

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

**SELECTIVE REMOVAL OF FAT FROM
ACID WHEY DURING WHEY PROTEIN
CONCENTRATE MANUFACTURE**

**A THESIS PRESENTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTERS OF TECHNOLOGY IN FOOD TECHNOLOGY**

BY

ARRON HARFORD

**INSTITUTE FOR FOOD, NUTRITION AND HUMAN HEALTH
MASSEY UNIVERSITY PALMERSTON NORTH**

2000

“The heavens declare the glory of God

The skies proclaim the work of his hands.”

Psalm 19:1 (NIV Bible)

..... as does all creation, great and *small*.

ABSTRACT

The purpose of this study was to develop a low cost technology for selective removal of lipids from acid whey during whey protein concentrate manufacture. Attention was focused on gaining a better understanding of the structure and composition of the lipids in whey and ultrafiltration retentate. The effects of varying dilutions, pH, salt concentration, temperature and holding time on the flocculation of lipids in the whey and retentate were investigated.

The composition and structure of lipids in acid whey and retentate were determined by ultracentrifugation, compositional analysis, integrated light scattering and confocal scanning laser microscopy (CLSM) techniques. Acid whey contained ~ 0.034% lipids. The size of the milk fat globules (MFG) in whey varied from 0.1 and 10 μm , with the majority of the globules < 1 μm in diameter. The retentate contained ~ 0.36% lipids. The size of the MFG in the retentate ranged between 0.1 and 20 μm , generally larger than the MFG in the acid whey.

Investigation into the removal of lipids from acid whey revealed that flocculation of MFG in the acid whey occurred at temperatures between 40 and 50 °C and at pH values from 5.8 to 7.0. It was observed that under these conditions, high-density lipid containing flocculent/precipitates was formed, which subsequently sedimented upon centrifugation (at 1126 g for 10 min). The MFG removed in the flocculent/precipitate appears to be either part of a calcium-MFG complex or MFG entrapped by precipitation of calcium precipitate.

Examination of the effects of physiochemical factors on the flocculation of MFG in between the retentate revealed that flocculation occurred upon dilution and at pH values between 4.5 and 4.7. It was found that at increasing dilutions, there was an increase in the removal of MFG and in the retention of protein in the supernatant. At retentate dilution of 1:6, the majority of the MFG was removed and a majority of protein was retained in the supernatant.

Flocculation of MFG in the diluted retentate was influenced by ionic strength (at Low pH values) of the system. This flocculation is thought to result from the hydrophobic association of proteins of MFGM, aggregates of serum proteins, lipoprotein complexes or individual denatured serum proteins.

Low fat whey protein concentrate powder (WPC) was produced on a pilot-scale plant using the process conditions determined at the laboratory scale to remove MFG from acid whey retentate. The resulting product contained ~ 1% fat, considerably less than the normal commercial WPC. On a dry basis the protein content was ~ 96% as compared to ~ 85% in the commercial WPC. Examination of the functionality of the low fat WPC revealed the heat-induced gels formed from 15% WPC were more elastic, had better water holding capacity, and were more "gelatinous" in nature. Their gelation properties were markedly superior to the commercial WPCs currently manufactured.

Based on the results of this study, recommendations are made on possible areas of process improvement and development opportunities.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my chief supervisor, Professor Harjinder Singh for his invaluable guidance, enthusiasm and expertise throughout all stages of this research. I would also like to thank Professor Singh for the time he always readily gave in assisting me with this project. I am also very thankful for the advice and warm support provided by my co-supervisors Dr Palatasa Havea and Dr Mike Taylor.

I would like to thank the staff of the Institute of Food, Nutrition and Human Health. In particular I would like to thank Mr Garry Radford, Mrs Michelle Tamehana, Miss Elizabeth Nicholson, Mrs Wibha Desai, Mr John Dawber, Mrs Geedah Reid and Mr Steve Glasgow for their technical support during the course of my study.

I wish also to thank the Whey Products Development Centre team (Anchor Products, Edgumbe). Particularly, Palatasa Havea, Brian Beach and Olaf Van Daleen for their invaluable assistance during the pilot plant trials. Also thanks to Jenny McVicker, for all her help in providing literature.

I wish also to thank the New Zealand Co-operative Dairy Company and the Foundation for Research, Science and Technology for the funding that made this project possible.

Thanks to all the postgrad students and researchers I have met during my study, in particular, Ping, Rogerio, Micheal, Carol, Fiona, Meili, Richard, Haden, and Maya.

Lastly, a special thanks to Nicola for her continued friendship and encouragement, and to my family (Dad, Mum, Joanna and Philip) for their support, encouragement and general family like behaviour.

TABLE OF CONTENTS

CHAPTER 1 INTRODUCTION.....	1
CHAPTER 2: LITERATURE RESEARCH.....	4
2.1 Introduction	4
2.2 Milk.....	5
2.2.1 <i>Composition of milk and milk lipids</i>	5
2.2.2 <i>Milk fat globule (MFG)</i>	7
2.2.3 <i>Effect of processing on milk fat globules (MFG)</i>	10
2.2.3.1 Agitation and shear force	11
2.2.3.2 Cooling.....	11
2.2.3.3 Heat treatment.....	12
2.2.3.4 Acidification	12
2.2.4 <i>Free fat</i>	13
2.3 Whey	14
2.3.1 <i>Composition of whey</i>	14
2.3.2 <i>Processing of whey</i>	15
2.3.3 <i>Production of WPC</i>	16
2.3.4 <i>General lipid composition and concentration in whey protein concentrate (WPC)</i>	20
2.3.4.1 Total Lipids.....	21
2.3.4.2 Triacylglycerols	21
2.3.4.3 Phospholipids.....	22
2.3.4.4 Fatty Acids.....	22
2.3.4.5 Minor simple lipid constituents	23
2.4 Removal of fat from whey.....	23
2.5 Characterisation of lipids in whey	26
2.5.1 <i>Fractionation of lipids in whey</i>	26
2.5.2 <i>Quantitative determination of lipids in whey</i>	27

2.5.2.1	Standardised Reference Methods.....	28
2.5.2.1	Cold Extraction Methods.....	28
CHAPTER 3: MATERIALS AND METHODS		31
3.1	Materials.....	31
3.2	Compositional analyses.....	31
3.3	Recovery and fractionation of whey and retentate lipids.....	32
3.4	Size distribution of fat globules.....	33
3.5	Microstructure of fat globules (Confocal Scanning Laser Microscopy).....	33
3.6	Spectrophotometry.....	34
3.7	WPC functionality.....	34
3.7.1	<i>Gel sample preparation</i>	34
3.7.2	<i>Gel strength</i>	35
3.7.3	<i>Gel syneresis</i>	35
CHAPTER 4: CHARACTERISATION AND SEPARATION OF LIPIDS FROM ACID WHEY.....		36
4.1	Introduction.....	36
4.1.1	<i>Characterisation of lipids in acid whey</i>	37
4.1.2	<i>Lipid content</i>	37
4.1.3	<i>Size distribution of fat globules</i>	39
4.1.4	<i>Microstructure of fat globules by confocal scanning laser microscopy(CSLM)</i>	42
4.1.5	<i>Discussion</i>	44
4.2	Removal of lipids from acid whey.....	46
4.2.1	<i>Effect of pH and temperature on separation of MFG</i>	46
4.2.2	<i>Effect of pH and temperature on amount of sediment</i>	48
4.2.3	<i>Composition of supernatant and sediment</i>	49
4.2.4	<i>Effect of pH re-adjustment on the turbidity of the supernatant</i>	51
4.2.5	<i>Discussion</i>	54

CHAPTER 5: THE CHARACTERISATION AND SEPARATION OF LIPIDS

FROM UF RETENTATE OBTAINED FROM ACID WHEY	57
5.1 Introduction	57
5.2 Characterisation of milk fat globules in acid whey retentate	58
5.2.1 <i>Lipid content</i>	58
5.2.2 <i>Size distribution of fat globules</i>	59
5.2.3 <i>Confocal Microscopy</i>	62
5.2.4 <i>Discussion</i>	64
5.3 MFG separation from UF retentate.....	66
5.3.1 <i>Preliminary experiments</i>	66
5.3.2 <i>Effect of dilution on separation of MFG</i>	68
5.3.3 <i>Effect of dilution and pH on MFG separation</i>	71
5.3.4 <i>Effect of NaCl addition on MFG separation</i>	74
5.3.5 <i>Effect of temperature and time</i>	77
5.4 Possible mechanisms of aggregation	79
5.5 Discussion.....	83

CHAPTER 6: PILOT-SCALE PLANT PRODUCTION OF A LOW FAT

WHEY PROTEIN CONCENTRATE POWDER.....	88
6.1 Introduction	88
6.2 Low fat WPC production process.....	88
6.2.1 <i>Process variables</i>	88
6.2.2 <i>Process flow outline</i>	90
6.3 Production of low fat WPC	91
6.3.1 <i>Composition of dilute retentate and its fractions after clarification</i> ..	91
6.3.2 <i>Composition of the 1° and 2° retentates</i>	94
6.3.3 <i>Composition of low fat WPC</i>	95
6.3.4 <i>Functionality of low fat WPC</i>	96
6.4 Discussion.....	99

CHAPTER 7: OVERALL CONCLUSIONS AND RECOMMENDATIONS..	103
7.1 Study approach	103
7.2 Experimental Methodology	103
7.3 Microstructural properties of fat in acid whey and its retentate	104
7.4 Factors affecting the flocculation of MFG in the acid whey	105
7.5 Factors affecting the flocculation of MFG in the retentate	105
7.5.1 <i>Dilution</i>	105
7.5.2 <i>pH</i>	106
7.5.3 <i>Ionic strength</i>	106
7.5.4 <i>Temperature and time of heat treatment</i>	106
7.6 Proposed mechanism of aggregation of MFG in the retentate	107
7.7 Production of low fat WPC	107
7.8 Further studies	108
REFERENCES	110

CHAPTER 1

INTRODUCTION

Whey is a by-product of cheese or casein manufacture. Cheese whey is produced after casein is separated from milk by the addition of the rennet enzyme in the initial stages of cheese manufacture. Acid whey is produced after casein is separated from milk by addition of mineral acid in the manufacture of casein. The whey streams are concentrated using ultrafiltration and evaporation, and then spray dried to produce whey protein concentrate powders (WPCs). These products are used as ingredients in many applications by the food industry. The ability of these products to form heat-induced gels makes them an attractive functional product for use as a gelling agent in many food applications.

Cheese WPC is produced in a much larger quantities by the New Zealand dairy industry but is sold at a lower price because the functional properties, especially gelling ability, of the WPC is inferior to that of the acid WPC. Recent research (Havea, 1998) showed that it may be possible to manipulate the manufacturing conditions to improve the functionality of cheese WPC, and possibly match the gelling abilities with that of acid WPC. It is forecasted that cheese WPC would compete with the acid WPC in the market place. This presents a threat to the acid WPC manufacturer. If the cheese WPC could provide the same functional properties as the acid WPC, then it would probably be sold at a lower price than that of acid WPC because of the larger quantities available. If this happened, the acid WPC would be phased out of the market.

It is therefore desirable that development work be conducted into the improvement of the acid WPC properties and thus its position in the world market. One possibility is to try to develop a new product from acid WPC that is similar to whey protein isolate (WPI), using a low cost technology. WPI is a relatively new whey protein product

produced by the New Zealand dairy industry. This product has a high content of protein (~ 94%) and a low fat content (< 1%), and is largely used in the nutritional and sports formulations. Current work indicates that WPIs are superior than the WPCs from a view point of lipid and protein content, protein solubility, foam expansion and stability, absence of protein denaturation and aggregation, and flavour rating (Morr and Foegeding, 1990). Compared to WPIs, the poor flavour and relatively high lactose, lipid and mineral content of WPCs represent a serious problem that undoubtedly limits their acceptance and use by the food industry, especially in nutritional applications. WPI is sold at a very high price in the world market. However, production of WPI involves very high cost technologies, such as micro-filtration and ion exchange.

In recent years, a greater understanding of the relationship between the basic physiochemical and functional properties of whey proteins as influenced by compositional and processing factors has been gained. Considerable effort has been focused on understanding and improving the functional properties of whey proteins. Significant improvements in the functional properties of whey products have been attributed to the lower proportion of non-protein components such as lipids, lactose and minerals. The development and production of a WPC with similar composition properties to those of WPI products, given proper handling and pretreatment of the whey prior to ultrafiltration (UF) and diafiltration (DF) processing and drying, may result in a lower cost (comparative to WPI), highly functional and nutritional protein product. The required reduction in minerals and lactose are achievable using the current ultrafiltration and diafiltration technologies. However, currently a process for reducing the lipid content from whey is not well established.

It is extremely difficult to remove all residual lipid from whey protein products (Kilara, 1994), because this material seems to be small in size and probably associated with proteins. Extensive studies have been carried out on the removal of lipid from both cheese and acid whey (Attebury, 1971; Hobman, 1992; Breslau *et al.*, 1975; Fauquant *et al.*, 1985a & b; Maubois *et al.*, 1987; Kim, *et al.*, 1989; Daufin *et al.* 1993; Hwang and Damodaran, 1994, 1995; Karleskind *et al.*, 1995a).

Investigations have successfully applied various pretreatments to produce acid whey protein concentrates with reduced lipid content and improved functional properties (Fauquant *et al.*, 1985*a* & *b*). However the majority of the work was conducted on cheese whey. Moreover, there are problems translating the results into industrial scale production of low fat WPC. To successfully remove the lipid material from acid whey during manufacture, a greater understanding of this material is required.

The purpose of this study was to develop a low cost technology for selective removal of lipids from acid whey during whey protein concentrate manufacture. This was achieved through the development of an understanding of the structure and composition of the lipids in whey and conducting an exploration into methods to induce flocculation of lipid material in whey. This study also sought to gain information on the effects of varying pH, temperature and salt concentration on the flocculation of lipid material. Knowledge gained throughout this study was used in the development of a process to remove lipids from whey and to produce a low fat WPC on a pilot-scale plant.
