^{40:60}$, the formation of fat aggregates also interfered with the growth of ice

crystals. Therefore, is no evidence available on the effect of fat agglomeration on ice crystals growth in ice cream, despite the SEM images suggest otherwise. It can be explained by the fact that native fat globules are relatively large (3-10 μm) as compared to conventional fat globules (0.5 -1.0 μm) in ice cream, and due to weak interfacial membrane, the native fat globules form very large size fat aggregates during freezing. These aggregates may hinder the movement of tiny ice crystals from the slush layer to the centre of the dasher, thus a small number of fine crystals are formed in ice cream. This leads to a decrease in the hardness of ice cream. The reduction in viscosity of the non-air ice phase due to less fat and relatively small sized ice crystals explains the softer ice cream in formulation with cream (till 40%).

For standard ice cream formulations with more than 40% non-homogenized cream, the hardness tends to go upwards and become comparable to positive control for $C_{AMF}^{45:55}$, $C_{AMF}^{50:50}$, and $C_{AMF}^{55:45}$, and even harder in the case of $C_{AMF}^{60:40}$ and $C_{AMF}^{80:20}$. This increase in hardness can be explained by the changes in the distribution of fat aggregates in both on-air cells and the matrix phase in these formulations. As evident from confocal images, the fat distribution becomes uneven after 40% of the cream formulation. The big, clustered fat aggregates and localized protein-protein aggregates are easily visible in the matrix phase. This localized concentration of fat may provide localized resistance to ice crystals growth, and at the same time, localized fat depletion will promote the formation of large ice crystals. Therefore, the concentration of fat particles and the formation of large ice crystals may increase the viscosity of the matrix phase; thus, an increasing trend in hardness is notable in formulations with more than 40% cream.

4.6 Melting stability

Melting, or drip-through rate, is one of the critical quality properties of ice cream. Melt properties of ice cream are important both from the material as well as sensory aspects. As heat transfers from the surrounding air, the ice crystals start to melt. The water from melted ice diffuses into the unfrozen serum phase and flows down through structural elements, including air cells and partially coalesced fat aggregates. During the initial stage of melting, water from the exterior of the product will be released. After sufficient heat penetration, the water from

the product's interior starts to drain, high matrix viscosity can slow down this drainage (Koxholt et al., 2001; Roland et al., 1999).

The ease of diffusion of melted water in the product determines the melting rate of ice cream. The presence of agglomerated fat in ice cream can cause obstruction to drainage of water and thus result in a lower melting rate. Thus, the melting rate of ice cream decreases with an increase in the degree of fat agglomeration. Another important aspect is the ease of heat penetration into the product. Being a poor heat conductor, the presence of air cells provides an insulation effect and result in melt stable ice cream. Thus, a high overrun results in a lower melting rate (Clarke, 2005; Roland et al., 1999).

For this study, constant volume blocks of ice cream with different formulations were analysed for melting rate at 20 °C. The results for both standard fat and low-fat ice creams are reported below. As the % final mass of melted ice cream is directly proportional to melting rate, so the comparison of melting rate has been outlined by comparing the weight of melted ice cream in a stipulated time.

Standard fat ice creams

Figure 4.12 shows the dripping rate curves for positive control, negative control, and formulations with different % of cream. The % final mass of ice cream melted (at the end of 2hrs) has been reported in table 4.6. A higher % final weight for negative control (56.1%) compared to a positive control (7.6%) indicates negative control ice cream melted quickly. The incorporation of non-homogenized ice cream resulted in an improvement of melting properties compared to the negative control. The maximum improvement or minimum melting rate was seen in the formulation with 40% cream which had % end mass equal to 18.3 while % final mass for all other formulations is in the range of 19.5 to 29.4.

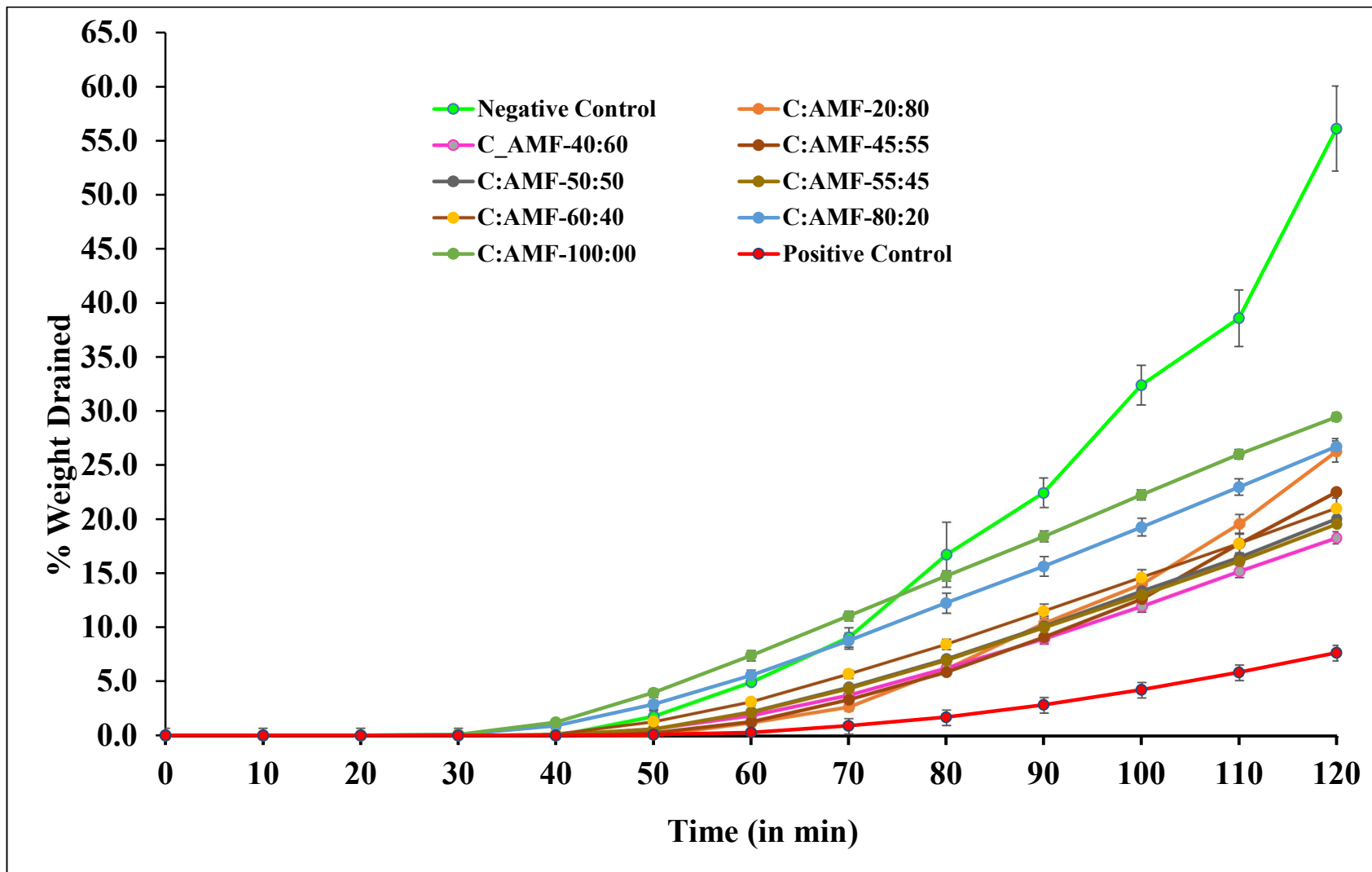


Figure 4.12 Dripping curves for different formulations of standard fat ice creams with various % of non-homogenized cream at 20°C

Table 4.6 % mass of melted ice cream (at the end of 2hrs) for standard fat ice cream formulations

Formulation	% the final mass of melted ice (m/m)
Positive Control	7.6
C:AMF-20:80	26.3
C:AMF-40:60	18.3
C:AMF-45:55	22.5
C:AMF-50:50	20.0
C:AMF-55:45	19.5
C:AMF-60:40	21.0
C:AMF-80:20	26.7
C:AMF-100:00	29.4
Negative Control	56.1

Low fat ice creams

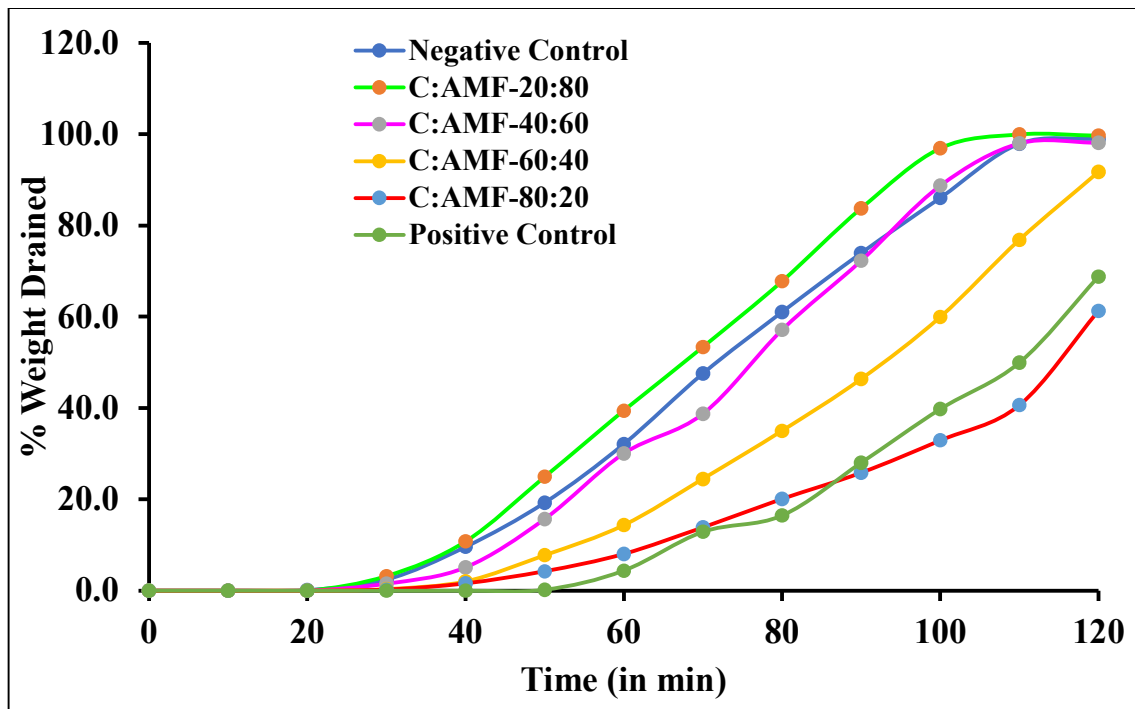


Figure 4.13 Dripping curves for different formulations of low-fat ice creams with cream.

Table 4.7 % mass of melted ice cream (at the end of 2hrs) for low-fat ice cream formulations

Formulation	% mass of melted ice cream (m/m)
Positive Control	68.8
C: AMF-20:80	99.7
C: AMF-40:60	98.1
C: AMF-60:40	91.7
C: AMF-80:20	61.3
Negative Control	99.7

Table 4.7 illustrates the % final mass of melted low-fat ice creams, and figure 4.13 depicts the dripping rate curves for low fat ice creams. A high melting rate was reported for negative control as compared to positive control. Up to 40% cream level incorporation in low-fat formulations results in a negligible improvement in melting rate. A notable improvement can be observed in $C_{AMF}^{60:40}$ with 91.7% and $C_{AMF}^{80:20}$ with 61.3% weight of melted ice cream.

From the above results, it can be illustrated that ice cream with MDG has higher melting resistance than ice cream without emulsifiers. This is because the inclusion of MDG in ice cream results in the partial coalescence of fat during freezing and results in the formation of fat aggregates. These fat aggregates provide resistance to the diffusion of water through the matrix, thus provide better melting stability.

In formulations with non-homogenized cream, the behaviour of melt stability is inconsistent. As reported in the literature, the melt stability of ice cream increase with the degree of fat destabilization. Therefore, melt stability should have shown an upward trend with the increasing amount of non-homogenized cream as increasing cream % in formulations result in a higher degree of destabilized fat. But the highest melt stable ice cream (as compared to negative control) was obtained with 40% cream, and this stability tends to reduce further with more cream. This can be explained by the fact that even though the amount of destabilized fat affects the melt stability, the distribution of this aggregated fat in the matrix is another critical aspect in determining the melting resistance.

As observable in confocal images for ice cream with non-homogenized cream, the distribution of fat changes with increasing % of cream. The fat aggregates are uniformly distributed on-air and matrix in $C_{AMF}^{20:80}$ and $C_{AMF}^{40:60}$. The increased fat destabilization and uniform distribution result in increased melting resistance. But in a formulation with more than 40% fat, the distribution of fat aggregates tends to become non-homogenous. A more localized clustering of partially coalesced fat is easily visible, and this also results in the formation of fat-free voids in the matrix. Even though a high degree of fat destabilization can be observed, the uneven distribution of fat results in the formation of fat-free areas, which provide less resistance to water diffusion, thus a high melting rate for ice cream.

4.7 Final discussion

The above-mentioned results and discussion suggest that the native fat globules undergo partial coalescence during the freezing of ice cream. The mechanism of fat agglomeration in ice cream with native globules is different from the conventional mechanism of partial coalescence in ice cream with monoglycerides. Figure 4.14 suggests the possible mechanism behind the fat agglomeration in ice cream partly having native milk fat globules.

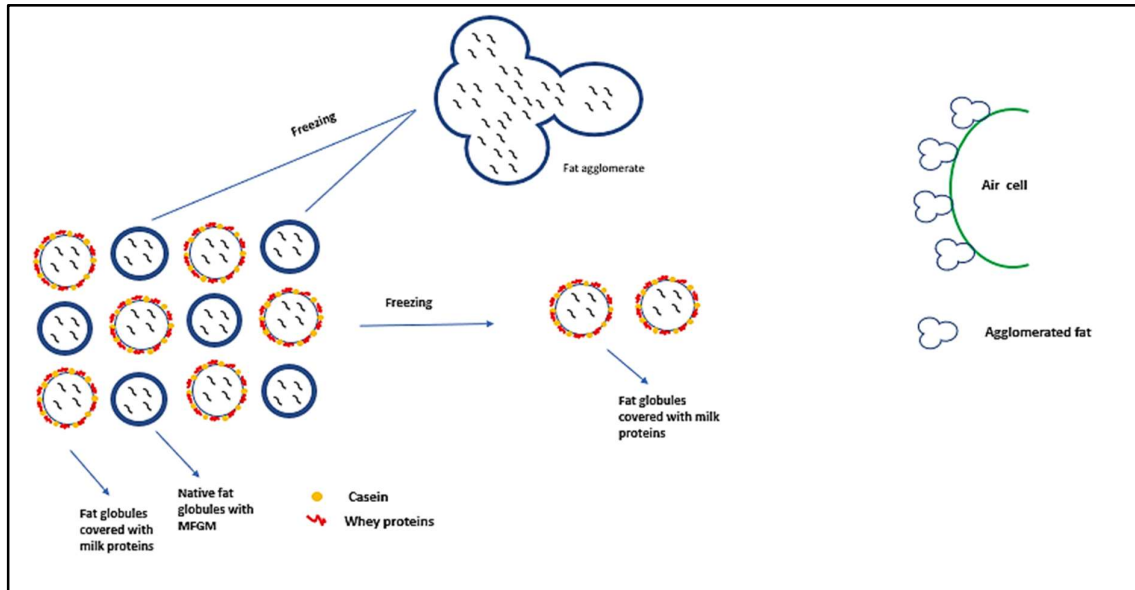


Figure 4.14 The mechanism of agglomeration in ice cream with native milk fat globules

As discussed previously, the inclusion of native fat globules in the ice cream mix results in the presence of two types of fat globules: 1. Fat globules having milk protein interfacial membrane and, 2. Fat globules having a native membrane composed of phospholipids, proteins, and triglycerides. These two types of fat globules behave differently when they are exposed to shear forces during freezing. Due to the nature of MFGM, the native milk fat globules undergo partial coalescence. In contrast, the fat droplets with protein-membrane remain stable during freezing. From the results, it can be suggested that these are the effects of the inclusion of native fat globules in ice cream:

- Only a fraction of the total fat of ice cream undergo agglomeration; thus, a limited number of fat aggregates are available to stabilize air cells. Therefore, air cells tend to be bigger in size in ice cream
- The native fat globules are covered with MFGM, which is fully susceptible to destabilization; thus, the size of aggregates formed is large in size. The relatively large

size of native fat globules compared to conventional fat droplets in ice cream also contribute to the formation of large size fat aggregates. The size of these aggregates increases with the increment amount of native fat globules

- The formation of large aggregates affects the mechanism of ice crystals growth; thus, ice cream becomes soft in texture (up to 40% cream)
- Increasing the amount of native fat globules to more than 40% of total fat result in non-uniform distribution of large aggregates; thus, a localized effect of these large fat aggregates is observed in ice cream
- The agglomeration of native fat globules has considerable improvement on melt stability of ice cream
- Replacement of 40% of total fat by native fat globules produces ice cream with optimum results in terms of melt stability and hardness

5. Effect of buttermilk powder addition on ice cream

5.1 Introduction

Findings from the first section of this research outline how important it is to have a weakened interfacial area on fat globules to create optimum partially coalesced fat aggregates during the freezing of ice cream. Following up on these results, a study was conducted to analyse the effect of adding purified buttermilk powder enriched in milk phospholipids on ice cream.

To create a homogenized emulsion with properties of native MFGM, we applied two approaches for incorporating the buttermilk powder. The first approach was to add buttermilk powder and milk powder into the ice cream mix, followed by homogenization of the mix. This approach is named traditional processing. The working hypothesis behind this approach was that the phospholipids (in buttermilk powder) will undergo competitive adsorption with milk proteins and will partially displace the milk protein from the fat globules interface during aging. It will create the required partially weak interfacial area in the same fashion as done by synthetic emulsifiers. The results for traditional processing are discussed in section 5.2.

The second approach was to add buttermilk powder in the absence of milk powder, followed by homogenization of mix. The lack of milk proteins during homogenization will lead to the preferential adsorption of phospholipids on the newly created fat interface. The milk proteins were then added after the homogenization of the mix. This approach is named selective adsorption. The idea behind this approach was to create a weaker interface by selectively adsorbing the phospholipids and proteins on the fat interface. The increasing amount of buttermilk powder will eventually lead to the formation of the desired partially weak interface. The results for selective adsorption are discussed in section 5.3.

5.2 Traditional processing

5.2.1 Overrun

Table 5.1 shows the overrun values for ice cream made with variable % of buttermilk powder using traditional processing. As reported, there is no significant difference between overrun values of positive control, negative control and ice cream formulations with 1 and 2% of BMP. Further increment in BMP% (at 3 & 4 %), results in a slightly low value of overrun as compared to controls.

Table 5.1 Overrun % for ice cream formulations made with traditional processing with different % of buttermilk powder

Ice creams	Overrun (%)
Negative Control	^{ab} 116 ± 2.48
BMP _{Trad} ^{1%}	^{bc} 112 ± 5.01
BMP _{Trad} ^{2%}	^{abc} 114 ± 0.98
BMP _{Trad} ^{3%}	^c 110 ± 3.42
BMP _{Trad} ^{4%}	^c 109 ± 1.80
Positive Control	^a 121 ± 0.55

Note: Mean of overrun values is followed by (±) standard deviation. Within rows, different superscripts in front of mean values indicate significance (p<0.05) The superscript along with values shows the grouping of mean values using Tukey methods and 95% confidence. Mean values followed by different superscripts are significantly different, n = 5.

In general, a variable trend among the overrun calculations was reported for all the formulations. Even though, a slight low overrun in the case of formulations with buttermilk powder indicates the inability to incorporate a high amount of air, as compared to the positive control. It is not conclusive evidence as it is challenging to control overrun with a batch freezer (Goff & Hartel, 2013b). Therefore, it can be inferred that the inclusion of buttermilk powder has no pronounced effect on the capacity of these formulations to incorporate air.

5.2.2 Microstructure

5.2.2.1 Confocal microscopy

The microstructure for melted positive and negative control is shown in figure 5.1. For the positive control, the fat globules can be seen distributed throughout the matrix. The larger red dots represent the agglomerated fat present in ice cream. As expected for positive control, agglomerated fat is uniformly distributed throughout the matrix. Some smaller size fat globules are present along with the protein particles at air lamella. In of negative control, few agglomerated fat particles are present in the matrix. It illustrates a low degree of fat destabilization. To a certain degree, the air lamella does have some fat particles present along with protein particles.

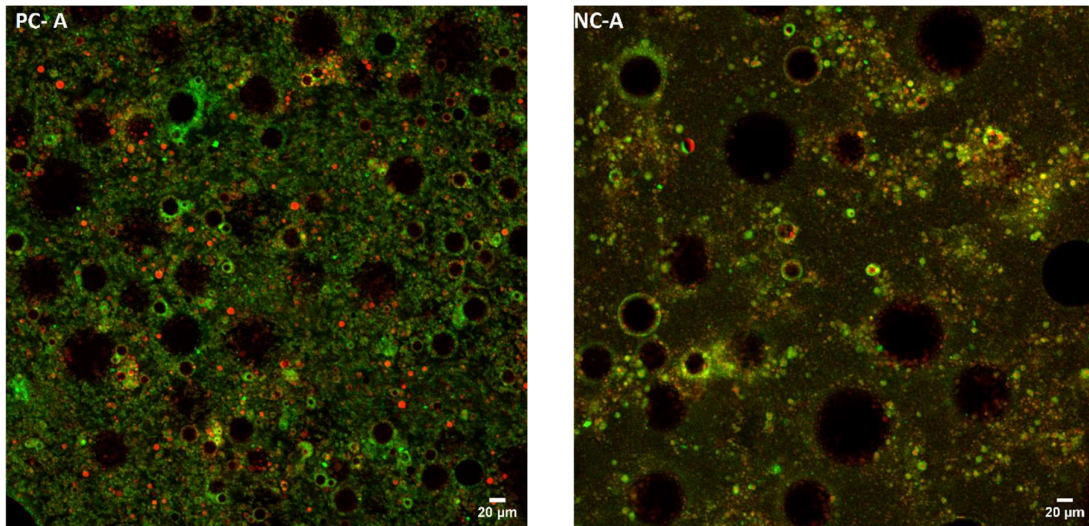


Figure 5.1 Confocal laser scanning microscopic images of melted ice cream for standard fat ice cream controls.

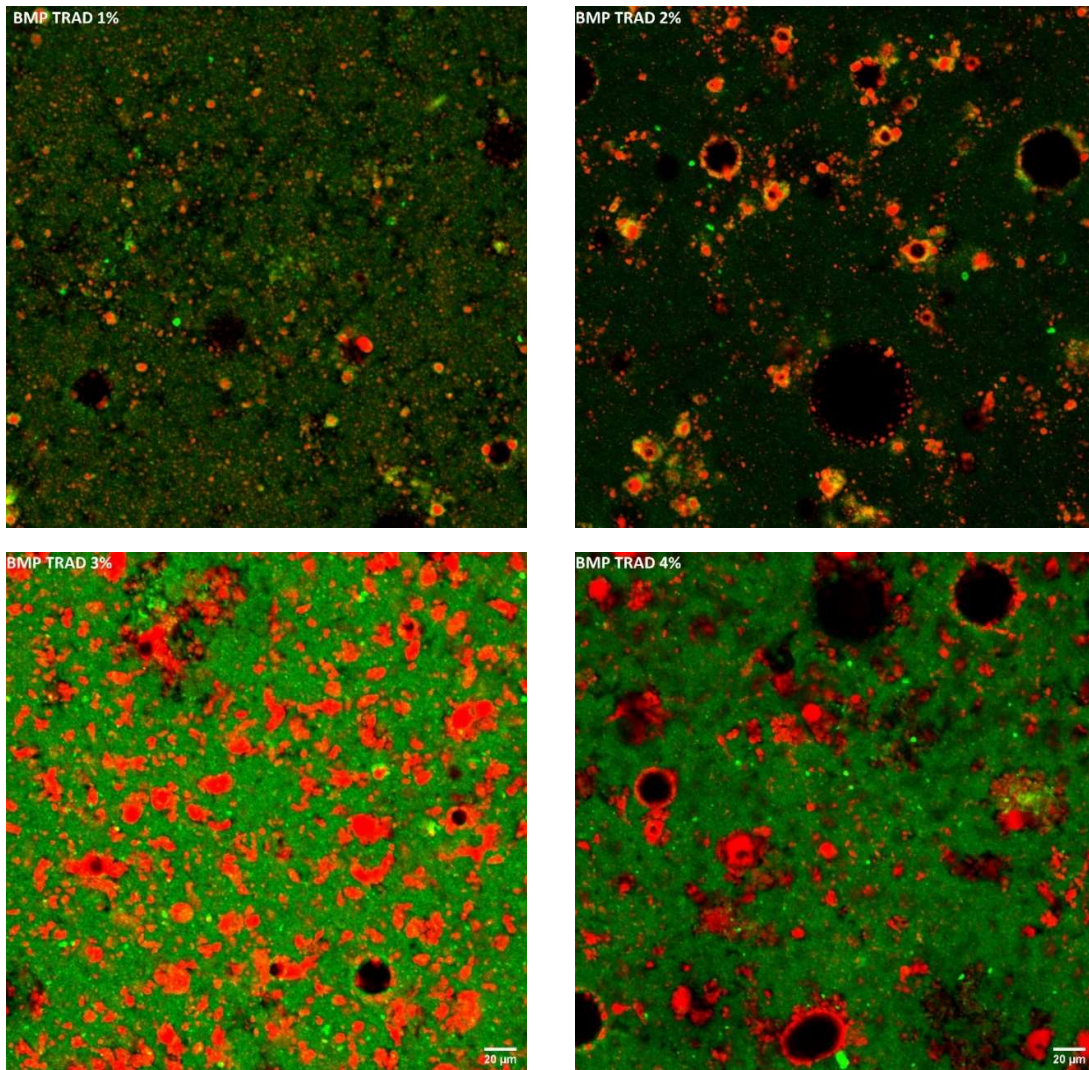


Figure 5.2 Confocal laser scanning microscopic images of melted ice cream with variable % of BMP

Figure 5.2 illustrates the confocal images for melted $\text{BMP}_{\text{Trad}}^{1\%}$, $\text{BMP}_{\text{Trad}}^{2\%}$, $\text{BMP}_{\text{Trad}}^{3\%}$, and $\text{BMP}_{\text{Trad}}^{4\%}$ ice creams. At the 1 % BMP level, the presence of a small number of agglomerated fats (seen as red dots) implies a low degree of partial coalescence. Few fat agglomerates, along with protein molecules, appears to be adsorbed on-air bubbles. With a 2% BMP level, fat is present as a mixture of small and large aggregated fat particles. The agglomerated fat is evenly distributed in the matrix, and air bubbles appear to be covered with both milk proteins and aggregated fat.

In $\text{BMP}_{\text{Trad}}^{3\%}$ formulation, the agglomerated fat particles appear to become more significant in size. This indicates the formation of larger fat aggregates during partial coalescence and a higher degree of fat destabilization. These large aggregates are uniformly distributed in a matrix. The air interfaces are partially covered with fat aggregates, and some smaller air

bubbles appear to be covered entirely with agglomerated fat. With further increase in BMP level, at 4%, the size of fat particles further enlarges significantly in size. Compared to the 3% BMP level, the distribution of these fat aggregates becomes non-uniform in the matrix. A few coalesced fat particles comparable to the size of air bubbles can also be seen in matrix. The distribution of fat particles on air bubbles also tends to be non-uniform with some bubbles fully covered and others partially covered.

In general, it is evident from results that increasing the level of buttermilk powder leads to the formation of larger coalesced fat particles indicating the increasing degree of fat destabilization. As the fat particles grow in size, the fat clusters also grow and tend to be bigger in size. It is important to acknowledge that the formation of large fat clusters also leads to the formation of localized fat depleted regions in the matrix, which is evident in the formulation with 4% BMP. In terms of distribution, with increasing size of coalesced fat molecules, the distribution of fat particles tends to become non-uniform on-air lamella (as seen in 3 and 4 % BMP level), with some of the air cells fully covered with fat while others only partially.

5.2.2.2 Scanning electron microscopy

Figure 5.3 represents the scanning electron microscopic images for positive control, negative control, BMP_{Trad}^{1%}, and BMP_{Trad}^{3%} ice creams at 250X magnification.

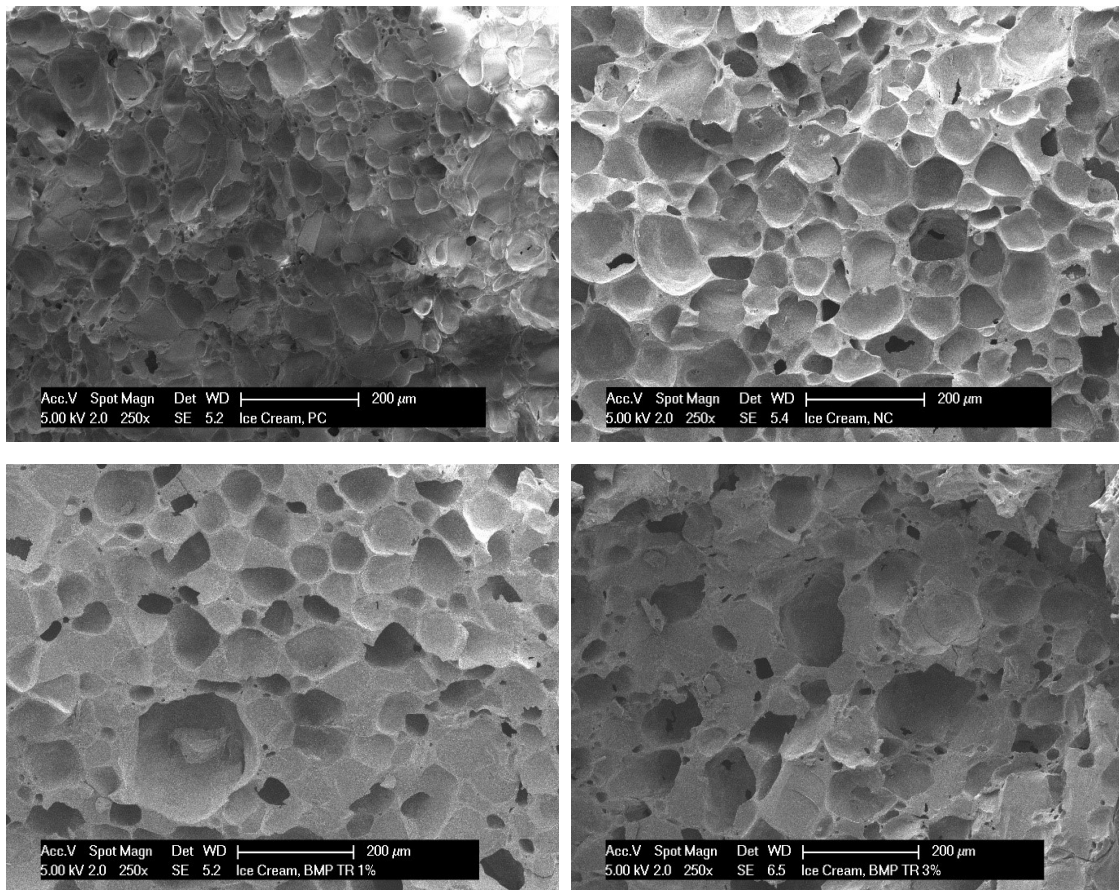


Figure 5.3 SEM images for controls and ice cream with 1 % and 3 % BMP at 250 x magnification

The size range of air cells in ice cream with buttermilk powder tends to be significantly different from the positive control. In formulation with 1% BMP, the air cells are larger in size with a low number of very fine air cells. Whereas in ice cream with 3% BMP, air cells' size distribution appears to almost be bimodal with large and small size air bubbles. It is also worth noting that the distribution of the ice phase is not uniform in ice cream with 3% buttermilk powder.

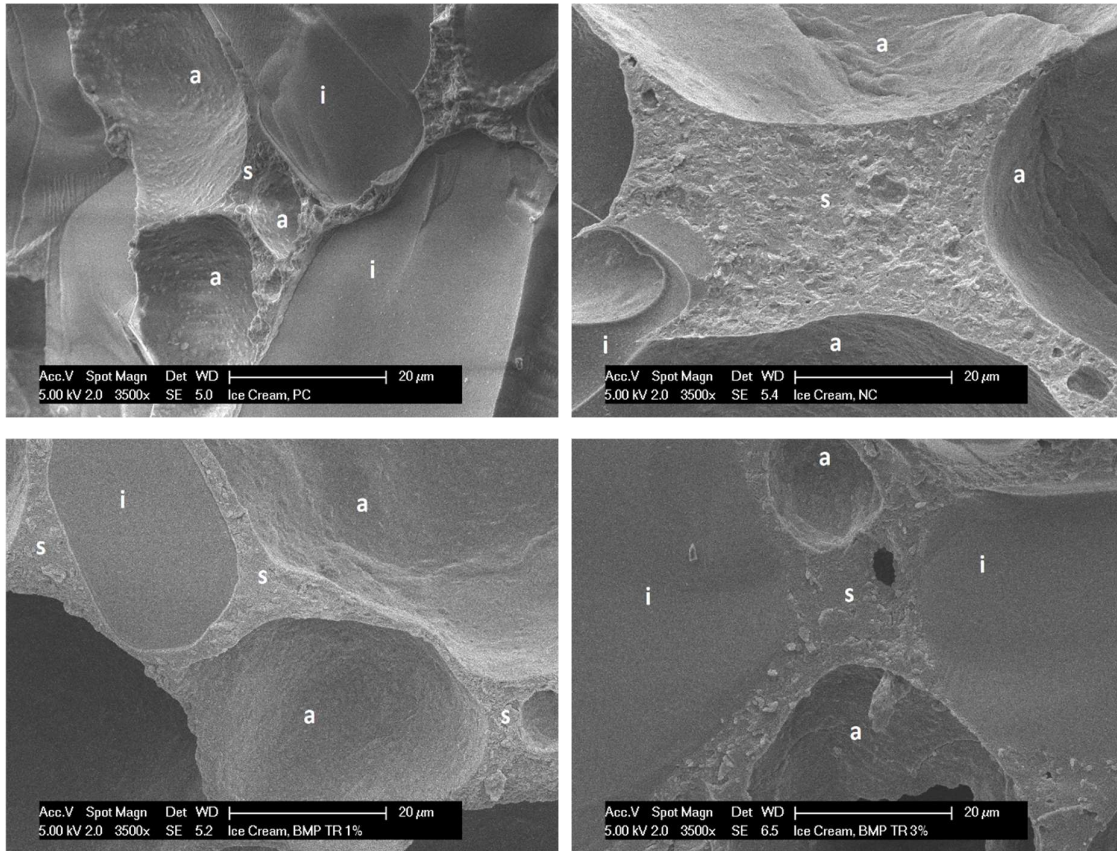


Figure 5.4 SEM images for controls and ice cream with 1 % and 3 % BMP at 3500 x magnification (s – serum phase, I – ice phase, a – air cells, and f – fat particles)

Figure 5.4 shows the microscopic images for positive control, negative control, BMP_{Trad}^{1%}, and BMP_{Trad}^{3%} ice creams at 3500X magnification. The aggregates of fat are evenly distributed on-air lamella and matrix for the positive control. Comparing the ice cream with buttermilk powder and positive control, it is evident that ice cream with 1% BMP level does not have structured fat, and air cells are not covered with fat agglomerates. In contrast, the air cells in the formulation with 3% BMP seemed to be covered with partially coalesced fat particles. These fat particles are also evident in the serum phase.

In general, it is apparent that the phospholipids in buttermilk powder can make fat globules susceptible to partial coalescence. In formulations with buttermilk powder, the air cells tend to be non-uniform in size, which may be due to poor stabilization of newly formed air cells by irregular fat aggregates during freezing. This non-uniform distribution of fat aggregates tends to increase with higher concentration of phospholipids. Thus, air cells tend to be more irregular in their size distribution. Another important observation is that the degree of fat destabilization increases with the amount of buttermilk powder which can be explained by the fact that the

increasing amount of surface-active material results in a weaker interface. Thus, more destabilization of fat during shearing.

5.2.3 Particle size distribution

Figure 5.5 shows the particle size distribution for melted positive control, negative control, and BMP_{Trad} 1% ice creams. The first peak, marked in the red box, represents the casein micelles. The casein micelles are evident in the negative control and the formulation with 1% BMP. In contrast, the particle distribution for positive control does not show any size distribution of casein micelles.

The second peak signifies the volume % of original fat globules which have not undergone partial coalescence during freezing of ice cream. As expected, the volume % of original fat globules is highest for the negative control, indicating a low degree of fat destabilization. Compared to the negative control, the formulation with 1% BMP has a low number (5%) of original fat globules. The lowest volume % (3) of original fat globules can be observed in positive control, indicating the high degree of partial coalescence.

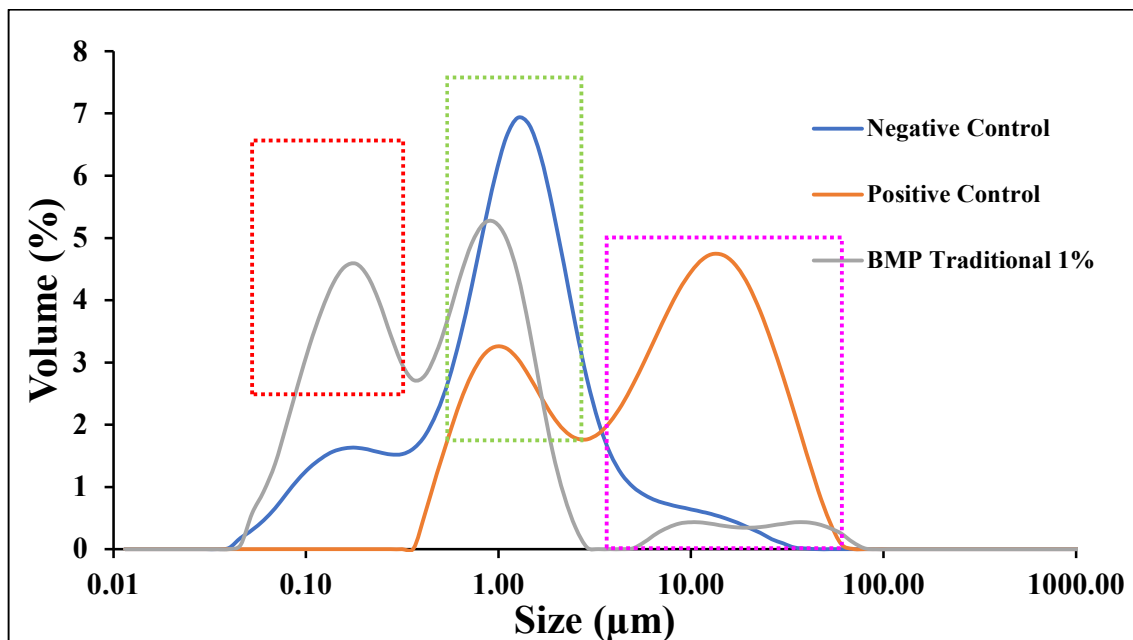


Figure 5.5 Model size distribution for melted controls and BMP Trad 1% ice creams

The third peak marked in the pink box represents the agglomerated fat particles. The fat aggregates in positive control are in the size range of 3-60 μm , with most of the fat aggregates having a size between 10-13 μm . A low volume % of fat aggregates with a wide size range (3-80 μm) can be observed in the formulation with 1% BMP.

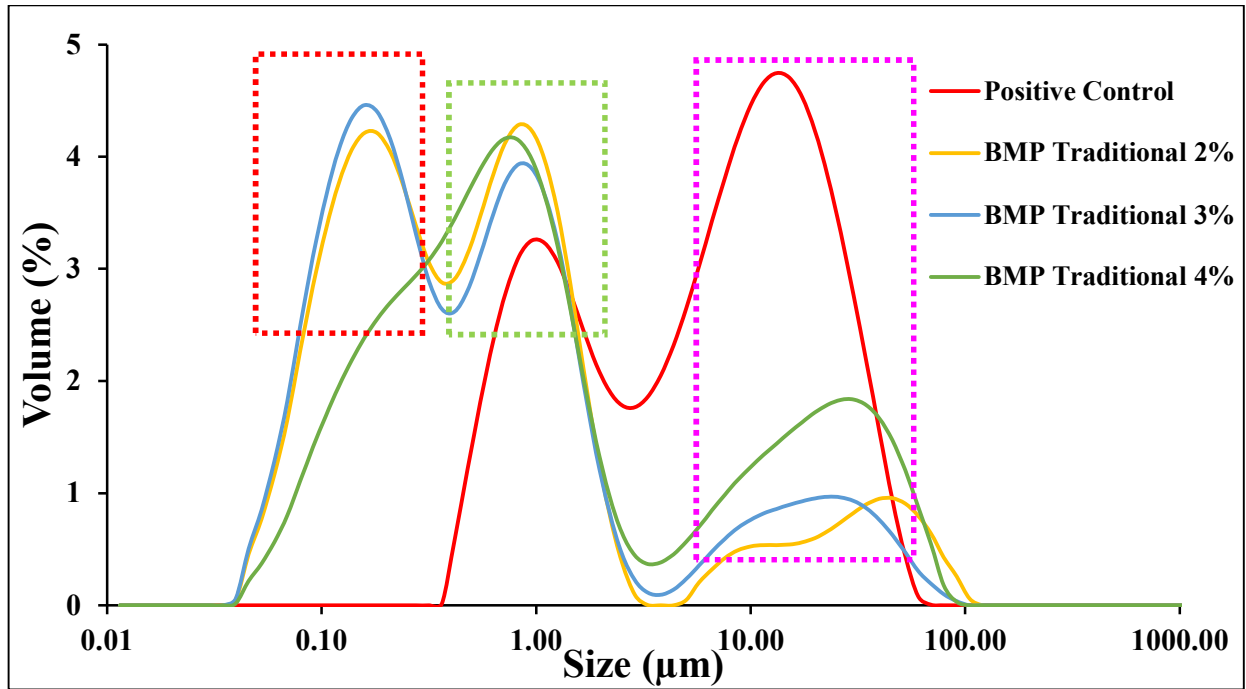


Figure 5.6 Model size distribution for melted positive control, BMP Trad 2%, 3 %, and 4% ice creams

Figure 5.6 illustrates the particle size distribution for melted positive control, BMP_{Trad}^{2%}, BMP_{Trad}^{3%}, and BMP_{Trad}^{4%} ice creams. The first peak marked in red indicates the casein micelles. The distribution of milk proteins is observable for all the formulations except BMP_{Trad}^{4%}. The second peak represents the fat globules that have not participated in partial coalescence during the freezing of ice cream. The volume % of original fat globules for all the formulations is in the range of 4-4.5%. The proportion of original fat globules is comparatively similar for BMP_{Trad}^{2%} and BMP_{Trad}^{4%}. Whereas a low volume % of original fat globules was reported for BMP_{Trad}^{3%}, indicating a higher degree of fat destabilization.

The third peak marked in the pink box signifies the agglomerated fat particles. The size range of these fat aggregates is between 4 to 104 μm . The highest peak height for BMP_{Trad}^{4%} indicates the highest degree of fat agglomeration. The peak height is relatively similar for both BMP_{Trad}^{2%} and BMP_{Trad}^{3%}. The majority of fat aggregates for BMP Trad 3% are around 23-26 μm . In contrast, bigger size aggregates (28-30 μm) have been reported for BMP_{Trad}^{4%}. The

presence of slightly bimodal distribution in the third peak for BMP_{Trad}^{2%} indicates a mixture of smaller and larger fat aggregates.

It is well known that displacement of milk proteins by mono-diglycerides during the aging of ice cream mix is responsible for partial coalescence of fat during freezing (Goff & Hartel, 2013b). As evident from results, this partial displacement of milk proteins by mono-diglycerides in the positive control leads to the formation of partially coalesced fat, which is absent in the negative control. Being enriched in surface-active phospholipids, the incorporation of buttermilk powder in ice cream is expected to displace the milk proteins during the aging of the mix. It is evident from size distribution results that incorporation of buttermilk powder leads to partial coalescence of fat during freezing. It is observed that an increasing number of phospholipids results in the formation of large size aggregates with a higher degree of fat destabilization.

The increase in the number and size of fat aggregates can be explained by the fact that an increasing amount of buttermilk powder increases the concentration of surface-active phospholipids in ice cream. This increase in the concentration of phospholipids displaces a higher amount of interfacial milk proteins. It results in the creation of a higher degree of weak interface susceptible to partial coalescence. There is a stark difference between the size of fat aggregates formed in the positive control and ice cream with phospholipids. It can be possibly explained by the argument that phospholipids are larger molecules as compared to mono-diglycerides. Thus, displacement of milk proteins by phospholipids results in a relatively larger weak interface than mono-diglycerides. This tends to increase the encounter frequency of fat globules during partial coalescence and result in the formation of a larger size of fat particles.

5.2.4 Hardness

The firmness values for ice cream with different % of BMP along with positive and negative control are shown in table 5.2. The hardness value for positive control is 59N, and hardness value, 69.5 N, was reported for negative control. In formulations with the buttermilk powder, a variable trend has been observed. The hardness values for both BMP 1 and 2% level are nearly similar, 55 N. With the increase in BMP % at 3% level, ice cream tends to become softer (hardness 48 N), and at 4% BMP level, ice cream tends to become harder as compared to BMP_{Trad}^{3%}.

Table 5.2 Hardness values (in N) for controls and ice cream with variable % of BMP made by using traditional processing

Ice creams	Hardness (N)
Negative Control	^a 69.5 ± 1.93
BMP Trad 1%	^{bc} 54.4 ± 1.83
BMP Trad 2%	^b 54.8 ± 1.70
BMP Trad 3%	^c 48.2 ± 4.08
BMP Trad 4%	^b 55.3 ± 2.28
Positive Control	^b 59.0 ± 5.43

Note: Mean of hardness values is followed by (±) standard deviation. Within rows, different superscripts in front of mean values indicate significance (p<0.05) The superscript along with values shows the grouping of mean values using Tukey methods and 95% confidence. Mean values followed by different superscripts are significantly different, n = 5.

It is well known that the hardness of ice cream is affected by the air, ice phase, and degree of fat agglomeration (Muse & Hartel, 2004). As explained previously, variation in one structural element of ice cream results in changes in other phases of ice cream. Thus, it is difficult to explain the differences in hardness in ice cream due to variation in one structural element of ice cream, as in the present study.

However, this trend in hardness values can be perhaps explained by the concept of viscosity of the matrix phase. This concept suggests that the hardness of ice cream increases with an increase in the viscosity of the non-air ice cream phase. The higher viscosity of the non-air ice cream phase can be due to the high number of ice crystals (high ice phase volume), larger ice crystals, agglomerated fat clusters, protein-protein aggregates, etc.

It can be observed that with the introduction of buttermilk powder as an emulsifier in ice cream, the product becomes relatively softer as compared to ice cream with monoglycerides. The results from particle size distribution suggest that the size of fat aggregates is relatively higher in formulations with BMP. It tends to increase with the increase in the concentration of phospholipids. As evident from confocal images, these fat particles appear to be covering the air cells. Therefore, a relatively lower proportion of fat is present in the matrix, resulting in a low viscous non-air ice cream phase. Thus, the hardness of ice cream tends to decrease with the incorporation of buttermilk powder. The relative decrease in hardness is dependent upon

the size of aggregates and their distribution on air cells. As the distribution of fat aggregates tends to become non-uniform, as seen in the 4% BMP formulation, an inverse effect is evident, and hardness tends to increase. It is essential to acknowledge that the above-mentioned mechanism is based on assumptions and needs to be verified.

5.2.5 Melting stability

The % mass of melted ice cream after 2hrs for various formulations with buttermilk powder and controls are outlined in table 5.3. A significantly lower mass% for the positive control (7.6%) than negative control shows the impact of fat agglomeration on melt stability of ice cream. The use of buttermilk powder has boosted the melt stability of ice cream compared to the formulation without emulsifier. The maximum melt stability has been reported at 3% BMP level with 21.7 remnant mass %. A similar melting rate has been reported for both 1 and 2% BMP levels. The mass % of formulation with 4% BMP level is 23.8%.

Table 5.3 % the final mass of melted ice cream for controls and formulations with different % of BMP

Formulation	% the final mass of melted ice (m/m)
Positive Control	7.6
BMP Trad 1%	25.3
BMP Trad 2%	25.2
BMP Trad 3%	21.7
BMP Trad 4%	23.8
Negative Control	56.1

Figure 5.7 illustrates the plot of mass % of melted ice cream at different time intervals. Comparing the onset of melting in various formulations, ice cream without emulsifier and with BMP start to melt in 30-40 min. In contrast, a late onset of melting around 60 min was reported for positive control.

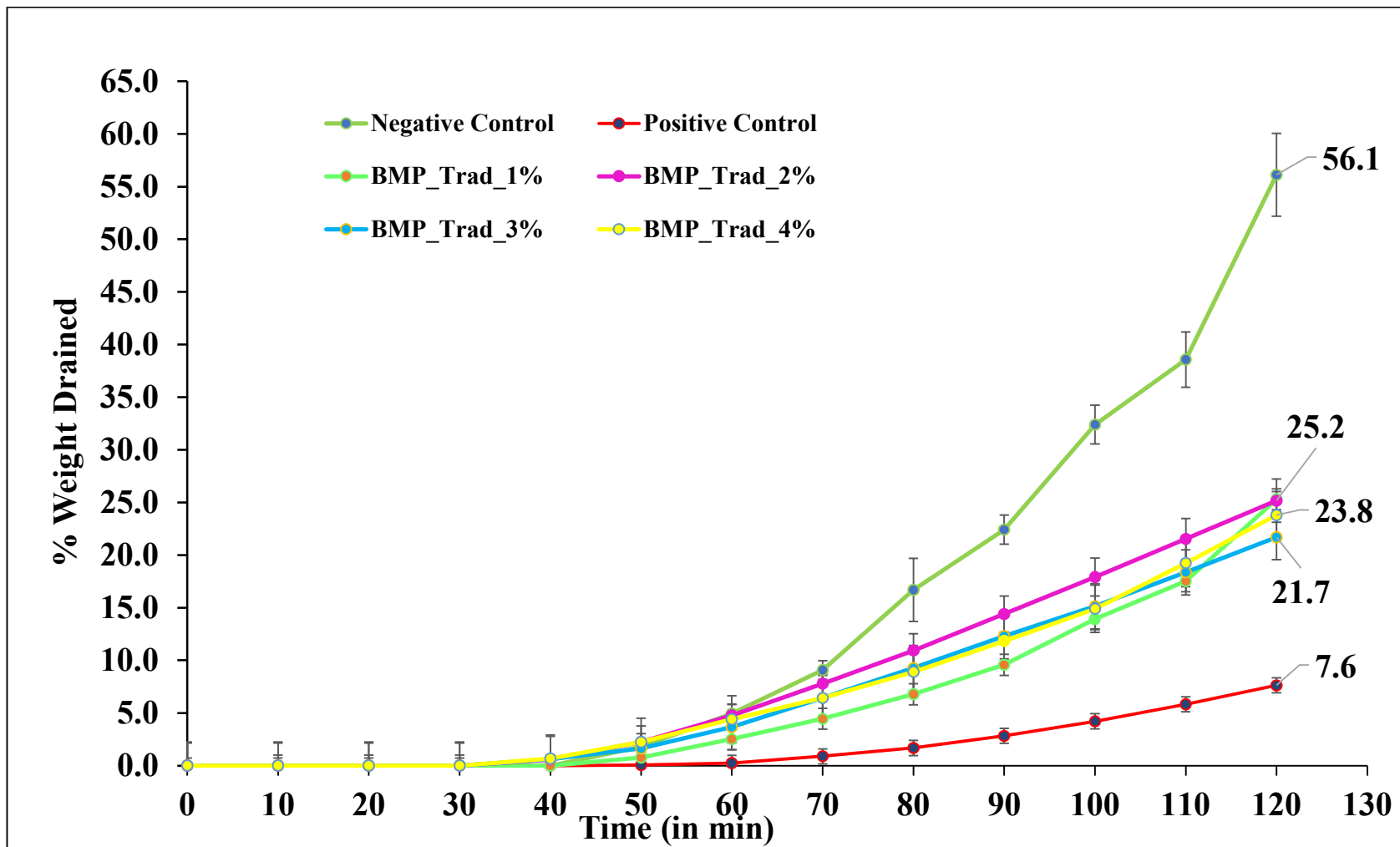


Figure 5.7 Dripping rate curves for controls and ice cream with variable % of buttermilk powder produce using traditional processing at 20°C

In general, the melting stability of ice cream depends upon how easily water will drain through the matrix. A uniformly distributed agglomerated fat provides good melt resistance. In ice cream with buttermilk powder, there is a significant improvement in melt stability of ice cream compared to the negative control. The incorporation of BMP resulted in a relatively higher degree of partial coalescence of fat compared to ice cream without monoglycerides during freezing. The melt stability is similar for 1 % and 2 % BMP levels. A slight improvement at 3% buttermilk level can be attributed to uniformly distributed large aggregates of fat, as visualized in confocal images. The uniform distribution of agglomerated fat is significant to provide resistance against the drainage of water. As the level of buttermilk powder reached 4%, the distribution of fat particles tends to become more localized and non-uniform in nature, so the aggregates become larger. This may result in the creation of fat-free voids in the matrix, thus decrease in melting resistance.

5.2.6 Final discussion

The different analyses suggest that with the conventional processing method, the monoglycerides in ice cream can be potentially replaced by buttermilk. Figure 5.8 presents the possible interfacial changes occurring on fat globules during aging.

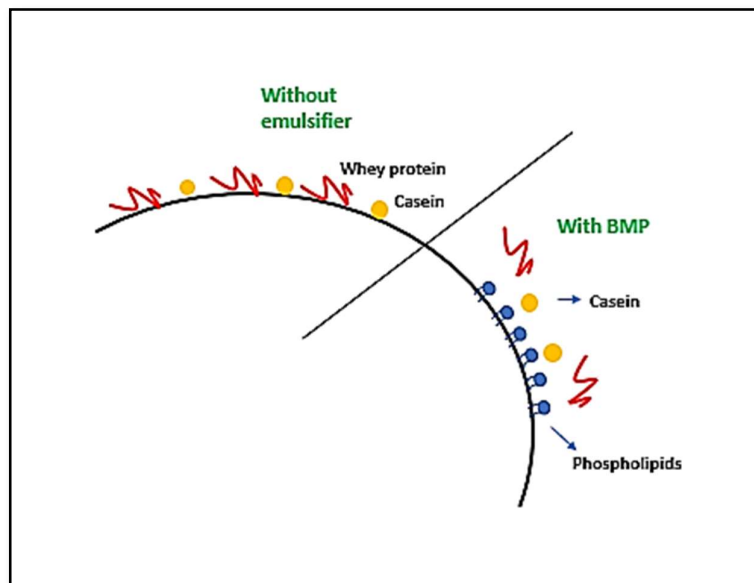


Figure 5.8 Fat globules interface with the incorporation of BMP using traditional processing

It can be suggested with conventional processing, the casein micelles and whey proteins get adsorbed on newly formed fat globules and stabilize them. Due to their high surface-active nature, the phospholipids undergo competitive adsorption with milk proteins. Thus, they displace a fraction of milk protein from the interfacial area. This displacement results in the creation of a weak interfacial area susceptible to partial coalescence. It is worth noting that phospholipid molecules are larger in size than SMS. Thus, they relatively may displace a high amount of milk proteins from the interfacial area, thus, providing more surface area for fat crystals protrusion. This leads to a higher capture efficiency during agglomeration and results in the formation of relatively large aggregates.

In summary, below are the effects of buttermilk addition in ice cream using conventional processing:

- Buttermilk powder displaces milk proteins and results in the formation of fat aggregates. These partially coalesced fat particles are large in size
- The size and degree of fat agglomerates increase with the increasing amount of buttermilk powder. With large aggregates, the distribution of fat changes rapidly in the ice cream system, thus producing some localized effect
- To some extent, the formation of large aggregates affects the number density of fat agglomerates to stabilize newly formed air cells during freezing. Thus, air cells tend to be non-uniform size in formulations with buttermilk powder
- To a limited extent, the large aggregates affect the growth of ice crystals in ice cream during freezing. This results in the formation of soft texture ice cream.
- The optimum results can be obtained by adding 3% of BMP in terms of melt stability and texture of ice cream

5.3 Selective adsorption

5.3.1 Overrun

The calculated % overruns for ice cream prepared with buttermilk powder using selective adsorption technique and controls are reported in table 5.4. The highest overrun (121%) was reported for positive control. Overrun values (~ 116%) were the same for ice cream without monoglycerides and all formulations with buttermilk powder except BMP_{Selective}^{1%}. The ice cream formulation with 1% BMP level was reported to have the lowest overrun (112%) as compared to positive control.

Table 5.4 Overrun % for controls and ice cream manufactured using selective adsorption with different level of buttermilk powder

Ice creams	Overrun (%)
Negative Control	^{ab} 116 ± 2.48
BMP _{Selective} ^{1%}	^b 112 ± 4.63
BMP _{Selective} ^{2%}	^{ab} 116 ± 4.04
BMP _{Selective} ^{3%}	^{ab} 116 ± 4.15
BMP _{Selective} ^{4%}	^{ab} 116 ± 1.37
Positive Control	^a 121 ± 0.55

Note: Mean of overrun values is followed by (±) standard deviation. Within rows, different superscripts in front of mean values indicate significance (p<0.05) The superscript along with values shows the grouping of mean values using Tukey methods and 95% confidence. Mean values followed by different superscripts are significantly different, n = 5.

In summary, there is small variations among overrun values for different formulations. As explained previously, it is difficult to control the amount of air incorporation in the batch freezer, so differences in overrun values are expected. Therefore, it can be implied that incorporation of buttermilk powder selectively as an emulsifier in ice cream does not impact the aeration capacity of ice cream and amount of air incorporated is similar to controls.

5.3.2 Microstructure

5.3.2.1 Confocal microscopy

The microstructures for melted positive and negative controls are shown in figure 5.9. As previously reported for the positive control, the fat globules can be seen distributed throughout the matrix. The big red dots represent the agglomerated fat present in ice cream. As expected for the positive control, agglomerated fat is uniformly distributed throughout the matrix. Some smaller size fat globules are present, along with protein particles at the air lamella. In negative control, few agglomerated fat particles are present in the matrix. This illustrates a low degree of fat destabilization. To a certain degree, air lamella does have some fat particles present along with protein particles.

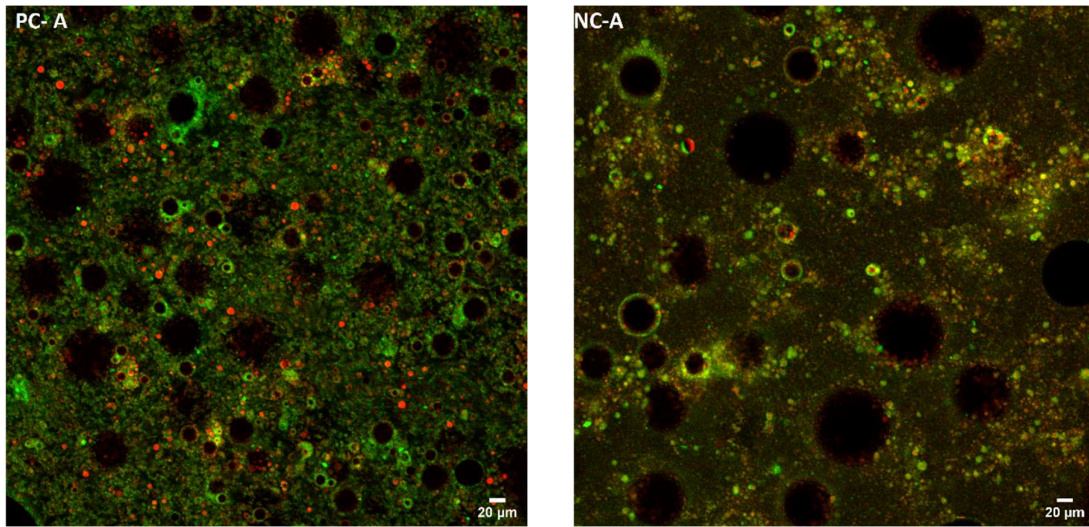


Figure 5.9 The confocal microscopic images for control ice creams

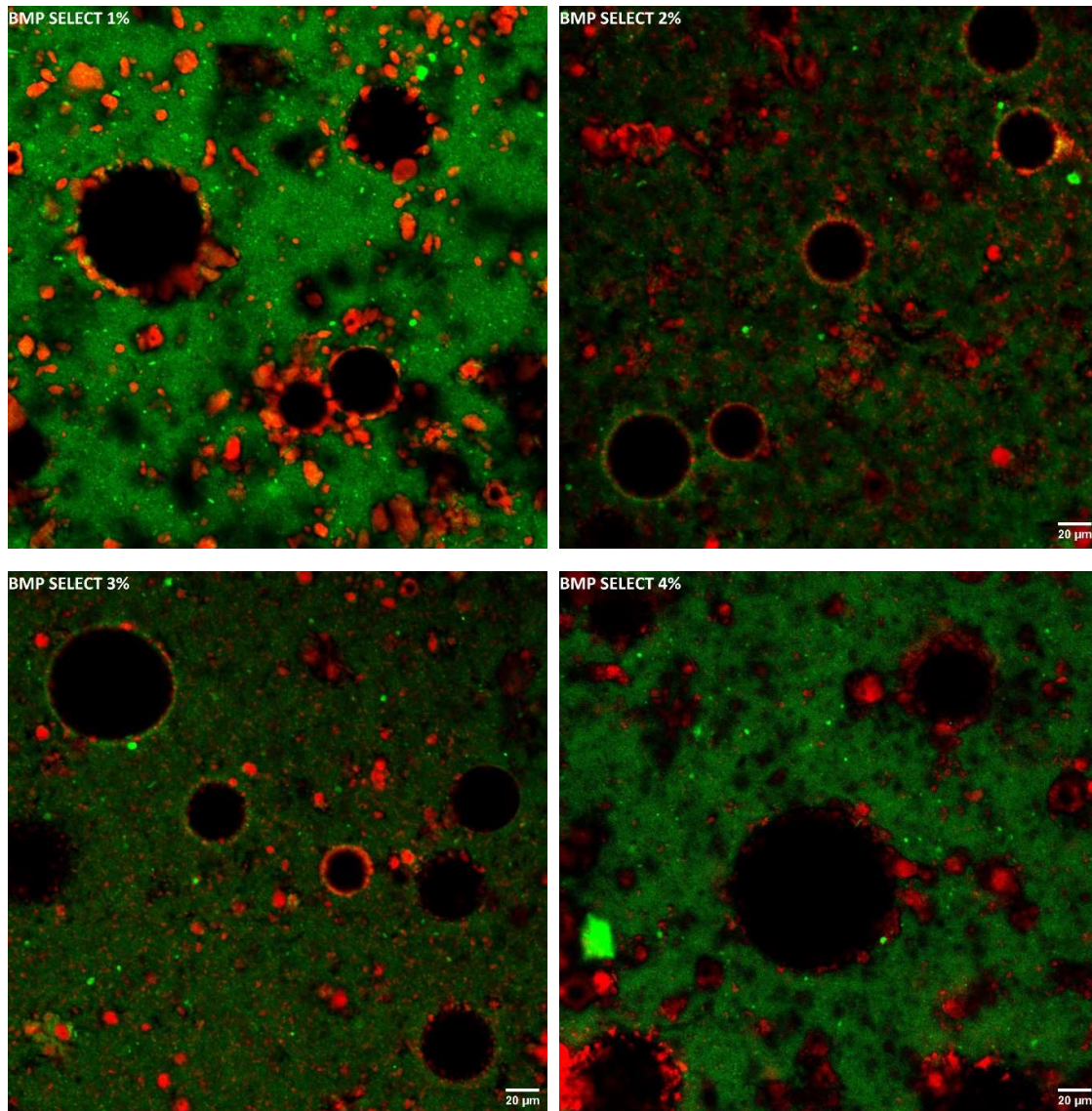


Figure 5.10 The confocal microscopic images for ice cream formulations with variable level of buttermilk powder incorporated by selective adsorption method

The images shown in figure 5.10 illustrates the microstructure of ice cream with different % of BMP. Many agglomerated fat particles as red dots can be seen in ice cream with 1% BMP level. These fat particles in the matrix have formed clusters and are unevenly distributed in the matrix. Thus, some localized protein-dense areas (green) can be observed. The interface of some air bubbles appears to be fully covered with fat particles, while other air bubbles are partially covered with both fat and protein molecules.

For ice cream with 2% BMP, coalesced fat molecules are more uniformly distributed in the matrix, with some of these fat molecules clustered together to form large aggregates. It is crucial to note that the interfacial area at the air cells is partially covered with both fat and

protein molecules. The size of fat molecules present on-air lamella is reasonably small compared to fat particles in the matrix. A similar fat and protein molecules distribution on air bubbles can be seen in both 2 and 3 % BMP formulation. The difference lies in the distribution of fat particles in the matrix. The fat particles are relatively small and uniformly distributed in the matrix for BMP _{Selective}^{3%}.

For the formulation with 4% buttermilk powder, a mixture of small and residual fat particles along with some clusters are present. The fat particles appear to be adsorbed on air cells along with protein. The distribution of fat particles in the matrix is non-uniform, along with localized protein-dense areas.

In general, it can be inferred that a higher degree of fat destabilization is evident in the ice cream system at a lower concentration of buttermilk powder. It is possible that this high intensity of fat agglomeration may be due to insufficient interfacial coating of newly formed fat globules, as less surface-active material is available during homogenization. As the amount of surface-active material increases in ice cream, the size of fat aggregates tends to be smaller. Their distribution tends to become more homogenous (until the 4% level). As the amount of buttermilk powder reaches 4%, the aggregates formed are a mixture of small and large size fat particles.

5.3.2.2 Scanning electron microscopy

Figure 5.11 represent the microstructure of controls and formulation BMP Selective^{1%} and BMP Selective^{3%}. As expected, the air cells tend to be a mixture of small and finer air bubbles in ice cream with monoglycerides. The air cells in negative control are relatively larger, fine air cells are absent. In the case of ice cream with 1% BMP, the air bubbles are connected with each other and have formed channels. It indicates a highly destabilized matrix formed due to excessive agglomeration of fat (i.e., over churning). The ice cream with 3% BMP tends to have a more comprehensive size range of air bubbles. Some air bubbles are larger in size (100-150 μm) while others are very small (10-20 μm).

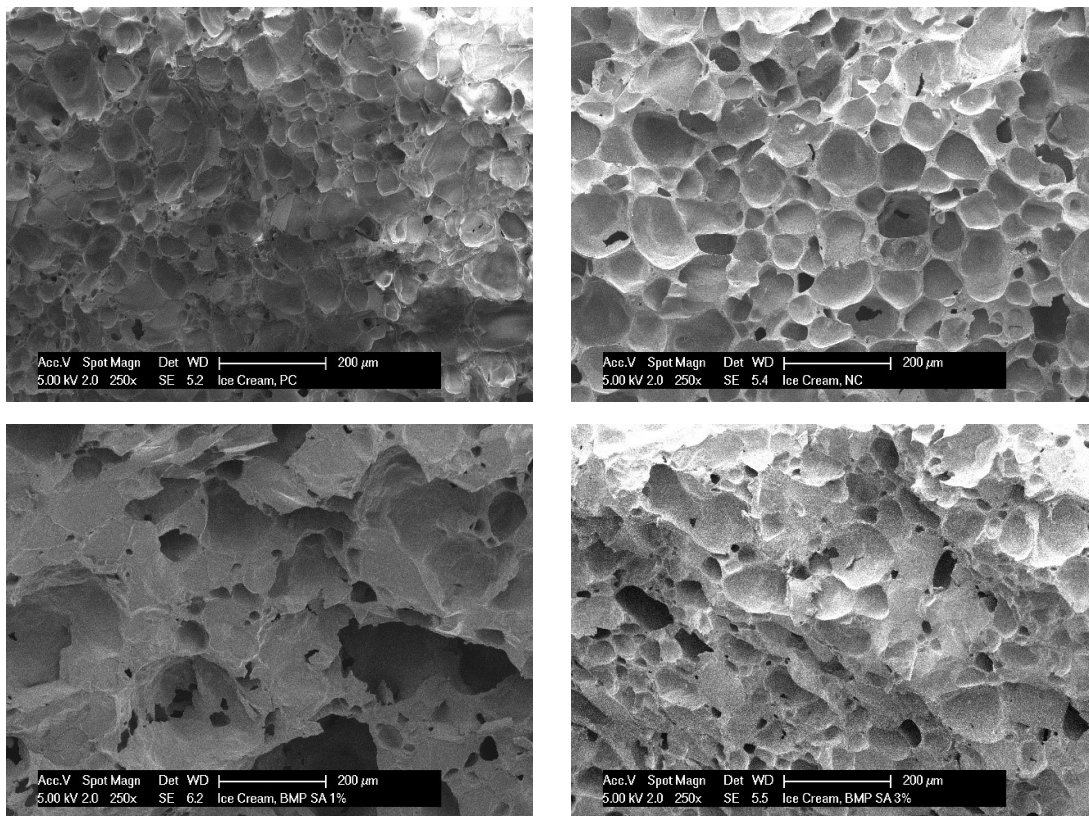


Figure 5.11 Scanning electron microscopic images for controls and formulation with 1 & 3 % buttermilk powder at 250x magnification

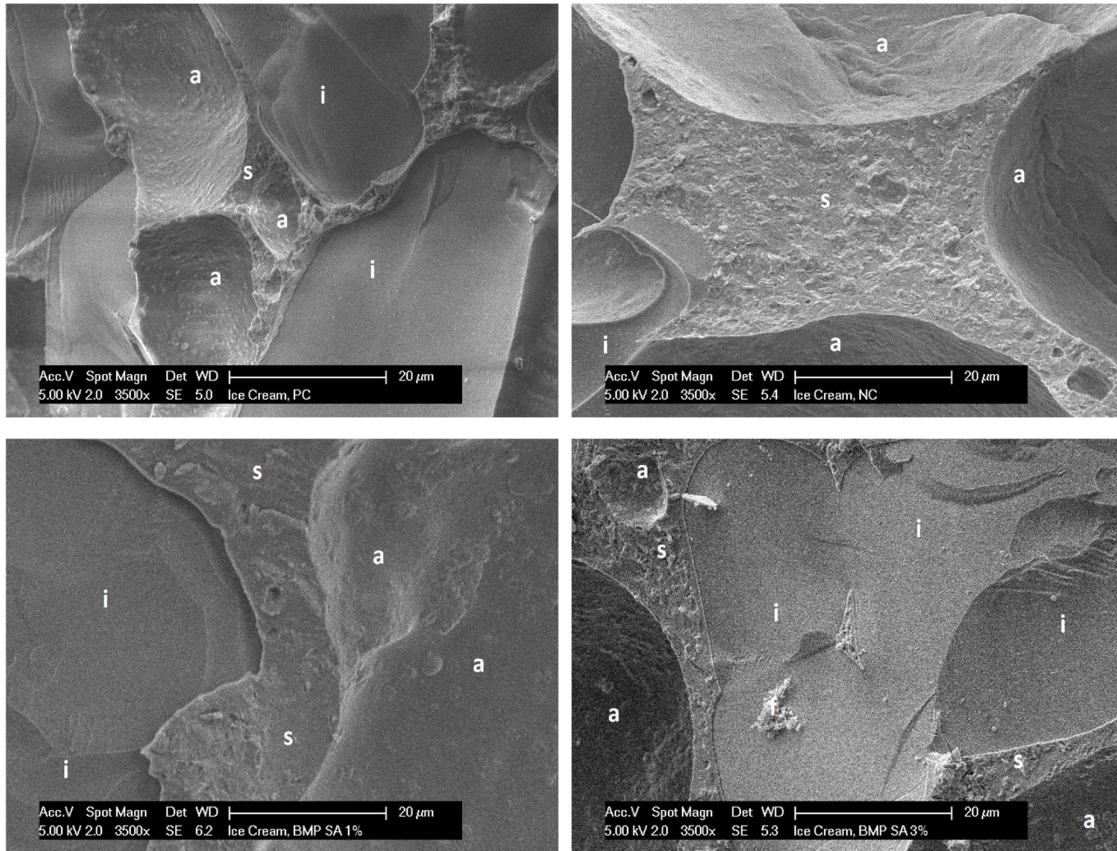


Figure 5.12 Scanning electron microscopic images for controls and formulation with 1 & 3 % buttermilk powder at 3500x magnification ((s – serum phase, I – ice phase, a – air cells and f – fat particles)

Figure 5.12 illustrates the microstructure of controls and ice cream with 1 and 3 % buttermilk powder at 3500x magnification. A reasonable amount of fat structuring is visible in both the formulations with buttermilk powder. Large-sized fat agglomerates covering both air bubble interface and matrix are present in BMP Selective^{1%}. In contrast, the partially coalesced fat particles are relatively small and are more uniformly distributed in matrix and on-air lamella in ice cream with 3 % buttermilk powder.

Overall, the results from scanning electron microscopy suggest that the singular use of buttermilk powder as an emulsifier affects the various phase components, including formation fat structure, formation of ice crystals and stabilization of newly formed air cells in ice cream. It can be suggested that air cells tend to form channels at lower concentration of emulsifier, which may be due to unstable fat globules in the ice cream mix resulting in excessive fat destabilization during freezing. As the concentration of buttermilk powder further increases, the fat globules in the ice cream mix get partially stabilized and resulting in fat agglomeration

appropriate to stabilize the air cells. However, the size distribution of air cells still remains non-uniform. It may be due to the formation of varying size fat agglomerates.

Another critical observation relevant to the texture of ice cream is the distribution of the ice phase. The ice phase tends to be distributed non-uniformly, suggesting that fat agglomeration affects the formation of ice crystals in ice cream. It is reported that fat agglomeration may hinder the ice crystal formation mechanism, but its effect is still unclear (Regand & Goff, 2003; Roland et al., 1999).

5.3.3 Particle size distribution

Figure 5.13 shows the particle size distribution curves for ice cream mix formulations with various level of buttermilk powder prepared using selective adsorption.

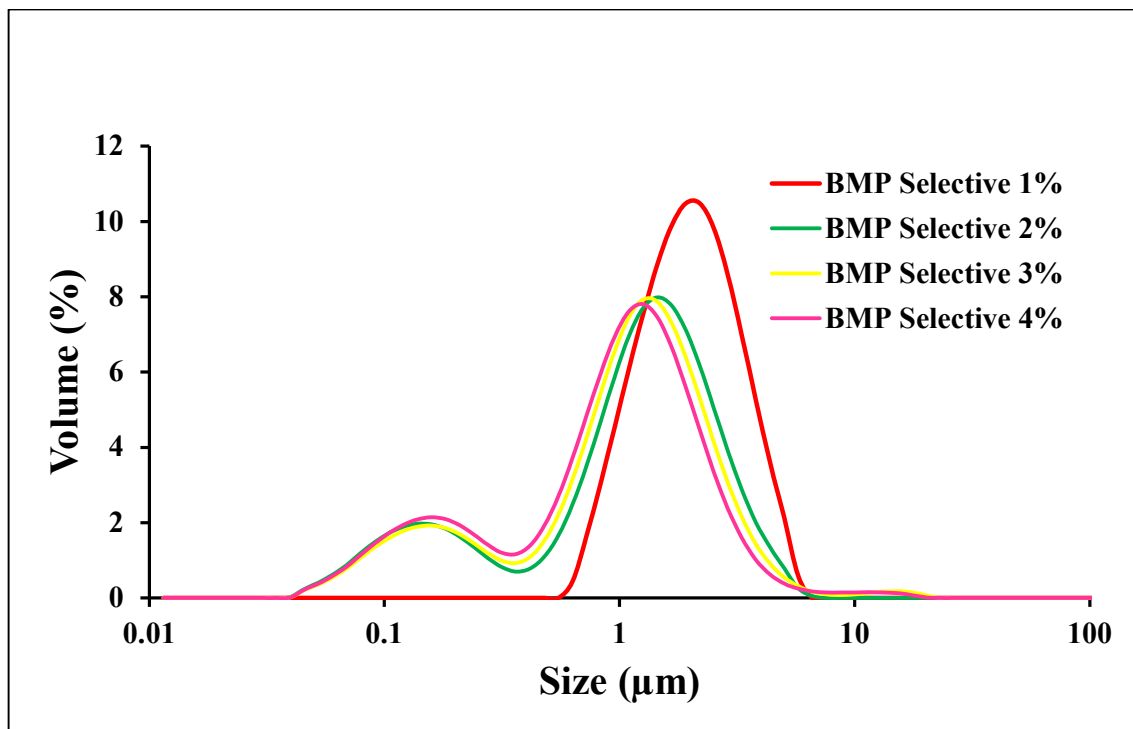


Figure 5.13 The model size distribution for ice cream mix for different formulations with BMP added using selective adsorption

The size of fat globules formed after homogenization range from 0.5 to 6 µm, with the majority of fat globules being of 2µm for formulation with 1% buttermilk powder. The fat globules for the remaining of the ice cream mix with BMP range from 0.5 to 5 µm, with a significant proportion of fat globules are of 1.25 to 1.5 µm.

It is evident that the size of fat globules in ice cream mix with 1% buttermilk powder is relatively bigger than others. This indicate that an insufficient surface-active material is available to provide full coverage to newly formed fat globules during homogenization.

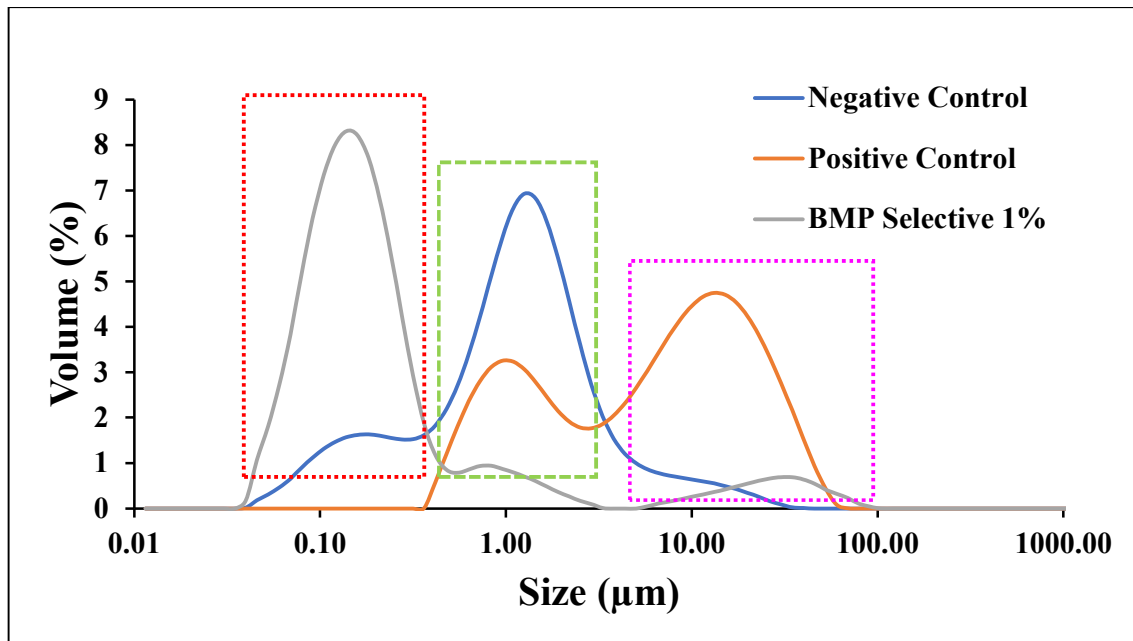


Figure 5.14 Model size distribution for melted positive control, negative control and *BMP Selective*^{1%}

The graphs shown in figure 5.14 illustrates the particle distribution curves for melted controls and ice cream with 1% BMP level using selective adsorption processing. The first peak demonstrates the distribution of milk proteins or casein micelles. The second and third plateaus indicate the proportion of original fat globules similar to ice cream mix and fat aggregates, respectively.

A large number of casein micelles can be seen in *BMP Selective*^{1%} compared to relatively low volume % in the negative control. Comparing peak height for original fat globules, the proportion of original fat globules is highest for negative control compared to positive control. Only a small proportion of original fat globules are present in *BMP Selective*^{1%}, which signifies a higher degree of fat destabilisation.

The fat aggregates formed during freezing have a size range of 3-60 μm for positive control, with the majority of particles in range of 10-13 μm . A low volume % fat aggregates in the size range of 4 -104 μm was reported for BMP_{Selective}^{1%}. It shows a low proportion of fat aggregates, but this interpretation can be misleading the volume % of original fat globules suggests a higher degree of fat agglomeration, which is evident in confocal images.

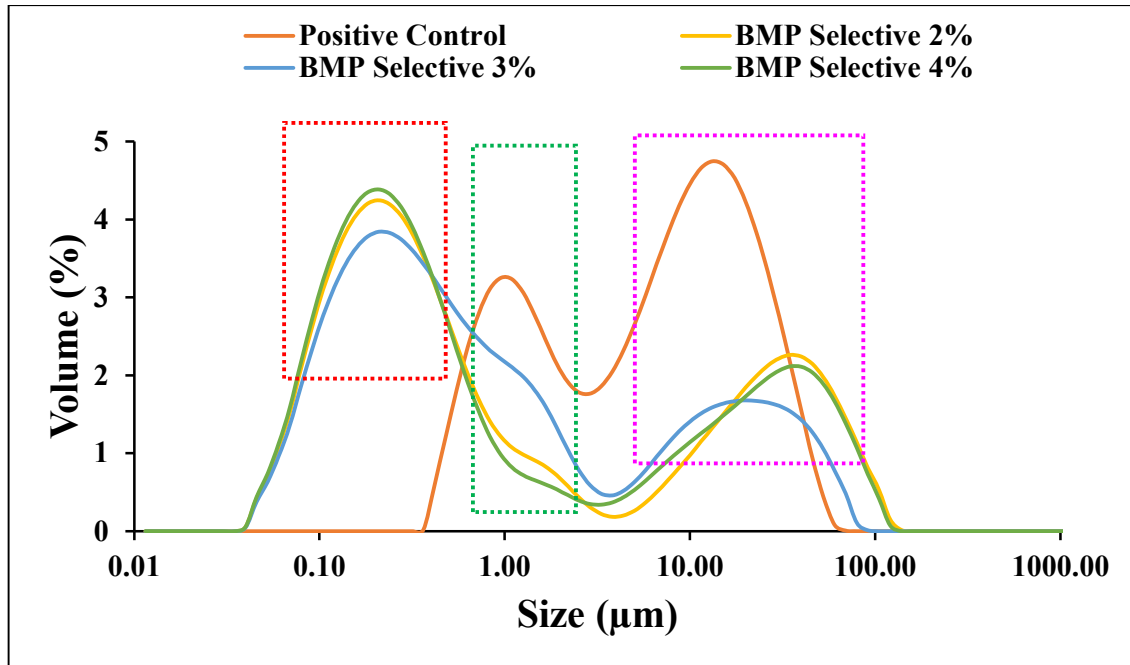


Figure 5.15 Particle size distribution for positive control, BMP_{Selective}^{2%}, BMP_{Selective}^{3%}, and BMP_{Selective}^{4%}

Figure 5.15 shows the modal size distribution for BMP_{Selective}^{2%}, BMP_{Selective}^{3%}, and BMP_{Selective}^{4%}. The peak marked in the green box indicates the distribution of original fat globules. As compared to controls, there is very low proportion of original fat globules present in ice cream prepared with buttermilk powder using selective adsorption technique. The low volume % of original fat globules signifies that most fat globules have undergone partial coalescence during freezing.

The peak marked in the pink box shows the modal distribution of coalesced fat molecules. The fat aggregates in formulations with 2 % and 4 % BMP level range size from 5 to 120 μm , with the majority of particles being of 36-40 μm . In the case of 3% BMP level, the fat aggregates are in size range of 5-80 μm , with most particles being between 16-20 μm .

The results from modal size distribution analysis imply that replacing the mono-diglycerides with buttermilk powder by selective adsorption leads to the formation of partially coalesced fat during freezing. But any variation in the degree of fat destabilization with different % of

phospholipids is unclear. The absence of original fat globules (similar to mix) is another abnormal behaviour. To understand these observations, it is essential to understand the mechanism of interface formation. As outlined in the process flow diagram, milk proteins were added after the homogenization of the mix. The buttermilk powder was the only surface-active component present during the formation of new fat globules during homogenization. The phospholipids and proteins in buttermilk powder were adsorbed on fat globules leading the formation of an interfacial membrane.

As buttermilk powder was the only available surface-active component during homogenization, therefore the quantity of BMP is also crucial to stabilize newly formed fat globules. In formulation with 1% buttermilk powder, a high degree of fat destabilization is due to the insufficient surface coverage of fat globules. The lack of surface-active material leads to the formation of a highly destabilized emulsion. Thus, it leads to a high degree of partial coalescence and air channelling (as seen in SEM Image) during the freezing of ice cream. This is further evident in the particle size distribution of ice cream mix with 1% BMP.

With 2% BMP formulation, the amount of buttermilk powder was sufficient to provide full coverage and form a full coverage interfacial layer. At such a low concentration, the phospholipids and milk protein may have formed a weak interfacial membrane that can readily undergo partial coalescence during freezing. As the whole interfacial membrane layer is fragile in nature, it can be easily penetrated by fat crystals, thus increasing the collision efficiency of partial coalescence. It leads to the formation of the large fat aggregates and non -uniform air bubbles evident in the microstructure image.

As the amount of buttermilk powder increases by more than 2%, the interfacial membrane tends to become thick due to the high concentration of proteins and phospholipids in BMP. It makes it challenging to be protruded by fat crystals, thus decreases the probability of partial coalescence. It explains the formation of uniformly sized smaller fat aggregates and uniform distribution of air bubbles with the increasing amount of buttermilk powder using selective adsorption technique.

5.3.4 Hardness

The table 5.5 shows the hardness values for ice cream formulations with different % of BMP and controls. The hardness is relatively the same for BMP_{Selective}^{1%} and BMP_{Selective}^{2%} and is in the range of 56-58 N. The ice cream become softer at 3% BMP level with the reported hardness of 49 N, and even softer with a higher level of BMP incorporation. In comparison with the positive control, ice creams produced with BMP have a soft texture.

Table 5.5 Hardness values for controls and formulations with different % of buttermilk incorporated using selective adsorption technique

Ice creams	Hardness (N)
Negative Control	^a 69.5 ± 1.93
BMP _{Selective} ^{1%}	^{bc} 56.2 ± 1.83
BMP _{Selective} ^{2%}	^b 57.5 ± 1.70
BMP _{Selective} ^{3%}	^c 49.3 ± 4.08
BMP _{Selective} ^{4%}	^d 46.3 ± 2.28
Positive Control	^b 59.0 ± 5.43

Note: Mean of hardness values is followed by (±) standard deviation. Within rows, different superscripts in front of mean values indicate significance (p<0.05) The superscript along with values shows the grouping of mean values using Tukey methods and 95% confidence. Mean values followed by different superscripts are significantly different, n = 5.

In summary, it can be seen that the addition of buttermilk as an emulsifier by selective adsorption technique produces relatively softer ice cream than ice cream with mono-diglycerides. The softness tends to increase with the increased concentration of phospholipids. As suggested previously, the degree of fat agglomeration and distribution of fat aggregates affect the viscosity of the matrix phase, which in turn affects ice cream's hardness. The same theory can be used here to explained why we see a decrease in the hardness of ice cream.

As evident from confocal images, the incorporation of buttermilk powder by selective adsorption methods results in the formation of larger size fat aggregates. The critical aspect here is analysing the uniformity of distribution of these fat aggregates on air bubbles. The structural images suggest that the relative concentration of fat particles on the air interface increases with increasing the amount of buttermilk powder. It implies more fat particles are sticking to the air interface; thus, a low proportion of fat is available in the matrix. Arguably, this possibly leads to a decrease in matrix viscosity, thus a higher amount of BMP increases

softness of ice cream with. The other possibility is the addition of BMP may also impact the freeze point depression of the ice cream mix.

5.3.5 Melting stability

Table 5.6 shows the final mass of melted ice cream for controls and ice cream with different levels of buttermilk powder. As expected, ice cream with monoglycerides has highest melt stability with 7.6 % remnant weight and negative control had the lowest melt stable (56.1 % final mass). The introduction of buttermilk powder using the selective adsorption method as replacement of MDG has improved melt stability compared to the negative control. The melt stability tends to increase with an increasing level of BMP in ice cream. The highest melt stability is reported for 4% BMP with 13.7 % final mass and lowest for 1% buttermilk powder level, with 27.6% remnant mass. The % final mass for BMP_{Selective}^{2%} and BMP_{Selective}^{3%} were 22.6 and 17.1, respectively.

Table 5.6 % the final mass of melted ice cream for controls and formulations with different % of BMP

Formulation	% the final mass of melted ice (m/m)
Positive Control	7.6
BMP _{Selective} ^{1%}	27.6
BMP _{Selective} ^{2%}	22.6
BMP _{Selective} ^{3%}	17.1
BMP _{Selective} ^{4%}	13.7
Negative Control	56.1

The dripping rate curves for ice cream with BMP and controls are shown in figure 5.16. Melting onset time among BMP formulations tends to increase with increasing buttermilk powder. The BMP_{Selective}^{1%} and BMP_{Selective}^{2%} started to melt after 30 min, whereas negative control, BMP_{Selective}^{3%}, and BMP_{Selective}^{4%} began to melt after 40 min. Melting onset time was highest (60 min) for positive control.

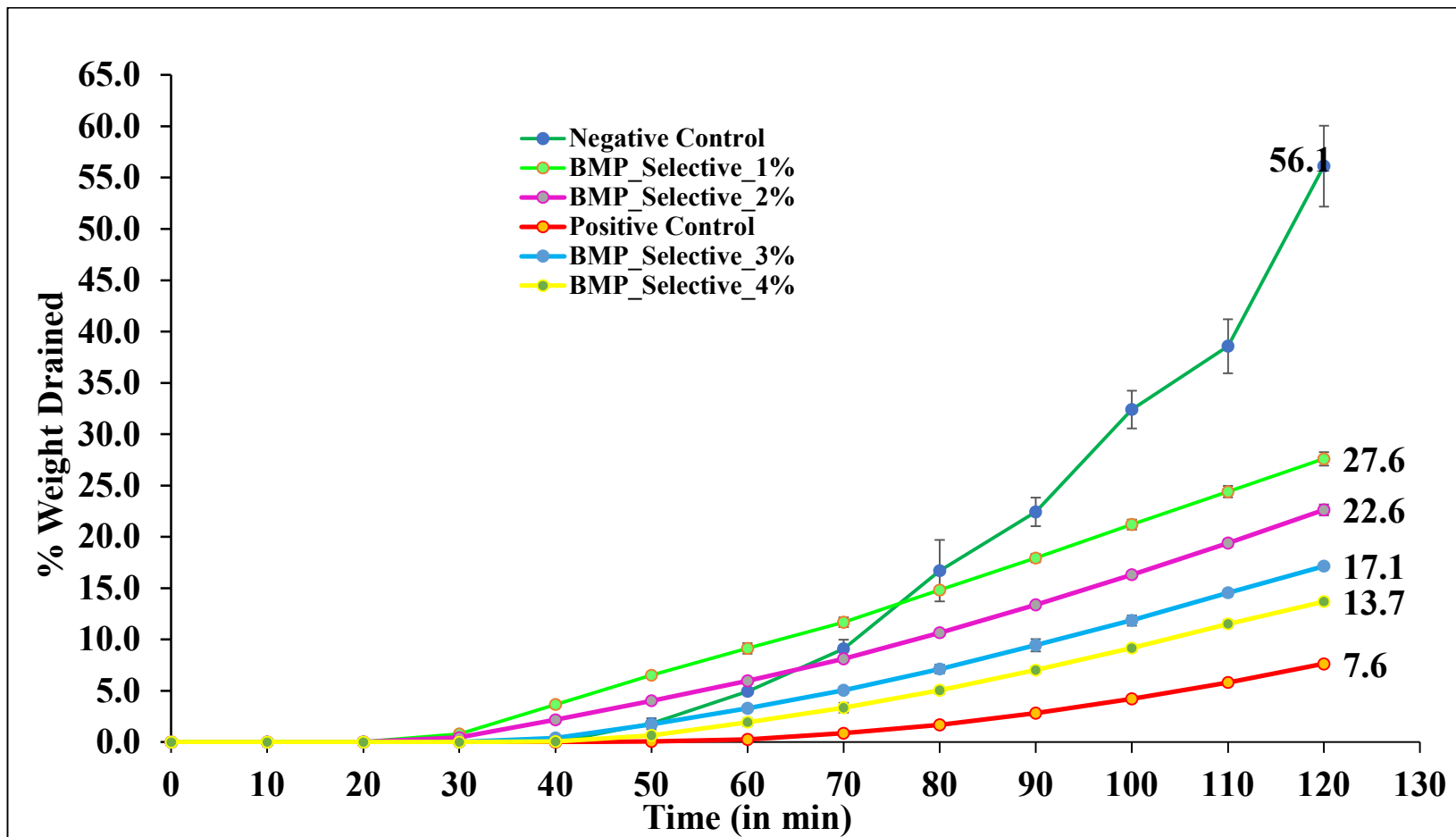


Figure 5.16 The melting rate curves for controls and ice cream formulations with different % of BMP added using selective adsorption at 20°C

In summary, compared to ice cream without monoglycerides, ice cream tends to become more melt stable with the increasing amount of buttermilk powder incorporated using the selective adsorption technique. As mentioned before, the melting resistance of ice cream depends upon how easily melted water diffuses through the matrix of ice cream. The agglomerated fat provides resistance to the flow of water, thus providing melt stability to ice cream. The increase in melt resistance by an increase in the amount of buttermilk powder can be explained by the fact that with increasing number of phospholipids, the distribution of fat agglomerates becomes more homogenous, whereas at a low level of buttermilk a localized clustering of fat is evident. This homogenous distribution provides resistance to the diffusion of water, thus increases the melt stability.

5.3.6 Final discussion

Figure 5.17 suggests the interfacial composition of fat globules in ice cream mix prepared using buttermilk powder by selective adsorption. The buttermilk powder is primarily composed of phospholipids and milk proteins. Both fractions are surface-active in nature. So, when the buttermilk powder is selectively added into the ice cream mix, the surface-active components of BMP get adsorbed on newly formed fat globules during homogenization.

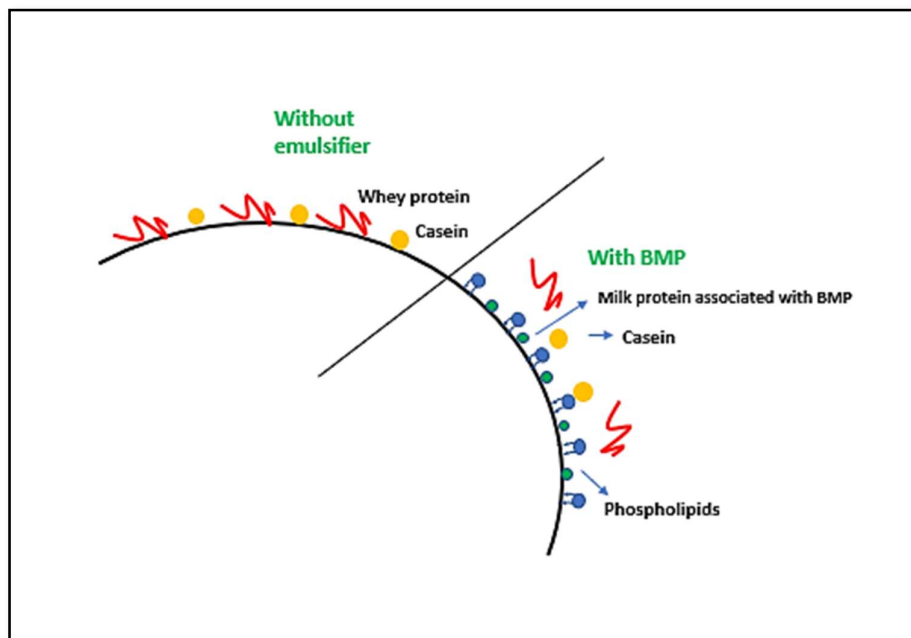


Figure 5.17 Aged fat globule interface with BMP incorporated using selective adsorption

The concentration of surface-active component is crucial to provide full surface coverage and determine the strength of the interfacial membrane. At low concentration of buttermilk powder, the number of surfactants is insufficient to provide full coverage. Thus, forming a highly destabilized ice cream system with non-uniformly distributed larger aggregates. As the concentration of buttermilk powder increases, the strength of the interfacial membrane increases, and fat globules undergo more homogenous agglomeration during freezing.

In summary, the effects of selective addition of buttermilk powder on the ice cream system are:

- At a low concentration of BMP, a destabilized system is obtained due to insufficient coverage, which results in the formation of air channels in ice cream
- As the concentration of BMP increases, the size of fat aggregates become smaller, and distribution air cells distribution become more uniform
- The agglomeration of fat affects the growth of ice cream; therefore, ice cream with BMP tends to become softer in texture
- The melt stability of ice cream increases with the increasing amount of selectively added BMP
- The optimum result in terms of texture and melt stability is obtained with 4% BMP level

The key difference with traditional and selective addition of buttermilk powder is the variation in behaviour of ice cream system with increasing concentration of BMP. It is evident that in selective adsorption method, the increasing amount of buttermilk powder provides a more uniform and stable system. Whereas with traditional technique, the stability of the ice cream system increases until a certain level of BMP, then tends to decrease with further increase in BMP concentration.

6. Conclusion and Future prospective

To conclude, the two hypotheses relating the use of native milk fat globules and buttermilk powder as natural emulsifiers in the ice cream were well validated. It can be suggested that the partial replacement of fat in ice cream with native milk fat globules can provide an approach to make a clean label product. The products obtained will have a soft texture and melt stability slightly lower than conventional ice creams. It is worth noting that non-homogenized cream provides more taste richness with the same level of fat. Thus, it can be added benefit to a clean label product. The simplicity of cream addition can make this technique well suited for commercial manufacturing of clean label ice cream.

Regarding the replacement of synthetic emulsifiers with buttermilk powder, results from traditional processing and selectively adsorption suggest that BMP can be a natural emulsifier for ice cream. The selective adsorption technique for BMP addition provides better quality ice cream as compared to traditional. But it is also worth noting that adaptation of selective adsorption technique at a commercial scale will require significant capital investment, making the production less economical.

Despite the encouraging results obtained from this research, there are many areas where a thorough scientific investigation is required to design an ice cream system that produces desired properties. These areas are:

- Understanding the effect of fat agglomerates size on the distribution of air cells in ice cream
- Understanding the effect of fat agglomeration of ice crystallization in ice cream
- Studying the effect of more than 4% BMP addition by selective adsorption and analyse the scope of further improvement in ice cream
- Using an alternative technique to laser scattering technique to measure the actual degree of fat destabilization and correlating it to properties of ice cream
- Conducting the consumer sensory panel study on selected formulations to see the consumer response in terms to check market viability
- Analysing the interfacial properties of fat globules after BMP addition for both traditional and selective adsorption techniques
- Analysing the effect of buttermilk powder addition in non-milkfat ice cream

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8. Appendices

Appendix A: Composition of raw materials

Skim milk powder

Table 8.1 Composition of SMP

Components	Value	Units
Fat content	1.25	%m/m maximum
Protein (SNF)	34	%m/m minimum
Protein (as is)	32.2-36.7	%m/m
Moisture	4.5	%m/m maximum

Glucose Syrup

Table 8.2 Composition of Glucose syrup

Components	Value	Units
DE value	38.0 – 42.0	
Sugar profile		(%w/w DB)(Typical)
Dextrose (DP1)	18	%
Maltose (DP2)	13	%
Maltotriose (DP3)	12	%
Other saccharides (DP4+)	57	%

Anhydrous Milk fat

Table 8.3 Composition of AMF

Components	Value	Units
Fat	99.9	%
Moisture	<0.1	%

Non- homogenized cream

Table 8.4 Composition of Non - homogenized cream

Components	Value	Units
Fat	37.3	g/100 ml
Protein	2.4	g/100 ml
Lactose	3	g/100 ml

Buttermilk Phospholipid powder

Table 8.5 Composition of buttermilk powder

Components	Value	Unit
Protein (6.38 x N)	29.574	%
Fat	20.494	%
Moisture	3.388	%
Total Solids	96.612	%
Polar Lipids Gravimetric %	7	%
Gangliosides GD3 (DCL100)	411.2	mg/100g
Total Phospholipid (PL)	7.7	g/100g
Phosphatidylcholine (PC)	2.10	g/100g
Phosphatidylinositol (PI)	0.70	g/100g
Phosphatidylserine (PS)	0.90	g/100g
Phosphatidylethanolamine (PE)	2.30	g/100g
Sphingomyelin (SM)	1.70	g/100g
Calcium	735	mg/Cg
Zinc	1.91	mg/Cg
Iodine	67	ug/Cg
Arsenic	< 0.010	mg/kg
Cadmium	< 0.0070	mg/kg
Mercury ICP-MS mg/kg	< 0.0030	mg/kg
Lead	< 0.010	mg/kg
Tin	< 0.050	mg/kg
Antimony	< 0.0050	mg/kg
Chromium	< 0.050	mg/kg

Appendix B: Formulations with non-homogenized cream

A fraction of total fat in standard ice cream with 10% fat was replaced by non-homogenized cream. The non-homogenized cream was 40% fat and 5.4 % milk solid not fat. The MSNF calculation for ice cream was adjusted to accommodate the addition MSNF from incorporation of non-homogenized cream. Table 8.6 shows the ingredient calculations for different formulation of ice cream with non-homogenized cream.

In case of low-fat ice cream, the MSNF and sugar content was set higher than standard fat ice cream to avoid the issue of excessive ice formation during processing of ice cream. Table 8.7 shows the mix calculations of low-fat ice cream with non-homogenized cream.

Table 8.6 Calculations for standard fat ice cream mix with non-homogenized cream

Standard fat ice cream mix calculation											
	Value	Units									
Final Fat in Ice cream mix	10	%									
Final MSNF in Ice cream mix	11	%									
Fat in Non-homogenized cream	40	%									
Fat in AMF	100	%									
MSNF in SMP	100	%									
MSNF in Cream	5.4	%									
Batch Size	6	Kg									
Non homogenized cream to AMF ratio		Positive Control	20/80	40/60	45/55	50/50	55/45	60/40	80/20	100/0	Negative Control
			0.2	0.4	0.45	0.5	0.55	0.6	0.8	1	
Ingredients	%	Qty. of Ingredients (In Kg)									
AMF		0.6	0.480	0.360	0.330	0.300	0.270	0.240	0.120	0.000	0.600
Non- homogenized cream		0.00	0.300	0.600	0.675	0.750	0.825	0.900	1.200	1.500	0.000
SMP		0.66	0.644	0.628	0.624	0.620	0.615	0.611	0.595	0.579	0.660
Sucrose	9	0.54	0.540	0.540	0.540	0.540	0.540	0.540	0.540	0.540	0.540
Avonsweet	4	0.24	0.240	0.240	0.240	0.240	0.240	0.240	0.240	0.240	0.240
Guar	0.1	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006
LBG	0.2	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012
Mono-diglycerides	0.3	0.018									
Flavour (vanilla)	0.1	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006
Water	65.3	3.912	3.766	3.602	3.561	3.521	3.480	3.439	3.275	3.111	3.930
TS	34.7										

Table 8.7 Calculations for low fat ice cream mix with non-homogenized cream

Low fat ice cream mix calculation								
	Value	Units						
Final Fat in Ice cream mix	5	%						
Final MSNF in Ice cream mix	14	%						
Fat in Non- homogenized cream	40	%						
Fat in AMF	100	%						
MSNF in SMP	100	%						
MSNF in Cream	5.4	%						
Batch Size	6	Kg						
Non homogenized cream to AMF ratio		Positive Control	20/80	40/60	60/40	80/20	100/0	Negative Control
			0.2	0.4	0.6	0.8	1	
Ingredients	%	Qty. of Ingredients (In Kg)						
AMF		0.3	0.240	0.180	0.120	0.060	0.000	0.300
Non- homogenized cream		0.00	0.150	0.300	0.450	0.600	0.750	0.000
SMP		0.84	0.832	0.824	0.816	0.808	0.800	0.840
Sucrose	10	0.6	0.600	0.600	0.600	0.600	0.600	0.600
Avonsweet	5	0.3	0.300	0.300	0.300	0.300	0.300	0.300
Guar	0.1	0.006	0.006	0.006	0.006	0.006	0.006	0.006
LBG	0.2	0.012	0.012	0.012	0.012	0.012	0.012	0.012
Mono-diglycerides	0.3	0.018						
Flavour (vanilla)	0.1	0.006	0.006	0.006	0.006	0.006	0.006	0.006
Water	65.3	3.912	3.848	3.766	3.684	3.602	3.521	3.930
TS	34.7							

Appendix C: Formulations with buttermilk powder

The table 8.8 shows calculations of ice cream mix formulations with different level of buttermilk powder. Traditional and selective adsorption were both used for incorporation of buttermilk powder in in ice cream. In case of selective adsorption processing 70% of total calculated water was used to dissolve the BMP, sugar and stabilizer. The remaining 30% of was used for dissolving skim milk powder. The amount of SMP was adjusted with consideration for protein supplied with incorporation of BMP.

Table 8.8 Calculations for ice cream mix with buttermilk powder

Ice cream mix calculation with BMP							
	Value	Units					
Final Fat in Ice cream mix	10	%					
Final MSNF in Ice cream mix	11	%					
Fat in AMF	100	%					
MSNF in SMP	100	%					
Batch Size	6000	g					
			Positive Control	Negative Control	1 % BMP	2% BMP	3 % BMP
			0	0	1	2	3
Ingredients	%	Qty. of Ingredients (In g)					
AMF		600	600	600	600	600	600
SMP		660	660	614	569	523	477
Sucrose	9	540	540	540	540	540	540
Avonsweet	4	240	240	240	240	240	240
Guar	0.1	6	6	6	6	6	6
LBG	0.2	12	12	12	12	12	12
Mono-diglycerides	0.3	18					
BMP		0	0	60	120	180	240
Flavour (vanilla)	0.1						
Water	65.3	3924	3942	3928	3913	3899	3885
TS	34.7	34.6	34.3	34.5	34.8	35.0	35.3
70 % Water				2749	2739	2729	2719
30 % Water				1178	1174	1170	1165

Appendix D: Confocal images

The following figures show confocal images of different ice cream formulations.

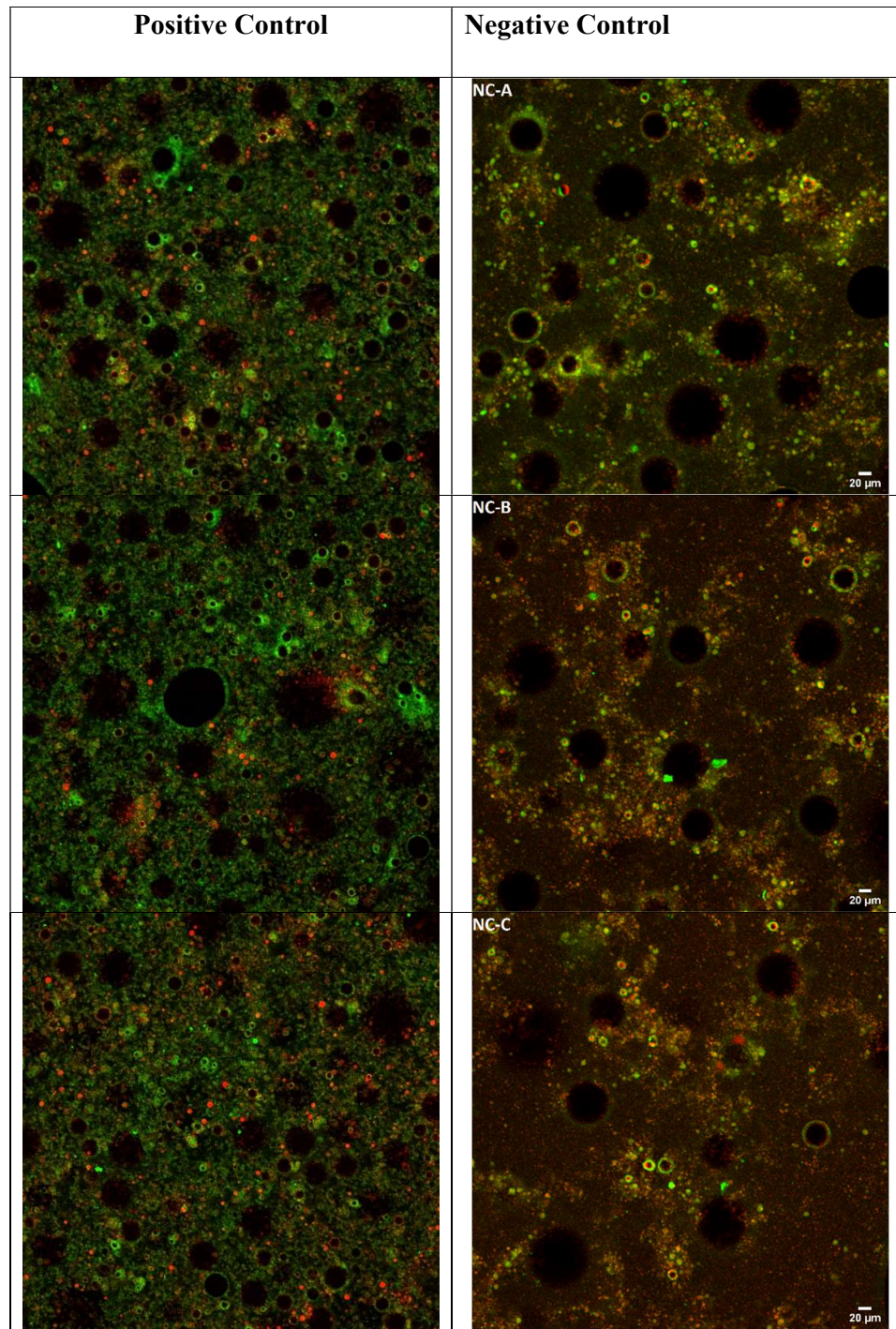


Figure 8.1 Confocal images for positive and negative controls

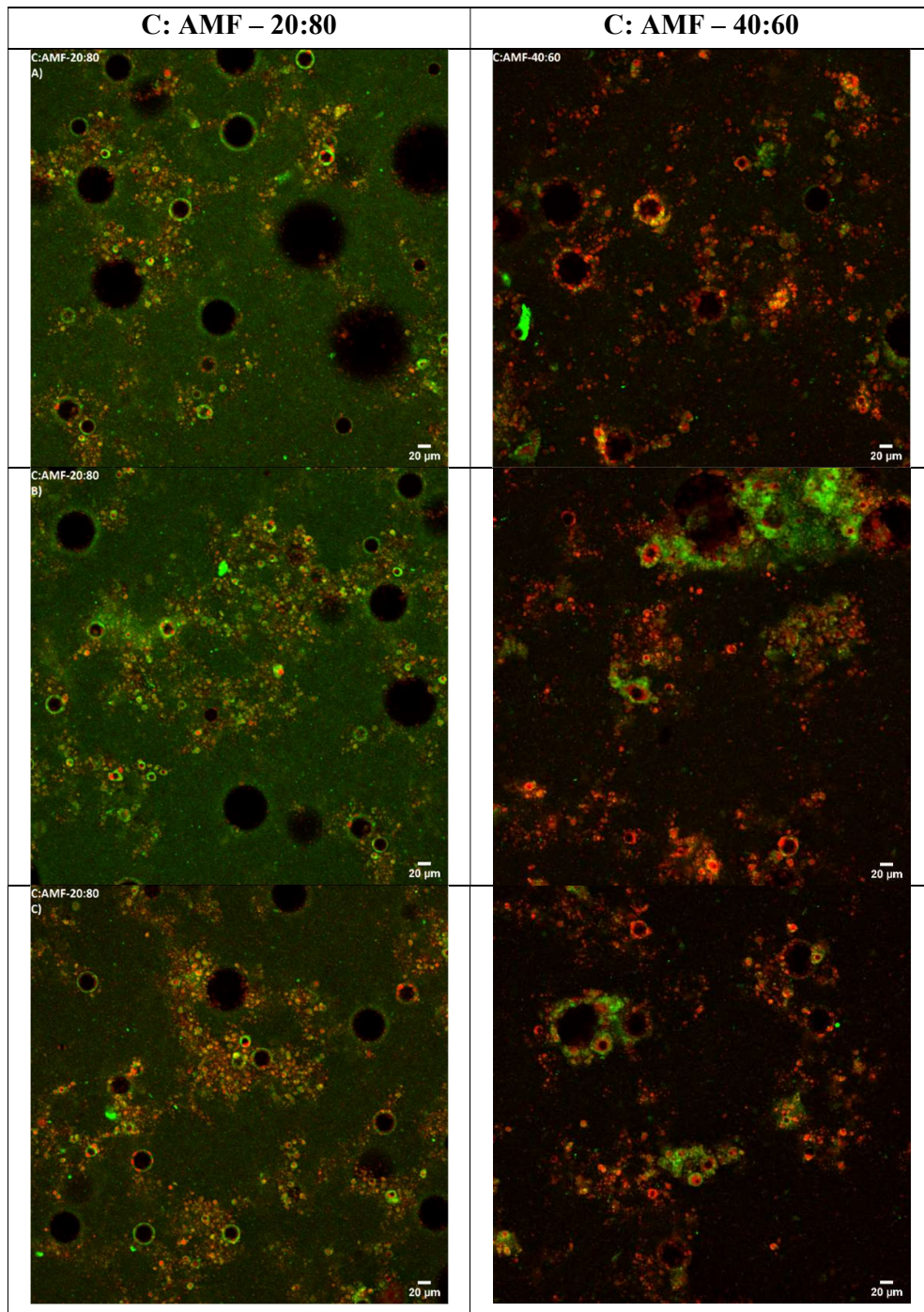


Figure 8.2 Confocal images for $C_{AMF}^{20:80}$ (left) and $C_{AMF}^{40:60}$ (right)

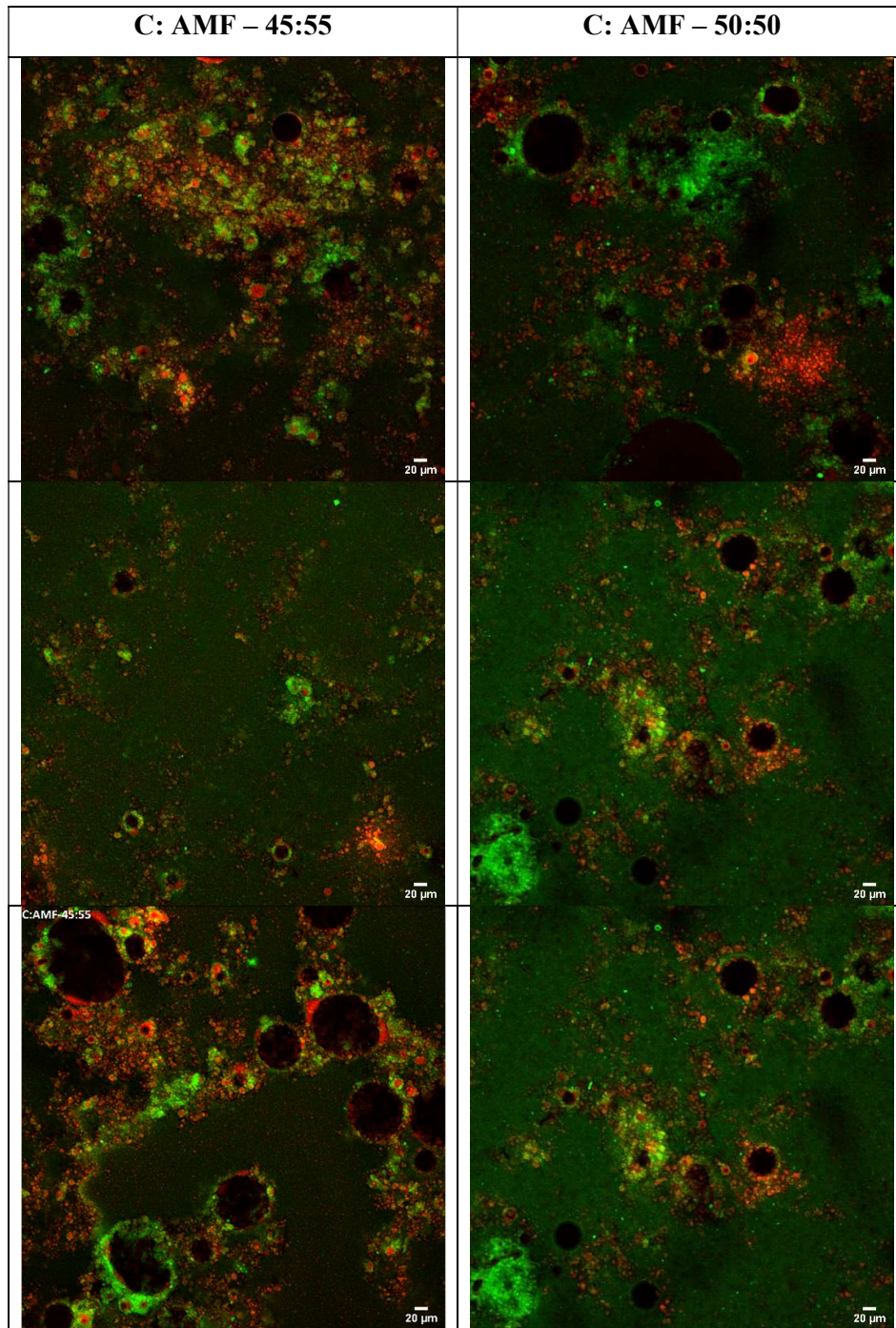


Figure 8.3 Confocal images for $C_{AMF}^{45:55}$ (left) and $C_{AMF}^{50:50}$ (right)

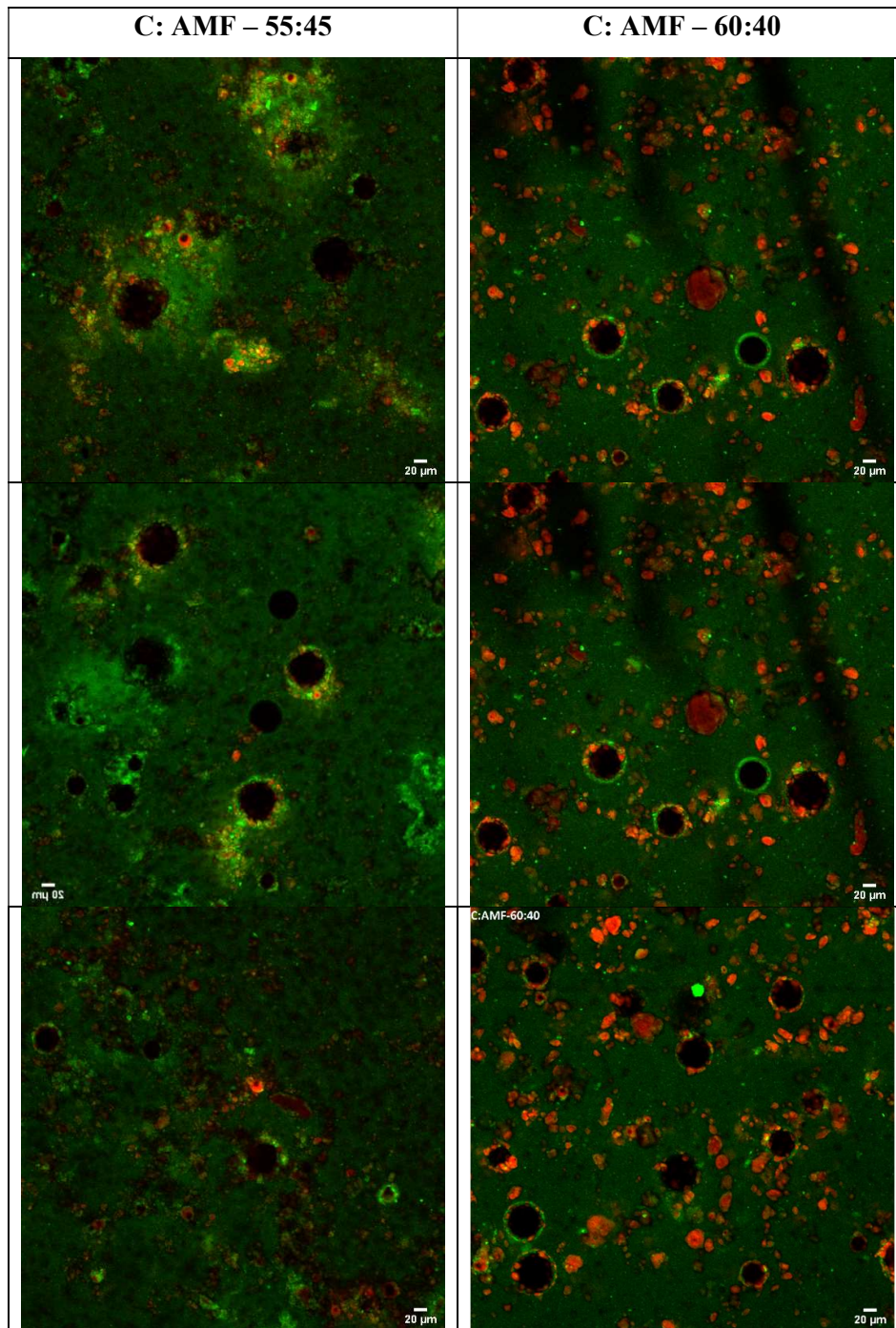


Figure 8.4 Confocal images for $C_{AMF}^{55:45}$ (left) and $C_{AMF}^{60:40}$ (right)

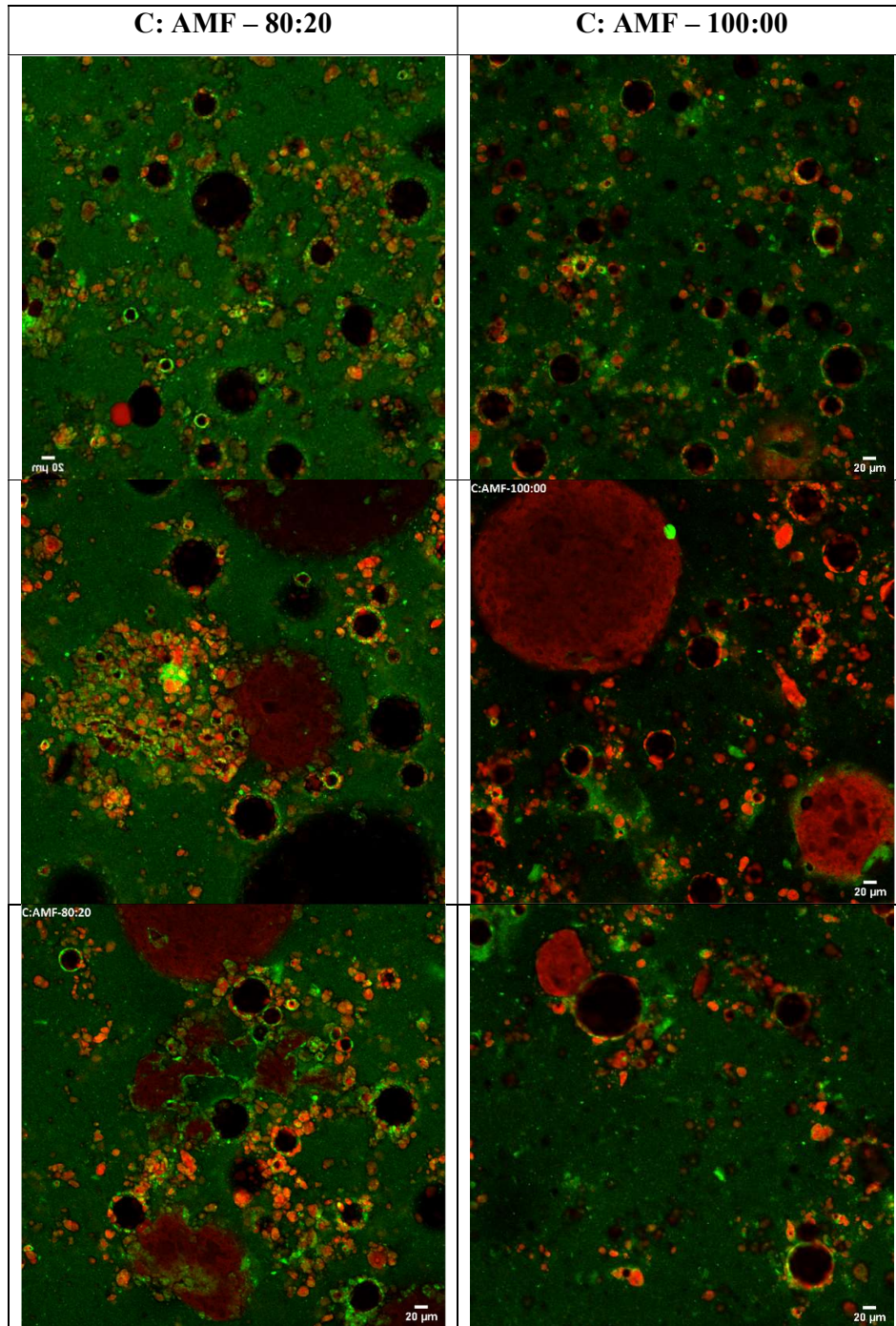


Figure 8.5 Confocal images for $C_{AMF}^{80:20}$ (left) and $C_{AMF}^{100:00}$ (right)

Appendix E: SEM Images

The following figures show SEM images of different ice cream formulations at different areas of sample.

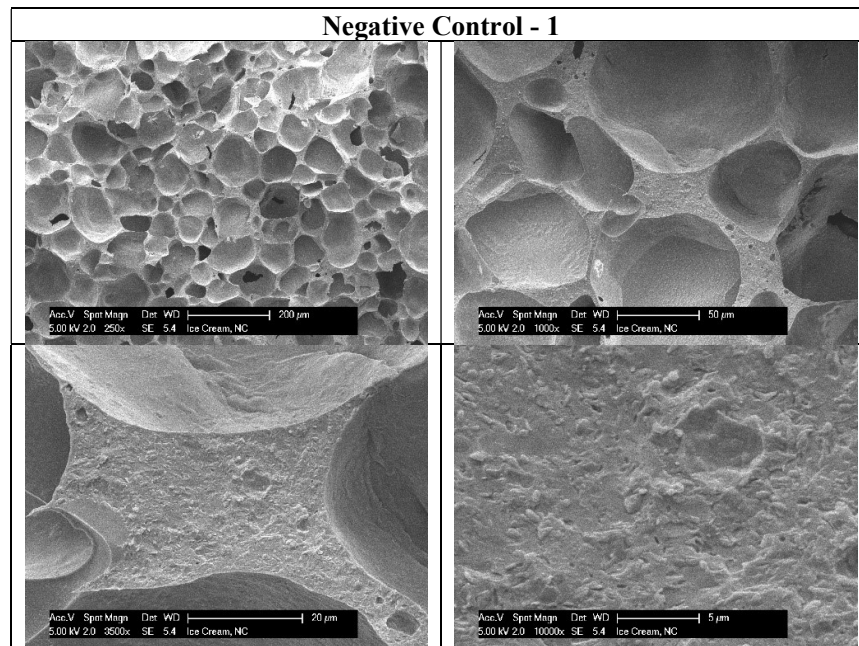


Figure 8.6 SEM images of negative control at different magnifications (area 1)

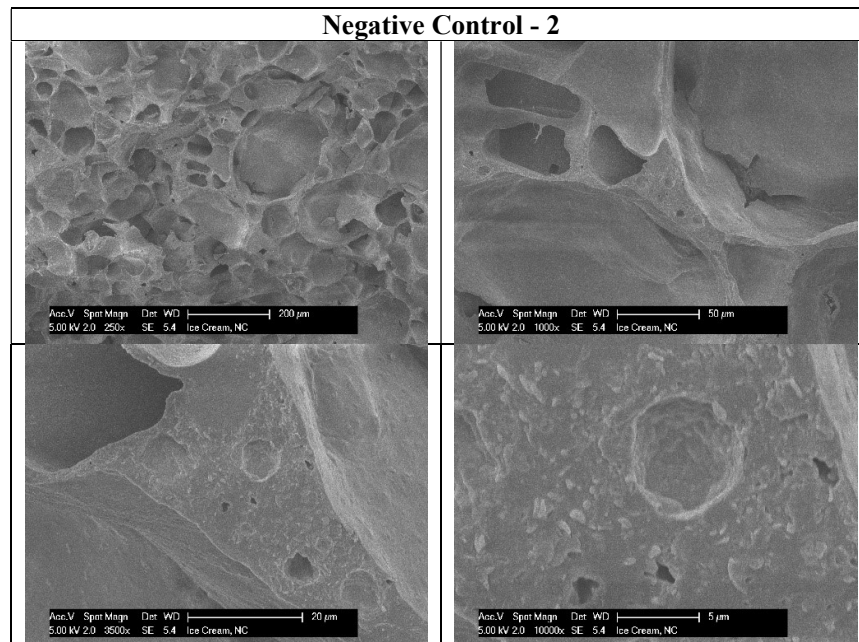


Figure 8.7 SEM images of negative control at different magnifications (area 2)

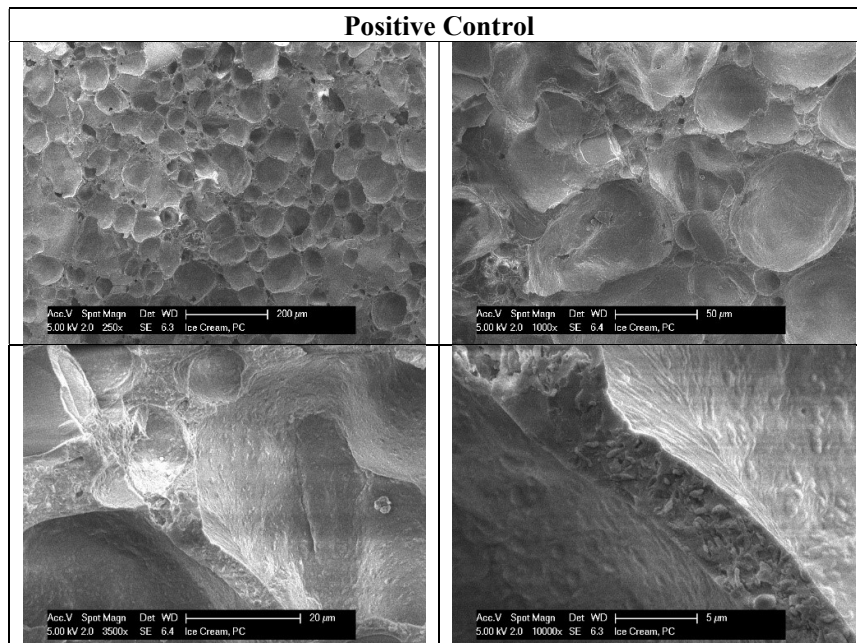


Figure 8.8 SEM images of positive control at different magnifications (area 1)

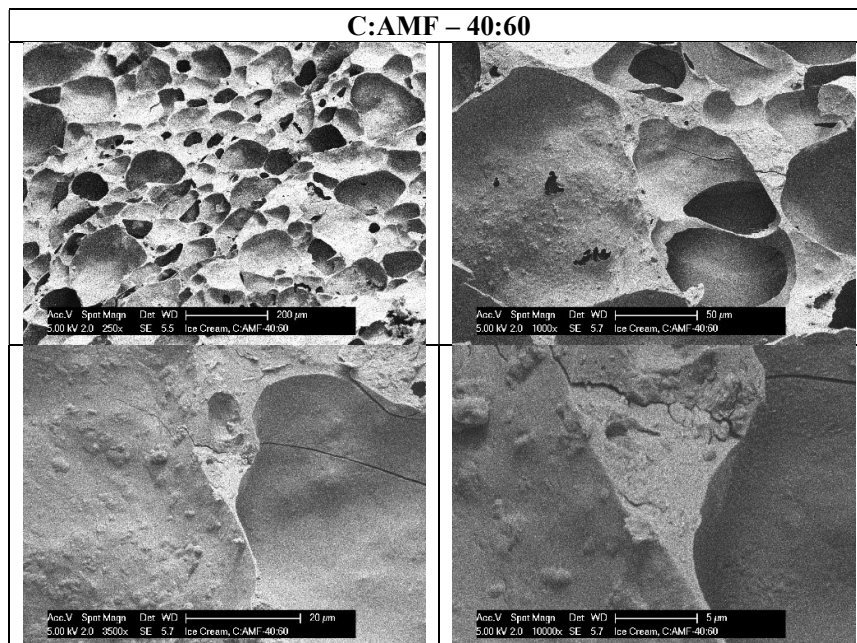


Figure 8.9 SEM images of C_{AMF}^{40:60} at different magnifications (area 1)

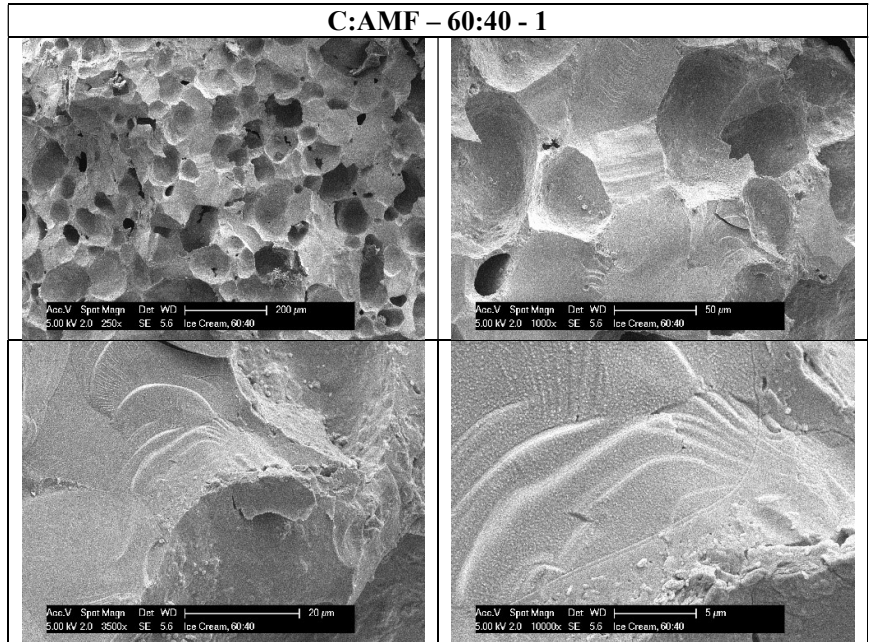


Figure 8.10 SEM images of $C_{AMF}^{60:40}$ at different magnifications (area 1)

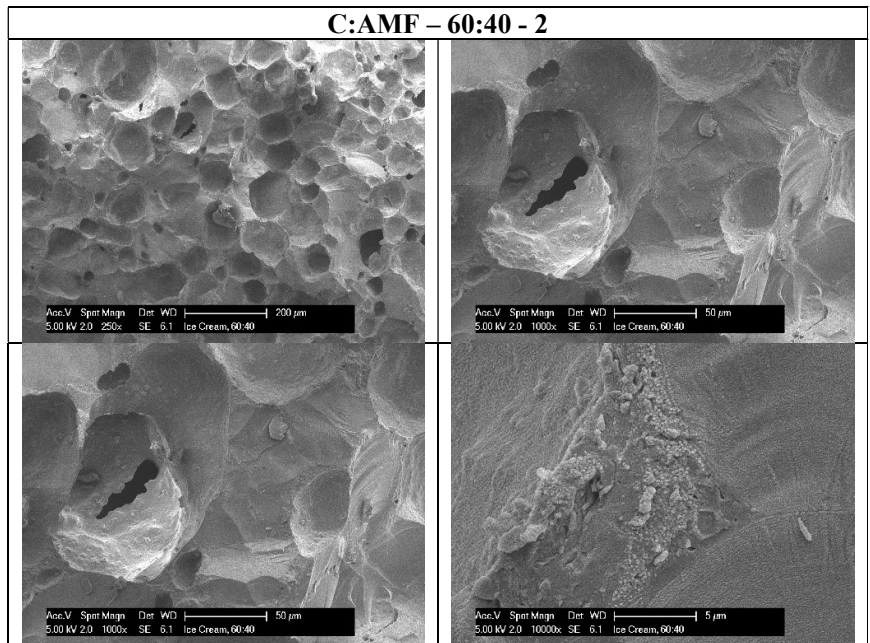


Figure 8.11 SEM images of $C_{AMF}^{60:40}$ at different magnifications (area 2)

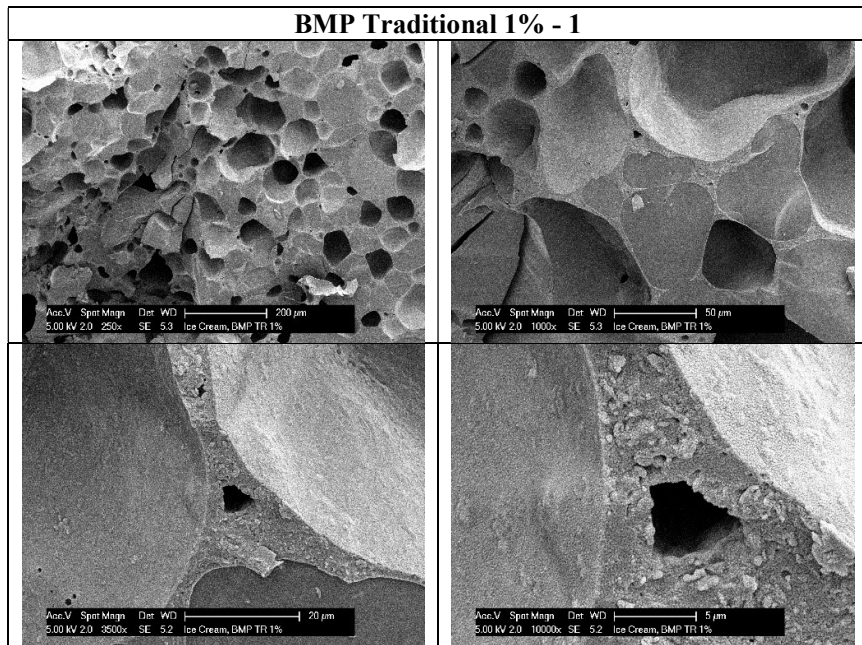


Figure 8.12 SEM images of BMP Trad 1% ice cream at different magnifications (area 1)

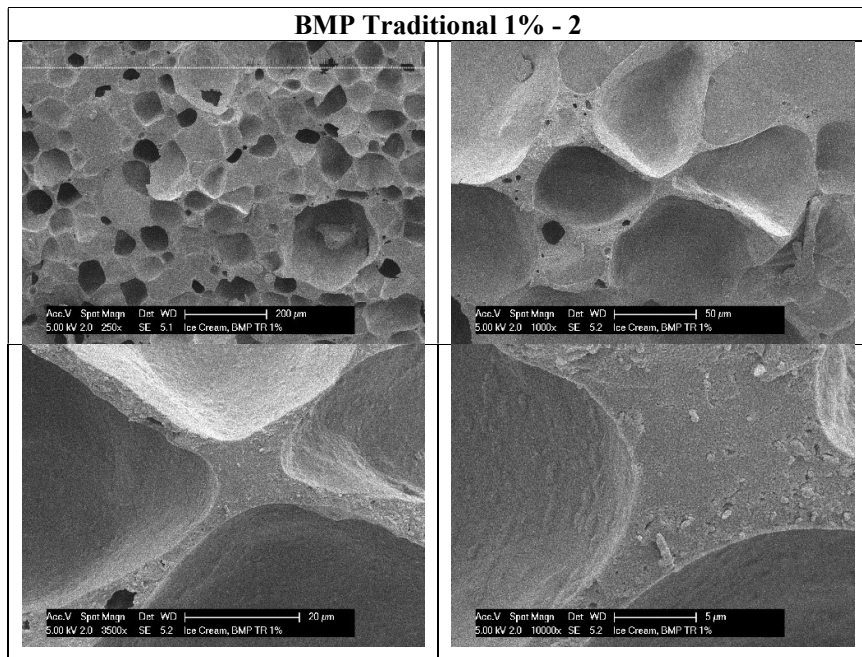


Figure 8.13 SEM images of BMP Trad 1% ice cream at different magnifications (area 2)

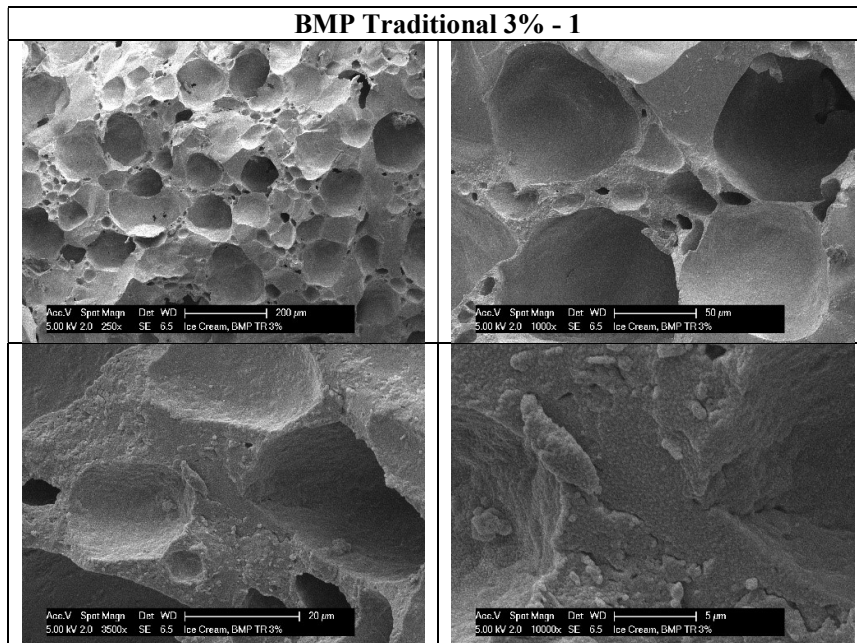


Figure 8.14 SEM images of BMP Trad 3% ice cream at different magnifications (area 1)

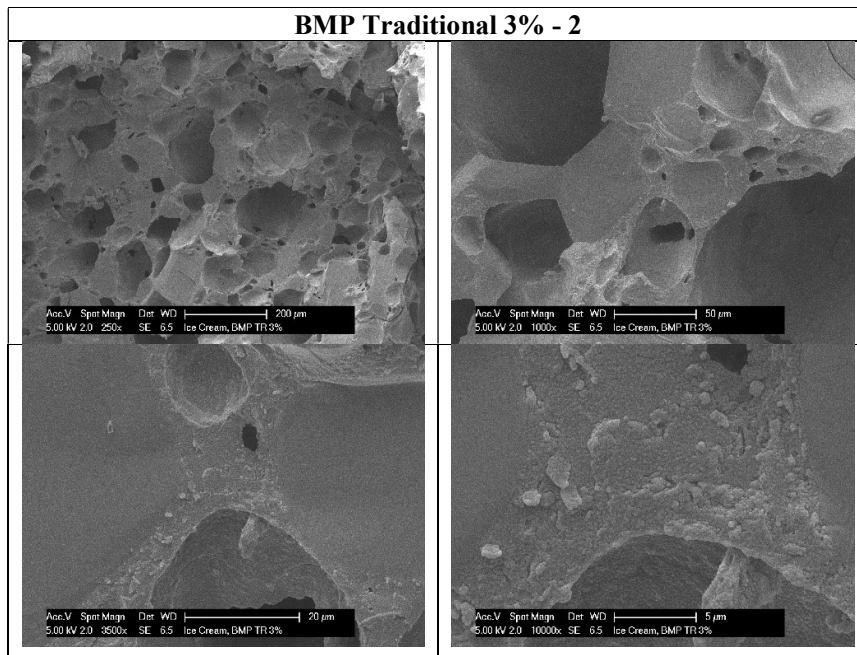


Figure 8.15 SEM images of BMP Trad 3% ice cream at different magnifications (area 2)

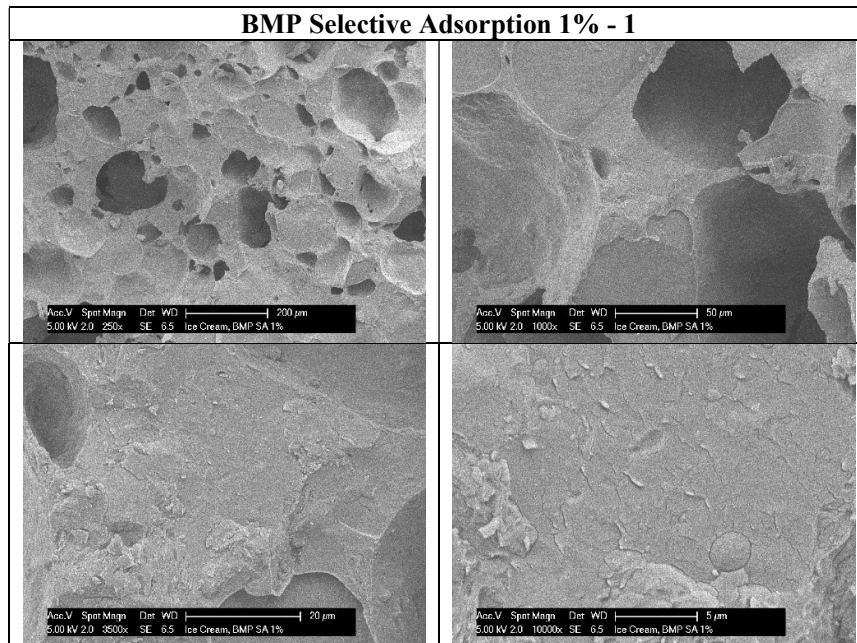


Figure 8.16 SEM images of BMP Selective adsorption 1% ice cream at different magnifications (area 1)

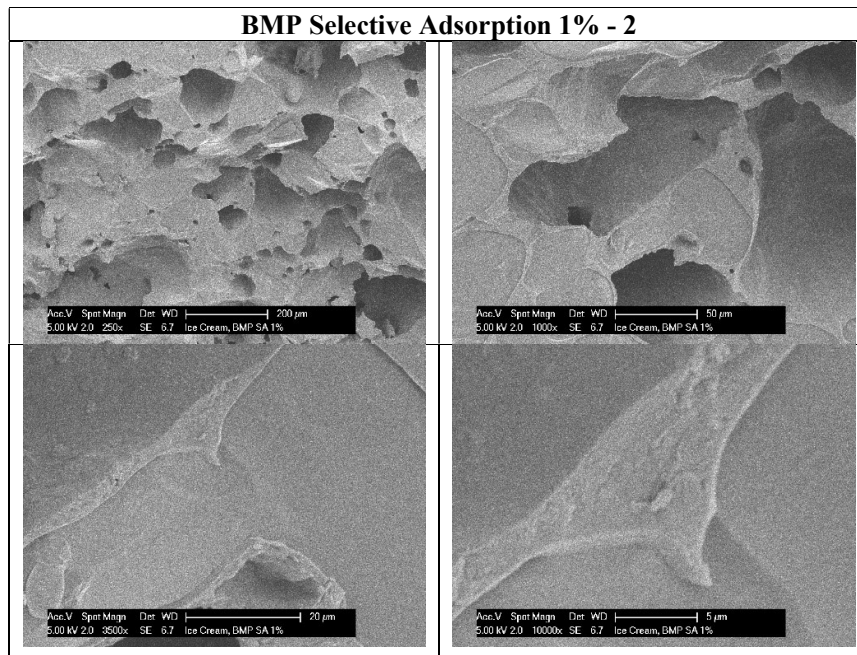


Figure 8.17 SEM images of BMP Selective adsorption 1% ice cream at different magnifications (area 2)

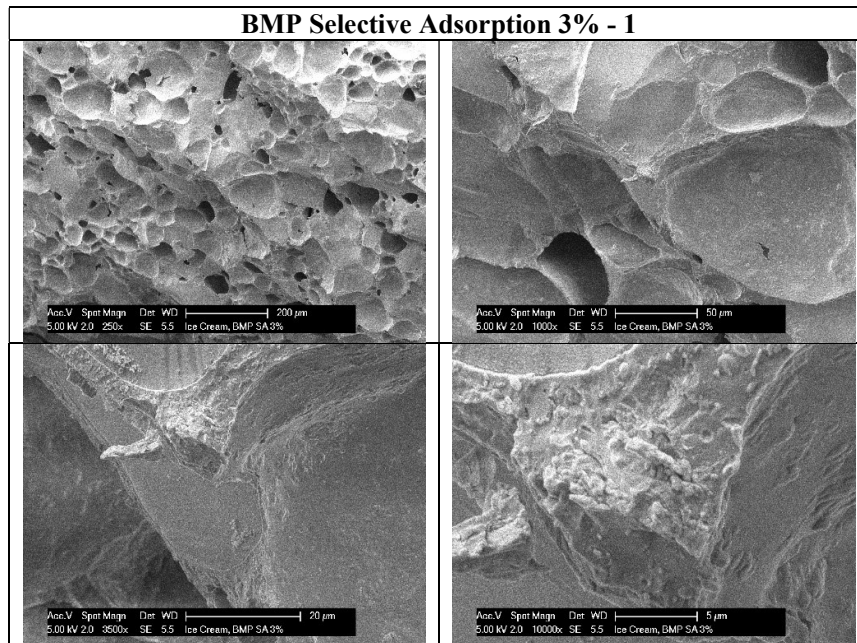


Figure 8.18 SEM images of BMP Selective adsorption 3% ice cream at different magnifications (area 1)

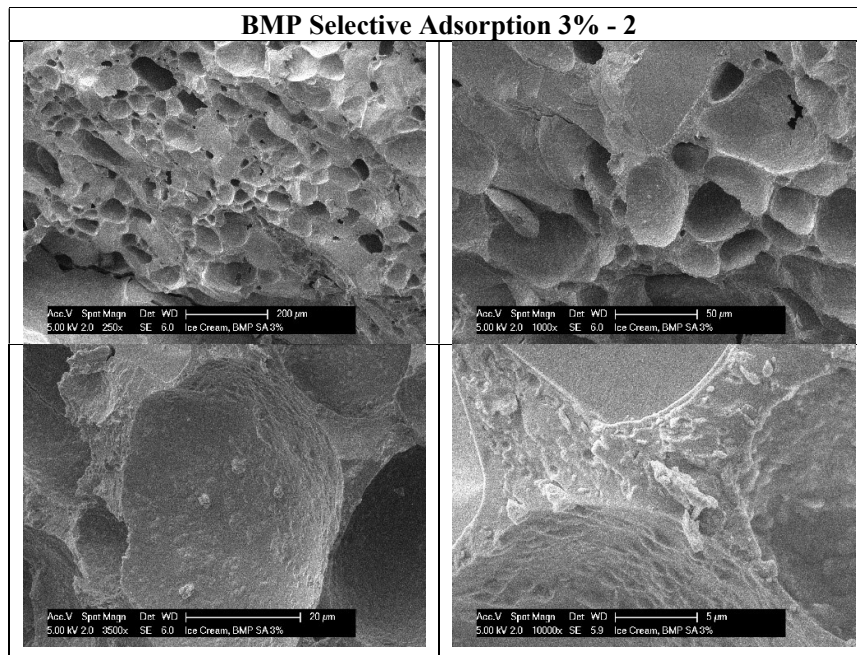


Figure 8.19 SEM images of BMP Selective adsorption 3% ice cream at different magnifications (area 2)