

**The Effect of Milkfat Melting Properties on Chemical and Physical Properties of
20% Reformulated Cream**

Lisa L. Scott

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
in
Food Science and Technology

Susan E. Duncan, Chair
Thomas W. Keenan
Susan S. Sumner

September 17, 1999
Blacksburg, Virginia

Keywords: Fractionated Butteroils, Emulsification, Cream Reformulation, Melting Range

Copyright 1999, Lisa L. Scott

The Effect of Milkfat Melting Properties on Chemical and Physical Properties of 20% Reformulated Cream

Lisa L. Scott

(ABSTRACT)

Skim, sweet buttermilk, and butter derived aqueous phase components were used to re-emulsify low-melt and medium-melt fraction butteroils to yield 20% milkfat creams. The implications of separation temperature in obtaining components, melting point characteristics, and formulation on the chemical and physical properties of reformulated and natural creams were analyzed. Transmission electron microscopy indicated that both reformulated and natural creams were oil-in-water emulsions, demonstrating lipid globules surrounded by surface material. Chemical analysis of components proved that sweet buttermilk and butter-derived aqueous phase components had significantly higher (p less than or equal to 0.01) amounts of cholesterol and phospholipid than skim milk, resulting in creams formulated with sweet buttermilk and butter-derived aqueous phase creams having significantly higher (p less than or equal to 0.01) amounts of cholesterol and phospholipid than creams formulated with skim milk. Butter-derived aqueous phase had higher (p less than or equal to 0.01) amounts of lipid, cholesterol, and phospholipid than sweet buttermilk. However, skim component had higher (p less than or equal to 0.01) amounts of protein than butter-derived aqueous phase. When compared to natural creams, creams consisting of sweet buttermilk and butter-derived aqueous phase components had similar amounts of total phospholipid and amount of phospholipid adsorbed to lipid globules than creams consisting of skim component. Creams consisting of skim component had higher (p less than or equal to 0.01) amounts of protein than natural cream. Reformulated creams having low-melt fraction butteroil had higher (p less than or equal to 0.01) amounts of cholesterol. For reformulated creams, creams processed from components obtained by 49°C separation had significantly higher (p less than or equal to 0.01) amounts of cholesterol than like creams manufactured from 55°C separation components.

Creaming stability, viscosity, feathering, and sensory quality of reformulated and natural creams were analyzed over a 13 day storage period at 3.3°C. Formulation, separation temperature, or melting point characteristics did not significantly (p greater than 0.01) affect creaming stability of reformulated and control creams homogenized at 13.6/3.4 MPa. The day within storage period, however, was a significant factor (p less than or equal to 0.01) in determining creaming stability of reformulated and natural creams. All creams displayed typical non-Newtonian behavior at 7°C, displayed by hysteresis curves in which viscosity decreased as shear rate increased. Formulation and separation temperature used to obtain components did not have a significant (p greater than 0.01) effect on viscosity; however, all creams formulated with medium-melt fraction butteroil had significantly (p less than or equal to 0.01) higher apparent viscosity values than

creams with low-melt fraction butteroil at shear rate 692.48 s^{-1} and at 1384.96 s^{-1} and 2769.92 s^{-1} for creams formulated with skim component. Regardless of formulation, separation temperature, and melting point characteristics, all creams feathered in a pH range of 4.70-5.09. Reformulated and natural creams met sensory quality specifications as determined by the In/Out Method of Specification, except for creams formulated with skim milk and low-melt fraction butteroil which were characterized as having oxidized flavors. Creams formulated with buttermilk and butter derived aqueous phase had more comparable physical properties to natural creams than skim milk creams.

Acknowledgments

I would like to express sincere gratitude to my graduate advisor, Dr. Susan E. Duncan, for her continuous support and assistance throughout the course of my research and graduate studies. Her guidance, support of my academic endeavors, and patience the past two years will not be forgotten. I also thank Dr. Thomas W. Keenan for providing his knowledge regarding the chemical aspects of the research, conducting the gel electrophoresis on components and cream samples, and helping with the formatting of the electron micrographs and gel photographs. I also thank Dr. Susan Sumner for contributing her expertise on the microbiology testing of natural and reformulated creams.

I would like to express an extra special thanks to Mrs. Kim Waterman for her invaluable assistance and support with the chemical analyses and processing of creams. Her friendship, support, and advice must not go unmentioned. I am also grateful to Mr. Walter Hartman for his time and dedication involved in the processing of creams. I would like to thank Mr. Brian Yaun for his support with the microbiological technique and analyses. Thanks are also extended to Mr. Matt Schaeffer of the Biological Systems Engineering Department for showing me how to use the Haake viscometer during the early phases of my research project. I also extend appreciation to the statistics team, Mr. Bob Noble and Mr. Seth Clark, for their help in establishing a statistical design and analyzing the data for this project. I also thank my fellow graduate peers, Ms. Kristen Matak, Mr. Cole Boling, Ms. Marleen Van Aardt, and Ms. Jodi Powell for being a great support group. I would also like to thank my best friend, Ms. Aretha Turner, who unfortunately was not here for most of my graduate research. However, her advice and undying support were greatly appreciated.

Dedication

I dedicate this thesis to my parents, Mrs. Anne E. Scott and the late Mr. Lawrence L. Scott. I am most grateful for their financial and spiritual support and guidance throughout my life. Thanks for believing in me and teaching me the power of believing in myself and prayer.

Table of Contents

	Page
Title	i
Abstract	iii
Acknowledgments	iv
Dedication	v
Table of contents	vi
List of Figures	ix
List of Tables	xi
Chapter 1. Introduction	1
Chapter 2. Review of Literature	3
2.1. Factors affecting milkfat melting range	3
Milkfat Composition and Melting Characteristics	3
Fatty Acid Composition	3
Distribution of Fatty Acids	4
Polymorphic State of Fat Crystals	5
2.2 Milkfat Modification	5
Overview of Fractionation	5
Dry Fractionation	6
Crystallization From Solvent Solution	6
Supercritical Fluid Extraction	7
Short Path Distillation	7
Applications of Milkfat Fractions	7
Oxidative Stability and Sensory Characteristics of Fractionated Milkfat	8
Challenges of Emulsifying Fractionated Butteroils	10
2.3. Emulsification of Milkfat into Aqueous Systems	10
Origin of Milkfat Globules	10
The Milkfat Globule Membrane	11
Recombination of Butteroil into Dairy Emulsions	13
Characteristics of Surface Active Molecules	14
Phospholipids	14
Proteins	16
2.4. Chemical Characteristics of Components Used for Emulsification	21
Purified Milkfat Globule Membrane Extract	21
Skim Component	22
Sweet Buttermilk and Butter-Derived Aqueous Phase	24
Milkfat Globule Surface Material in Processed Creams	26
Electron Microscopy of Milkfat Emulsions	27
2.5. Influence of Processing Parameters	28
Dairy Destabilization	28
Pasteurization	29

Homogenization	31
2.6. Definition and Physical Characteristics of Cream	33
Definition of Cream	33
Creaming Stability	33
Viscosity	34
Feathering.....	36
Sensory Quality	37
2.7. References	39
Chapter 3. The Effect of Milkfat Fraction Melting Properties on Chemical Properties of 20% Reformulated Cream	
3.1. Abstract	48
3.2. Introduction	49
3.3. Materials and Methods	51
Separation of Cream and Skim	51
Preparation of Sweet Buttermilk and Butter-Derived Aqueous Phase	52
Processing of Components.....	52
Characterization of Low-Melt and Medium-Melt Fractionated Butteroils...	52
Cream Reformulation	53
Fat, Protein, Cholesterol, and Phospholipid Determination of Components and Creams.....	54
Transmission Electron Microscopy of Creams	54
Total Fatty Acid Profiles of Components and Creams	55
Determination of Types of Proteins Associated with Lipid Globules	55
Analysis of Milkfat Surface Material	56
Statistical Analyses	56
3.4. Results and Discussion	57
Component Composition and Potential Emulsifying Characteristics	57
Visual Characterization of Natural and Reformulated Creams	64
Chemical Characterization of Natural and Reformulated Creams.....	65
Characterization of Milkfat Globule Surface Material	74
3.5. Conclusions	78
3.6. References	81
Chapter 4. The Effect of Milkfat Fraction Melting Properties on Physical Properties 20% Reformulated Cream	
4.1. Abstract	84
4.2. Introduction	85
4.3. Materials and Methods	88
Separation of Cream and Skim.....	88
Preparation of Skim, Sweet Buttermilk, and Butter-Derived Aqueous Phase Components	88
Cream Reformulation.....	88
Creaming Stability	88

Viscosity.....	88
Feathering Stability	88
Microbiological Analyses	89
Sensory Evaluation of Cream Quality	89
Statistical Analyses	89
4.4. Results and Discussion	
Creaming Stability.....	91
Viscosity.....	96
Feathering Stability	102
Microbiological Analysis	102
Sensory Evaluation of Cream Quality.....	103
4.5. Conclusions	105
4.6. References	107
Appendix A	110
Appendix B	115
Appendix C	118
Appendix D	125
Vitae	131

List of Figures

- | Title | Page |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Figure 3-1. Polypeptide profiles of pilot plant produced components obtained .. 60 at two different separation temperatures (49°C and 55°C) and commercially obtained components (1, 49°C butter-derived aqueous phase) (2, 55°C butter-derived aqueous phase) (3, commercial butter-derived aqueous phase) (4, commercial sweet buttermilk) (5, 49°C sweet buttermilk) (6, 55°C sweet buttermilk) (7, unpasteurized commercial sweet buttermilk) (8, 49°C skim component) (9, 55°C skim component) (m, milkfat globule membrane extract) (dashes from top to bottom: xanthine oxidase, CD36, butyrophilin, and glycoprotein B) (arrowheads from top to bottom: casein and whey proteins, β -lactoglobulin and α -lactalbumin, respectively) | 60 |
| Figure 3-2. Thin layer chromatographs of individual phospholipids (sphing- ... 62 myelin, phosphatidyl choline, phosphatidyl serine, phosphatidyl inisitol, and phosphatidyl ethanolamine) for pilot plant processed components obtained at two separation temperatures (49oC and 55oC) and commercially components (GI BM and GI Ap) | 62 |
| Figure 3-3. Electron micrographs of reformulated creams processed from 66 components (skim component or sweet buttermilk and commercial butter-derived aqueous phase) obtained at two different separation temperatures (49°C and 55°C) and low-melt or medium-melt fraction butteroil (a, 49°C skim component, low-melt fraction butteroil, magnification x 8100) (b, 55°C skim component, low-melt fraction butteroil, magnification x 12,000) (c, 55°C skim component, medium-melt fraction butteroil, magnification x 10,473) (d, 49°C sweet buttermilk, commercial butter-derived aqueous phase, low-melt fraction butteroil, magnification x 18,327). | 66 |
| Figure 3-4. Electron micrographs of natural and reformulated creams 66 processed from components (sweet buttermilk and commercial butter-derived aqueous phase or control) obtained at two different separation temperatures (49°C and 55°C) and low-melt or medium-melt fraction butteroil (e, 55°C sweet buttermilk, commercial butter-derived aqueous phase, low-melt fraction butteroil, magnification x 12,800) (f, 55oC sweet buttermilk, commercial butter-derived aqueous phase, medium-melt butteroil, magnification x 10,560) (g, 49°C natural cream, magnification x 15,467) (h, 55°C natural cream, magnification 12,800). | 66 |
| Figure 3-5. Thin layer chromatographs of individual phospholipids (sphingo- .. 72 | 72 |

myelin, phosphatidyl choline, phosphatidyl serine, phosphatidyl inisitol, and phosphatidyl ethanolamine) for natural and reformulated creams processed from components obtained at two separation temperatures (49°C and 55°C) and low-melt and medium-melt fraction butteroils (a, 49°C skim, low-melt butteroil) (b, 55°C skim, low-melt butteroil) (c, 55°C skim, medium-melt butteroil) (d, 49°C sweet buttermilk, commercial butter-derived aqueous phase, low-melt butteroil) (e, 55°C sweet buttermilk, commercial butter-derived aqueous phase, low-melt butteroil) (f, 55°C sweet buttermilk, commercial butter-derived aqueous phase, medium-melt butteroil) (g, 49°C natural cream) (h, 55°C natural cream)

Figure 3-6. Polypeptide profiles of surface material (SM) isolated from lipid... 75 globules from natural and reformulated creams processed from components obtained at two different separation temperatures (49°C and 55°C) and low-melt and medium-melt fraction butteroils. (a, 49°C skim component, low-melt butteroil) (b, 55°C skim component, low-melt butteroil) (c, 55°C skim component, medium-melt butteroil) (d, 49°C sweet buttermilk, commercial butter-derived aqueous phase, low-melt butteroil) (e, 55°C sweet buttermilk, commercial butter-derived aqueous phase, low-melt butteroil) (f, 55°C sweet buttermilk, commercial butter-derived aqueous phase, medium-melt butteroil) (g, 49°C natural cream) (h, 55°C natural cream)

Figure 4-1. Creaming stability (percent change in the fat content of the top 92 layer of natural and reformulated creams formulated with low-melt and medium-melt butteroils over a two week storage period at 3.3°C.

Figure 4-2. Creaming stability (percent change in the fat content of the bottom 94 layer) of natural and reformulated creams formulated with low-melt and medium-melt butteroils over a two week storage period at 3.3°C.

Figure 4-3. Hysteresis curve at 7°C of apparent viscosity of natural creams 97 and creams formulated with low-melt butteroil (LM) and components obtained at two different separation temperatures on Day 1 of storage (-- increasing shear; - - decreasing shear).

Figure 4-4. Hysteresis curve at 7°C of apparent viscosity of natural cream 100 and creams formulated with low-melt (LM) and medium-melt (MM) butteroils and components obtained at separation temperature of 55°C on Day 1 storage (-- increasing shear; - - decreasing shear).

Figure 4-5. Apparent viscosity (shear rate = 692 s⁻¹) at 7°C of natural creams 101 and reformulated creams with low-melt or medium-melt butteroil over a two week storage period at 3.3°C.

List of Tables

Title	Page
Table 3-1. Fatty acid analysis (wt. %) of low-melt and medium-melt fractionated butteroils.	53
Table 3-2. Description of 20% natural and reformulated creams processed from components obtained at two different separation temperatures (49°C and 55°C) and butteroils having different melting range characteristics (low-melt and medium-melt fractions)	53
Table 3-3. Chemical composition ¹ (% lipid, protein, cholesterol, and phospholipid) of pilot plant produced components (skim, sweet buttermilk, and butter-derived aqueous phase) obtained at two different separation temperatures 49°C and 55°C) and commercially processed components (sweet buttermilk and butter-derived aqueous phase).	58
Table 3-4. Free fatty acid analysis ¹ of components used for cream reformulation obtained at two different separation temperatures (49°C and 55°C).	63
Table 3-5. Globule count, diameter range, and mean diameter (µm)+standard deviation values for natural and reformulated creams indicated by transmission electron microscopy.	68
Table 3-6. Chemical composition ¹ (% lipid, protein, cholesterol, and phospholipid) of natural and reformulated creams consisting of components obtained at two different separation temperatures (49°C and 55°C) and butteroils different melting point range characteristics (low-melt fraction and medium-melt fraction)	69
Table 3-7. Free fatty acid analysis of natural and reformulated creams processed from components obtained at two different separation temperatures (49°C and 55°C).	73
Table 3-8. Composition ¹ of milkfat surface material (SM) isolated from lipid globules of natural and reformulated creams processed from components obtained two different separations (49°C and 55°C) and butteroils having different melting range characteristics (low-melt and medium-melt fractions).	77

Title	Page
Table 3-9. Phosphodiesterase activity ¹ of natural and reformulated creams.....	79
consisting of components obtained at two different separation temperatures (49°C and 55°C) and butteroils having different melting point range characteristics (low-melt and medium-melt fractions).	
Table 4-1. Creaming stability (total percent change in fat content of top layer)	93
for reformulated creams formulated with low-melt and medium-melt butteroils and natural creams processed from components obtained at two separation temperatures (49°C and 55°C).	
Table 4-2. Creaming stability (total percent change in fat content of bottom	95
layer) for reformulated creams formulated with low-melt and medium-melt butteroils and natural creams processed from components obtained at two separation temperatures (49°C and 55°C).	
Table 4-3. Percent of "In" specification responses for 20% natural and.....	104
reformulated creams within 4 days of refrigerated storage at 3.3°C.	
Table B-1. Phospholipid analysis ¹ (% in sample, mg/g lipid, and % in lipid	115
portion)of pilot plant processed components (skim, sweet buttermilk, and butter derived aqueous phase) obtained at two different separation temperatures (49°C and 55°C) and commercially processed components (sweet buttermilk and butter-derived aqueous phase).	
Table B-2. Percent lipid and phospholipid (% in sample, mg/g lipid, and	116
% in lipid portion) composition ¹ of natural and reformulated creams consisting of components obtained at two different separation temperatures (49°C and 55°C) and butteroils having different melting point range characteristics (low-melt fraction and medium-melt butteroils).	
Table B-3. Protein and phospholipid content ¹ of a given amount of surface...	117
material isolated from the lipid globules of natural and reformulated creams processed from components obtained at two different separation temperatures (49°C and 55°C) and butteroils having different melting range characteristics (low-melt and medium-melt butteroils).	
Table D-1. Creaming stability (percent change in fat content of top layer) for.	125
natural and reformulated creams processed from components obtained at two different separation temperatures (49°C and 55°C) stored over a two week period at 3.3°C.	

Title	Page
Table D-2. Creaming stability (percent change in fat content of bottom layer) For natural and reformulated creams processed from components obtained at two different separation temperatures (49°C and 55°C) stored over a two week period at 3.3°C.	126
Table D-3. Apparent viscosity (shear rate = 692 s ⁻¹) of natural and re- Formulated creams at 7°C processed from components obtained at two separation temperatures (49°C and 55°C) stored at 3.3°C for a two week storage period.	127
Table D-4. Incidence of feathering (pH of buffer at which feathering occurred) of reformulated creams consisting of low-melt and medium-melt fractionated butteroil and natural creams processed form components obtained at two separation temperatures (49°C and 55°C) over a two week storage period at 3.3°C.	128
Table D-5. Aerobic, modified psychrotrophic, and coliform plate counts ¹ For 10 ⁰ dilution of natural and reformulated creams processed from components obtained at two different separation temperatures (49°C and 55°C) stored at 3.3°C on Day 0 of reformulation.	129
Table D-6. Number of "In" and "Out" of specification responses for reformulated and natural creams within 4 days of refrigerated storage at 3.3°C.	130

