

ABSTRACT

EVANS, JOSHUA PETER. Comparison of Composition, Sensory Properties and Volatile Components of Whey Protein and Serum Protein Concentrates. (Under the direction of Dr. MaryAnne Drake.)

Whey proteins are highly functional and nutritious proteins used in a variety of products.

Whey protein concentrate (WPC) is one of the most commonly used value-added forms of whey protein. Whey proteins should ideally have a bland flavor to facilitate application in foods, but flavor of these products is highly variable due to the original whey source, processing, and storage. Recent research has highlighted removal of whey proteins from skim milk prior to cheese making. These proteins removed from milk before the cheese making process are referred to as serum or “native” whey proteins and serum protein concentrate (SPC) when further processed to 34-89% protein. Since SPC are not exposed to the cheese make-process, enzymatic and/or chemical reactions that can lead to off-flavors are reduced. The objectives of this research were to characterize and compare the composition, sensory properties, and flavor chemistry of both whey protein concentrate and serum protein concentrate at protein concentrations of 34 and 80% (SPC34, WPC34, SPC80, WPC80). A second objective was to compare the composition, sensory properties and flavor chemistry of the serum protein and whey protein concentrates made in our study with those of commercial WPC34 and WPC80. SPC and WPC were manufactured in triplicate with each pair of serum and traditional whey protein manufactured from the same lot of milk. At each replication, spray-dried (SD) product from each protein source was collected. Commercial WPC34 and WPC80 were also collected for sensory and volatile analyses. A trained sensory panel documented the sensory profiles of the rehydrated powders. Volatile components were

extracted by solid phase micro-extraction (SPME) and solvent extraction followed by solvent assisted flavor evaporation (SAFE) with gas chromatography-mass spectrometry and gas chromatography-olfactometry. Consumer acceptance testing with 6 % protein beverages was conducted with SPC80 and WPC80, as well as commercial WPC80. Fat content of SPC34 and SPC80 was lower and pH was higher than WPC34 and WPC80 ($p < 0.05$). Few sensory differences were documented between the rehydrated SPC and WPC at both protein levels manufactured in this study, but their flavor profiles were distinct from flavor profiles of rehydrated commercial WPC ($p < 0.05$). WPC34 and WPC80 generally had higher concentrations of lipid oxidation products than SPC34 and SPC80 ($p < 0.05$). Lipid oxidation product concentrations were also higher in commercial products compared to pilot plant products ($p < 0.05$). Fifty-six aroma-active compounds were identified in the four products manufactured in this study (WPC34, SPC34, WPC80, SPC80), eighteen of these compounds were found in all four products. Overall, aroma-active compounds were primarily lipid oxidation products, followed by fermentation, Strecker degradation, Maillard browning, and caramelization products. More aroma-active compounds were identified in the 80% protein samples (46) than in the 34% protein samples (29). Free fatty acid and soapy flavors and bitter taste were identified in beverages made with SPC80, and soapy flavor and bitter taste were documented in beverages made with WPC80 manufactured in this study; these flavors were not present in beverages made with commercial WSPC80. Overall consumer acceptance scores were highest for beverages made with one commercial WPC80 followed by beverages made with the WPC80 manufactured in this study. Differences between products manufactured in this study and commercial samples, as well as variability among commercial samples alone, suggests that additional processing parameters contribute to

flavor and flavor variability in WPC. Composition, physical properties and volatile compound composition of SPC are distinct from WPC, and these differences may contribute to flavor differences in ingredient applications.

Comparison of Composition, Sensory Properties and Volatile Components of Whey Protein and Serum Protein Concentrates

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Food Science

Raleigh, North Carolina

2009

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DEDICATION

This work is dedicated to my beautiful wife, Jennifer. Jen I never could have done all of this without your constant support and love.

BIOGRAPHY

Joshua Peter Evans was born September 9, 1982, in Ocala, FL to James and Judy Evans. He has two successful and supportive brothers, Jacob and James Evans, whom both live in Tallahassee, FL. His parents have been married for 32 years and still reside in Ocala, FL.

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ACKNOWLEDGMENTS

The path to my Masters has been eventful and interesting to say the least. It has not been the most conventional path but I know I could not have gotten to where I am without the help of others.

My family is by far the greatest gift God has blessed me with. My parents have been a great source of encouragement and guidance. They have served as amazing role models and inspirations, and have helped to shape me into the man I have become. My brothers are two people I know I can count on for anything, no matter what, when or where. I don't know if I could have gotten through my Masters if it was not for the support of my wife. She has shared in each success and failure. She is my main support, and inspiration and I could not imagine being with someone more amazing.

I would like to thank Dr. MaryAnne Drake for her unending patience, and encouragement. Thank you for working so hard with me to finish, and for not firing me after breaking; 6 centrifuge tubes, five computers, one SAFE head, a pH meter, and all the other small stuff, oh, and the \$400 chair. I would like to thank Evan Miracle for answering so many questions (some more than once), and being patient when I broke things. I would like to thank Jessica Childs for her statistical knowhow and constant source of laughter. If I didn't know better I would say that the two of you were being paid to help me. And last but not least, thank you to the MAD lab and fellow graduate students for: our long lunches, your practical jokes, our office discussions, your crude sense of humor, and the other little things, like all the work you put in on my project. I will truly not forget them.

TABLE OF CONTENTS

List of Tables	vii
List of Figures.	xii
Chapter 1. Literature Review	1
Whey defined	2
Whey Processing	4
Whey Protein Concentrate	7
Whey Protein Isolate	8
Native Whey Proteins	9
Whey Flavor	9
Milk	11
Starter Culture	11
Chemical Reactions	13
Lipid Oxidation	13
Maillard Browning	15
Processing	16
Storage	18
Research Objectives	20
References	21
 Chapter 2. 34% Whey Protein and Serum Protein Concentrate Manuscript	 25
<i>Comparison of composition, sensory, and volatile components of 34%</i> <i>whey protein and serum protein concentrates</i>	 26
Abstract	27
Introduction	29
Materials and Methods	30
Results	49
Conclusions	60
Acknowledgements	60
References	61
 Chapter 3. 80% Whey Protein and Serum Protein Concentrate Manuscript	 86
<i>Comparison of composition, sensory, and volatile components of 80%</i> <i>whey protein and serum protein concentrates</i>	 87
Abstract	88
Introduction	90

Materials and Methods	92
Results.	107
Conclusions	121
Acknowledgments	122
References	123

LIST OF TABLES

Chapter 1

Table 1.1	Typical Composition and pH of Sweet (Rennet Casein and Cheddar Cheese) Whey and Acid (Lactic or Mineral Acid) Whey	3
Table 1.2	Differences between reverse osmosis and ultrafiltration	5

Chapter 2

Table 2.1	Mean (n = 3) composition (percent by weight) of the serum protein concentrate (SPC) and whey protein concentrate (WPC) liquids after UF and before drying	65
Table 2.2	Mean (n = 3) moisture content of spray dried and freeze dried serum protein concentrate (SPC) and whey protein concentrate (WPC)	66
Table 2.3	Mean (n = 3) composition (percent by weight) of spray dried serum protein concentrate (SPC) and whey protein concentrate (WPC) calculated on a dry basis and pH.	67
Table 2.4	Mean (n = 3) mineral composition (percent by weight) of spray dried serum protein concentrate (SPC) and whey protein concentrate (WPC) as calculated on a dry basis	68
Table 2.5	Composition (percent by weight) and pH of spray dried (n = 3) serum protein concentrate (SPC), spray dried (n = 3) whey protein concentrate (WPC) produced in this study and six commercial (n = 2) 34% whey protein concentrates	69
Table 2.6	Mean (n = 3) relative proportions of casein to serum proteins for 34% WPC and 34% SPC powders by the densitometry analysis of the SDS-PAGE gels	70
Table 2.7	Mean (n = 3) relative proportions of β -lactoglobulin and α -lactalbumin for 34% WPC and 34% SPC powders by the densitometry analysis of the SDS-PAGE gels	71

Table 2.8	Mean (n = 3) Hunter L, a, b color values of spray dried and freeze dried powders	72
Table 2.9	Mean (n = 3) Hunter L, a, b color values of spray dried and freeze dried serum protein concentrate (SPC) and whey protein concentrate (WPC)	73
Table 2.10	Mean (n = 3) sensory attributes of spray dried serum protein concentrate (SPC) and spray dried whey protein concentrate (WPC) produced in this study.	74
Table 2.11	Mean (n = 3) sensory attributes of spray dried and freeze dried serum protein concentrate (SPC) and whey protein concentrate (WPC) produced in this study	75
Table 2.12	Mean sensory attributes of spray dried (n = 3) serum protein concentrate (SPC), spray dried (n = 3) whey protein concentrate (WPC) produced in this study and six commercial (n = 2) 34% whey protein concentrates	76
Table 2.13	Mean (n = 3) concentrations of selected aroma compounds (µg/L) of spray dried serum protein concentrate (SPC) and spray dried whey protein concentrate (WPC) produced in this study isolated using solid phase microextraction (SPME)	77
Table 2.14	Mean (n = 3) concentrations of selected aroma compounds (µg/L) of spray dried (SD) and freeze dried (FD) serum protein concentrate (SPC) and spray dried whey protein concentrate (WPC) produced in this study isolated using solid phase microextraction (SPME)	78
Table 2.15	Mean concentrations of selected aroma compounds (µg/L) of spray dried (n = 3) serum protein concentrate (SPC), spray dried (n = 3) whey protein concentrate (WPC) produced in this study and six commercial (n = 2) 34% whey protein concentrates isolated using solid phase microextraction (SPME)	79
Table 2.16	Aroma active compounds detected in spray dried (SD) and freeze dried (FD) pilot plant SPC34 and WPC34 by gas chromatography olfactometry with aroma extract dilution analysis (AEDA)	80

Chapter 3

Table 3.1	ANOVA type III SS and <i>P</i> values for ultrafiltration (UF) (after first 30 min of processing run) and diafiltration (DF) flux across the time of processing. Treatment is the comparisons of processing whey versus MF permeate .	127
Table 3.2	Mean (n = 3) composition (percent by weight) of the serum protein concentrate (SPC) and whey protein concentrate (WPC) liquids after UF/DF and before spray drying .	128
Table 3.3	Mean (n = 3) composition (percent by weight) of spray dried serum protein concentrate (SPC) and whey protein concentrate (WPC) calculated on a wet basis .	129
Table 3.4	Mean (n = 3) composition (percent by weight) of spray dried serum protein concentrate (SPC) and whey protein concentrate (WPC) calculated on a dry basis .	130
Table 3.5	Mean (n = 3) mineral composition (percent by weight) of serum protein concentrate (SPC) and whey protein concentrate (WPC) as calculated on a dry basis .	131
Table 3.6	Mean (n = 3) composition (percent by weight) of spray dried serum protein concentrate (SPC) and whey protein concentrate (WPC) calculated on a non fat dry basis .	132
Table 3.7	Composition (percent by weight) and pH of (n = 3) 80% serum protein concentrate (SPC), whey protein concentrate (WPC) and five commercial (n = 2) 80% whey protein concentrates calculated on a wet basis .	133
Table 3.8	Composition (percent by weight) and pH of (n = 3) 80% serum protein concentrate (SPC), whey protein concentrate (WPC) and five commercial (n = 2) 80% whey protein concentrates calculated on a dry basis .	134
Table 3.9	Composition (percent by weight) and pH of (n = 3) 80% serum protein concentrate (SPC), whey protein concentrate (WPC) and five commercial (n = 2) 80% whey protein concentrates calculated on a non fat dry basis .	135

Table 3.10	Mean (n = 3) relative proportions of casein to serum proteins for skim milk and 80% WPC and 80% SPC powders by the densitometry analysis of the SDS-PAGE gels	136
Table 3.11	Mean (n = 3) relative proportions of β -lactoglobulin to α -lactalbumin for skim milk and 80% WPC and 80% SPC powders by the densitometry analysis of the SDS-PAGE gels	137
Table 3.12	Means sensory attributes of serum protein concentrate (SPC80) and whey protein concentrate (WPC80) produced in this study (n=3)	138
Table 3.13	Mean sensory attributes of SPC80 and WPC80 produced in study (n=3) and 5 commercial WPC80 (n=2)	139
Table 3.14	Mean (n=3) concentrations of selected aroma-active compounds ($\mu\text{g/L}$) of spray dried serum protein concentrate (SPC) and spray dried whey protein concentrate produced in this study isolated using solid phase microextraction (SPME)	140
Table 3.15	Mean concentrations of selected volatile compounds ($\mu\text{g/L}$) of spray dried (n = 3) serum protein concentrate (SPC) and spray dried (n = 3) whey protein concentrate produced in this study and five commercial (n=2) 80% whey protein concentrates isolated using solid phase microextraction (SPME)	141
Table 3.16	Aroma active compounds detected in SPC80 and WPC80 by solvent assisted flavor evaporation with gas chromatography olfactometry with aroma extract dilution analysis (AEDA)	142
Table 3.17	Mean sensory attributes of 6% protein peach beverages manufactured (n=2) from SPC80 and WPC80 manufactured in this study and three commercial WPC80	144

Table 3.18	Consumer (n=77) liking scores of 6% protein peach beverages manufactured from SPC80 and WPC80 manufactured in this study and three commercial WPC80	145
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LIST FIGURES

Chapter 2

- | | | |
|------------|--|----|
| Figure 2.1 | Mean (n = 3) UF flux during the production of 34% WPC and 34% SPC using a spiral wound 10 kDa polyethersulfone membrane | 81 |
| Figure 2.2 | Sodium dodecyl sulfate-PAGE electrophoretogram of proteins in 34% serum protein concentrate (SPC), 34% whey protein concentrate (WPC) and skim milk. The loading of the samples was 7 μ l (8.5 μ l for milk) and samples were run in triplicate. Bands in lanes are identified on the gel: SP1, SP2, SP3 = serum proteins, Cn1 = α_s -Cn (combination of α_{s1} and α_{s2} -Cn), Cn2, Cn3 = casein proteolysis products, β -lg (β -lactoglobulin), α -la (α -lactalbumin), Cn4 = proteolysis products of casein | 82 |
| Figure 2.3 | Rehydrated at 10% solids the liquid 34% whey protein cocentrate (WPC) and 34% serum protein concentrate (SPC). From left to right: WPC replicate 1, SPC replicate 1, WPC replicate 2, SPC replicate 2, WPC replicate 3, SPC replicate 3 | 83 |
| Figure 2.4 | Principal component biplot of sensory attributes of whey and serum protein concentrates and commercial WPC34 | 84 |
| Figure 2.5 | SPME PCA biplot of instrumental analysis results for whey and serum protein concentrates and commercial WPC34 | 85 |

Chapter 3

- | | | |
|------------|---|-----|
| Figure 3.1 | Mean (n = 3) ultrafiltration (UF) and diafiltration (DF) flux during the production of 80% WPC and 80% SPC using a spiral wound 10 kDa polyethersulfone membrane | 146 |
| Figure 3.2 | Sodium dodecyl sulfate-PAGE electrophoretogram of proteins in experimental 80% serum protein concentrate (SPC), 80% whey protein concentrate (WPC) and skim milk. Bands are identified on the gel: SP1, SP2, SP3 = high molecular weight serum proteins, Cn1 = α_s -Cn (combination of α_{s1} and α_{s2} -Cn), Cn2 = casein proteolysis products, β -lg (β -lactoglobulin), α -la (α -lactalbumin), β -Cn (β -casein) and κ -Cn (κ -casein) Cn3 = proteolysis products of casein | 147 |

Figure 3.3	Liquid 80% whey protein concentrate (WPC) (on the left) and 80% serum protein concentrate (SPC) (on the right) after diafiltration	148
Figure 3.4	Principal component biplot of sensory attributes of whey and serum protein concentrates manufactured in this study and 5 commercial WPC80	149
Figure 3.5	PCA biplot of SPME volatile compound results for whey and serum protein concentrates manufactured in this study and 5 commercial WPC80	150

CHAPTER 1: LITERATURE REVIEW

Whey Defined

Whey is an opaque greenish yellow serum produced during manufacture of cheese after separation of casein and fat by milk coagulation (Kosikowski et al., 1997). The primary milk protein, casein, is coagulated (or gelled) via acid (decreases pH to isoelectric point of casein) and/or via enzymatic destabilization (e.g. addition of rennet). Fat is trapped in the casein coagulum. The liquid released from the coagulated casein and fat is called whey. The greenish yellow color is caused by riboflavin (Spreer, 1998) Riboflavin is a B vitamin found naturally in milk and is also known as lactoflavin or vitamin B₂. Water, protein, and lactose are the primary components in liquid whey.

Sweet whey and acid whey are the two main categories of whey. The type of whey formed depends on the method used for milk coagulation (Spreer, 1998). Acid whey is commercially formed from skimmed milk in the manufacture of acid casein or acid-coagulated cheese in which starter culture and rennet are not added. Because it is formed from skimmed milk it is essentially free of fat (Fox et al., 2000). A mineral acid (HCL) or lactic acid is used to directly coagulate the milk. Cottage and Ricotta are examples of direct set cheeses (Kosikowski et al., 1997). When cheese or caseins are produced by enzymatic coagulation of milk, sweet whey is produced (Spreer, 1998).

Acid whey and sweet whey differ in their composition. Acid whey contains a much higher concentration of calcium, magnesium, phosphate, and citrate than sweet whey (Fox et al., 2000). Acid whey has an average pH of 4.6 and a titratable acidity of 0.4 % lactic acid. When acid is used in direct set cheese, calcium is removed from the calcium-casein complex,

resulting in the formation of calcium lactate. Sweet whey in contrast, has a pH of 5.8-6.3 and a titratable acidity of 0.1 % lactic acid (Kosikowski et al., 1997). During the enzymatic coagulation of casein and formation of sweet whey, calcium remains bound in the coagulated protein, resulting in no formation of calcium lactate. A comparison of the component differences between acid and sweet whey are listed in Table 1.

Table 1.1 Typical Composition and pH of Sweet (Rennet Casein and Cheddar Cheese) Whey and Acid (Lactic or Mineral Acid) Whey

Component	Sweet Whey		Acid Whey	
	Rennet Casein (g/L)	Cheddar Cheese	Lactic Acid Casein (g/L)	Mineral Acid Casein (g/L)
Total solids	66	67	64	63
Total protein (N x 6.38)	6.6	6.5	6.2	6.1
Nonprotein nitrogen (NPN)	0.37	0.27	0.4	0.3
Lactose	52	52	44	47
Milk fat	0.2	3	0.3	0.3
Minerals (ash)	5	5.2	7.5	7.9
Calcium	0.5	0.4	1.6	1.4
Phosphate	1	0.5	2	2
Sodium	0.53	0.5	0.51	0.5
Lactate	/	2	6.4	/
pH	6.4	5.9	4.6	4.7

Fox et al., 2000

Traditionally, whey was thought of as a waste product. Manufacturers of cheese often had to pay to have it discarded. In order to dispose of liquid whey it was fed to animals, used in irrigation of land, dumped into waterways, or treated as effluent. Each of these disposal methods besides dumping in waterways is still used today (Fox et al., 2000). Dumping in waterways is strictly enforced because the whey organic material uses up much of the available oxygen, causing waterways to become uninhabitable for aquatic life (Kosikowski et

al., 1997). Well over 150 million tons of whey are manufactured in the world each year.

Improved technology and knowledge of whey and its potential have turned a waste product in to a highly profitable industry (Fox et al., 2000). Foods, cosmetics, dietetics, sports foods, baby food, and cleaning agents all use products formed from liquid whey, and applications of whey products are increasing constantly (Spreer, 1998). Most of the whey used comes from liquid whey that has undergone some form of processing. Concentrated whey, whey powder, lactose, lactalbumin, whey protein fractions, whey protein concentrate, and whey protein isolate are a few of the products formed from processing whey.

Whey Processing

Clarification is the first step in processing whey. A vibrating screen separator or a centrifugal separator is used most often to separate any excess curd particles that may be present in the whey. Small amounts of fat that are present at about 0.3% (w/w) are recovered using centrifugal separators. This fat is then used to make whey butter or whey cream. Phospholipids are present in whey fat and may not be removed efficiently by centrifugal separation. These compounds can inhibit membrane processing and need to be removed. This is done by adding CaCl_2 , and raising the pH to approximately 7.5. Microfiltration separates out the flocculated calcium phosphate-lipoprotein particles. The separated lipoproteins are used in food applications due to their excellent emulsifying properties. By removing fat and lipoproteins the whey is less prone to lipid oxidation and spoilage.

After clarification and fat removal, whey is pasteurized and cooled rapidly in order to kill all lactic acid bacteria. Pasteurization takes place at 71-74°C for 15 seconds (Spreer,

1998). The presence of excess lactic acid will make the whey difficult to dry and prone to problems following spray drying. If a large amount of lactic acid is present in the dried whey ingredient, the product will be sticky and hygroscopic. Pasteurization may also help to slow other enzymatic processes such as lipid oxidation that occur during fluid whey storage (Carunchia Whetstine et al., 2003; Tomaino et al., 2004). After pasteurization, the liquid whey is further processed to achieve a product with specific characteristics.

After the whey has been processed it can be sold as a concentrated liquid or further processed to become a powder. When processing whey to make powders, selective membrane filtration steps are applied. Types of membrane filtration that are used include: reverse osmosis, ultrafiltration, and microfiltration. Each of these methods utilizes different levels of pressure and different pore sizes to selectively separate components based on molecular weight. Differences in reverse osmosis and ultrafiltration can be found in Table 2. The product that does not pass through the membrane is called retentate. The permeate is the portion of the whey that passes through the membrane and consists of different levels of water, lactose, salts, vitamins, and amino acids depending on the method being used.

Table 1.2. Differences between reverse osmosis and ultrafiltration.

Properties	Reverse Osmosis	Ultrafiltration
Size of Retained Molecules mass M in kg/kmol	M = 5000	M = 1000
Operating pressure in bar	50 bar	1-10 bar
Type of Membrane Effect	Diffusion (0.4 nm pore size)	Sieving Process (2 nm pore size)
Nature of Permeate	Water, perhaps with traces of low-molecular-weight components	Low-molecular-weight components, present at similar concentration to that in the feed

Adapted from Spreer (1998) and Sienkiewicz et al. (1990)

During reverse osmosis water passes through a pore small enough that only it can pass through, forming a more concentrated liquid whey retentate. Because it requires high amounts of energy, it is not economical beyond concentrations of 25% solids. This method is often used as a preliminary step prior to ultrafiltration or evaporation before drying (Kosikowski et al., 1997). Evaporation occurs under vacuum at temperatures lower than 100°C. The temperature prevents proteins from becoming denatured, and allows water to evaporate. Ultrafiltration utilizes a pressure drop over membranes with small pores (2 nm) to remove water and dissolved smaller molecules, while retaining colloidal and large molecules, namely protein (Spreer, 1998).

Spray drying is the most commonly used method for producing dried food products (Reineccius, 2004). During spray drying, the liquid is atomized into a stream of hot air. As the liquid is exposed to the hot air, the water evaporates rapidly. This process alone yields a high moisture powder with 10 to 14% moisture. In a two stage process lactose is allowed to crystallize further after leaving the spray drier before being dried to a moisture content of 3 to 5% in a vibrating fluid bed (Kosikowski et al., 1997).

Whey powder contains all of the components found in liquid whey, except water. Liquid whey is concentrated to 50-60% total solids via reverse osmosis and evaporation, and seeded with lactose crystals to induce crystallization. When the lactose is crystallized, the concentrate is then dried (Kosikowski, 1997). Lactose represents about 70% of the total solids in whey. Lactose is a sugar unique to milk with low solubility, low sweetness, difficulty with crystallizing and a tendency to absorb flavors and pigments. Because bovine

milk has higher concentrations of salts than human milk, electrodialysis and/or ion exchange are used to reduce the concentration of ions which produces demineralized whey. This step enables bovine whey to be added to skim milk in human infant formulas to provide a product that will not apply such a high renal load on the baby (Fox et al., 2000).

Whey Protein Concentrate

Whey proteins consist of 50% β -lactoglobulin, 25% α -lactalbumin, and 25% other proteins (Spreer, 1998). Whey protein concentrate and whey protein isolate are the most often used whey products in industry due to their higher concentrations of proteins and functional applications. While whey powders are dried whey and contain all of the original components of liquid whey besides water, whey protein concentrates and isolates are produced by removing non-protein components in order to achieve a specific protein concentration (Fox et al., 2000). Whey protein concentrates (WPC) are found in protein concentrations from 30 to 90% protein and isolates (WPI) contain > 90 % protein (Fox et al., 2000).

Whey proteins have many functional characteristics such as high solubility, dispersibility, water binding, foaming, whipping, emulsification, gelation, and buffering power (Kosikowski et al., 1997). Ultrafiltration is a processing step used to produce whey protein concentrates and isolates. Ultrafiltration not only allows for the removal of water, but lactose and small amounts of minerals as well. By removing lactose, whey can be concentrated to different protein levels, without the concern of lactose crystallization (Kosikowski et al., 1997). Diafiltration involves the addition of potable water at approximately a 1:1 ratio into

the liquid whey concentrate with continuing ultrafiltration – essentially washing the retentate. This process is utilized in order to remove considerable lactose and soluble materials in order to achieve a high protein concentration (Kosikowski et al., 1997). Ultrafiltration and diafiltration are carried out at $\leq 50^{\circ}\text{C}$ to prevent protein denaturation. The product retained by ultrafiltration and diafiltration is often referred to as retentate.

Permeate is the by-product of whey protein manufacturing and it is what is allowed to pass through (or permeate) the membrane during filtration steps. It contains high amounts of lactose and minerals, and is most often fermented to make ethyl alcohol that is concentrated for spirits. Spreading on crop land and feeding to cattle are the next two most economically viable practices (Kosikowski et al., 1997). Other applications for permeate have also been explored such as in beverages (Beucler et al., 2005; Geilman et al., 1992) but applications for this by-product of whey protein processing remain limited.

Whey Protein Isolate

Whey protein concentrates are referred to as WPI when the protein concentration reaches 90% or more (Kosikowski et al., 1997). To produce WPI that is low in lactose, fat, and minerals, an anion exchange or microfiltration method must be used to further concentrate and purify the protein and remove residual fat and lactose. In anion exchange, the liquid concentrated whey is reduced to an acidic pH (which creates a positive charge on the proteins) and pumped into a tank containing negatively charged resin beads. The proteins attach to the resin beads while the lactose, fat and minerals do not and are removed. To release the proteins, the pH of the tank is then brought up to alkaline pH, ultrafiltration and

drying then follow (Huffman, 1996). Whey protein isolates are more expensive to produce and therefore are not used as widely as whey protein concentrates.

Native Whey Proteins

Proteins found in whey are called whey proteins. These same proteins are referred to as serum proteins when they are in milk. Native whey proteins are simply whey proteins separated from milk before the cheese making process. They may also be referred to as serum proteins. Marcelo et al. (2007) studied the functional behavior of 8 % liquid virgin whey protein concentrate and found the native whey to have a higher thermal stability and improved rheological properties in relation to commercial whey. Little published research has evaluated native whey protein concentrates and isolates. Because native whey proteins are being looked at as a product of their own, it is important to know if the milk can still be used to make quality cheese once the milk serum proteins have been removed. Nelson et al. (2005) studied the yield and aging of Cheddar cheese manufactured from milks with different milk serum protein contents. Cheese produced with low serum protein milk had higher fat recovery, and cheese yield. The study demonstrated that quality cheese could still be made from milk that had serum protein removed (Nelson et al., 2005).

Whey Flavor

Whey has become a valuable and profitable ingredient and additive. However, whey is limited in its use by flavor variability and off-flavors. Manufacturing differences and raw product variations result in inconsistencies between batches. These differences cause

variations in the amount of lactose, minerals, and lipids (Morr et al., 1990; Schmidt et al., 1984).

WPC gets its flavor from a combination of volatile flavor compounds. Aliphatic hydrocarbons, aromatic hydrocarbons, aldehydes, methyl ketones, esters, furans, alkyl pyrazines, pyrroles, saturated fatty acids, carboxylic acids, alcohols, pyridines, imidazoles and sulfur compounds are present in the volatile fraction of whey protein concentrate (Quach et al., 1998). Whey powders develop flavor compounds from the original source and through chemical reactions, processing, and storage (Mahajan et al., 2004; Gallardo-Escamilla et al., 2005). In ingredient applications, brothy, diacetyl, bitter, acidic, cabbage and free fatty acid (rancid) off-flavors are most objectionable (McGugan et al., 1979; Quach et al., 1998; Drake, 2006).

In order to standardize and better evaluate dried milk powders and dairy ingredients, Drake et al. (2003) developed a descriptive language. The language included twenty-one descriptors for dried dairy ingredients. Russell et al. (2006) later developed a more specific sensory language for whey and soy proteins (WPC80, WPI, SPC70, SPI). Sweet aromatic, cardboard/wet paper, animal/wet dog, soapy, brothy, cucumber, cabbage, cooked milky, diacetyl flavors along with basic tastes salty and bitter, and the feeling factor astringency have been documented in whey protein concentrates, isolates, and powders (McGugan et al., 1979; Carunchia-Whetstine et al., 2005; Wright et al., 2006).

Isleten et al. (2006) found a negative impact on sensory characteristics of whey protein isolate fortified yogurt. Drake (2006) and Wright et al. (2009) demonstrated that flavors in

WPI and WPC80 did carry through into ingredient applications (beverages) and consumer acceptance was negatively impacted. Therefore it is important to understand flavor development in whey powders, their source, and what can be done to minimize these flavors. Ideally, the more bland a whey ingredient is, the better.

Milk

The overall quality of milk impacts the flavor of whey protein concentrate. Because whey is traditionally a by product, in order to understand flavor development in whey, it is important to first understand the flavor components of milk (all milk references mentioned in this paper pertain to bovine milk). Milk is primarily composed of water, lipids (3.7%), protein (total protein 3.4%, casein 2.8%, and whey protein 0.6%), lactose (4.8%) and minerals or ash (0.7%) (Fox et al., 2000). Fat content varies the most of all the components of milk, ranging from 3.2%-6%. Breed, feed type, and health play a role in the fat content of the milk (Spreer, 1998). The other components of milk are influenced by the breed of cow, health of the animal, stage of lactation, season, feed, and individuality of the cow (Tobias, 1990). Feed type also influences milk flavor (Croissant et al., 2007).

Impact of Starter Culture

The type of cheese the whey comes from influences whey flavor. Whey, like milk is often pooled from many sources prior to processing, including different types of cheese. Whey from cheese produced by a starter culture is different in flavor from whey from cheese produced by direct acidification (Gallardo Escamilla et al., 2005). Karagul-Yuceer et al. (2003) used gas chromatography-olfactometry and aroma extract dilution analysis to

compare fresh Cheddar cheese whey from different starter culture rotations and found that the use of different mesophilic starter cultures influenced volatile aroma components within that single cheese type (Cheddar). Tomaino et al. (2001) used solid phase micro extraction techniques to monitor the lipolytic activity of different starter cultures in whey. Differences in fatty acid concentrations were observed from liquid whey samples produced from different starter cultures. Whey produced with a *Lactococcus lactis* sub species *lactis* starter culture contained the highest level of total free fatty acids compared to those produced with *Lactococcus lactis* sub species *cremoris*, a combination of the two, and the control utilizing *glucono- δ -lactone* (Tomaino et al., 2001). Tomaino et al. (2004) reported that starter cultures significantly influenced the level of volatile lipid oxidation products. Using instrumental and sensory methods, Carunchia Whetstine et al. (2003) also concluded that the flavor of liquid industrial Cheddar whey was variable and impacted by starter culture rotation and milk source.

Gallardo-Escamilla et al. (2005) suggested that starter cultures influenced flavor of whey to different degrees. In whey produced by starter culture compared to whey produced without a starter culture, samples with starter culture had higher scores for yogurt odor and flavor. This is believed to be due to the production of acetaldehyde and diacetyl (Gallardo-Escamilla et al., 2005). Variability and differences in liquid whey from starter culture rotation and milk source will be increased when the whey is further processed into whey protein concentrate and whey protein isolate.

Chemical reactions: Riboflavin

Chemical reactions that occur in whey and whey powder affect the flavor profile of the product. Vitamin B₂, also known as riboflavin, occurs naturally in whey. In milk, riboflavin is found at a level of 0.2ppm, and in whey 0.1-0.27ppm (Spreer, 1998). Riboflavin is activated by light. Riboflavin is believed to be responsible for light-induced oxidized flavor in fluid milk (Morr et al., 1991). Riboflavin is an effective photosensitizer that produces singlet oxygen, as well as a good reactant for singlet oxygen (Rongmin et al., 1991). The pH of the solution affects the mechanism for the photolysis of riboflavin (Morr et al., 1991). Lumichrome and lumiflavin are formed from riboflavin under light. Once the riboflavin is activated, it is capable of breaking down peptides. In whey, proteolysis may result in bitterness (Carunchia-Whetstine et al., 2003).

Heat treatment in skim milk and homogenization of whole milk increase riboflavin photostability (Saidi et al., 1995). While studying the mechanism of the photolysis of riboflavin in a model system, Toyosaki et al. (1990) found that hydroxyl radicals and active oxygen were involved in the decomposition of riboflavin. They also reported that the process was accelerated in the presence of β -lactoglobulin, and α -lactalbumin (Toyosaki et al., 1990). Off-flavors from riboflavin photolysis have not been determined in whey. However, activated riboflavin and formation of free radicals can aid in lipid oxidation.

Lipid oxidation

In food, free radicals or singlet oxygen mechanisms generate a series of autocatalytic free radical reactions causing lipid oxidation (Kubow, 1992). Lipid oxidation is responsible for

undesirable flavors in milk and whey products (Morr et al., 1991). Lipid oxidation purportedly imparts a cardboard flavor to whey protein powders (Hammond, 1989). Pure proteins have little flavor, however they have the ability to bind other flavorants and influence perceived flavor by binding off-flavors (Damodoran et al., 1980). By products of lipid oxidation therefore can bind with proteins and produce further off-flavors in whey. Carunchia Whetstine et al. (2005) failed to find a correlation in WPC80, and WPI with fat content and lipid oxidation flavors. With increased storage time, possible differences in fat content among whey protein isolate and concentrate might contribute to differences in flavor stability. Carunchia Whetstine et al. (2005) attributed cardboard/wet paper, cucumber, doughy/fatty, and pasta water flavors to lipid oxidation. Cabbage off flavors from dimethyl trisulfide were also believed to be caused by secondary lipid and protein oxidation reactions in whey proteins (Wright et al., 2006)

Mahajan et al. (2004) identified unsaturated aldehydes in sweet whey powder as products of the autooxidation of unsaturated fatty acids. The higher amounts of lactose in sweet whey powder compared to whey protein concentrate or isolate mean most of the flavor compounds found were associated with Maillard browning and Strecker degradation instead of lipid oxidation (Mahajan et al., 2004). Tomaino et al. (2004) showed that lipid oxidation was initiated during cheese-making and whey pasteurization, and escalated during storage. When comparing fresh and stored liquid whey, the stored liquid whey showed a significant increase in volatile lipid oxidation products and a significant increase in cardboard aroma, flavor, and aftertaste (Tomaino et al., 2004). Tomaino et al. (2004) concluded that many different

compounds or combinations of compounds formed by lipid oxidation can induce cardboard flavors in fluid whey.

Although milk lipases can cause off-flavors in milk, they are typically inactivated during pasteurization. Fluorescent light and temperature abuse after pasteurization are two ways in which lipid oxidation reactions are initiated. Polyunsaturated fatty acids are susceptible to oxidation in milk and whey powders. When polyunsaturated fatty acids react with oxygen they form secondary products such as epoxy-hydroperoxides, oxo-hydroperoxides, hydroperoxy epidioxides, dihydroperoxides, hydroperoxy bis-epidioxides, and hydroperoxy bicycloendoperoxides (Frankel, 2003). The secondary products then decompose and form aldehydes, carbonyl compounds, hydrocarbons, furans, and other related compounds (Frankel, 2003). Each of these compounds indicate lipid oxidation, with straight chain aldehydes such as hexanal, pentanal, propanal, and butanal being the main indicators (Hidalgo et al., 1989). These compounds contribute to the flavor deterioration of food including fatty, stale, painty, and cardboard flavors (Frankel, 2003). The compounds formed are also believed to be involved in Maillard browning reactions (Hammond, 1989).

Maillard Browning

Maillard browning reactions in whey mainly involve aldehyde groups of lactose and reactive amino groups of whey proteins. Maillard reactions coupled with Strecker degradation of amino acids form heterocyclic compounds. These compounds have distinct flavors and aromas. Heterocyclic compounds also have low orthonasal thresholds (Farmer, 1996). Temperature, water activity, pH and availability of reactant affect the rate of reaction.

In order to bring the Maillard reaction to a standstill, the moisture content must fall to 3%, however this value is not reached in dried dairy ingredients (Sienkiewicz et al., 1990). Maillard browning reactions occur faster at higher temperatures, but can occur at refrigerated temperatures. Increased pH levels increase the rate of reaction (Morr et al., 1990). Due to the high concentration of protein and presence of lactose in whey protein concentrate, Maillard reactions are increased. An aged-stale flavor is the most criticized off-flavor in dried whey products (Morr et al., 1991). Mahajan et al. (2004) described lipid oxidation and Maillard browning as the two main causes of off-flavors in whey powders.

Sithole et al. (2005) studied the rate of Maillard browning in sweet whey powder under accelerated shelf life testing and under normal storage conditions (21°C and 35% relative humidity) to validate the use of Maillard browning in determining shelf life. Results showed that the rate of brown pigment formation was comparable between the accelerated shelf-life test and the test under normal conditions for two of the three samples tested. Samples had increased free moisture content and acidity values with storage. Sensory evaluation of the samples after 19 months of storage showed no significant differences in odor and flavor (Sithole et al., 2005).

Processing

Processing and storage of milk and whey products greatly influence the flavor. As discussed previously, light can greatly affect the flavor of whey products through light induced lipid oxidation, and riboflavin decomposition. When manufacturing whey protein

from colored Cheddar cheese, the whey goes through a bleaching process using hydrogen or benzyl peroxide. This step can lead to off-flavors (Drake et al., 2009).

Using gas chromatography mass spectrometry and gas chromatography olfactometry to identify aroma compounds of sweet whey powder, Mahajan et al. (2004) suggested that the aroma of whey powder could be formed during the manufacturing process. Whey powders are expected to have different flavor profiles than liquid whey due to their more extensive processing (Mahajan et al., 2004). The additional steps may act to reduce and or generate flavor compounds.

McGugan et al. (1979) reported that a major portion of fresh cheese whey flavor was readily removed during ultrafiltration and diafiltration steps in WPC manufacturing. Further processing by spray drying resulted in excess loss from volatilization in fresh whey protein concentrate. WPI undergo additional processing than WPC. Theoretically, WPI are more pure and have reduced lactose, lipids, and minerals and should have a superior flavor compared to WPC (Morr et al., 1990).

Improvements have been made in processing technology of whey, increasing the quality (Onwulata et al., 2004). Ultrafiltration and vacuum evaporation followed by lactose crystallization and spray drying, vacuum evaporation followed by lactose crystallization and spray drying, ultrafiltration and vacuum-evaporation followed by spray drying, and ultrafiltration followed by spray drying were four processing methods used by Ji et al. (2003) to study the effects of processing on compositional and functional properties of WPC. The process used strongly affected the moisture content, functionality, and thermostability.

Sensory or volatile analysis was not conducted, however flavor would logically be impacted as well. However, without linking instrumental analysis and sensory analysis, key flavor differences cannot be known for certain (Drake et al., 2007).

Storage

Many producers of WPC80 suggest a shelf life anywhere from 12-24 months, although this does not mean that off-flavors will not form before that time (Drake et al., 2009). Sithole et al. (2005) suggest that off-flavors do not develop in whey powders for up to 19 months. Similar studies are currently underway with WPC80 and WPI. Transportation and storage conditions are two areas where off-flavor development can occur. Under poor storage conditions, all of the reactions that are believed to cause off-flavors in whey powders and proteins (lipid oxidation, Maillard browning, riboflavin decomposition) can occur. Tomaino et al. (2001) reported that there were significant declines in oleic, linoleic, and palmitic acids and increases in lipid oxidation compounds during storage of liquid whey. Lee et al. (1996) studied the formation of volatile compounds in WPC during elevated temperature storage as a function of water activity. A WPC with 75% protein was stored for six days at an elevated temperature of 60°C in the dark. The samples used ranged in water activity levels from 0.11 to 0.79. Water activity strongly influenced formation of volatile compounds such as aldehydes, ketones, furans, aromatic hydrocarbons, and sulfur compounds. The research showed that by storing in an elevated temperature for only a short period of time, the concentration of key volatile compounds was increased (Lee et al., 1996). The results indicate that the storage stability of whey protein concentrate is affected by water activity.

To investigate the chemical nature of off-flavors that developed during storage Javidipour et al. (2008) stored WPC80, and WPI samples at room temperature for 12 months under accelerated storage temperatures. Using solid phase microextraction-gas chromatography-olfactometry –mass spectrometry, volatile compound changes were analyzed. The results indicated that during storage, lipid and protein oxidation compounds were formed (Javidipour et al., 2008). More specifically, lipid oxidation products such as ketones aldehydes, and free fatty acids, along with protein degradation and Maillard reaction played a role in storage reactions (Javidipour et al., 2008). The authors also stored instantized and regular WPC80 at 35, 45 and 55°C, and analyzed samples weekly for a period of three months using headspace solid-phase microextraction-gas chromatography. The temperature at which the samples were stored influenced the formation of volatile compounds mentioned above. Unfortunately, sensory analysis was not conducted so the impact of storage time, temperature and changes of these volatile compounds on actual sensory quality is still unknown.

Originally whey was a by-product of cheese production, but with a better understanding and improved technology whey products have become a highly profitable industry. Whey products have become so profitable that they are now being looked at as a product of their own. Nelson et al (2005) designed a new processing technique that effectively removes 95% of serum proteins from raw milk. Further research by Nelson et al. (2005) has shown that serum proteins can be removed from milk before cheese production, and a quality cheese can still be produced. Because they are not exposed to as many processing techniques that can

possibly cause off-flavor formation, native whey proteins (serum proteins) have the potential to be a superior product.

The objectives of this research were to characterize and compare the composition, sensory properties, and flavor chemistry of both whey protein concentrate and serum protein concentrate at a protein concentration of 34 and 80% (SPC34, WPC34, SPC80, WPC80).

The composition, sensory properties and flavor chemistry of the serum protein and whey protein concentrates made in our study were also compared with those of commercial WPC34 and WPC80.

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CHAPTER 2: 34% MANUSCRIPT

COMPARISON OF COMPOSITION, SENSORY AND VOLATILE COMPONENTS OF
34% WHEY PROTEIN AND SERUM PROTEIN CONCENTRATE

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*Submitted for publication in Journal of Dairy Science

ABSTRACT

Serum or “native” whey protein concentrates (SPC) are whey proteins that are removed from milk before the cheese making process. Since SPC are not exposed to the cheese make-process, enzymatic and/or chemical reactions that can lead to off-flavors are reduced. The objectives of this study were to identify and compare the composition, flavor, and volatile components of 34% protein SPC and WPC (SPC34, WPC34). SPC34 and WPC34 were manufactured in triplicate with each pair of serum and traditional whey protein manufactured from the same lot of milk. At each replication, liquid retentate was collected as were spray-dried (SD) and freeze-dried (FD) product from each protein source. Commercial WPC34 were also collected for sensory and volatile analyses. A trained sensory panel documented the sensory profiles of rehydrated spray or freeze-dried powders. Volatile components were extracted by solid phase micro-extraction (SPME) and solvent extraction followed by solvent assisted flavor evaporation (SAFE) with gas chromatography-mass spectrometry and gas chromatography-olfactometry. WPC34 had higher fat content and Ca content compared to SPC34 ($p < 0.05$). Color differences (L, a, b) were not evident between the two powders ($p > 0.05$), but when rehydrated, SPC34 solutions were clear while WPC34 solutions were cloudy. No consistent differences were documented in sensory profiles of SD and FD SPC34 and WPC34 ($p > 0.05$). Spray dried WPC34 had low but distinct buttery and cardboard flavors while spray dried SPC34 did not. Sensory profiles of both rehydrated SD products were bland and lower in overall aroma and cardboard flavor compared to the commercial WPC34. Twenty-nine aroma impact compounds were identified in the SPC34 and WPC34. Lipid and protein oxidation products were present in both products. SPC34 and WPC34

manufactured in this study had lower total volatiles and lower concentrations of many lipid oxidation compounds compared to commercial WPC34. Our results suggest that when manufactured under controlled conditions in a similar manner, there are few sensory differences between SPC34 and WPC34, but distinct compositional differences and differences in physical properties which may influence functionality. Further, flavor (sensory and instrumental) properties of both pilot-scale manufactured protein powders were distinct from commercial powders suggesting the role of other influencing factors.

Key words: serum protein, whey protein, flavor, composition, color

INTRODUCTION

Whey proteins are highly functional and nutritious proteins used in a variety of products. Whey proteins can be found in sports and nutrition bars and beverages, infant formula, dairy foods, meat, and other foods (National Dairy Council, 2008). Concentrated whey, whey powder, lactose, whey protein fractions, whey protein concentrate (WPC), and whey protein isolate (WPI) are a few of the products formed from processing of liquid whey. The two most commonly used forms of whey in industry are WPC and WPI. WPC contains 34-89 % protein and WPI contains at least 90 % protein. Whey proteins have many functional characteristics such as high solubility, dispersibility, water binding, foaming, whipping, emulsification, gelation, and buffering power (Davis et al., 2007) and are used frequently in an increasing number of food applications (National Dairy Council, 2008). Freeze drying and spray drying are two methods used to dry WPC into a powder. The freeze drying method in a plant operation requires a lengthy process time and considerable cost (Snowman, 1997). As such, spray drying is the most common method of drying and has been in use since the early 1900's (Masters, 1997).

Whey proteins should ideally have a bland flavor to facilitate application in foods (Drake et al., 2008), but flavor of these products is highly variable due to many sources, including the original whey source, processing, and storage (Carunchia Whetstine et al., 2005a; Mahajan et al., 2004; Gallardo-Escamilla et al., 2005; Wright et al., 2006; Wright et al., 2009). Off-flavors may carry through into ingredient applications and limit food applications (Drake, 2006; Drake et al., 2008; Wright et al., 2009). Recent research has highlighted removal of whey proteins from skim milk prior to cheese making (Nelson and

Barbano, 2005a; 2005b). Prior to cheese making, these proteins are called serum proteins (SP). Due to a lack of familiarity with the term SP, these proteins have also been referred to as “native” whey proteins. Nelson and Barbano (2005a) developed a microfiltration and ultrafiltration method to remove SP from skim milk and subsequently demonstrated that SP removal from fluid milk had no impact on quality of Cheddar cheese (Nelson and Barbano, 2005b). It is believed that serum protein concentrates (SPC), because they are not subjected to a cheese make procedure, are not exposed to as many processing techniques that may alter flavor formation and thus may have the potential to offer a more bland flavor profile compared to WPC. To our knowledge, no studies have compared SPC and WPC flavor. The objective of this research was to compare the composition and sensory properties of 34% whey protein concentrates and 34% serum protein concentrates made from the same milk. A second objective was to compare the composition and sensory properties of the serum protein and whey protein concentrates made in our study with those of six commercial 34% WPC.

MATERIALS AND METHODS

Experimental Design

One batch of whole raw bovine milk (ca 1780 kg) was received from the Cornell University dairy farm. Processing was done at Cornell University. The milk was divided into two portions. One portion of the milk was used for Cheddar cheese manufacture to produce 34%WPC. The other portion was centrifugally separated at 4°C into raw cream and raw skim and the skim milk was used to produce 34%SPC. This was replicated 3 times with different batches of raw milk. The time line for the processing was as follows: raw whole milk was

received on Monday, split into two portions. On Monday, one portion of the whole raw milk was pasteurized and used for Cheddar cheese making and by the end of the day Monday, pasteurized, separated whey was produced. The other portion of raw milk was cold separated to produce raw skim milk. On Tuesday, the raw skim milk from Monday was pasteurized, cooled to 4°C and stored and the separated pasteurized whey was ultrafiltrated (UF) to produce a liquid 34% WPC that was cooled and split into two batches: one to be freeze dried (this material was immediately frozen in trays) and one to be spray dried. On Wednesday, the pasteurized skim was microfiltrated (MF) to produce 65% SP reduced casein concentrate and MF permeate and the liquid WPC was spray dried. The characteristics of the 65% SP reduced native micellar casein concentrate will be reported in a separate publication. On Thursday, MF permeate was UF to produce 34% liquid SPC that was cooled and split into two batches: one to be freeze dried (this material was immediate frozen in trays) and one to be spray dried. On Friday, the liquid 34% SPC was spray dried. Six commercial 34% WPC were obtained in duplicate (2 kg each, < 2 mo old, from different production dates) and analyzed under the same conditions for comparison of sensory and flavor characteristics to the samples produced in the controlled experiment.

Whey Protein Concentrate manufacture

Raw whole milk for Cheddar cheese production was pasteurized with a plate heat exchanger (Model 080-S, AGC Engineering, Manassas, VA) at 72°C and a holding time of 16s. The pasteurized milk was cooled to 31°C and weighed into a cheese vat (Model DLHD8SSS, Kusel Equipment Company, Watertown, WI). If necessary, the milk was

standardized to a ratio of 0.70 casein to fat by cream addition. Samples of milk were taken for chemical and microbiological analysis before and after the standardization for Cheddar cheese making. The average fat and protein content of the milk for cheese making were $3.44\% \pm 0.03$ and $2.98\% \pm 0.03$. The milk was inoculated with the starter culture including *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* (911 DVS, Chr Hansen, Inc., Milwaukee, WI) at the rate of 0.1 g/kg. The milk was agitated for 5 min and allowed to ripen for 30 min. A small amount of annatto food color (AFC WOS 550, Rhodia Inc., Madison, WI) was added (0.0033 ml/kg of milk) to provide a consistent color to the white Cheddar. The ripened milk, 31°C, was coagulated with double strength chymosin (Chymax Extra, Chr Hansen Inc., Milwaukee, WI) for 30 min at a rate of 0.1 ml/kg of milk. The coagulum was cut with 1.6-cm wire knives, and the curd and whey was allowed to rest for 5 min and then the curd plus whey was gently stirred for 10 min without added heat. The temperature was increased gradually from 31 to 33°C over 15 min and then from 33 to 38°C over an additional 15 min. The curd was continuously stirred at 38°C until the target whey draining pH of 6.35 was attained. The whey was drained and immediately pasteurized using a plate heat exchanger (three sections regeneration, heating, and cooling: Model 080-S, AGC Engineering, Manassas, VA) at 72°C for 16 s. The whey was cooled to 50°C at the exit of the pasteurizer and immediately processed with a cream separator (Model 619, DeLaval, Inc., Kansas, MO) to reduce the fat content. The fat content of the whey before separation was $0.21\% \pm 0.02$ and after separation was $0.06\% \pm 0.01$. After separation, the whey was cooled to 4°C and held overnight at $\leq 4^\circ\text{C}$. The whey was mixed and sampled directly before and

after cream separation. During cheese manufacturing, the pH of whey and cheese were measured with an electrode (model HA 405, Mettler Toledo, Columbus, OH) that was standardized at pH 6.97 and 4.03 at 38°C and kept immersed in 3M KCl at 38°C between readings in order to keep its temperature equal to the temperature of the buffers and samples. All samples were at 35 to 38°C at the time of measurement.

On the next day, the separated whey was weighed into a vat and heated to 50°C using a plate heat exchanger (Model A3, DeLaval, Inc, Kansas, MO). The separated Cheddar cheese whey was fractionated using a pilot plant UF system equipped with a polyethersulfone spiral wound membrane (Model 3838, GEA NIRO Inc., Hudson WI, nominal separation cutoff: 10,000 Daltons, surface area: 6.8 m²). The inlet pressure was 276 kPa and the retentate outlet pressure was 103 kPa with no permeate back pressure on the permeate side and the processing temperature was 50°C.

Immediately prior to processing, the spiral wound UF membrane was given a short cleaning cycle. First, the soak solution was flushed out from the system until the flush water was at neutral pH. The membrane was then washed for 20 min at 276 kPa inlet pressure and no permeate back pressure with a combination of Ultrasil 110, liquid alkaline membrane cleaner, and XY-12, liquid sanitizer, (both Ecolab Inc., Food and Beverage Division, St Paul, MN) and RO water. The wash solution was at 50°C and pH of 10 to 11. After the wash cycle was completed the membrane system was flushed with 50°C RO water until neutral pH was obtained. The membrane was cooled to < 24°C and sanitized with a solution of Ultrasil 110, liquid alkaline membrane cleaner and XY-12, liquid sanitizer, at pH 10 to 11 and a

chlorine level of 120 ppm, and RO water. This solution was circulated through the membrane for 10 min at 276 kPa inlet pressure and no permeate back pressure. The membrane was then flushed out with 50°C RO water to neutral pH and the clean water flux was determined by operating only the inlet pump with an inlet pressure of 172 kPa. The flux (kg/m²/h) was calculated based on the weight of permeate collected in 30 s and the filtration area.

Approximately 573 kg of separated whey was heated to 50°C and processed with the UF system in batch recirculation mode. When starting the process, prior to directing the retentate back to the feed tank, approximately 10 L of liquid was collected and discarded. After that the retentate was returned to the feed vat and the process continued until 34% protein concentration as a percentage of solids was reached. The flux was determined by weight every 15 min and samples of permeate and retentate in the feed vat were taken for analysis using an infrared milk analyzer (Lactoscope FTIR, Delta Instruments, The Netherlands) to control the ratio of protein to lactose. The total time of UF was approximately 110 min. The infrared was calibrated using modified milk samples as described by Kaylegian et al. (2006). After UF was complete, the UF retentate left in the feed vat was combined with the UF retentate drained from the dead volume of the UF system, mixed, and sampled. A sample of permeate from UF process was taken at the end of UF. The final concentration factor was about 5.2 X and the total time of UF processing was about 1.9 h. The final liquid UF retentate protein concentration was weighted and divided into two portions. One portion was frozen at -40°C in stainless steel freeze dryer pans in

preparation for freeze drying. The other portion was cooled to 4°C and held overnight at $\leq 4^{\circ}\text{C}$ for spray drying.

After whey processing, the UF system was cleaned as follows: first, the UF system was rinsed with two 70 L lots of 50°C RO water at 276 kPa inlet pressure and 103 kPa retentate outlet pressure with no back pressure on the permeate side. During the second rinse the recirculation pump was turned off and the inlet pressure was adjusted to 172 kPa. The fouled water flux ($\text{kg}/\text{m}^2/\text{h}$) was calculated based on the weight of permeate collected in 30 s and the filtration area. After determination of the fouled water flux the membrane was washed for 30 min with a combination of Ultrasil 110, liquid alkaline membrane cleaner, Ultrasil 01, liquid high surfactant cleaner, (both Ecolab Inc., Food and Beverage Division, St Paul, MN) and 50°C RO water at 276 kPa inlet pressure and 103 kPa retentate outlet pressure with no back pressure on the permeate side. These inlet and outlet pressures were used throughout all cleaning procedure unless indicated differently. The pH of the wash solution was 10.5 to 11. After the 30 min wash, the membrane was flushed to a neutral pH with 50°C RO water. The membrane was then washed with a combination of Ultrasil 76, liquid acid cleaner, (Ecolab Inc., Food and Beverage Division, St Paul, MN) and 50°C RO water for 30 min. The pH of the wash solution was 2.0 to 2.2. After the 30 min wash, the membrane was flushed to a neutral pH with 50°C RO water. The membrane was then washed for 30 min with a combination of Ultrasil 110, liquid alkaline membrane cleaner, XY-12, liquid sanitizer, and 50°C RO water. The pH of the wash solution was 10 to 11 and the chlorine level was 150 to 180 ppm. After the 30 min wash, the membrane was flushed to a neutral pH

with 50°C RO water. When the rinse water pH was neutral, the clean water flux was determined by operating only the inlet pump with an inlet pressure of 172 kPa. The flux (kg/m²/h) was calculated based on the weight of permeate collected in 30 s and the membrane surface area. After the clean water flux was determined the membrane was cooled to < 24°C and sanitized with a solution of Ultrasil 110, liquid alkaline membrane cleaner and XY-12, liquid sanitizer, and < 24°C RO water at pH 10 to 11 and a chlorine level of 120 ppm. This solution was circulated through the membrane for 10 min at 276 kPa inlet pressure and no permeate back pressure. The membrane was then flushed with room temperature RO water to a neutral pH. After a neutral pH was obtained, a storage solution of Ultrasil MP, soak solution, (Ecolab Inc., Food and Beverage Division, St Paul, MN) and room temperature RO water at pH 3.5 to 4.0 was circulated through the membrane for 10 min. After 10 min, the pumps were shut off and all the valves on the membrane housing were closed so that the soak solution stayed in contact with the membrane until the next processing day.

Serum Protein Concentrate manufacture

Raw whole milk was separated in the Cornell University dairy plant at 4°C using a Model 590 Air Tight Centrifuge, DeLaval Co., Chicago, IL. The raw skim milk was pasteurized (1380.6 kg/h) on the following day with a plate heat exchanger (three sections regeneration, heating, and cooling: Model 080-S, AGC Engineering, Manassas, VA) at 72°C and a holding time of 16 s. The milk was cooled to 4°C and stored refrigerated overnight at

$\leq 4^{\circ}\text{C}$. On the day of processing the pasteurized skim milk was heated to 50°C with a DeLaval Model A3 plate heat exchanger.

The milk was microfiltered by a bleed and feed process using a pilot scale, uniform transmembrane pressure MF system (Tetra Alcross M, TetraPak Filtration Systems, Aarhus, Denmark) equipped with ceramic Membralox membranes (pore diameter: $0.1\mu\text{m}$; surface area: 1.7m^2). Immediately prior to the MF processing, the MF membrane was given a short clean. The storage solution (0.74% nitric acid solution, Fisher Scientific, NJ) was flushed out of the system with room temperature RO water to reach neutral pH. The membrane was then heated with RO water up to 80°C and Ultrasil 20, liquid alkaline membrane cleaner (Ecolab Inc., Food and Beverage Division, St Paul, MN) was added to the water to reach pH 11. The solution was allowed to recirculate for 20 min. After the cleaning, the membrane was cooled down to room temperature and flushed with RO water until neutral pH was reached. The membrane was cooled down to 50°C with RO water, the clean water flux was determined and the membrane was ready for production.

Skim milk (ca 1040 kg) was fractionated at 50°C . At a concentration factor of 3X, the retentate and permeate removal rates were 45 and 90 L/h, respectively, with the transmembrane pressure in the range of 24 to 28 kPa during the processing run. The inlet pressure of retentate was about 414 kPa and the outlet pressure was about 235 kPa. MF retentate and permeate were collected continuously and cooled to 4°C as they were collected and kept refrigerated at $\leq 4^{\circ}\text{C}$. The flux of the membrane was determined every 15 min and samples of permeate and the retentate were taken for analysis by infrared spectrophotometer

(Lactoscope FTIR, Delta Instruments, The Netherlands) to monitor the percentage of SP removal. At the end of MF run the final retentate and permeate were stirred and sampled. The total time of milk processing was about 8 h. The MF permeate was cooled to 4°C and held overnight.

On the following day MF permeate (ca 670 kg) was weighed into a vat, heated to 50°C using a DeLaval Model A3 plate heat exchanger and UF processed. Prior to processing the UF membrane was cleaned following the same procedure as mentioned previously for whey processing. The MF permeate was processed using a polyethersulfone spiral wound UF membrane (Model 3838GEA, NIRO Inc., Hudson, WI) with a nominal separation cutoff of 10,000 Daltons. The conditions and parameters of UF processing of MF permeate remained the same as for whey concentration and the processing time was about 155 min at a concentration factor 6.5X. After producing the 34 % SPC liquid concentrate the UF system was cleaned as described above after 34 % WPC processing.

Spray drying

The 34% WPC, 34% SPC, and 3X MF retentate were spray dried in a spray dryer (Model 1, Niro Atomizer Inc., Columbia, MD). The feed material (ca 50 kg) was kept at or below 7°C. The spray dryer was equipped with a FU11 atomizer rotating at 23,000 rpm and the feed rate was 16 kg/h. The inlet temperature was 200°C and the outlet temperature was 95°C. The powder from the first 10 min of the run was discarded. Thereafter, the dried product was collected and packaged every half hour. The total time of the drying run was approximately 3.2 h. The material for flavor (sensory and volatile) examination was

packaged in VWR Trace Clean 950 ml amber glass WM jars with PTFE-lined caps (VWR International, West Chester, PA) and the material for functional testing was packaged in high density polyethylene bottles with screw top lids (Consolidated Plastics, Twinsburg, OH). The material for chemical and microbiological analysis was stored at 21°C in clear 84-ml plastic vials (Capitol Vial, Auburn, AL).

Freeze drying

The liquid 34 % WPC and 34% SPC were held at -40°C until freeze dried. The freeze drying was carried out in a freeze dryer (Model 101-SRC-5, VirTis Company, Gardiner, NY). The material for freeze drying was transferred from -40°C freezer to the freeze drier where the shelves had been cooled to -30 to -40°C. When the vacuum reached approximately *100 microns*, the shelf temperature was raised to 30 to 35°C and was maintained throughout the drying. The time of freeze drying was approximately 72 to 96 h for WPC and for SPC.

After freeze drying, the dried material was removed from the drier pans and blended. The material for flavor (sensory and volatile) examination was packaged in 950 ml amber glass jars with a PTFE-lined caps and the material for functional testing was packaged in high density polyethylene bottles with screw top lids. The material for chemical and microbiological analysis was stored 21°C in clear 84-ml plastic vials.

Chemical analyses

Samples of milk for cheese processing were analyzed using an infrared spectrophotometer (IR) (Lactoscope FTIR, Delta Instruments, The Netherlands) for fat

content and true protein content (Kaylegian et al., 2006). The fat content of unseparated and separated whey was determined by ether extraction (AOAC, 2000; 33.2.26, 989.05).

The samples of final liquid 34 % WPC and liquid 34 % SPC were analyzed for fat, TS, total N, and NPN content using ether extraction (AOAC, 2000; method number 989.05; 33.2.26), forced air oven drying (AOAC, 2000; method number 990.20; 33.2.44), Kjeldahl (AOAC, 2000; method number 991.20; 33.2.11), and Kjeldahl (AOAC, 2000; method number 991.21; 33.2.12), respectively. The glycomacropeptide (which is soluble in 12% TCA) content of WPC was calculated as the absolute difference of nonprotein nitrogen between SPC and WPC. All samples were analyzed fresh. The six commercial 34 % WPC samples were reconstituted to 10% solids and the liquids were analyzed for fat and TN by the method indicated above. The samples of 34% WPC and 34% SPC powders were reconstituted to 10% solids and pH was measured with an electrode (model Electrolyte 9823, Mettler Toledo, Columbus, OH) that was standardized at pH 7.01 and 4.00 at RT (22°C).

The spray and freeze dried 34 % WPC and SPC powder, and 6 commercial 34 % WPC powders were analyzed for moisture using a vacuum oven drying for 5 h (Wehr and Frank, 2004; 15.111).

Microbiological analyses

Samples of pasteurized skim milk for cheese making before and after standardization, pasteurized whey from cheese making, pasteurized skim milk for MF, liquid WPC, SPC and 3X MF retentate, spray dried and freeze dried powders were taken for total bacterial and coliform counts. (Wehr and Frank, 2004; 6.020 and 7.020).

Color analysis of powders

The Hunter L, a, b values for the spray and freeze dried powders were determined in duplicate with a MacBeth Color-Eye spectrophotometer (Model 2020; Kollmorgen Instruments, Corp., Newburgh, NY) with Optiview software from the same company. Hunter values were computed from the diffuse reflectance data in the 360 to 740 nm range, at 20-nm intervals, based on illuminant A. The measurements were done at 23 to 25°C.

Serum and whey proteins

Products were shipped on ice by overnight carrier to North Carolina State University (Raleigh, NC). Subsamples (1 kg) were stored in glass jars at -80°C and analyzed within three months of receipt. For all analyses, samples were reconstituted at 10% solids. Sensory analysis samples were reconstituted using deodorized water (prepared by boiling 4L of distilled water until its volume was decreased by one third). Instrumental analysis samples were reconstituted using HPLC water (EMD Chemicals Inc., Gibbstown, NJ). Six commercial WPC34 (less than 2 mo old) were also received on 2 separate occasions and reconstituted using the same methods above for comparison to pilot plant WPC34 and SPC34.

Descriptive sensory analysis

Sensory testing was conducted in compliance with NCSU Institutional Review Board (IRB) for human subjects approval. A trained sensory panel (n=10, 7 females, 3 males, ages 22-37 y) evaluated the flavor attributes of the reconstituted serum and whey proteins using a

previously published lexicon for dried dairy ingredients (Drake et al., 2003). Each panelist had over 150 h of experience with descriptive analysis of dried dairy ingredients, and additional training with WPC and SPC aroma and flavor. Consistent with Spectrum™ descriptive analysis training, panelists were presented with reference solutions of sweet, sour, salty, and bitter tastes to learn to use the universal intensity scale (Meilgaard et al., 1999; Drake et al., 2003b). Panelists then evaluated and discussed flavor attributes of rehydrated dairy ingredients with a focus on WPC80 and WPC34 using an established sensory language (Drake et al., 2003a; Russell et al., 2006; Wright et al., 2009). Analysis of variance of data collected in preliminary sessions confirmed that the panel and the panelists could consistently identify and scale flavor attributes. Attribute intensities were scaled using the 0 to 15-point universal intensity scale characterized by the Spectrum™ descriptive analysis method (Meilgaard et al., 1999; Drake et al., 2003a).

Reconstituted products (ca 30 ml) were dispensed into lidded 58 ml soufflé cups with three-digit codes and stored at 3°C overnight. Products were tempered to 20°C and served at this temperature with spring water and unsalted crackers for palate cleansing. Panelists evaluated each sample individually in booths in a positive air pressure room dedicated to sensory analysis. Each product replication was evaluated by each panelist in duplicate in a randomized balanced block design on separate occasions. Products were scored using paper ballots or computerized ballots using Compusense™ version 5.0 (Compusense, Guelph, Canada).

Volatile compound extraction

SPME GC-MS of WPC/SPC powders

SPME GC-MS was conducted using a modified method of Wright et al. (2006). Spray dried and freeze dried powders were reconstituted at 10 % solids, with 10 % NaCl, and 10 ul internal standard solution (2-methyl-3-heptanone in methanol at 81 ppm) in 20 ml autosampler vials with steel screw tops containing silicone septa faced in Teflon (Microliter Analytical, Sawanee, FL). Samples were injected using a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) attached to an Agilent 6890N GC with 5973 inert MSD (Agilent Technologies Inc., Santa Clara, CA). Samples were maintained at 5°C prior to fiber exposure. Samples were equilibrated at 40°C for 25 min before 30 min fiber exposure of a 1 cm DVB/CAR/PDMS fiber at 31 mm with 4 sec pulsed agitation at 250 rpm. Fibers were injected for 5 min at a depth of 50 mm. The GC method used an initial temperature of 40°C for 3 min with a ramp rate of 10°C/min to 250°C held for 5. SPME fibers were introduced into the split/splitless injector at 250°C. An Rtx-5ms column (Rtx-5ms 30 m length \times 0.25 mm inner dia \times 0.25 μ m film thickness; Restek, Bellefonte, Pa., U.S.A) was used for all analysis at a constant flow rate of 1 ml/min. Purge time was set at 1 min. The MS transfer line was maintained at 250°C with the Quad at 150°C and Source at 250°C.

Direct solvent extraction of WPC/SPC powders.

Solvent extraction was conducted using the modified methods of Carunchia Whetstine et al. (2005a). One hundred grams of powder were divided into four Teflon bottles (Nalgene, Rochester, NY, capacity of 250 ml) with Tefzel closures (Nalgene,

Rochester, NY). Five ml of HPLC water (Gibbstown, NJ) was added to each bottle, along with 20 μ l of internal standard at 81 ppm (2-methyl-3-heptanone, 2-methylpentanoic acid in methanol, Sigma Aldrich, Milwaukee, WI). Seventy-five ml of diethyl ether (EMD Chemicals Inc., Gibbstown, NJ) was added to each bottle. The bottles were then shaken for 30 min on a Roto mix (Type 50800: Thermolyne Dubuque, IA) at high speed and centrifuged at 1459 \times g for 10 min in order to separate the solvent phase from the mixture. After centrifugation, the solvent phase containing the extracted volatile components was removed by pipette to an amber glass jar. This procedure was repeated twice with the successive addition of 40 ml of diethyl ether to each bottle, each time. After the third round of solvent was removed, the bottles were centrifuged a fourth time and any remaining solvent was removed. The solvent extracted from the bottles was then concentrated to 150 ml using a Vigreux column placed inside a water bath at 40°C.

Solvent assisted flavor evaporation (SAFE).

Volatile compounds from serum and traditional whey protein extracts were distilled using SAFE (Ace Glassware, Vineland, NJ). The assembly used was similar to that described by Engel et al. (1999). A rough pump/diffusion pump combination was used as the vacuum source. The SAFE apparatus was connected to a separate receiving tube and a waste tube, both submerged in liquid nitrogen. The distillation procedure was carried out over 2 h under vacuum (10^{-5} Torr). The liquid sample was poured into the SAFE apparatus and introduced drop-wise into the vacuum until all of the liquid extract had been placed

under vacuum conditions. The SAFE apparatus was kept at 40°C with a circulating water bath.

Phase Separation

Following SAFE, the distillate was concentrated under a nitrogen stream to 20 ml. The concentrated distillate was then washed twice with 3 ml of sodium bicarbonate (Fischer Scientific, Fairlawn, NJ), mixed thoroughly and the bottom (water phase) removed to a separate test tube. The concentrated solvent was then washed with 2 ml of saturated sodium chloride solution three times. Each time, the solution was mixed thoroughly and the water phase removed to the same test tube. The upper layer (ether) containing the neutral/basic fraction was collected, dried over anhydrous sodium sulfate (VWR International, West Chester, PA) and concentrated to 0.5 ml under a gentle stream of nitrogen gas. Acidic volatiles were recovered by acidifying the bottom layer (aqueous phase) with hydrochloric acid (18 w/v %) (Sigma Aldrich, Milwaukee, WI) to a pH of 2-2.5 and extracting the sample three times with 15 ml ethyl ether. The acidified extract was dried over anhydrous sodium sulfate before concentration to 0.5 ml under a nitrogen gas stream.

Gas chromatography-mass spectrometry (GC-MS) of SAFE extracts

An Agilent 6890N GC with 5973 inert mass selector detection (MSD) (Agilent Technologies Inc., Santa Clara, CA) was used to analyze neutral basic solvent extracts. Separations were performed on a fused silica capillary column (Rtx-5ms 30 m length \times 0.25 mm inner dia \times 0.25 μ m df; Restek, Bellefonte, PA). A carrier gas (helium) at a constant flow rate of 1ml/min was used. The oven temperature was programmed from 40°C to 200°C

at a rate of 5°C per min with initial and final hold times of 5 min, respectively. Each extract (2ul) was injected in the split less mode. Duplicate analyses were performed on each sample.

A Varian CP-3380 GC with Saturn 2000 inert mass selector detection (MSD) (Varian Inc., Palo Alto, CA) was used to analyze acidic solvent extracts. Separations were performed on a fused silica capillary column (Rtx-wax 30 m length \times 0.25 mm inner dia \times 0.25 μ m df; Restek, Bellefonte, PA). A carrier gas (helium) at a constant flow rate of 1ml/min was used. The oven temperature was programmed from 40°C to 200°C at a rate of 5°C per min with initial and final hold times of 5 min, respectively. Each extract (2ul) was injected in the split less mode. Duplicate analyses were performed on each sample.

Gas chromatography-olfactometry (GC-O)

GC-O analysis was performed on neutral/basic and acidic extracts from SAFE using an HP 5890 series II gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif., U.S.A.) equipped with a flame ionization detector (FID), a sniffing port, and a split/splitless injector. Neutral/basic and acidic fractions were analyzed from each solvent extraction. Two ul were injected onto a polar capillary column (Rtx-Wax 30 m length \times 0.25 mm inner dia \times 0.25 μ m film thickness; Restek, Bellefonte, Pa., U.S.A.) and a nonpolar capillary column (Rtx-5ms 30 m length \times 0.25 mm inner dia \times 0.25 μ m df; Restek). Column effluent was split 1:1 between the FID and the sniffing port using deactivated fused silica capillaries (1-m length \times 0.25-mm inner dia). The GC oven temperature was programmed from 40°C to 200°C at a rate of 10 °C/min with an initial hold for 3 min and final hold of 20 min. The FID was maintained at a temperature of 300°C. The sniffing port was maintained at a temperature 105°C. The

sniffing port was supplied with humidified air at 30 ml/min. The post-peak intensity of aroma active compounds was evaluated (Grosch 1993; van Ruth, 2001). Each extract was sniffed in duplicate by two experienced sniffers, each with >50 h training on GC-O of dairy products on both polar and nonpolar columns.

Aroma Extract Dilution Analysis (AEDA)

AEDA was performed under the same conditions as those for post peak intensity GC-O. Representative samples of each of the pilot plant products (SDWPC34, SDSPC34, FDWPC34, FDSPC34) were analyzed. NB fractions were injected onto the DB-5ms (Rtx-5ms 30 m length \times 0.25 mm inner dia \times 0.25 μ m df; Restek, Bellefonte, Pa., U.S.A.) capillary column and acid fractions were injected onto the DB-WAX (Rtx-Wax 30 m length \times 0.25 mm inner dia \times 0.25 μ m film thickness; Restek, Bellefonte, Pa., U.S.A.) capillary column. Each sample was diluted stepwise at a ratio of 1:3 (vol/vol) with diethyl ether. Samples were evaluated by two experienced sniffers until no odors were detected. The greatest dilution in which a compound was sniffed was reported as the flavor dilution (\log_3 FD) factor (Grosch, 1993).

Volatile Compound Identification

Volatile compounds from SAFE and SPME were identified using the NIST 2005 library of spectra and comparison of spectra of authentic standards injected under identical conditions. Relative abundance of compounds was calculated using the calculated recovery of the internal standard concentration to determine relative concentrations of each compound. Retention indices were calculated using an alkane series (Sigma Aldrich, Milwaukee, WI)

(Van den Dool et al., 1963). For aroma active compound identity verification, retention index and aroma properties were also compared to authentic standards injected under identical conditions.

Statistical analyses

To determine if there were significant differences in UF flux, color, composition, sensory, or functional properties between WPC and SPC, all data were analyzed by ANOVA using the Proc GLM procedures of SAS (SAS version 8.02, 1999-2001, SAS Institute Inc., Cary, NC). The GLM model for flux data after 60 min of UF and for the direct comparison of composition from WPC34 and SPC34 produced in this study was dependent variable = treatment (WPC or SPC) + replicate + error. The GLM model for analysis of sensory data for the direct comparison of composition from WPC and SPC produced in this study was treatment (WPC, SPC) + replicate + panelist + error. The GLM model for comparison of composition among samples produced in this study and the commercial powders was dependent variable = treatment (WPC, SPC, commercial powders 1, 2, 3, 4, 5, 6) + error. For descriptive sensory analysis and volatile compound relative abundance, the GLM model was dependent variable = treatment (WPC, SPC, commercial powders 1, 2, 3, 4, 5, 6) + replicate + panelist (sensory only) + error. If the F-test for the model was significant (i.e., $P < 0.05$), then the least square means were compared to determine if there were significant differences. Principal component analysis using the Proc Princomp command using the correlation matrix was also applied to sensory and volatile component data of products produced in this study and commercial powders to visualize how products were differentiated

across sensory attributes or volatile components (SAS version 9.2, SAS Institute Inc., Cary, NC).

RESULTS

Processing

The largest decrease in the UF flux occurred during the initial 30 min of the processing for both for Cheddar cheese whey and MF permeate. After initial 15 min of the run, the flux decreased approximately 33 %, with additional 10 % decrease during the subsequent 15 min. No difference in UF flux when processing whey ($15.71 \pm 0.23 \text{ kg/m}^2/\text{h}$) or MF permeate from skim milk ($14.62 \pm 0.74 \text{ kg/m}^2/\text{h}$) using a spiral wound 10 kDa polyethersulfone membrane was detected ($P > 0.05$). All 34% WPC and 34 % SPC spray and freeze dried products conformed to the Dry Whey Grade Standards (ADPI, 2008) of $<30,000 \text{ cfu/g}$ SPC and $<10 \text{ cfu/g}$ coliform. The actual maximum counts for our products were 3300 cfu/g for SPC and $<1 \text{ cfu/g}$ for coliform.

Composition

No significant difference in the total solids content of SPC and WPC liquids was detected (Table 2.1). The fat content of liquid WPC (Table 2.1) was higher ($P < 0.05$) than the fat content of liquid SPC, because most of the fat left in the skim milk by the cream separator was retained in the MF retentate during MF processing of milk using a membrane with a pore size of $0.1 \mu\text{m}$. The fat that was not removed from the whey by the cream separator was concentrated in the liquid WPC by the UF. The difference in fat content

between SPC and WPC may influence the sensory and functional properties. WPC contained more ($P < 0.05$) nonprotein nitrogen than SPC (Table 2.1), which was due to the presence of glycomacropeptide (GMP) in the whey (which is soluble in 12 % TCA) as a result of κ -casein hydrolysis by rennet. The true protein (TP) content, expressed as a difference of TN minus nonprotein nitrogen, was higher ($P < 0.05$) for SPC liquid (Table 2.1) than WPC liquid.

There was no significant difference detected ($P > 0.05$) in moisture content of WPC versus SPC (Table 2.2) within each drying method. Spray dried WPC contained more fat and glycomacropeptide ($P < 0.05$) on a dry basis than SPC, while the SPC contained more protein on a TN basis (Table 2.3). The pH of the reconstituted liquid WPC was lower ($P < 0.05$) than liquid SPC (Table 2.3) due to the lactic acid from cheese making that was present in the WPC. The lower pH of the WPC would be expected to cause some differences between WPC and SPC in mineral content. The calcium content of the WPC powder on a dry basis was higher than SPC ($P < 0.05$) (Table 2.4). A higher calcium content in WPC would be expected because calcium was released from casein micelles into the cheese whey as pH decreases due to lactic acid production by the starter culture during cheese making. There was a trend for higher phosphorous content in WPC ($P < 0.07$).

Commercial 34% whey protein concentrates were obtained from six different cheese plants and their composition was compared with experimental 34% SPC and WPC. No significant difference was detected ($P > 0.05$) in moisture content among powders (Table 2.5). Spray dried 34% WPCs produced commercially had an average fat content of 3.08%

and were higher ($P > 0.05$) in fat than the SPC (0.25%) and WPC (1.93%) produced in this study. All powders contained more than 34% protein. Most of the commercial powders had significantly higher total nitrogen content than experimental 34% SPC and WPC. The pH of 34% WPC produced in our study was similar to pH of all of the commercial WPCs. The lower fat content of SPC may have impacts on flavor and functionality. The 34% SPC had the highest pH but was not different ($P > 0.05$) from the pH of several of the commercial WPCs. The WPC from plants 1, 2 and 3 were from Cheddar cheese whey that had been bleached, the WPC from plants 4 and 5 were from Mozzarella whey that was not bleached and the WPC from plant 6 was a blend of Mozzarella and bleached Cheddar whey. It is not known if the commercial WPC products were neutralized.

A typical SDS-PAGE gel for 34% WPC and SPC is shown in Figure 2. 2. There was a significant difference in relative proportion of casein to serum protein between SPC and WPC (96.03 and 97.11%, 3.97 and 2.89%, respectively) (Table 2.6). The difference resulted mostly from the presence of α_{s1} -casein (band Cn1 in Figure 2. 2) in SPC, whereas WPC did not contain any α_{s1} -casein. Both 34% SPC and 34% WPC contained casein proteolysis products (band Cn2 in Figure 2. 2), however, a higher content of Cn2 was found in SPC (3.53 vs 2.89 for SPC and WPC, respectively). Verdi et al. (1987) studied the somatic cell associated proteolysis and presumed that as a result of plasmin action on β -casein, the casein-proteolysis products consisted predominantly of γ -caseins. Based on comparison of the mobility of band Cn2 with the mobility of proteolysis products reported by Verdi et al. (1987), it is likely that Cn2 represents secondary proteolysis products of γ_1 -casein = amino

acids 28 to 209 of β -casein (MW 20 520 Da) and a fragment of α_{s1} -casein (MW 20 500 Da) (Eigel and Keenan, 1979). Significant difference in β -lactoglobulin to α -lactalbumin proportions was detected between WPC and SPC (Table 2.7) with higher β -lactoglobulin content estimated for WPC. The typical proportions of β -lactoglobulin to α -lactalbumin for the milk run on the same gels (not milk used in this study) were 78.07 to 21.93 and were similar to WPC. The presence of GMP in WPC and not SPC (Table 2.3) and the difference in fat content may have more impact on functional properties than the small differences in the proportions of β -lactoglobulin to α -lactalbumin between SPC and WPC.

Color

There was an impact ($P < 0.05$) of drying method (spray drying versus freeze drying) on the L, a, b values of the powders (Table 2.8). Freeze dried powders were less white (i.e., lower L-value) and more brown ($P < 0.05$) than spray dried powder for both WPC and SPC. This was not expected. The longer time of drying for the freeze drying versus the spray drying and a shelf temperature of 35°C during the freeze drying process probably caused more non-enzymatic browning in the freeze dried powders than in the spray dried powders. No differences ($P > 0.05$) in L, a, b color values were detected between WPC and SPC powders within the spray dried or freeze dried powders (Table 2.9), however when the powders were reconstituted in water at 10% solids the liquid solutions produced from the 34% WPC and SPC powders looked very different. The liquids produced from the SPC powders were clear and the liquids produced from the WPC powders were cloudy (Figure 2.

3). These visual differences may be due to the differences in fat content between the two powders.

Sensory Analysis

Serum and whey proteins

Both WPC34 and SPC34 products were characterized by low flavor intensities (Table 2.10) all of which have been previously reported in dried whey ingredients (Drake et al., 2003b, 2008; Wright et al., 2008). Sweet aromatic, cooked flavor and sweet taste were the most intense attributes found in both of the pilot plant products. Diacetyl flavor was absent from SPC34, but was detected in the WPC34. Diacetyl flavor, associated with the chemical compound diacetyl, may be due to starter culture fermentation and has been previously documented in the volatile profiles of sweet whey powders and WPC80 (Mahajan et al., 2004; Carunchia Whetstine et al., 2005a). Both products also displayed low intensities (scores of 1 on a 15 point scale) of cereal flavors, and WPC34 also had a low but detectable cardboard flavor.

Spray dried and Freeze dried Samples

The process of drying the products by either spray drying or freeze drying method had minimal effects on the sensory profiles of the pilot plant products (Table 2.11). Previous research has focused on functional properties of freeze-dried and spray-dried samples. Cooked/milky flavor was significantly lower in SD products compared to FD products while sweet taste was higher in SD products compared to FD products. Cooked/milky flavors are

typically associated with Maillard browning and caramelization products. It is possible that the process of spray drying drives off volatiles and decreases perception of cooked/milky flavor, while also causing increased caramelization and increased sweetness. Alternatively, it is possible that the prolonged time for freeze drying the samples at 35C, just as color was impacted, also resulted in additional nonenzymatic browning which imparted additional cooked flavor. Cardboard flavor was also detected in both freeze dried products and only detected in spray dried WPC which may also have been due to the prolonged time required to freeze dry the samples. Typically freeze drying is a more costly process on a large scale (Snowman et al., 1997). However, spray drying can be difficult to perform on a small sample size in a bench top process. Since flavor properties of FD and SD products were similar, FD may serve as an acceptable alternative for screening bench top processes for flavor.

Commercial and Pilot plant Products

Commercial WPC34 were also evaluated for comparison to pilot plant products. The six commercial samples demonstrated similar aroma and flavor characteristics as the pilot plant products but generally with greater intensities (Table 2.12). One exception was cereal flavor which was absent in the commercial WPC34 but present in pilot scale products. This flavor has been previously documented in commercial WPC80 (Russell et al., 2006) so it was not unique to the pilot plant products. The principal component analysis (PCA) biplot of the sensory profiles (Figure 2. 4) further demonstrated differences between the pilot plant products and commercial products. Previous studies have demonstrated wide variability

among commercial fluid whey, whey powders and whey proteins (Carunchia Whetstine et al., 2003; Karagul-Yuceer et al., 2003; Mahajan et al., 2004; Carunchia Whetstine et al., 2005a). These differences were attributed to different milk supplies, whey sources, and processing parameters as well as minor but distinct differences in composition and these may also represent sources of difference between pilot plant and commercial products. Fat content of commercial samples was higher than the fat content in pilot products (Table 2.5). The higher fat content may lead to a higher amount of lipid oxidation products, purportedly responsible for many of the off-flavors found in whey proteins (Morr et al., 1991; Carunchia Whetstine et al., 2005a).

Instrumental Volatile Analysis:

SPME

Selected volatile compounds in commercial and pilot scale products were quantified by SPME (Tables 2.13, 2.14). Compounds were selected based on previous literature and because they represented a range of lipid oxidation or fermentation (diacetyl) compounds and they were consistently detected within quantification limits from three or more whey proteins. SD WPC had higher concentrations of diacetyl, acetic acid, pentanal, hexanal, heptanal, octanal, and nonanal compared to SD SPC while SD SPC had higher concentrations of decanal (Table 2.13). Diacetyl, and acetic acid were not detected in the SD SPC34. These compounds are strongly associated with cheese production. Starter culture fermentation has also been associated with lipid oxidation flavors and lipid oxidation volatile compounds in fluid cheese whey (Tomaino et al., 2004; Gallardo Escamilla et al., 2005).

The higher concentration of lipid oxidation compounds in the SD WPC compared to SD SPC was not surprising, given the higher fat concentration in the WPC (Table 2.3).

Concentrations of 2,3-butanedione were higher in FD WPC compared to SD WPC, respectively, and lipid oxidation compounds (pentanal, hexanal, and nonanal) were higher in FD SPC compared to SD SPC. It is possible that the longer time to freeze dry the powders allowed or promoted lipid oxidation. Cardboard flavors were detected (at threshold) in FD SPC but not in SD SPC (Table 2.11), and lipid oxidation products have been associated with cardboard flavors in whey and whey protein (Tomaino et al., 2004; Wright et al., 2009) which is in agreement with our results.

Commercial products were characterized by higher total concentrations of volatile compounds based on peak areas (results not shown) and generally had higher concentrations of pentanal, hexanal, and heptanal compared to pilot scale products manufactured in this study (Table 2.15, Figure 2. 5). SD SPC34 and SD WPC34 were distinct from commercial products and there was a large amount of variability among the commercial samples (Table 2.15, Figure 2. 5). These results are consistent with sensory profiles of these products as well as the differences in composition and different processing histories. Both sensory and instrumental volatile compound variability among commercial samples has been previously documented among commercially produced whey proteins (Drake et al., 2008; Wright et al., 2009; Carunchia Whetstine et al., 2005).

GC-O

GC-O was conducted on the SD and FD manufactured pilot scale powders to more closely evaluate and compare flavor-contributing compounds. Twenty-nine aroma-active compounds were detected in the pilot plant SD and FD WPC34 and SPC34 (Table 2.16). The compounds identified were aldehydes, ketones, esters, sulfur-containing compounds, and free fatty acids. Of these compounds, fourteen were positively identified by mass spectra, retention index, and odor properties of authentic standards, 13 compounds were tentatively identified by comparing retention index (RI) and odor properties with authentic standards, 1 was tentatively identified by RI and previously published data and 1 remained unknown. These compounds are lipid or protein oxidation products and have been previously documented in dried whey products (Gallardo-Escamilla et al., 2005; Mahajan et al., 2004; Wright et al., 2006; Carunchia-Whetstine et al., 2005a; Javidipour et al., 2008; Wright et al., 2008). It is important to keep in mind that AEDA is a semi-quantitative technique and therefore the values do not represent actual concentrations of compounds, only their aroma activity in the extracts. As such, AEDA results provide a snapshot or profile of the most potent aroma (flavor) contributing compounds in a given extracted sample (FD WPC, SD WPC, FD SPC, SD SPC) (Audouin et al., 2001). \log_3 FD factors for the same compound differing by equal to or more than 2 \log_3 are considered suggestive of concentration differences. Similarly, SAFE is a different volatile compound extraction approach and recovers different classes of compounds compared to SPME so that compounds not recovered by SPME may be recovered by SAFE with GC-O.

1-octen-3-one, (E, Z)-2-6-nonadienal, (E)-2-undecanal, and tridecanal, all lipid oxidation compounds, were detected in all 4 products (Table 2.16). Other compounds were variable or were specific to FD/SD products or WPC/SPC. Five sulfur containing compounds (dimethyl disulfide, dimethyl trisulfide, methional, dimethyl tetrasulfide, thenythiol) were identified in the pilot whey products. Each of these compounds with the exception of dimethyl tetrasulfide and thenythiol have previously been documented in whey products (Wright et al., 2006; Wright et al., 2008; Mortenson et al., 2008; Carunchia-Whetstine et al., 2005; Mahajan et al., 2004). These sulfur containing compounds have aromas associated with garlic, cabbage, and potato. Methional has been identified in dairy products as a Strecker degradation product formed from the degradation of methionine. Dimethyl disulfide and dimethyl trisulfide are degradation products of sulfur-containing amino acids (Wright et al., 2006). Thenythiol has been demonstrated to be a reaction product of hydrogen sulfide (product of Strecker degradation of amino acid and 2-furfural carbohydrate caramelization product) in meat (Shibamoto, 1980). Dimethyltetrasulfide, which has a garlic aroma, and has been identified in Cheddar cheese, is the result of further degradation of methionine (Milo et al., 1997).

Some compounds were more prevalent (based on FD factors) in SD products compared to FD products. Specifically, 2/3methylbutanal, dimethyl sulfide, and dimethyl trisulfide were more apparent in SD products compared to FD products. These differences may reflect the extreme heat that products are exposed to during spray drying. Thermal denaturation of the two main whey proteins β -lactoglobulin and α -lactalbumin, occurs

between 50°C and 75°C causing the protein to unfold and unmask the SH group (van der Linden et al., 1999). The increase in sulfur compounds in the SD products is most likely caused by their greater exposure of the SH groups from denaturation from the higher temperature exposure during drying (inlet temp 200°C, outlet temp 95°C). In contrast, hexanal and phenethanol were only detected by GC-O in FD products and 2-methoxy phenol and (E, Z)-2-6-nonadienal were present at higher FD factors on average in FD products compared to SD products. Hexanal and (E, Z)-2-6-nonadienal are lipid oxidation products while 2-phenethanol and 2-methoxyphenol are formed by the Strecker degradation of aromatic amino acids, especially phenylalanine (Singh et al., 2003; Carunchia-Whetstine et al., 2005b). Once again, the prolonged time required for freeze-drying at 50°C may have promoted formation of these compounds.

Acetic acid, diacetyl, butanoic acid, and methional generally had higher FD factors in WPC or were not detected in SPC. All four of these compounds are potent odorants in Cheddar cheese and likely represent differences due to starter culture fermentation (Singh et al., 2003). 1-octen-3-one, thenylthiol, dimethyl tetrasulfide and 2-methylbutyl acetate were more prevalent in SPC. 1-octen-3-one is a lipid oxidation compound, previously reported in whey products (Carunchia Whetstine et al., 2005) while thenylthiol, dimethyl tetrasulfide, 2-methylbutyl acetate have not been previously identified in dried whey, however they have been identified in other dairy products including ewe's milk cheeses (Larrayoz et al., 2001), Cheddar cheese (Carunchia-Whetstine et al., 2005; Avsar et al., 2005), Parmesan cheese (Qian et al., 2003) and Gorgonzola cheese by Moio et al. (2000).

CONCLUSIONS

Manufactured SPC34 and WPC34 were distinct in composition. Although color differences were not evident between the two powders, when rehydrated at 10% solids, SPC solutions were clear while WPC solutions were cloudy. Sensory differences were minor, but distinct as were volatile compound differences. However, both products were bland in flavor compared to commercially manufactured WPC34. Lipid oxidation products were also generally lower in the products manufactured in this study compared to commercially manufactured WPC34. Our results suggest that when manufactured under controlled conditions in a similar manner, there are few sensory differences between SPC34 and WPC34, but distinct compositional differences and differences in physical properties which may influence functionality. Further, flavor (sensory and instrumental) properties of both pilot-scale manufactured protein powders were distinct from commercial powders suggesting the role of other influencing factors.

ACKNOWLEDGEMENTS

Funded in part by Dairy Management, Inc. (Rosemont, IL). The use of tradenames does not imply endorsement nor lack of endorsement by those not mentioned.

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Table 2.1. Mean (n = 3) composition (percent by weight) of the serum protein concentrate (SPC) and whey protein concentrate (WPC) liquids after UF and before drying.

	Total Solids	Fat	TN ¹	NPN ²	TP ³
SPC	9.71	0.02 ^b	3.43	0.23 ^b	3.20 ^a
WPC	9.68	0.19 ^a	3.33	0.37 ^a	2.96 ^b
R-Square	0.59	0.99	0.87	0.99	0.97
SE	0.068	0.003	0.026	0.003	0.023

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹TN = total protein x 6.38.

²NPN = nonprotein nitrogen x 6.38.

³TP = true protein (TN minus NPN).

Table 2.2. Mean (n = 3) moisture content of spray dried and freeze dried serum protein concentrate (SPC) and whey protein concentrate (WPC).

Treatments	Moisture (%)	
	Spray dried	Freeze dried
SPC	4.60	3.33
WPC	4.08	3.62
R-Square	0.24	0.38
SE	0.55	0.40

Table 2.3. Mean (n = 3) composition (percent by weight) of spray dried serum protein concentrate (SPC) and whey protein concentrate (WPC) calculated on a dry basis and pH.

	Fat	TN ¹	NPN ²	TP ³	GMP ⁴	pH ⁵
SPC	0.25 ^b	35.29 ^a	2.37 ^b	32.92 ^a	0.00 ^b	6.74 ^a
WPC	1.93 ^a	34.38 ^b	3.82 ^a	30.56 ^b	1.48 ^a	6.44 ^b
R-Square	0.99	0.99	0.99	0.99	0.99	0.99
SE	0.03	0.06	0.03	0.03	0.004	0.011

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹TN = Total nitrogen x 6.38.

²NPN = Nonprotein nitrogen x 6.38, includes GMP for WPC.

³TP = True protein (TN minus NPN), for WPC this does not include GMP.

⁴GMP = Glycomacropeptide, calculated as NPN of WPC minus NPN of SPC.

⁵pH of the reconstituted powders (10% w/v).

Table 2.4. Mean (n = 3) mineral composition (percent by weight) of spray dried serum protein concentrate (SPC) and whey protein concentrate (WPC) as calculated on a dry basis.

	Ca	P	K	Mg	Na	S
SPC	0.44 ^b	0.56	1.89	0.12	0.43	0.57
WPC	0.55 ^a	0.63	1.93	0.12	0.45	0.52
R-Square	0.99	0.95	0.81	0.48	0.84	0.91
SE	0.006	0.008	0.022	0.003	0.005	0.009

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

Table 2.5. Composition (percent by weight) and pH of spray dried (n = 3) serum protein concentrate (SPC), spray dried (n = 3) whey protein concentrate (WPC) produced in this study and six commercial (n = 2) 34% whey protein concentrates.

Source	Product	Moisture	Fat	TN	pH ¹
Experimental	SPC	4.60 ^a	0.25 ^e	35.29 ^{cd}	6.74 ^a
Experimental	WPC	4.08 ^a	1.93 ^f	34.38 ^d	6.44 ^{bcd}
Commercial	1	5.05 ^a	3.41 ^{ab}	39.08 ^a	6.13 ^d
WPCs	2	4.93 ^a	3.52 ^a	36.16 ^{bc}	6.42 ^{acd}
	3	4.86 ^a	3.23 ^{abc}	36.53 ^{bc}	6.28 ^{cd}
	4	5.84 ^a	2.81 ^{cd}	36.81 ^b	6.64 ^{ab}
	5	5.63 ^a	3.03 ^{bc}	35.61 ^{bcd}	6.68 ^{ab}
	6	5.08 ^a	2.50 ^d	36.29 ^{bc}	6.57 ^{abc}
R-Square		0.65	0.98	0.89	0.73
SE Commercial		0.3745	0.1371	0.4312	0.1113
SE Experimental		0.3058	0.1119	0.3521	0.0909

¹pH of the reconstituted powders (10% w/v).

^{a,b,c,d,e,f} Means in the same column not sharing a common superscript are different ($P < 0.05$).

Table 2.6. Mean ($n = 3$) relative proportions of casein to serum proteins for 34% WPC and 34% SPC powders by the densitometry analysis of the SDS-PAGE gels.

	Casein	Serum Proteins
SPC	3.97 ^a	96.03 ^b
WPC	2.89 ^b	97.11 ^a
R-Square	0.89	0.89
SE	0.11	0.11

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

Table 2.7. Mean ($n = 3$) relative proportions of β -lactoglobulin and α -lactalbumin for 34% WPC and 34% SPC powders by the densitometry analysis of the SDS-PAGE gels.

	β -lactoglobulin	α -lactalbumin
SPC	77.24 ^b	22.76 ^a
WPC	78.54 ^a	21.46 ^b
R-Square	0.80	0.80
SE	0.16	0.16

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

Table 2.8. Mean (n = 3) Hunter L, a, b color values of spray dried and freeze dried powders.

Treatments	Hunter values		
	L	a	B
Spray dried	90.15 ^a	-0.46 ^b	6.16 ^b
Freeze dried	83.37 ^b	1.55 ^a	11.41 ^a
R-Square	0.99	0.99	0.99
SE	0.19	0.03	0.21

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

Table 2.9. Mean (n = 3) Hunter L, a, b color values of spray dried and freeze dried serum protein concentrate (SPC) and whey protein concentrate (WPC).

Treatments	Spray dried			Freeze dried		
	L	a	b	L	A	b
SPC	90.05	-0.50	5.92	83.59	1.32	10.68
WPC	90.25	-0.42	6.40	83.15	1.77	12.15
R-Square	0.71	0.94	0.79	0.52	0.95	0.83
SE	0.16	0.01	0.15	0.23	0.05	0.35

Table 2.10. Mean (n = 3) sensory attributes of spray dried serum protein concentrate (SPC) and spray dried whey protein concentrate (WPC) produced in this study.

Product	Aroma intensity	Sweet aromatic	Buttery	Crdbrd	Cereal	Cooked	Sweet taste	Astringent
SPC	1.7 ^a	0.7 ^b	ND	ND	1.0 ^a	1.5 ^a	2.0 ^a	1.7 ^a
WPC	1.8 ^a	1.1 ^a	0.5	0.6	0.7 ^a	1.4 ^a	2.0 ^a	1.7 ^a
R-Square	0.26	0.36	0.43	0.23	0.20	0.25	0.02	0.18
SE	0.06	0.08	0.06	0.08	0.06	0.08	0.08	0.09

Means in the same column not sharing a common superscript are different ($P < 0.05$).

Intensities were scored on a 0 to 15-point universal scale where 0 = none and 15 = very high intensity (Meilgaard et al., 1999).

ND = Not detected. Dried whey ingredient intensities generally fall between 0 and 4 (Drake et al., 2003; Wright et al., 2009).

Attributes not listed were not detected

Crdbrd – cardboard flavor

Table 2.11. Mean (n = 3) sensory attributes of spray dried and freeze dried serum protein concentrate (SPC) and whey protein concentrate (WPC) produced in this study.

Product	Aroma intensity	Sweet aromatic	Buttery	Crdbrd	Cereal	Cooked	Sweet taste	Astringent
1 SD WPC	1.8 ^b	1.1 ^b	0.5 ^a	0.6 ^a	0.7 ^b	1.4 ^a	2.0 ^a	1.7 ^a
2 SD SPC	1.7 ^{bc}	0.7 ^c	ND	ND	1.0 ^a	1.5 ^a	2.0 ^a	1.7 ^a
3 FD WPC	2.3 ^a	1.9 ^a	0.5 ^a	0.7 ^a	1.0 ^a	2.0 ^a	1.6 ^b	1.5 ^a
4 FD SPC	1.6 ^c	1.2 ^b	0.5 ^a	0.5 ^a	1.0 ^a	2.0 ^a	1.5 ^b	1.8 ^a
R-Square	0.31	0.48	0.14	0.14	0.19	0.25	0.24	0.17
SE	0.07	0.08	0.08	0.08	0.06	0.08	0.08	0.09

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

Intensities were scored on a 0 to 15-point universal scale where 0 = none and 15 = very high intensity (Meilgaard et al., 1999).

ND = Not detected. Dried whey ingredient intensities generally fall between 0 and 4 (Drake et al., 2003; Wright et al., 2009).

Attributes not listed were not detected

Crdbrd – cardboard flavor

Table 2.12. Mean sensory attributes of spray dried (n = 3) serum protein concentrate (SPC), spray dried (n = 3) whey protein concentrate (WPC) produced in this study and six commercial (n = 2) 34% whey protein concentrates.

Source	Trt	Aroma intensity	Sweet aromatic	Buttery	Crdbrd	Cereal	Cooked	oxidized	Swt taste	Sour taste	Salty taste	Astgt
Experimental	SPC	1.7 ^{bc}	0.7 ^d	ND	ND	1.0 ^a	1.5 ^d	ND	2.0 ^b	ND	ND	1.7 ^c
	WPC	1.8 ^{bc}	1.1 ^c	0.5 ^c	0.6 ^e	0.7 ^b	1.4 ^d	ND	2.0 ^b	ND	ND	1.7 ^c
Commercial WPCs	1	2.0 ^b	1.0 ^d	1.0 ^b	2.0 ^b	ND	1.4 ^d	1.5 ^a	1.4 ^c	0.8 ^a	0.5 ^c	3.0 ^a
	2	1.4 ^c	1.5 ^b	ND	1.4 ^d	ND	1.9 ^{bc}	1.6 ^a	1.9 ^b	ND	ND	2.0 ^c
	3	2.3 ^{ab}	2.0 ^a	1.9 ^a	1.7 ^d	ND	2.0 ^b	ND	2.0 ^b	0.7 ^a	2.0 ^a	2.2 ^{bc}
	4	2.0 ^b	1.7 ^b	1.8 ^a	2.9 ^a	ND	1.7 ^{cd}	ND	1.5 ^c	ND	ND	2.5 ^b
	5	2.4 ^a	1.7 ^b	1.0 ^b	2.9 ^a	ND	2.0 ^b	ND	1.5 ^c	ND	1.2 ^b	2.5 ^b
	6	1.7 ^{bc}	2.2 ^a	ND	0.5 ^e	ND	2.3 ^a	ND	2.4 ^a	ND	ND	2.5 ^b
R-Square		0.27	0.53	0.76	0.78	0.47	0.35	0.17	0.30	0.22	0.72	0.44
SE		0.11	0.10	0.08	0.11	0.08	0.10	0.06	0.10	0.05	0.08	0.11
Commercial SE		0.08	0.08	0.06	0.09	0.06	0.08	0.06	0.08	0.05	0.08	0.09
Experimental												

Means in the same column not sharing a common superscript are different ($P < 0.05$).

Intensities were scored on a 0 to 15-point universal scale where 0 = none and 15 = very high intensity (Meilgaard et al., 1999).

ND = Not detected. Dried whey ingredient intensities generally fall between 0 and 4 (Drake et al., 2003; Wright et al., 2009).

Crdbrd – cardboard, swt taste – sweet taste, astgt - astringent

Table 2.13. Mean (n = 3) concentrations of selected aroma compounds (µg/L) of spray dried serum protein concentrate (SPC) and spray dried whey protein concentrate (WPC) produced in this study isolated using solid phase microextraction (SPME).

Product	Diacetyl	Acetic acid	Pentanal	Hexanal	Heptanal	Octanal	Nonanal	Decanal
WPC	0.82	0.24	0.06 ^a	0.72 ^a	0.14 ^a	0.40 ^a	0.72 ^a	0.33 ^b
SPC	ND	ND	0.02 ^b	0.19 ^b	0.04 ^b	0.29 ^b	0.31 ^b	1.04 ^a
R-Square	0.30	0.55	0.72	0.82	0.70	0.75	0.61	0.90
SE	0.13	0.07	0.01	0.06	0.02	0.02	0.12	0.06

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

ND - not detected

Table 2.14. Mean (n = 3) concentrations of selected aroma compounds (µg/L) of spray dried (SD) and freeze dried (FD) serum protein concentrate (SPC) and spray dried whey protein concentrate (WPC) produced in this study isolated using solid phase microextraction (SPME).

Product	Diacetyl	Acetic acid	Pentanal	Hexanal	Heptanal	Octanal	Nonanal	Decanal
SD WPC	0.82 ^b	0.24 ^a	0.06 ^b	0.72 ^b	0.14 ^b	0.40 ^a	0.72 ^a	0.33 ^b
SD SPC	ND	ND	0.02 ^b	0.19 ^b	0.04 ^b	0.29 ^a	0.31 ^b	1.04 ^a
FD WPC	7.03 ^a	0.07 ^a	0.03 ^b	0.18 ^b	ND	0.32 ^a	0.82 ^a	0.96 ^a
FD SPC	ND	ND	0.43 ^a	4.56 ^a	ND	0.52 ^a	0.88 ^a	0.34 ^b
R-Square	0.88	0.41	0.54	0.42	0.53	0.28	0.34	0.73
SE	0.37	0.06	0.06	0.84	0.98	0.05	0.13	0.09

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

ND – not detected

Table 2.15. Mean concentrations of selected aroma compounds ($\mu\text{g/L}$) of spray dried ($n = 3$) serum protein concentrate (SPC), spray dried ($n = 3$) whey protein concentrate (WPC) produced in this study and six commercial ($n = 2$) 34% whey protein concentrates isolated using solid phase microextraction (SPME).

Source	Treat	Diacetyl	Pentanal	Acetic acid	Hexanal	Heptanal	Octanal	Nonanal	Decanal
Experimental	WPC	0.82 ^a	0.06 ^d	0.24 ^a	0.72 ^b	0.14 ^c	0.40 ^a	0.72 ^{ab}	0.33 ^a
	SPC	ND	0.02 ^d	ND	0.19 ^b	0.04 ^c	0.29 ^a	0.31 ^a	1.04 ^a
Commercial	1	12.11 ^a	1.35 ^{bc}	1.97 ^a	42.71 ^a	1.25 ^{ab}	0.59 ^a	0.11 ^b	1.04 ^a
	2	19.21 ^a	2.19 ^{ab}	4.71 ^a	39.90 ^a	1.29 ^{ab}	0.81 ^a	0.19 ^b	1.84 ^a
	3	6.25 ^a	2.23 ^a	0.62 ^a	39.04 ^a	1.41 ^a	1.08 ^a	0.36 ^{ab}	0.80 ^a
	4	5.05 ^a	2.39 ^a	0.36 ^a	48.76 ^a	1.18 ^{ab}	0.94 ^a	0.15 ^b	0.25 ^a
	5	20.02 ^a	1.57 ^{abc}	9.72 ^a	36.04 ^a	0.46 ^{bc}	0.98 ^a	0.20 ^b	1.73 ^a
	6	1.95 ^a	1.07 ^c	0.21 ^a	36.85 ^a	0.46 ^{bc}	0.69 ^a	0.14 ^b	0.12 ^a
R-Square		0.23	0.64	0.18	0.38	0.37	0.13	0.29	0.17
SE Experimental		4.9	0.25	2.3	9.1	0.25	0.26	0.12	0.47
SE Commercial		6.6	0.34	3.1	12.3	0.34	0.35	0.16	0.63

^{a,b,c,d} Means in the same column not sharing a common superscript are different ($P < 0.05$).

ND – not detected

Table 2.16: Aroma active compounds detected in spray dried (SD) and freeze dried (FD) pilot plant SPC34 and WPC34 by gas chromatography olfactometry with aroma extract dilution analysis (AEDA)

No	Compound	Fraction	Odor ¹	Log ₃ Flavor dilution ² (Aroma intensity) ³				Retention Index ⁴		Methods of Identification ⁵
				SD SPC34	FD SPC34	SD WPC34	FD WPC34	DB-5	DB-Wax	
1	2/3-methylbutanal	NB	malty/chocolate	<1 (1.5)	<1 (2.5)	2 (1.75)	ND ⁶	664		RI ^f , Odor
2	acetic Acid	AC	vinegar/sour	ND	1 (1.5)	1 (1)	2 (2)		1510	RI, MS, Odor
3	diacetyl	NB	buttery/green	ND	ND	2 (3)	1 (1.5)	704	954	RI, Odor
4	pentanal	NB	peanut/roasted/sweet	1 (2.5)	<1 (2)	2 (2)	ND	731	937	RI, MS, Odor
5	dimethyl disulfide	NB	rubbery/garlic/sweet	3 (1)	1 (1)	2 (2.5)	<1 (2.25)	732	1073	RI, MS, Odor
6	ethyl butanoate	NB	fruity/catty	<1 (1.75)	ND	<1 (2.5)	<1 (1.5)	815	1192	RI, Odor
7	hexanal	NB	grassy/plastic	ND	1 (1.5)	ND	1 (2)	821	1050	RI, MS, Odor
8	butanoic acid	AC	cheesy/rancid/buttery	ND	<1 (1.75)	2 (1)	<1 (1.5)		1721	RI, MS, Odor
9	2-methylbutyl acetate	NB	fruity	1 (2)	<1 (2.5)	ND	ND	886		RI, MS, Odor
10	Z-4-heptanal	NB	plastic/green/fishy	5 (2.5)	ND	<1 (3)	4 (2.25)	909	1224	RI, MS, Odor
11	methional	NB	potato/green/veg	3 (2.5)	<1 (1.5)	3 (2.5)	3 (1.5)	931	1434	RI, Odor
12	2-acetyl-1-pyrroline	NB	popcorn/fatty	4 (3.5)	<1 (1.5)	<1 (2)	4 (2)	947	1319	RI, Odor ⁶
13	dimethyl trisulfide	NB	garlic/sulfur/citrus	6 (2.5)	ND	3 (3)	1 (1.5)	965	1365	RI, Odor
14	1-octen-3-one	NB	mushroom	5 (2)	3 (2)	3 (2.5)	1 (2.25)	1001	1285	RI, MS, Odor
15	octanal	NB	citrus	1 (2)	ND	ND	1 (2.5)	1028	1277	RI, MS, Odor
16	unknown	NB	cooked/cheesy/bread	1 (3)	<1 (2)	ND	ND	1054		Odor
17	thienylthiol	NB	garlic	6 (3)	2 (2)	2 (3.5)	ND	1087		RI, Odor
18	2-methoxy phenol	NB	smokey/burnt/fatty	<1 (2)	3 (2.5)	ND	2 (2.5)	1119	1466	RI, MS, Odor
19	2,3-diethyl-5-methyl pyrazine	NB	cabbage/potato	1 (1)	<1 (2)	<1 (2.5)	ND	1159	1540	RI, Odor
20	2-phenethanol	AC	fruity	ND	<1 (1.5)	ND	<1 (1.5)	1167	1880	RI, Odor
21	(E,Z)-2-6-nonadienal	NB	cucumbers burnt	1 (2.5)	3 (2.5)	<1 (2.5)	2 (2.5)	1173	1565	RI, MS, Odor
22	(E)-2-nonenal	NB	sweet/carpet/cucumber	<1 (2)	<1 (2)	ND	<1 (2.5)	1188	1580	RI, MS, Odor
23	dimethyltetrasulfide	NB	garlic	4 (2)	<1 (2.5)	ND	ND	1202		RI, MS, Odor
24	phenylethyl acetate	AC	cucumbers/rosy	ND	3 (2)	<1 (1.75)	<1 (3)	1292	1815	RI, Odor
25	(E)-2-undecenal	NB	rubber	4 (2)	1 (1)	3 (1)	5 (2)	1377		RI, Odor
26	decanoic acid	AC	fatty	ND	<1 (1.5)	ND	3 (2.5)	1418	2411	RI, MS, Odor
27	2-dodecenal	NB	sweet, green, fat	<1 (2)	1 (1.5)	ND	1 (1.5)	1454		RI, Odor
28	tridecanal	NB	sweet, must	4 (2.25)	3 (1)	3 (3)	2 (2)	1511		RI, Odor
29	(E,E)-farnesyl acetate	NB	waxy	<1 (1.5)	ND	<1 (1)	ND	1925		RI, Odor

¹Odor description at the gas chromatograph (GC) sniffing port

²Flavor dilution factors were determined on a DB-5MS column for neutral/basic (N/B) compounds, and on a DB-Wax column for acidic (AC) compounds.

³Aroma intensity at the GC sniffing port, on a scale from 1-5

⁴Retention indices were calculated from gas chromatography/olfactory data

⁵Compounds were identified by comparison with the authentic standards on the following criteria; retention index (RI) on DB-Wax and DB-5MS columns, odor property at the GC-sniffing port, and mass spectra in the electron impact mode. Positive identifications indicate that mass spectral data was compared with authentic standards.

⁶Compound identified by comparing RI and aroma with literature (Avsar et al., 2004)

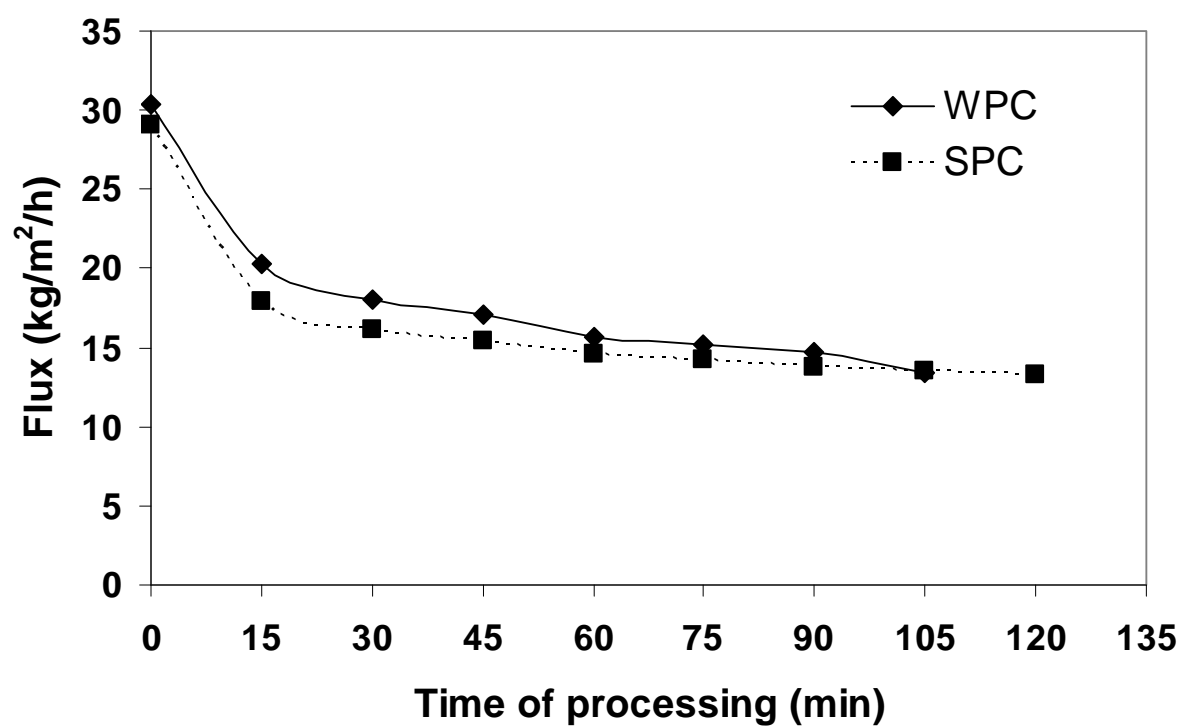


Figure 2. 1. Mean ($n = 3$) UF flux during the production of 34% WPC and 34% SPC using a spiral wound 10 kDa polyethersulfone membrane.

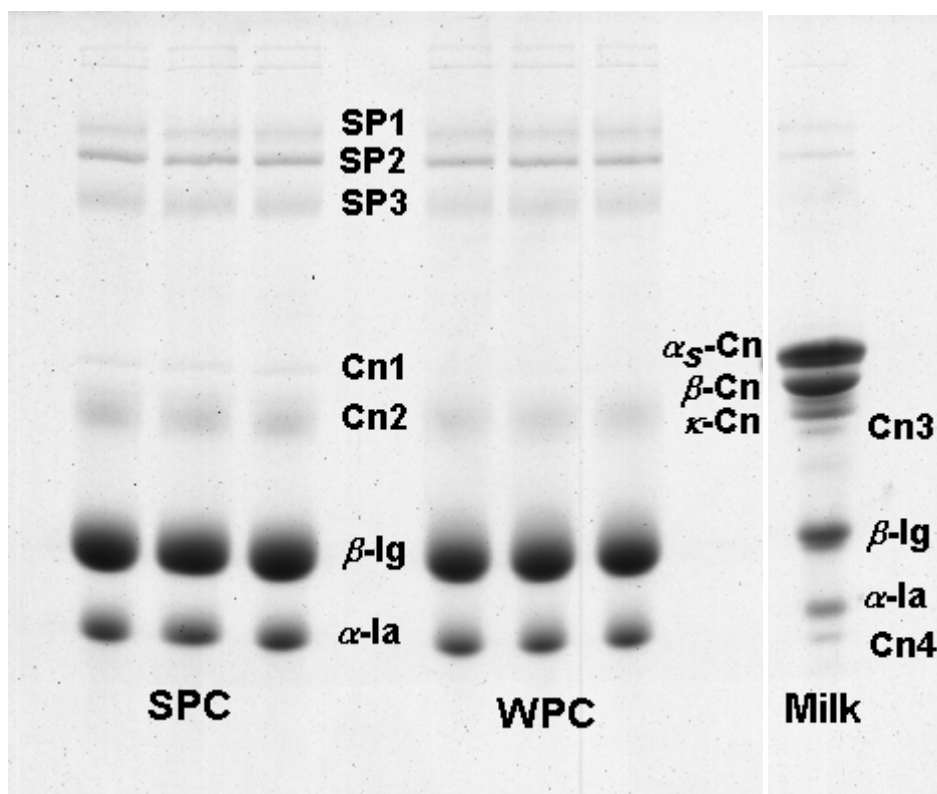


Figure 2. 2. Sodium dodecyl sulfate-PAGE electrophoretogram of proteins in 34% serum protein concentrate (SPC), 34% whey protein concentrate (WPC) and skim milk. The loading of the samples was 7 μ l (8.5 μ l for milk) and samples were run in triplicate. Bands in lanes are identified on the gel: SP1, SP2, SP3 = serum proteins, Cn1 = α_s -Cn (combination of α_{s1} and α_{s2} -Cn), Cn2, Cn3 = casein proteolysis products, β -Ig (β -lactoglobulin), α -la (α -lactalbumin), Cn4 = proteolysis products of casein.

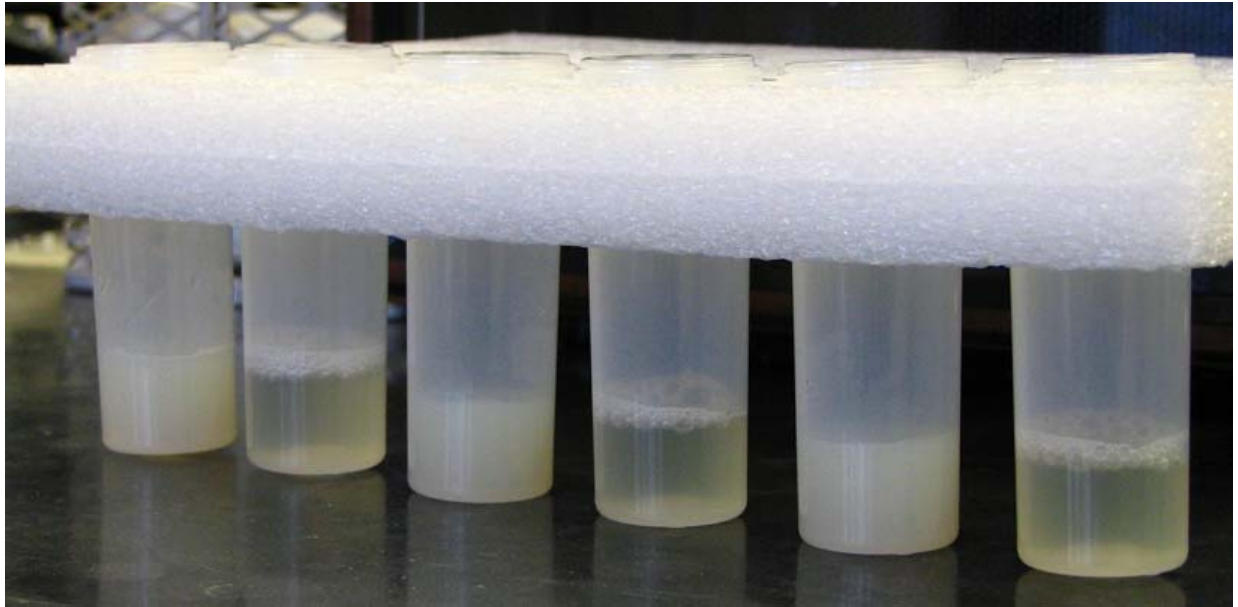
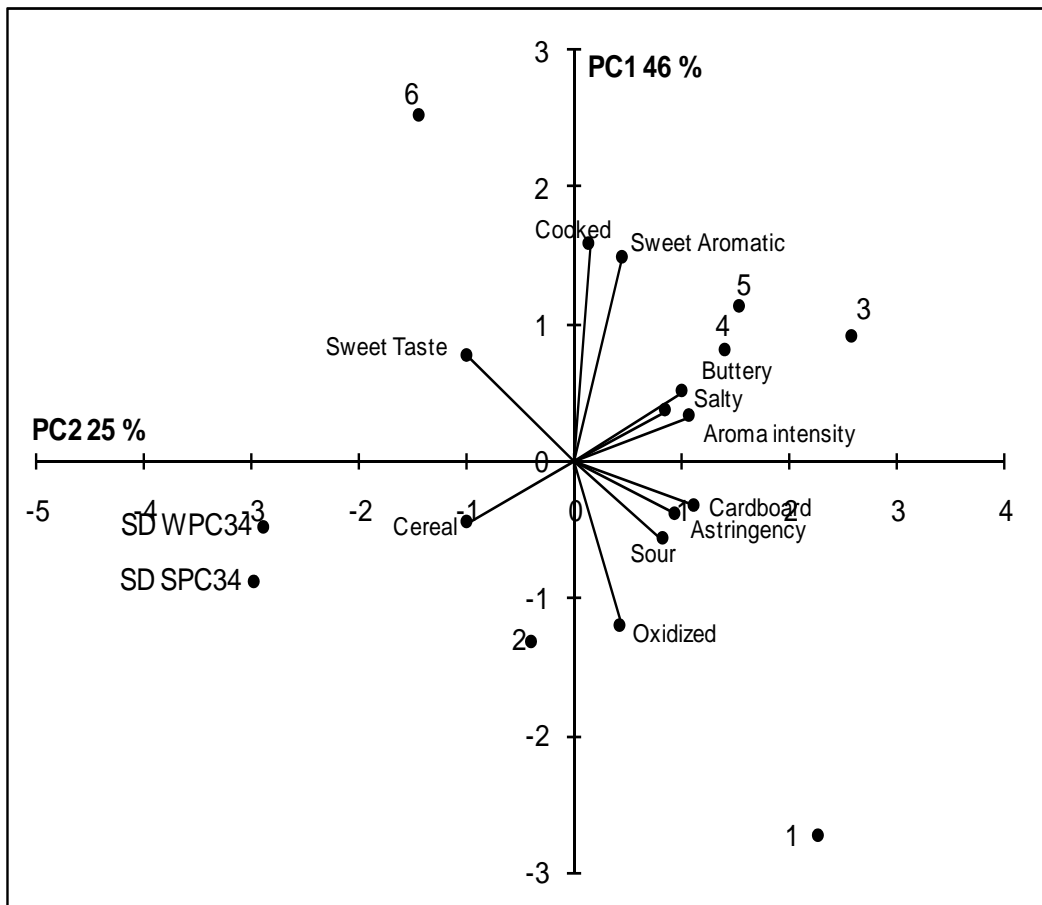
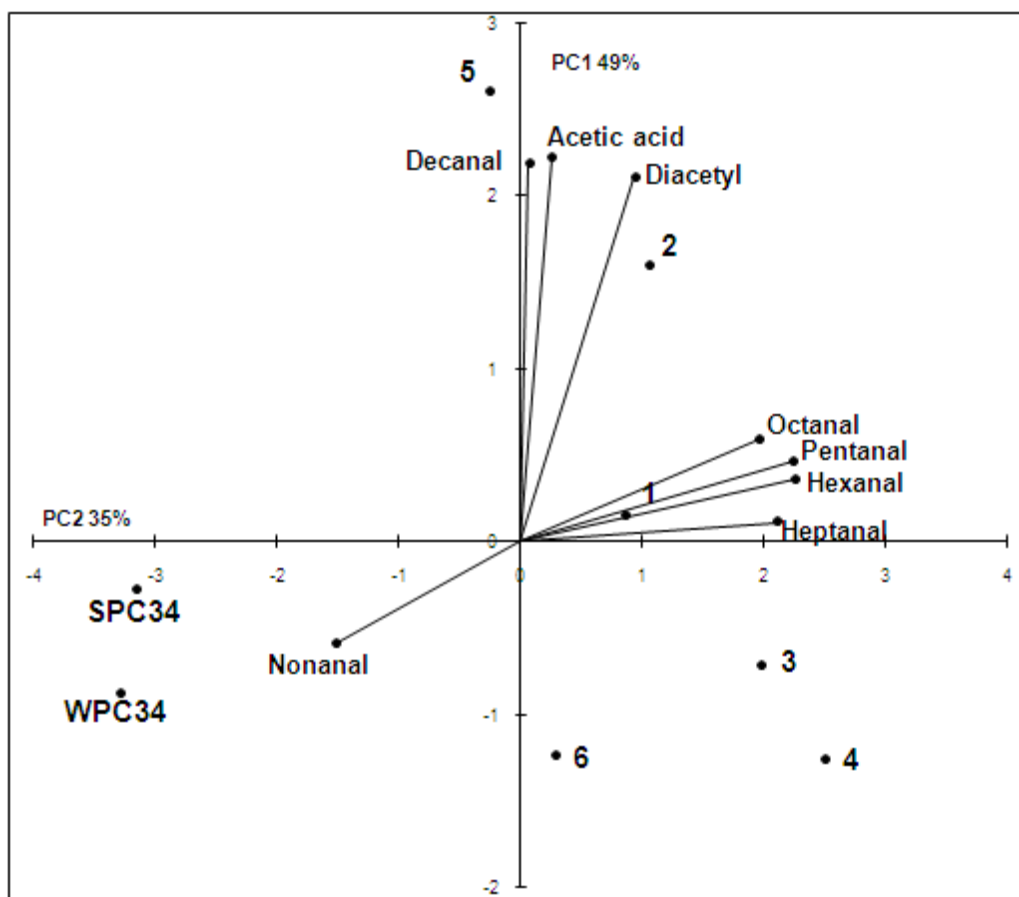


Figure 2. 3. Rehydrated at 10% solids the liquid 34% whey protein cocentrate (WPC) and 34% serum protein concentrate (SPC). From left to right: WPC replicate 1, SPC replicate 1, WPC replicate 2, SPC replicate 2, WPC replicate 3, SPC replicate 3.



PC1 and PC2 - principal components 1 and 2. SD-spray dried. WPC34- Whey Protein Concentrate 34, SPC34- Serum Protein Concentrate 34, Numbers 1-6 represent six commercial WPC34.

Figure 2. 4. Principal component biplot of sensory attributes of whey and serum protein concentrates and commercial WPC34.



PC1 and PC2 - principal components 1 and 2. SD-spray dried. WPC34- Whey Protein Concentrate 34, SPC34- Serum Protein Concentrate 34, Numbers 1-6 each represent one of the six commercial samples.

Figure 2. 5: SPME PCA biplot of instrumental analysis results for whey and serum protein concentrates and commercial WPC34.

CHAPTER 3: 80% MANUSCRIPT

COMPARISON OF COMPOSITION, SENSORY AND VOLATILE COMPONENTS OF
80% WHEY PROTEIN AND SERUM PROTEIN CONCENTRATE

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*Submitted for publication in Journal of Dairy Science

ABSTRACT

Serum or “native” whey protein concentrates (SPC) are whey proteins that are removed from milk before the cheese making process. Since SPC are not exposed to the cheese making process, enzymatic and/or chemical reactions that can lead to off-flavors are reduced. The objectives of this study were to identify and compare the composition, flavor, and volatile components of 80% protein SPC and WPC (SPC80, WPC80). SPC80 and WPC80 were manufactured in triplicate with each pair of serum and traditional whey protein manufactured from the same lot of milk. At each replication, spray-dried (SD) product from each protein source was collected. Commercial WPC80 were also collected for sensory and volatile analyses. A trained sensory panel documented the sensory profiles of the rehydrated powders. Volatile components were extracted by solid phase micro-extraction (SPME) and solvent extraction followed by solvent assisted flavor evaporation (SAFE) with gas chromatography-mass spectrometry and gas chromatography-olfactometry. Consumer acceptance testing with 6 % protein beverages was conducted with SPC80 and WPC80, as well as commercial WPC80. SPC80 was lower in fat and had a higher pH than pilot plant manufactured WPC80 and commercial WPC80 ($p<0.05$). Few sensory differences were documented between the rehydrated SPC80 and WPC80 manufactured in this study, but their flavor profiles were distinct from flavor of rehydrated commercial WPC80 ($p<0.05$). WPC80 manufactured in this study had higher concentrations of lipid oxidation products than SPC80. Concentrations of lipid oxidation products in commercial WPC80 were generally higher than concentrations in SPC80 or WPC80 manufactured in this study ($p<0.05$). Protein

beverages had distinct flavors associated with protein source. Protein beverages made with SPC80 were not liked as well protein beverages made with WPC80 manufactured in this study or one commercial WPC80 ($p < 0.05$). These results suggest that composition, physical properties and volatile compound composition of SPC80 are distinct from WPC80. These differences may contribute to differences in flavor in ingredient applications.

Key words: serum protein, whey protein, sensory, composition, flavor

INTRODUCTION

Whey proteins are highly functional and nutritious proteins used in a variety of products. The two most commonly used value-added forms of whey protein in industry are whey protein concentrate (WPC) and whey protein isolate (WPI). WPC contains 34-89 % protein and WPI contains at least 90 % protein (USDEC, 2008). Whey proteins have many functional characteristics such as high solubility, dispersibility, water binding, foaming, whipping, emulsification, gelation, and buffering power (Davis et al., 2007) and are used frequently in an increasing number of food applications (National Dairy Council, 2008).

Whey proteins should ideally have a bland flavor to facilitate application in foods (Drake et al., 2008), but flavor of these products is highly variable due to the original whey source, processing, and storage (Carunchia Whetstine et al., 2005a; Mahajan et al., 2004; Gallardo-Escamilla et al., 2005; Wright et al., 2006; Wright et al., 2009). Off-flavors may carry through into ingredient applications and limit food applications (Drake, 2006; Drake et al., 2008; Wright et al., 2009). Recent research has highlighted removal of whey proteins from skim milk prior to cheese making (Nelson et al., 2005a; 2005b). Prior to cheese making, these proteins are called serum proteins (SP). Due to a lack of familiarity with the term SP, these proteins have also been referred to as “native” whey proteins. Nelson et al. (2005a) developed a microfiltration and ultrafiltration method to remove SP from skim milk and subsequently demonstrated that SP removal from fluid milk had no impact on quality of Cheddar cheese (Nelson et al., 2005b). Serum protein concentrates (SPC), because they are not subjected to a cheese make procedure, are not exposed to as many processing techniques

that may alter flavor formation and thus may have the potential to offer a blander flavor profile. Evans et al. (In Press) reported few sensory or volatile component differences between SPC34 and WPC34, but distinct compositional differences between these two products which might impact functionality were noted and there were visible appearance differences between the two proteins when rehydrated in solution. WPC80 are distinct in composition and application from WPC34. More obvious differences between whey and serum protein concentrates may be evident. Punidadas et al. (1998) demonstrated foaming properties of a 5% concentrated serum protein product comparable to values cited for egg white and better than those cited for products made from cheese whey. They also reported better gelation properties compared to cheese whey (Punidadas et al., 1998). Nelson et al. (2005b) reported higher fat recovery and cheese yield in cheeses made from milk with a 65% reduced serum protein content. The objective of this research was to characterize and compare the composition and sensory properties of 80% whey protein concentrate and 80% serum protein concentrates (WPC80, SPC80) made from the same milk. Sensory and instrumental methods were applied to pilot plant-manufactured WPC80 and SPC80. A second objective was to compare the composition and sensory properties of the serum protein and whey protein concentrates made in our study with those of five commercial 80% WPC.

MATERIALS AND METHODS

Experimental Design

One batch of whole raw bovine milk (ca 1900 kg) was received from the Cornell University dairy farm. The milk was divided into two portions. One portion of the milk was used for Cheddar cheese manufacture to produce 80% WPC. The other portion was centrifugally separated at 4°C into raw cream and raw skim and the skim milk was used to produce 80% SPC. This was replicated 3 times with different batches of raw milk. The time line for the processing was as follows: raw whole milk was received on Monday, split into two portions. On Monday, one portion of the whole raw milk was pasteurized and used for Cheddar cheese making. The Cheddar whey was pasteurized and run through a cream separator and cooled to 4°C. The other portion of raw milk was cold separated to produce raw skim milk. On Tuesday, the raw skim milk from Monday was pasteurized, cooled to 4°C and stored and the separated pasteurized whey was first UF (stage 1) and then diafiltered (stage 2) to produce a liquid 80% WPC that was frozen at -40°C. On Wednesday, the pasteurized skim was microfiltered to produce 65% SP reduced casein concentrate and MF permeate. The MF permeate was cooled to 4°C and held overnight. MF retentate was further diafiltered twice to produce 95% SP reduced native micellar casein concentrate and its characteristics will be reported in a separate publication. On Thursday, MF permeate was UF (stage 1) and diafiltered (DF) (stage 2) to produce 80% liquid SPC that was frozen at -40°C in 20L plastic containers. Frozen 80% liquid SPC and WPC were thawed and then spray dried. Commercial samples of 80% WPC were obtained from five different companies (2

different lots per company) and analyzed under the same conditions for comparison to the samples produced in this study.

Whey Protein Concentrate Manufacture

The cheese making procedure for WPC production was as described in Evans et al. (In Press). The average fat and protein content of the milk for cheese making were $3.73\% \pm 0.05$ and $3.05\% \pm 0.04$, respectively. Whey was drained at pH of 6.35 and immediately pasteurized at 72°C for 16 s and continuously cooled to 50°C and processed with a cream separator. The fat content of the whey before separation was $0.24\% \pm 0.03$ and after separation was $0.05\% \pm 0.02$. After separation, the whey was cooled to 4°C and held overnight at $\leq 4^\circ\text{C}$.

Approximately 640 kg of separated whey was heated to 50°C and processed with the UF system in batch recirculation mode using a polyethersulfone spiral wound UF membrane (Model 3838GEA, NIRO Inc., Hudson, WI) with a nominal pore size of 10,000 Da. Prior to processing the UF membrane was cleaned following the same procedure described in Evans et al. (In Press). The water flux was typically about 52 kg/m²/h. Whey was UF for 2.3 h to achieve concentration factor of approximately 5.2 X and the protein content of the retentate measured by infrared spectrophotometer was 47% of protein plus lactose in the retentate. The UF retentate was diluted with pasteurized RO water at 50°C to bring the weight back to the original total weight of the starting whey for DF. The membrane was not cleaned before proceeding to the DF stage. The mixture was recirculated through the membrane for 5 min to ensure complete mixing. Then, the diluted UF retentate was DF to achieve concentration

factor of 6.9 X. Diafiltration was continued until the protein content of the retentate measured by infrared spectrophotometer was 90% of protein plus lactose in the retentate. The total time of DF was 2.4 h. At the end of processing, the retentate drained from the dead volume of the system was mixed with the retentate in the feed vat, mixed, and sampled. The final liquid retentate protein concentrate was weighed, frozen at -40°C in 20 L plastic containers and stored until spray drying. After producing the 80% WPC liquid concentrate the UF system was cleaned as described in Evans et al. (In Press). The fouled water flux (kg/m²/h) was typically about 32% of the clean water flux (17 vs 52 kg/m²/h). It was possible to restore the flux after cleaning. The clean water flux after final cleaning was typically about 52 kg/m²/h.

Serum Protein Concentrate Manufacture

Raw whole milk was processed using the procedures described in Evans et al. (In Press) to produce serum protein concentrate. Skim milk (about 1100 kg) was heated to 50°C with a DeLaval Model A3 plate heat exchanger and microfiltered using a pilot scale, uniform transmembrane pressure (UTP) MF system (Tetra Alcross MFS-7, TetraPak Filtration Systems, Aarhus, Denmark) equipped with ceramic Membralox membranes (pore diameter: 0.1µm; surface area: 1.7m²). For a more detailed description of the UTP system and process see Zulewska et al. (In Press). The total time of milk processing was about 8.3 h. The MF permeate was cooled to 4°C and held overnight.

On the following day, MF permeate (705 kg) was weighed into a vat, heated to 50°C using a DeLaval Model A3 plate heat exchanger and UF processed. The conditions and parameters of UF processing of MF permeate were the same as for whey processing. The

total processing time of first stage was about 3 h at a concentration factor 5.5 X and the protein content of the retentate measured by infrared spectrophotometer was 41% of protein plus lactose in the retentate. The DF time was 3.3 h and DF (6.9 X) was continued until the protein content of the retentate measured by infrared spectrophotometer was 88% of protein plus lactose in the retentate. The final retentate was frozen at -40°C in 20 L plastic containers and stored until spray drying.

Spray drying

The conditions for spray drying were the same as described in Evans et al. (In Press) except that feed material (ca 55 kg) was kept frozen at -40°C prior to spray drying. Before drying, the frozen retentate in 20 L plastic containers was thawed at 4°C for 48h and after that the containers were immersed in 50 to 55°C water for 1 to 2h to allow for complete thawing. The total time of the spray drying run was approximately 4 h.

Chemical analyses

Milk for cheese making was analyzed using infrared spectrophotometer (Lactoscope FTIR, Delta Instruments, The Netherlands) for fat content and true protein content (Kaylegian et al., 2006). The fat content of unseparated and separated whey was determined by ether extraction (AOAC, 2000; 33.2.26, 989.05).

Fresh samples of the final liquid 80% WPC and liquid 80% SPC were analyzed for fat, TS, total N, and NPN content using ether extraction (AOAC, 2000; method number 989.05; 33.2.26), forced air oven drying (AOAC, 2000; method number 990.20; 33.2.44), Kjeldahl (AOAC, 2000; method number 991.20; 33.2.11), and Kjeldahl (AOAC, 2000;

method number 991.21; 33.2.12), respectively. The pH of final liquid 80% WPC and 80% SPC was measured with an electrode (model HA 405, Mettler Toledo, Columbus, OH) that was standardized at pH 6.97 and 4.06 at 50°C and kept immersed in 3M KCl at 50°C between readings in order to keep its temperature equal to the temperature of the buffers and samples.

The commercial 80% WPCs and experimental 80% WPC and 80% SPC were reconstituted to 10% solids and the liquids were analyzed for fat and total N by the methods indicated above. The pH was measured with an electrode (model Electrolyte 9823, Mettler Toledo, Columbus, OH) that was standardized at pH 7.01 and 4.00 at RT (22°C). The reconstituted samples (80% WPC, 80% SPC and five 80% commercial powders) were analyzed for TS content by forced air oven drying (AOAC, 2000; method number 990.20; 33.2.44) and moisture content was calculated. Experimental 80% WPC and 80% SPC were analyzed for NPN using Kjeldahl (AOAC, 2000; method number 991.21; 33.2.12). The glycomacropeptide content (which is soluble in 12% TCA) of WPC was calculated as a difference in NPN content between WPC and SPC powders on a dry basis.

Microbiological analyses

Samples of pasteurized skim milk for cheese making before and after standardization, pasteurized whey from cheese making, pasteurized skim milk for MF, liquid WPC, liquid SPC and spray dried powders were taken for total bacterial and coli counts (Wehr and Frank, 2004; methods 6.020 and 7.020, respectively).

SDS-PAGE Electrophoresis

The gels and the milk samples were prepared using the same approach as described in Zulewska et al. (In Press). Fresh liquid (after second DF) samples of 80% SPC and 80% WPC were diluted with sample buffer to keep the protein content per sample similar to milk sample. Milk was loaded at 8.5 μ L onto an SDS-PAGE gel and the samples of 80% SPC and 80% WPC were loaded to achieve the content of proteins per lane similar to milk loading. The gels were scanned and analyzed with USB GS 800 Densitometer using Quantity One 1-D Analysis Software (BIO-RAD Laboratories, Inc., Hercules, CA). The maximum optical density (OD) of the predominant protein in each sample was in the range of 1.0 to 1.4 OD. For each lane the background was adjusted separately using the rolling disk method of subtraction to obtain a flat base on the pop-up trace. The line that defines each lane was adjusted using the lane tools functions (add/adjust anchors) in the software, so that the red lane line crossed each band at the center. The bands for all lanes were detected using the same detection settings, which were chosen to detect the main bands in the milk sample. The adjust band function of the software was used with brackets to set the leading and trailing edge for each band as visually observed on the image of the gel, not based on the beginning and end of the peak in the pop-up trace. The width of the brackets within each lane was set for all bands in that lane to be slightly wider than the widest band in that lane.

Color analysis of powders

The Hunter L, a, b values for the spray and freeze dried powders were determined in duplicate with a MacBeth Color-Eye spectrophotometer (Model 2020; Kollmorgen

Instruments, Corp., Newburgh, NY) with Optiview software from the same company. Hunter values were computed from the diffuse reflectance data in the 360 to 740 nm range, at 20-nm intervals, based on illuminant A. The measurements were done at 23 to 25°C.

Serum and whey proteins

Products were shipped on ice by overnight carrier to North Carolina State University (Raleigh, NC). Subsamples (1 kg) were stored in glass jars at -80°C and analyzed within three months of receipt. For all analyses, samples were reconstituted at 10% solids. Sensory analysis samples were reconstituted using deodorized water (prepared by boiling 4L of distilled water until its volume was decreased by one third). Instrumental analysis samples were reconstituted using HPLC water (EMD Chemicals Inc., Gibbstown, NJ). Five commercial WPC80 (less than 2 mo old) were also received on two separate occasions and reconstituted using the same methods for comparison to pilot plant WPC80 and SPC80.

Descriptive sensory analysis

Sensory testing was conducted in compliance with NCSU Institutional Review Board (IRB) for human subjects approval. A trained sensory panel (n=10, 7 females, 3 males, ages 22-37 y) evaluated the flavor attributes of the reconstituted serum and whey proteins and commercial powders using a previously published lexicon for dried dairy ingredients (Drake et al., 2003a; Drake et al., 2008; Wright et al., 2009). Each panelist had over 150 h of experience with descriptive analysis of dried dairy ingredients, and additional training and experience with WPC and SPC aroma and flavor. Consistent with Spectrum™ descriptive analysis training, panelists were presented with reference solutions of sweet, sour, salty, and

bitter tastes to learn to use the universal intensity scale (Meilgaard et al., 1999; Drake et al., 2003b). Panelists then evaluated and discussed flavor attributes of rehydrated dairy ingredients with a focus on 80 % WPC and SPC. Analysis of variance of data collected in preliminary sessions confirmed that the panel and the panelists could consistently identify and scale flavor attributes. Attribute intensities were scaled using the 0 to 15-point universal intensity scale characterized by the SpectrumTM descriptive analysis method (Meilgaard et al., 1999; Drake et al., 2003b).

Reconstituted products (ca 30 ml) were dispensed into lidded 58 ml soufflé cups with three-digit codes and stored at 3°C overnight. Products were tempered to 20°C and served at this temperature with spring water and unsalted crackers for palate cleansing. Panelists evaluated each sample individually in booths in a positive air pressure room dedicated to sensory analysis. Each product replication was evaluated by each panelist in duplicate in a randomized balanced block design. Products were scored using paper ballots or computerized ballots using CompusenseTM version 5.0 (Compusense, Guelph, Canada).

Volatile compound extraction

SPME GC-MS of WPC/SPC powders.

Volatile compounds were extracted by solid phase microextraction (SPME) and subsequently separated and identified by gas chromatography-mass spectrometry (GC-MS) using a modified method of Wright et al. (2006). Both products manufactured in this study (pilot plant manufactured SPC80 and WPC80) as well as commercial products were evaluated by SPME GC-MS. Spray dried powders were reconstituted at 10 % solids, with 10

% NaCl (Fischer Scientific, Fairlawn, NJ), and 10 μ l internal standard solution (2-methyl-3-heptanone in methanol at 81 ppm (SigmaAldrich, Milwaukee, WI)) in 20 ml autosampler vials with steel screw tops containing silicone septa faced in Teflon (Microliter Analytical, Sawanee, FL). Samples were injected using a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) attached to an Agilent 6890N GC with 5973 inert MSD (Agilent Technologies Inc., Santa Clara, CA). Samples were maintained at 5°C prior to fiber exposure. Samples were equilibrated at 40°C for 25 min before 30 min fiber exposure of a 1 cm DVB/CAR/PDMS fiber at 31 mm with 4 sec pulsed agitation at 250 rpm. Fibers were injected for 5 min at a depth of 50 mm.

The GC method used an initial temperature of 40°C for 3 min with a ramp rate of 10°C/min to 250°C held for 5. SPME fibers were introduced into the split/splitless injector at 250°C. An Rtx-5ms column (Rtx-5ms 30 m length \times 0.25 mm inner dia \times 0.25 μ m film thickness; Restek, Bellefonte, Pa., U.S.A) was used for all analyses at a constant flow rate of 1 ml/min. Purge time was set at 1 min. The MS transfer line was maintained at 250°C with the quad at 150°C and source at 250°C. Compounds were identified using the NIST 2005 library of spectra and comparison of spectra of authentic standards injected under identical conditions. Relative abundance for each compound was calculated using the calculated recovery of the internal standard concentration to determine relative concentrations of each compound. Retention indices were calculated using an alkane series (Sigma Aldrich, Milwaukee, WI) (Van den Dool et al., 1963).

Direct solvent extraction of WPC/SPC powders.

Solvent extraction of pilot plant manufactured SPC80 and WPC80 was conducted using the modified methods of Carunchia Whetstine et al. (2005a). One hundred grams of powder were divided into four Teflon bottles (Nalgene, Rochester, NY, capacity of 250 ml) with Tefzel closures (Nalgene, Rochester, NY). Five ml of HPLC water (Gibbstown, NJ) was added to each bottle, along with 20 μ l of internal standard at 81 ppm (2-methyl-3-heptanone, 2-methylpentanoic acid in methanol, Sigma Aldrich, Milwaukee, WI). Seventy-five ml of diethyl ether (EMD Chemicals Inc., Gibbstown, NJ) was added to each bottle. The bottles were then shaken for 30 min on a Roto mix (Type 50800: Thermolyne Dubuque, IA) at high speed and centrifuged at 1459 x g for 10 min in order to separate the solvent phase from the mixture. After centrifugation, the solvent phase containing the extracted volatile components was removed by pipette to an amber glass jar. This procedure was repeated twice with the addition of 40 ml of diethyl ether to each bottle, each time. After the third round of solvent was removed, the bottles were centrifuged a fourth time and any remaining solvent was removed. The solvent extracted from the bottles was then concentrated to 150 ml using a Vigreux column placed inside a water bath at 40°C.

Solvent assisted flavor evaporation (SAFE).

Volatile compounds from serum and traditional whey protein extracts were distilled using SAFE (Ace Glassware, Vineland, NJ). The assembly used was similar to that described by Engel et al. (1999). A rough pump/diffusion pump combination was used as the vacuum source. The SAFE apparatus was connected to a separate receiving tube and a

waste tube, both submerged in liquid nitrogen. The distillation procedure was carried out over 2 h under vacuum (10^5 Torr). The liquid sample was poured into the SAFE apparatus and introduced drop-wise into the vacuum until all of the liquid extract had been placed under vacuum conditions. The SAFE apparatus was kept at 40°C with a circulating water bath.

Phase Separation

Following SAFE, the distillate was concentrated under a nitrogen stream to 20 ml. The concentrated distillate was then washed twice with 3 ml of sodium bicarbonate (Fischer Scientific, Fairlawn, NJ), mixed thoroughly and the bottom (water phase) removed to a separate test tube. The concentrated solvent was then washed with 2 ml of saturated sodium chloride solution three times. Each time, the solution was mixed thoroughly and the water phase removed to the same test tube. The upper layer (ether) containing the neutral/basic fraction was collected, dried over anhydrous sodium sulfate (VWR International, West Chester, PA) and concentrated to 0.5 ml under a gentle stream of nitrogen gas. Acidic volatiles were recovered by acidifying the bottom layer (aqueous phase) with hydrochloric acid (18 w/v %) (Sigma Aldrich, Milwaukee, WI) to a pH of 2-2.5 and extraction of the sample three times with 15 ml ethyl ether. The acidified extract was dried over anhydrous sodium sulfate before concentration to 0.5 ml under a nitrogen gas stream.

Gas chromatography-mass spectrometry (GC-MS) of SAFE extracts

An Agilent 6890N GC with 5973 inert mass selector detection (MSD) (Agilent Technologies Inc., Santa Clara, CA) was used to analyze neutral basic solvent extracts.

Separations were performed on a fused silica capillary column (Rtx-5ms 30 m length \times 0.25 mm inner dia \times 0.25 μ m df; Restek, Bellefonte, PA). A carrier gas (helium) at a constant flow rate of 1ml/min was used. The oven temperature was programmed from 40°C to 200°C at a rate of 5°C per min with initial and final hold times of 5 min, respectively. Each extract (2ul) was injected in the split less mode. Duplicate analyses were performed on each sample.

A Varian CP-3380 GC with Saturn 2000 inert mass selector detection (MSD) (Varian Inc., Palo Alto, CA) was used to analyze acidic solvent extracts. Separations were performed on a fused silica capillary column (Rtx-Wax 30 m length \times 0.25 mm inner dia \times 0.25 μ m df; Restek, Bellefonte, PA). A carrier gas (helium) at a constant flow rate of 1ml/min was used. The oven temperature was programmed from 40°C to 200°C at a rate of 5°C per min with initial and final hold times of 5 min, respectively. Each extract (2ul) was injected in the split less mode. Duplicate analyses were performed on each sample.

Gas chromatography-olfactometry (GC-O)

GC-O was performed on neutral/basic and acidic solvent extracts using an HP 5890 series II gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif., U.S.A.) equipped with a flame ionization detector (FID), a sniffing port, and a split/splitless injector. Neutral/basic and acidic fractions were analyzed from each solvent extraction. Two ul were injected onto a polar capillary column (Rtx-Wax 30 m length \times 0.25 mm inner dia \times 0.25 μ m film thickness; Restek, Bellefonte, Pa., U.S.A.) and a nonpolar capillary column (Rtx-5ms 30 m length \times 0.25 mm inner dia \times 0.25 μ m df; Restek). Column effluent was split 1:1 between

the FID and the sniffing port using deactivated fused silica capillaries (1-m length \times 0.25-mm inner dia). The GC oven temperature was programmed from 40°C to 200°C at a rate of 10 °C/min with an initial hold for 3 min and final hold of 20 min. The FID was maintained at a temperature of 300°C. The sniffing port was maintained at a temperature 105°C. The sniffing port was supplied with humidified air at 30 ml/min. The post-peak intensity of aroma active compounds was evaluated (Grosch 1993; van Ruth, 2001). Each extract was sniffed in duplicate by two experienced sniffers, each with >50 h training on GC-O of dairy products on both polar and nonpolar columns.

Aroma Extract Dilution Analysis (AEDA)

AEDA was performed under the same conditions as those for post peak intensity GC-O. Representative samples of each of the pilot plant products were analyzed. NB fractions were injected onto the DB-5ms (Rtx-5ms 30 m length \times 0.25 mm inner dia \times 0.25 μ m df; Restek, Bellefonte, Pa., U.S.A.) capillary column and acid fractions were injected onto the DB-WAX (Rtx-Wax 30 m length \times 0.25 mm inner dia \times 0.25 μ m film thickness; Restek, Bellefonte, Pa., U.S.A.) capillary column. Each sample was diluted stepwise at a ratio of 1:3 (vol/vol) with diethyl ether. Samples were evaluated by two experienced sniffers until no odors were detected. The greatest dilution in which a compound was sniffed was reported as the flavor dilution (\log_3 FD) factor (Grosch, 1993).

Volatile Compound Identification

Volatile compounds from SAFE and SPME were identified using the NIST 2005 library of spectra and comparison of spectra of authentic standards injected under identical

conditions. Relative abundance of compounds was calculated using the calculated recovery of the internal standard concentration to determine relative concentrations of each compound. Retention indices were calculated using an alkane series (Sigma Aldrich, Milwaukee, WI) (Van den Dool et al., 1963). For aroma active compound identity verification, retention index and aroma properties were also compared to authentic standards injected under identical conditions.

Consumer Testing

Flavor of SPC80 and WPC80 in an ingredient application was determined using an acidified peach beverage formulation as described by Wright et al. (2009). Both pilot-plant manufactured proteins and three representative commercial whey proteins were evaluated in beverages. Beverages were prepared by first combining all of the dry ingredients then slowly blending into de-ionized water with agitation from an Oster 2614 hand blender (Sunbeam Products Inc., Boca Raton, FL) until fully blended. Coloring and flavoring were then added and then blended again until combined using the hand blender. Descriptive analysis was conducted on each of the peach beverages using the methods previously described. Beverages were evaluated for overall aroma intensity, the flavor attributes sweet aromatic, fruity, cardboard, cereal, free fatty acid, and soapy and sour, sweet and bitter tastes.

For consumer testing, approximately 30 ml of peach beverage were dispensed into 58 ml plastic cups with fitted lids (Sweetheart Cup Co., Owings Mills, MD) labeled with three digit codes. Samples were prepared one day in advance and stored under refrigeration (5 °C). Samples were served at 5 °C. Self-reported beverage consumers (n = 77) were

recruited through university listservs and paper fliers. To insure no allergic reactions occurred, consumers were required to sign informed consent forms. Each panelist was first asked a series of demographic questions, and then presented samples in a monadic, random and balanced order. Each beverage was evaluated for overall liking, appearance liking, flavor liking, and texture/mouthfeel liking using the 9-point hedonic scale. Consumers were provided with ambient temperature de-ionized water for palate cleansing between samples and received food treats for their participation. Evaluations were conducted in individual sensory booths using Compusense® five (Compusense, Guelph, Canada) software for data collection.

Statistical analyses

To determine if there were significant differences in UF flux, color, composition, or sensory between WPC and SPC, all data were analyzed by ANOVA using the Proc GLM procedures of SAS (SAS version 8.02, 1999-2001, SAS Institute Inc., Cary, NC). The GLM model was used to determine if flux changed with the time of processing run after the first 30 min of running UF and during DF between treatments (WPC, SPC). Time was treated as a continuous variable in the split-plot ANOVA models. Distortion of the ANOVA by multicollinearity in the model was minimized by centering the time of running using a mathematical transformation (Glantz et al., 2001). Time was transformed as follows: time transformed = running time – [(last time – first time) / 2]. This transformation made the data set orthogonal with respect to time. The model was dependent variable (UF flux, DF flux) = treatment + replicate + treatment x replicate + time + time x replicate + time x treatment +

error, with treatment and replicate as category variables and time as a continuous variable. The GLM model for the direct comparison of composition from WPC and SPC produced in this study was treatment (WPC, SPC) + replicate + error. The GLM model for analysis of sensory data for the direct comparison of composition from WPC and SPC produced in this study was treatment (WPC, SPC) + replicate + panelist + error. The GLM model for comparison of composition among samples produced in this study and the commercial powders was dependent variable = treatment (WPC, SPC, commercial powders 1, 2, 3, 4, 5) + error. For descriptive sensory analysis the GLM model was dependent variable = treatment (WPC, SPC, commercial powders 1, 2, 3, 4, 5) + panelist + error. If the F-test for the model was significant (i.e., $P < 0.05$), then the least square means were compared to determine if there were significantly different. Principle component analysis using the correlation matrix was also applied to descriptive sensory and volatile compound data.

RESULTS

Processing

The largest decrease in the UF flux occurred during the initial 30 min of processing for both for Cheddar cheese whey and MF permeate. After the initial 15 min of the run, the flux decreased at about 38 and 31% during WPC and SPC processing, respectively, with additional 10 and 23% decrease during the subsequent 15 min for WPC and SPC, respectively (Figure 3. 1). During first 30 min of DF the flux improved slightly and then gradually decreased by 8 and 13% at the end of WPC and SPC processing, respectively.

Time dependent differences ($P < 0.05$) in UF and DF flux after 30 minutes of processing when processing MF permeate from skim milk and whey were detected (Table 3.1). The mean flux during the time of UF and DF processing were higher for WPC than SPC (Figure 3. 1), however flux was decreasing at higher rate per hour for the WPC than for the SPC (i.e., Table 3.1 significant time x treatment interaction) and with longer running times the flux for processing WPC may have been lower than for SPC (Figure 3. 1). All 80% WPC and 80% SPC products conformed to the Dry Whey Grade Standards (ADPI, 2002) of <30,000 cfu/g standard plate count and <10 cfu/g coliform. The actual maximum counts for our products were 4000 cfu/g for standard plate count and <1 cfu/g for coliform.

Composition

The liquid WPC before drying had a higher ($P < 0.05$) total solids content than the liquid SPC (Table 3.2), which might be explained by a difference in fat and NPN content along with a difference in final concentration factor. Fat and NPN content of the liquid WPC were significantly higher ($P < 0.05$) than SPC (Table 3.2). The residues of fat remaining in skim milk after cream separation was retained in MF retentate during microfiltration of skim milk using a ceramic UTP membrane with a pore size of 0.1 μm . While the fat that was not removed from the whey by the cream separator was concentrated in the liquid WPC by the UF and DF. Because the high content of fat in whey protein concentrates (WPC) obtained from whey after cheese production has a negative effect on flavor (Morr et al., 1991), the low fat content in SP products produced directly from skim milk using MF may produce serum protein concentrate (SPC) products with different flavor and functional properties than whey

protein concentrates (WPC). A higher ($P < 0.05$) total nitrogen based protein content was detected (Table 3.2) for WPC than SPC. The nonprotein nitrogen content of the WPC was higher ($P < 0.05$) than SPC (Table 3.2), which was caused by the presence of glycomacropeptide (GMP) in the whey. The pH of the liquid WPC was lower ($P < 0.05$) than liquid SPC (Table 3.2) because of the lactic acid from cheese making that is present in the WPC.

The wet and dry basis compositions of the spray dried powders are presented in Tables 3.3 and 3.4, respectively. No significant difference was detected ($P > 0.05$) in moisture content of spray dried experimental WPC versus SPC (Table 3.3). There was a difference ($P < 0.05$) in fat content between WPC and SPC (8.12 vs. 0.53, respectively, on a dry basis) (Table 3.4). Spray dried SPC had a higher ($P < 0.05$) content of total nitrogen than WPC. Glycomacropeptide, calculated as a difference in NPN content between WPC and SPC, was higher ($P < 0.05$) for WPC than SPC (Table 3.4). No difference ($P > 0.05$) in calcium content on a dry basis was detected between SPC and WPC (Table 3.5). There was a trend for higher phosphorous content in WPC ($P < 0.07$) (Table 3.5). SPC had significantly higher ($P < 0.05$) content of potassium, sodium and sulfur than WPC (Table 3.5).

From the functionality point of view, it might be valuable to compare nitrogen fractions of WPC and SPC based on non-fat basis and this was done in Table 3.6. There was no significant difference ($P > 0.05$) in total nitrogen content for WPC versus SPC (Table 3.6) on a non-fat basis. However, true protein content (calculated as a difference between total

nitrogen and nonprotein nitrogen) (Table 3.6) was significantly higher ($P < 0.05$) for SPC than WPC which resulted from the presence of glycomacropeptide in WPC.

The compositions of experimental 80% SPC and WPC were compared with five commercial 80% WPC on a wet basis (Table 3.7) and on a dry basis (Table 3.8). The moisture content of the experimental SPC was higher ($P < 0.05$) than the commercial WPC products (Table 3.7). There was no difference in moisture content between four commercial WPC (plants: 1, 2, 3, and 4) and the experimental 80% WPC, while the WPC from plant 5 had a lower moisture. The average total nitrogen based protein contents on a wet basis of all and experimental powders were less than 80% (Table 3.7) and the experimental WPC was lower ($P < 0.05$) than the commercial WPC powders. The average fat content on a wet basis for the commercial powders was 6.01% (Table 3.7) and only the powders from plant 2 and 5 were not significantly lower in fat content than the experimental 80% WPC (Tables 7 and 8). The SPC produced in this study had a higher pH ($P < 0.05$) than the experimental and commercial WPC (Table 3.7). The experimental 80% WPC with pH of 6.43 was not different than the pH of WPC from plants 1, 2, 3, and 4. The WPC from plant 5 was lower ($P < 0.05$) than all others. The total nitrogen based protein content, as calculated on a non-fat dry basis, of all commercial WPC powders was significantly higher ($P < 0.05$) than WPC and SPC produced in this study (Table 3.9).

A typical SDS-PAGE gel for 80% WPC and SPC is shown in Figure 3. 2. The relative proportions of casein to serum protein in WPC and SPC are presented in Table 3.10. The casein content of SPC was higher ($P < 0.05$) than WPC (3.76 vs. 1.98%). As was

discussed in Evans et al. (In Press), band Cn2 refers to casein proteolysis products and was found in both SPC and WPC. A higher content of Cn2 was present in SPC (3.48 vs 1.98% for SPC and WPC, respectively) and this accounted for most of the difference in casein content between SPC and WPC. The higher level of proteolysis products of casein in the SPC versus WPC could be due to a difference in partitioning of these products between cheese and whey in cheese making versus partitioning between retentate and permeate during MF. Also, there could be some casein proteolysis that occurred due to native milk protease activity at 50°C during MF processing of the milk that did not occur in the cheese making. The relative β -lactoglobulin content of WPC was higher ($P < 0.05$) than SPC (Table 3.11). This could be due to a small amount of rejection of β -lactoglobulin by the MF membrane or due to a minor amount of heat induced denaturation of β -lactoglobulin and interaction with casein micelles during heating of the skim milk to 50°C for MF. The mean relative proportions of β -lactoglobulin and α -lactalbumin in the milk used in our study were 79.77 and 20.23, respectively, and were different ($P < 0.05$) than for SPC and WPC (Table 3.11). The small differences in β -lactoglobulin to α -lactalbumin proportion between SPC and WPC should have only a minor, if any, influence on functional properties of the WPC versus SPC compared to other large composition differences in composition (e.g. fat content) that we have reported in earlier tables.

Color

There was a very noticeable difference in the appearance of the WPC and SPC liquid concentrates (Figure 3. 3). The liquid concentrates obtained after UF and DF processing

looked very different; the 80% SPC was clear and 80% WPC was cloudy and opaque. Such a difference in clarity may limit use of WPC for fortification of non-dairy beverages where clarity of the final beverage is required. When commercial WPC powders were reconstituted, they also were opaque. Evans et al. (In Press) reported similar results between rehydrated SPC34 and WPC34, and hypothesized that differences in fat content contributed to the differences in clarity. Similar distinctions in fat content were observed between SPC80 and WPC80 in this study and may also be the reason for the differences in appearance.

Sensory Analysis:

Serum and Traditional Whey proteins

Pilot plant manufactured SPC80 and WPC80 were characterized by low flavor intensities (Table 3.12), similar to results reported previously for pilot plant manufactured WPC34 and SPC34 (Evans et al., 2009). WPC80 had a higher overall aroma intensity and cereal flavor than SPC80, as well as the presence of sweet aromatic which was absent from SPC80. Diacetyl was previously documented in WPC34 and absent from SPC34, however diacetyl flavor was not detected by sensory panelists in either WPC80 or SPC80.

Commercial and Pilot plant Products

Flavor profiles of commercial WPC80 were similar to pilot plant products with the addition of the flavors diacetyl and potato/brothy (Table 3.13). All of these flavors have been previously reported in WPC80 and WPI (Wright et al., 2009; Drake et al., 2008). No consistent differences were noted between pilot plant and commercial products except the

presence of cooked/milky flavor in pilot samples, which was absent from commercial samples. Similar to results reported by Evans et al. (In Press) with WPC34 and SPC34, pilot plant manufactured WPC80 and SPC80 were distinguished from commercial WPC80 (Figure 3. 4). Additionally, the two pilot products were similar in flavor profile, while there was a large amount of flavor variability among commercial samples. Previous studies have demonstrated wide variability among commercial fluid whey, whey powders and whey proteins (Carunchia Whetstine et al., 2003; Karagul-Yuceer et al., 2003; Mahajan et al., 2004; Carunchia Whetstine et al., 2005a; Wright et al., 2009). These differences were attributed to different milk supplies, whey sources, and processing parameters as well as minor but distinct differences in composition, and these distinctions may also represent sources of differences between pilot plant and commercial products.

Instrumental Volatile Analysis:

SPME GC-MS

SPC vs. WPC

Consistent with GC-O differences, differences were also noted in relative abundance of selected compounds (Table 3.14). WPC80 had higher aldehyde concentrations than SPC80 except for decanal. Pentanal, heptanal, or Z-4-heptanal were not detected in SPC80. WPC80 had significantly higher fat content compared to SPC80, which may have contributed to higher concentrations of lipid oxidation products in WPC80 (Table 3.2). These results are consistent with relative abundance results from WPC34 and SPC34 (Evans et al., In Press), with a lower overall number of aldehydes calculated in SPC80. Diacetyl not detected in

SPC80, which was also expected since this compound is produced during cheesemaking, and this difference was also observed with WPC34 and SPC34. GC-O results (FD factors from AEDA) suggested that hexanal played a larger role in SPC80 flavor compared to its role in WPC80 flavor, while relative abundance calculations showed lower concentrations of hexanal in SPC80 compared to WPC80. GC-O results identify aroma-active compounds and suggest which compounds play the largest role in flavor. Since sensory thresholds for compounds vary, aroma activity is not related to concentration when different compounds are compared.

Pilot vs. Commercial

Differences were noted between pilot and commercial products (Table 3.15, Figure 3. 5) Pilot samples were generally lower in diacetyl, 2-methyl butanal, hexanal, heptanal and pentanal compared to most of the commercial WPC80 (Table 3.15). Commercial sample 3, a Mozzarella WPC80, was the exception. This WPC80 was distinct in volatile profile from the other WPC80 which were sourced from Cheddar whey. Carunchia-Whetstine et al. (2005a) and Wright et al. (2009) demonstrated a range of flavor (sensory and instrumental) among commercial WPC80 and WPI and distinctions between Cheddar and Mozzarella-sourced whey proteins. A previous study comparing SPC34 and WPC34 flavor also demonstrated distinct volatile compound differences between pilot plant manufactured and commercial samples (Evans et al., In Press). Processing and handling differences, time required for processing, different raw product streams (e.g. Mozzarella vs white Cheddar vs colored Cheddar) and composition differences are all possible source of these differences.

GC-O

Forty-six aroma active compounds were detected in the pilot plant WPC80 and SPC80 by GC-O (Table 3.16). The compounds identified were aldehydes, ketones, esters, sulfur-containing compounds, and free fatty acids. Of these compounds, thirteen were positively identified by mass spectra, retention index (RI), and odor properties of authentic standards, 26 compounds were tentatively identified by comparing RI and odor with standards, 3 were tentatively identified by RI and previously published standards and 4 remained unknown (Table 3.16). These compounds are primarily products of lipid or protein oxidation. Most of the compounds identified have been previously identified in whey, WPC, and or WPI (Carunchia-Whetstine et al., 2005a, Evans et al., In Press; Karagul-Yuceer et al., 2003; Mahajan et al., 2004; Mortenson et al., 2006; Wright et al., 2006, Wright et al., 2008).

Saturated and unsaturated aldehydes were detected in all samples (Table 3.16). These compounds are lipid oxidation products and have been previously documented in dried whey products (Gallardo-Escamilla et al., 2005; Mahajan et al., 2004; Wright et al., 2006; Carunchia-Whetstine et al., 2005; Javidipour et al., 2008; Wright et al., 2008). Five acids (acetic acid, butanoic acid, pentanoic acid, decanoic acid, and 4-methyl octanoic acid) were identified in the pilot plant products. Of these five fatty acids, butanoic, 4-methyl octanoic acid, and decanoic acid showed high aroma activity (3 or greater) (Table 3.16). These three acids have been previously reported as aroma active compounds in whey products (Wright et

al., 2006; Mahajan et al., 2004; Gallardo-Escamilla et al., 2005; Mortenson et al., 2007; Carunchia-Whetstine et al., 2005a). Free fatty acids can be formed from the action of milk or bacterial lipase reacting with triglycerides and phospholipids found in the milk (Ferretti et al., 1971; Kim et al., 2003). Five ketones, diacetyl (buttery), 1-hexen-3-one (rubbery), 1-octen-3-one (mushroom), (Z)-1,5-octadien-3-one (geranium), and 1-nonen-3-one (mushroom) were identified in both proteins.

Four sulfur containing compounds (dimethyl disulfide, dimethyl trisulfide, methional, and thenythiol) were identified in the pilot whey products. Each of these compounds have previously been documented in whey products (Wright et al., 2006; Wright et al., 2008; Mortenson et al., 2007; Carunchia-Whetstine et al., 2005a; Mahajan et al., 2004; Evans et al., In Press). These sulfur containing compounds have aromas associated with garlic, cabbage, and potato. Thermally generated compounds were also identified in both products. 2-Acetyl-1-pyrroline was tentatively identified by RI and aroma, and has been documented in previously published work on fluid whey (Karagul-Yuceer et al., 2003) and dried whey proteins (Carunchia-Whetstine et al., 2005a; Wright et al., 2006) as has 2-methyl-3-furanthiol. 2-Isobutyl-3-methoxypyrazine, 2-acetylpyridine, 2-acetyl pyrroline, 2-methyl-3-furanthiol, and sotolon are thermally generated compounds from Maillard and caramelization reactions (Friedman, 1996; Blank et al., 1996; Mahajan et al., 2004). Three esters were identified in the proteins; ethyl propionate, 2-methylbutyl acetate, and ethyl octanoate. These compounds had sweet fruity aromas and are formed by an enzymatic or chemical pathway resulting in the esterification of fatty acids with primary alcohols (Dumont et al., 1979). 2-

methylbutyl acetate has been previously reported in serum protein concentrate 34 (SPC34) (Evans et al., In Press). Ethyl propionate and ethyl octanoate have not been previously identified in whey or whey proteins, however they have been reported in various cheeses (Qian et al., 2003 (Parm); Larrayoz et al., 2001 (Ewe's milk cheese); Moio et al., 2000 (Gorgonzola)).

2-Phenethanol, 2-methoxyphenol and skatole were identified in the proteins and the compounds have been previously identified in dried whey and whey proteins (Carunchia-Whetstine et al., 2005a; Mahajan et al., 2004). Lactones, with fruity, dairy, and tropical aromas, were identified in both products. Lactones are believed to be formed from triglycerides containing a hydroxyl acid moiety (Parlimentt et al., 1966) during the pasteurization of milk (Dimick et al., 1969). Lactones have been previously reported in liquid Cheddar whey (Karagul-Yuceer et al., 2003), whey protein concentrate and isolate (Carunchia-Whetstine et al., 2005a; Wright et al; 2006), and Cheddar cheese (Carunchia-Whetstine et al., 2005b).

SPC vs. WPC

Five compounds were more prevalent in SPC80 than WPC80 (higher FD factors) or were not detected in WPC80 (Table 3.16). Hexanal (green), 2-methoxy phenol (smokey), and E-2-nonenal (cucumber) had higher FD factors in SPC80 compared to WPC80, and thenythiol (garlic) and 4,5-dimethyl thiazole (burnt) were not detected in WPC80 but present in SPC80. These compounds most likely result from the alternative processing parameters, especially the multiple filtration steps associated with separating out the serum proteins.

SPC80 was significantly lower in fat content compared to the pilot plant WPC80. The lower fat content means the lipid oxidation products that do form play a more significant role in the overall flavor, as well as compounds not formed by lipid oxidation. Eighteen compounds were more prevalent in WPC80 or absent altogether from SPC80 by GC-O (Table 3.16). A majority of these compounds can be associated as products formed during the cheese making process and starter culture fermentation (Singh et al., 2003), along with lipid oxidation products. The increased number of lipid oxidation products in WPC80 might also be associated with the higher fat content in WPC80 compared to SPC80 (Table 3.3) or due to starter culture fermentation (Tomaino et al., 2004).

Evans et al. (In Press) evaluated aroma-active compounds in pilot plant manufactured SPC34 and WPC34. Eighteen of the 29 compounds identified in the pilot plant manufactured WPC34 and SPC34 were identified in the pilot plant manufactured WPC80 and SPC80 (diacetyl, acetic acid, 2/3-methylbutanal, dimethyl disulfide, hexanal, butyric acid, 2-methylbutyl acetate, Z-4-heptanal, methional, 2-acetyl-1-pyrroline, dimethyl trisulfide, 1-octen-3-one, octanal, thenylthiol, 2-methoxy phenol, E,Z-2-6-nonadienal, 2-phenethanol, and E-2-nonenal). More aroma-active compounds were identified in the WPC80 and SPC80 (46) than in the SPC34 and WPC34 (29). The additional compounds identified in the 80% protein samples were primarily aldehydes (5), lactones (5), and ketones (4). Similar to WPC34 and SPC34, WPC80 had higher FD factors for diacetyl, acetic acid, and butyric acid. These compounds are associated with starter culture fermentation (Singh et al., 2003). The differences observed between WPC80 and SPC80 were greater than those

observed in WPC34 and SPC34. These more pronounced differences can be attributed to further processing and concentration of the whey and serum proteins and resulting differences in composition.

Consumer Testing:

An acidified 6.8% protein peach flavored beverage was selected as an ingredient application. Previous studies have demonstrated that this application was generally liked by consumers compared to other protein beverages while also being sensitive to ingredient flavor carry-through (Drake et al., 2008; Childs et al., 2007; Wright et al., 2009).

Representative commercial WPC80, selected by descriptive sensory profiles, WPC80 2, 3, and 4, were also incorporated into beverages and compared to beverages made with pilot plant proteins.

Serum vs. Traditional

Descriptive sensory analysis of peach beverages revealed differences between beverages made from pilot plant SPC80 and WPC80, as well as differences between beverages made from pilot plant products and beverages made from commercial WPC80 (Table 3.17). One of the main differences between pilot plant SPC80 and WPC80 beverages was the presence of FFA flavor in beverages made from SPC80 and the absence of free fatty acid flavor in beverages made from WPC80. Reconstituted SPC80 at neutral pH had no detectable levels of FFA flavor (Table 3.12). However, a shift to a lower pH (beverage pH was 3.83-3.9) may increase volatility of free fatty acids with pKa values of 4.75, 4.82, 4.86 and 4.84 for acetic, butyric, propionic and hexanoic acid, respectively. SPC80 also had

lower fat content compared to WPC80 which may have provided a reservoir for protonated free fatty acids, allowing them to be undetected by sensory analysis. Our volatile compound analyses were not conducted on acidified solutions, meaning concentrations of these volatile free fatty acids in these proteins may have been overlooked. Both pilot SPC80 and WPC80 also contained soapy flavor and bitter taste intensities (Table 3.17). Soapy flavor has been previously noted in WPI rehydrated under neutral conditions and has been associated with medium chain fatty acids (Carunchia Whetstine et al., 2005a). Free fatty acids (short and medium chain length) may also be the source of bitter taste. SPC80 had higher intensities of soapy flavor and bitter taste compared to WPC80. Consistent with trained panel descriptive profiles, overall liking of beverages made from pilot WPC80 was higher than scores for beverages made from pilot SPC80 (Table 3.18).

Pilot vs. Commercial

Trained panel profiles of beverages made from commercial and pilot scale proteins were also distinct (Table 3.17). Cereal and soapy flavors and bitter taste were documented in SPC80 and WPC80 pilot plant beverages and were not present in the beverages made with commercial proteins. In contrast, cardboard flavor was present in all three beverages made from commercial whey protein, but this flavor was not detected in beverages made with pilot plant SPC80 and WPC80. Cardboard and brothy flavors have been previously documented in beverages with whey protein (Childs et al., 2007; Drake et al., 2008; Wright et al., 2009).

Consumer liking scores were highest for beverages made with one commercial protein (product 3) (Table 3.18). Beverages made with pilot plant WPC80 and commercial

protein sample 2 were liked second best. Both beverages made with pilot scale products scored significantly higher in appearance liking than beverages made with the commercial proteins. Beverages made with commercial sample 4 scored the lowest in overall liking and liking attributes. This protein had the highest cardboard flavor intensity when rehydrated in water (Table 3.13).

CONCLUSIONS

SPC80 had lower fat content and higher pH compared to pilot manufactured and commercial WPC80. Pilot manufactured rehydrated SPC80 and WPC80 were similar in flavor profile. Volatile compound differences were noted; pilot WPC80 generally had higher concentrations as well as a higher number of lipid oxidation products than pilot SPC80. Similarly, lipid oxidation products were generally higher in commercial samples compared to the SPC80 and WPC80 produced in this study. These results suggest that additional commercial processing parameters contribute to flavor and flavor variability in WPC80. Acidified peach protein beverages made with pilot or commercial proteins were distinct in sensory profiles and consumer liking and revealed differences that were not identified in rehydrated protein solutions, emphasizing the need for ingredients to be evaluated in representative ingredient applications. Differences between pilot plant products and commercial samples, and the variability between commercial samples alone, suggests other influencing factors play a role in flavor of WPC80.

ACKNOWLEDGEMENTS

Funded in part by Dairy Management, Inc. (Rosemont, IL). The use of tradenames does not imply endorsement nor lack of endorsement by those not mentioned.

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Table 3.1. ANOVA type III SS and *P* values for ultrafiltration (UF) (after first 30 min of processing run) and diafiltration (DF) flux across the time of processing. Treatment is the comparison of processing whey versus MF permeate.

	UF flux		DF flux	
	SS	<i>P</i>	SS	<i>P</i>
Treatment (n = 2)	66.05	0.02	128.41	0.04
Replicate (n = 3)	3.64	0.04	5.41	0.67
Treatment x Replicate	2.67	0.04	10.94	< 0.01
Time	101.24	< 0.01	30.47	< 0.01
Time x Replicate	2.70	0.04	0.30	0.65
Time x Treatment	12.33	< 0.01	3.89	< 0.01
R-square	0.90		0.88	

Table 3.2. Mean (n = 3) composition (percent by weight) of the serum protein concentrate (SPC) and whey protein concentrate (WPC) liquids after UF/DF and before spray drying.

	Total Solids	Fat	TN ¹	NPN ²	TP ³	pH ⁴
SPC	6.81 ^b	0.02 ^b	5.41 ^b	0.10 ^b	5.31 ^b	6.87 ^a
WPC	8.57 ^a	0.79 ^a	6.26 ^a	0.36 ^a	5.90 ^a	6.51 ^b
R-square	0.98	0.82	0.98	0.98	0.98	0.97
SE	0.25	0.10	0.10	0.01	0.10	0.03

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹TN = total protein x 6.38.

²NPN = nonprotein nitrogen x 6.38.

³TP = true protein (TN minus NPN).

⁴pH of the liquid WPC and SPC.

Table 3.3. Mean (n = 3) composition (percent by weight) of spray dried serum protein concentrate (SPC) and whey protein concentrate (WPC) calculated on a wet basis.

	Moisture	Fat	TN ¹	NPN ²	TP ³
SPC	7.08	0.49 ^b	72.39 ^a	2.18 ^b	70.22 ^a
WPC	5.99	7.63 ^a	67.85 ^b	6.79 ^a	61.06 ^b
R-square	0.53	0.88	0.82	0.99	0.93
SE	0.29	0.73	0.70	0.10	0.71

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹TN = Total nitrogen x 6.38.

²NPN = Nonprotein nitrogen x 6.38.

³TP = True protein (TN minus NPN), for WPC this does not include GMP.

Table 3.4. Mean (n = 3) composition (percent by weight) of spray dried serum protein concentrate (SPC) and whey protein concentrate (WPC) calculated on a dry basis.

	Fat	TN ¹	NPN ²	TP ³	GMP ⁴
SPC	0.53 ^b	77.90 ^a	2.34 ^b	75.56 ^a	0.00 ^b
WPC	8.12 ^a	72.17 ^b	7.22 ^a	64.95 ^b	4.88 ^a
R-square	0.87	0.88	0.99	0.94	0.99
SE	0.78	0.64	0.10	0.68	0.09

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹TN = Total nitrogen x 6.38.

²NPN = Nonprotein nitrogen x 6.38.

³TP = True protein (TN minus NPN), for WPC this does not include GMP.

⁴GMP = Glycomacropeptide, calculated as NPN of WPC minus NPN of SPC.

Table 3.5. Mean (n = 3) mineral composition (percent by weight) of serum protein concentrate (SPC) and whey protein concentrate (WPC) as calculated on a dry basis.

	Ca	P	K	Mg	Na	S ¹
SPC	0.41	0.32	0.95 ^a	0.07	0.21 ^a	1.26 ^a
WPC	0.51	0.38	0.82 ^b	0.07	0.19 ^b	1.08 ^b
R-square	0.93	0.95	0.99	0.99	0.99	0.96
SE	0.02	0.01	0.01	0.00005	0.002	0.02

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹The P value for sulfur was $P = 0.0543$

Table 3.6. Mean (n = 3) composition (percent by weight) of spray dried serum protein concentrate (SPC) and whey protein concentrate (WPC) calculated on a non fat dry basis.

	TN ¹	NPN ²	TP ³
SPC	78.32	2.36 ^b	75.97 ^a
WPC	78.66	7.87 ^a	70.79 ^b
R-square	0.96	0.99	0.96
SE	0.33	0.12	0.41

^{a,b} Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹TN = Total nitrogen x 6.38.

²NPN = Nonprotein nitrogen x 6.38.

³TP = True protein (TN minus NPN).

Table 3.7. Composition (percent by weight) and pH of (n = 3) 80% serum protein concentrate (SPC), whey protein concentrate (WPC) and five commercial (n = 2) 80% whey protein concentrates calculated on a wet basis.

Product	Moisture	Fat	TN ¹	pH ²
1	5.22 ^{bc}	5.03 ^b	77.14 ^a	6.41 ^b
2	3.99 ^{bc}	7.72 ^a	77.43 ^a	6.36 ^b
3	4.24 ^{bc}	4.32 ^b	76.52 ^a	6.36 ^b
4	4.53 ^{bc}	4.95 ^b	77.30 ^a	6.37 ^b
5	3.58 ^c	6.76 ^{ab}	75.92 ^a	6.01 ^c
SPC	7.08 ^a	0.49 ^c	72.40 ^b	6.77 ^a
WPC	5.99 ^{ab}	7.63 ^a	67.85 ^c	6.43 ^b
R-square	0.68	0.74	0.80	0.82
SE Commercial	0.47	0.85	1.01	0.09
SE Pilot	0.39	0.69	0.83	0.08

^{a,b,c} Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹TN = Total nitrogen x 6.38.

²pH of the reconstituted (10% w/v) commercial and experimental powders.

Table 3.8. Composition (percent by weight) and pH of (n = 3) 80% serum protein concentrate (SPC), whey protein concentrate (WPC) and five commercial (n = 2) 80% whey protein concentrates calculated on a dry basis.

Product	Fat	TN ¹
1	5.31 ^b	81.38 ^a
2	8.02 ^a	80.65 ^a
3	4.51 ^b	79.91 ^{ab}
4	5.19 ^b	80.97 ^a
5	7.02 ^{ab}	78.77 ^{ab}
SPC80	0.53 ^c	77.90 ^b
WPC80	8.12 ^a	72.17 ^c
R-square	0.74	0.76
SE Commercial	0.90	1.02
SE Pilot	0.74	0.83

^{a,b,c} Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹TN = Total nitrogen x 6.38.

Table 3.9. Composition (percent by weight) and pH of (n = 3) 80% serum protein concentrate (SPC), whey protein concentrate (WPC) and five commercial (n = 2) 80% whey protein concentrates calculated on a non fat dry basis.

Product	TN ¹
1	85.94 ^{ab}
2	87.70 ^a
3	83.68 ^b
4	85.40 ^{ab}
5	84.73 ^{ab}
SPC	78.32 ^c
WPC	78.66 ^c
R-square	0.70
SE Commercial	1.33
SE Pilot	1.08

^{a,b,c} Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹TN = Total nitrogen x 6.38.

Table 3.10. Mean ($n = 3$) relative proportions of casein to serum proteins for skim milk and 80% WPC and 80% SPC powders by the densitometry analysis of the SDS-PAGE gels.

	Casein	Serum Proteins
Milk	83.23	16.77
SPC	3.76 ^a	96.24 ^b
WPC	1.98 ^b	98.02 ^a
R-square	0.93	0.93
SE SPC and WPC	0.18	0.18

^{a,b,c} Means for SPC and WPC in the same column not sharing a common superscript are different ($P < 0.05$).

Table 3.11. Mean ($n = 3$) relative proportions of β -lactoglobulin to α -lactalbumin for skim milk and 80% WPC and 80% SPC powders by the densitometry analysis of the SDS-PAGE gels.

	β -lactoglobulin	α -lactalbumin
Milk	79.77 ^a	20.23 ^c
SPC	76.05 ^c	23.95 ^a
WPC	77.39 ^b	22.61 ^b
R-square	0.91	0.91
SE Milk	0.33	0.33
SE SPC, WPC	0.19	0.19

^{a,b,c} Means in the same column not sharing a common superscript are different ($P < 0.05$).

Table 3.12. Means sensory attributes of serum protein concentrate (SPC80) and whey protein concentrate (WPC80) produced in this study (n=3).

Product	Aroma Intensity	Cardboard	Cereal/grainy	Cooked/Milky	Sweet Aromatic	Astringency
SPC80	1.2 ^B	0.8 ^A	1.2 ^B	1.1 ^A	ND	1.6 ^A
WPC80	1.6 ^A	1.3 ^A	1.9 ^A	1.1 ^A	0.8 ^A	1.9 ^A
R-square	0.74	0.65	0.81	0.09	NA	0.67
SE	0.11	0.11	0.07	0.14	NA	0.12

Means in the same column not sharing a common superscript are different (P<0.05)

Intensities were scored on a 0 to 15-point universal scale where 0 = none and 15 = very high intensity (Meilgaard et al., 1999).

Dried whey ingredient intensities usually fall between 0 and 4 on this scale (Drake et al., 2003; Wright et al., 2009).

Attributes not listed were not detected. ND – not detected

SE – standard error, NA – not applicable

Table 3.13. Mean sensory attributes of SPC80 and WPC80 produced in study (n=3) and 5 commercial WPC80 (n=2).

Product	Aroma Intensity	Sweet Aromatic	Cereal/grainy	Cooked/ Milky	Diacetyl	Cardboard	Potato	Astringency
SPC80	1.2 ^D	ND	1.2 ^B	1.1 ^A	ND	0.8 ^D	ND	1.6 ^D
WPC80	1.6 ^{BC}	0.7 ^B	1.9 ^A	1.1 ^A	ND	1.3 ^{BCD}	ND	1.9 ^C
1	1.8 ^{BC}	ND	1.1 ^B	ND	ND	1.6 ^B	ND	2.3 ^{AB}
2	1.2 ^D	0.8 ^B	ND	ND	ND	1.1 ^{CD}	ND	2.1 ^B
3	1.4 ^{CD}	1.5 ^A	1.1 ^B	ND	ND	1.0 ^{CD}	ND	2.3 ^{AB}
4	2.3 ^A	ND	ND	ND	ND	2.2 ^A	1.4	2.2 ^{AB}
5	1.0 ^D	0.8 ^B	0.8 ^C	ND	1.1	1.3 ^{BCD}	ND	2.5 ^A
R-square	0.74	0.65	0.91	0.77	NA	0.70	NA	0.56
SE Comm	0.14	0.16	0.09	0.17	NA	0.15	NA	0.14
SE Pilot	0.11	0.13	0.07	0.14	NA	0.11	NA	0.12

Means in the same column not sharing a common superscript are different ($P < 0.05$)

Intensities were scored on a 0 to 15-point universal scale where 0 = none and 15 = very high intensity (Meilgaard et al., 1999).

Dried whey ingredient intensities usually fall between 0 and 4 on this scale (Drake et al., 2003; Wright et al., 2009).

Attributes not listed were not detected. ND = Not Detected.

Comm = Commercial. Pilot = Pilot plant products, SE – standard error, NA – not applicable

Table 3.14. Mean (n=3) concentrations of selected aroma-active compounds (µg/L) of spray dried serum protein concentrate (SPC) and spray dried whey protein concentrate produced in this study isolated using solid phase microextraction (SPME).

Product	Diacetyl	2-Methyl butanal	Pentanal	Hexanal	Heptanal	Z-4-Heptanal	Octanal	Nonanal	Decanal
SPC80	ND	0.26 ^B	ND	1.06 ^B	ND	ND	1.04 ^A	0.5 ^B	2.32 ^A
WPC80	0.50 ^A	0.77 ^A	0.66 ^A	6.58 ^A	0.43 ^A	2.3 ^A	0.78 ^A	1.14 ^A	0.74 ^B
R-Square	0.38	0.80	0.88	0.72	0.90	0.81	0.24	0.79	0.92
SE	0.07	0.05	0.04	0.74	0.02	0.20	0.10	0.07	0.08

^{A, B} Means in same column not sharing a common superscript are different (P < 0.05).

SE – standard error

Table 3.15. Mean concentrations of selected volatile compounds ($\mu\text{g/L}$) of spray dried ($n = 3$) serum protein concentrate (SPC) and spray dried ($n = 3$) whey protein concentrate produced in this study and five commercial ($n=2$) 80% whey protein concentrates isolated using solid phase microextraction (SPME).

Product	Diacetyl	2-Methyl butanal	Pentanal	Hexanal	Heptanal	Z-4-Heptanal	Octanal	Nonanal	Decanal
1	1.16 ^{BC}	2.78 ^{AB}	6.11 ^A	61.2 ^A	4.25 ^A	2.66 ^A	1.18 ^A	2.25 ^A	0.09 ^C
2	2.92 ^A	3.15 ^A	3.9 ^B	26.5 ^B	2.15 ^B	2.23 ^A	0.70 ^{BC}	1.92 ^A	0.27 ^C
3	0.57 ^{BCD}	1.17 ^{CD}	1.03 ^C	1.4 ^C	0.63 ^C	2.39 ^A	0.42 ^C	0.73 ^C	0.78 ^B
4	1.37 ^B	1.99 ^{ABC}	3.36 ^B	44.6 ^{AB}	2.57 ^B	2.31 ^A	1.1 ^{AB}	1.63 ^{AB}	0.08 ^C
5	2.86 ^A	1.72 ^{ABC}	3.66 ^B	40.01 ^B	2.04 ^B	2.50 ^A	0.53 ^C	1.83 ^A	0.15 ^C
SPC80	ND	0.26 ^C	ND	1.06 ^C	ND	ND	1.04 ^{AB}	0.5 ^C	2.32 ^A
WPC80	0.50 ^{CD}	0.77 ^{CD}	0.66 ^C	6.58 ^C	0.43 ^C	2.32 ^A	0.78 ^{ABC}	1.14 ^{BC}	0.74 ^B
R-square	0.67	0.43	0.63	0.65	0.73	0.02	0.33	0.54	0.86
SE Comm	0.31	0.50	0.68	7.1	0.35	0.41	0.16	0.24	0.14
SE Pilot	0.25	0.40	0.55	5.8	0.29	0.34	0.13	0.20	0.11

^{A, B} Means in same column not sharing a common superscript are different ($P < 0.05$).

ND = Not Detected, Comm = Commercial, Pilot = Pilot plant products produced in this study

SE – Standard Error, NA – Not Applicable, DMDS – Dimethyl disulfide, DMTS – Dimethyl trisulfide

Table 3.16. Aroma active compounds detected in SPC80 and WPC80 by solvent assisted flavor evaporation with gas chromatography olfactometry with aroma extract dilution analysis (AEDA).

No	Compound	Fraction	Odor ¹	Log ₃ Flavor Dilution ² (Aroma Intensity ³)		Retention Index ⁴		Methods of Identification ⁵
				WPC80	SPC80	DB-5	DB-Wax	
1	Diacetyl	NB	buttery	3 (1)	ND	604		RI, Odor
2	Acetic Acid	AC	vinegar	<1 (1.5)	ND	643	1365	RI, Odor
3	2/3-Methylbutanal	NB	malty	<1 (2.4)	<1 (1)	647		RI, Odor
4	Ethyl propionate	NB	fruity	1 (1.5)	<1 (1)	708	945	RI, Odor
5	Dimethyl disulfide (DMS)	NB	garlic	<1 (1.2)	<1 (1.5)	744	1101	RI, Odor, MS
6	1-Hexen-3-one	NB	rubber	2 (1.3)	ND	759		RI, Odor
7	Hexanal	NB	green/earthy	<1 (1.8)	4 (1)	791	922	RI, Odor, MS
8	Butyric Acid	AC	butyric/cheesy/sweaty/malty	3 (2)	1 (2.5)	807	1630	RI, Odor
9	2-Methyl-3-furanthiol	NB	vitamin	<1 (3)	<1 (4)	853		RI, Odor, MS
10	2-Methylbutyl acetate	NB	dried fruit	<1 (3.5)	ND	888	1061	RI, Odor
11	Z-4-Heptanal	AC	fishy/fatty	1 (1)	1 (1)	891	1138	RI, Odor, MS
12	Methional	AC	potato	4 (2)	3 (1.5)	899	1358	RI, Odor, MS
13	Pentanoic acid	NB	sour/sweetish /swiss cheese	<1 (3.2)	<1 (2)	906		RI, Odor
14	2-Acetyl-1-pyrroline ⁷	NB	popcorn	4 (2.5)	3 (2)	914	1290	RI, Odor
15	4, 5-Dimethyl thiazole	AC	burnt	ND	3 (1)	939		RI, Odor, MS
16	Dimethyl trisulfide (DMTS)	NB	garlic	2 (3.4)	3 (3.25)	945	1379	RI, Odor
17	1-Octen-3-one	AC	mushroom	4 (1.5)	3 (2)	972	1225	RI, Odor, MS
18	(Z)-1,5-octadien-3-one ⁶	NB	geranium	2 (3)	<1 (1.75)	978		RI
19	Octanal	NB	citrus	2 (3)	<1 (2.5)	995	1157	RI, Odor, MS
20	2-Acetylpyridine	NB	popcorn	<1 (2)	<1 (2)	1019		RI, Odor
21	Phenylacetaldehyde	NB	honey/floral	<1 (1.5)	ND	1041		RI, Odor
22	Thienylthiol	NB	weeds/sulfur	ND	<1 (2)	1050	1504	RI, Odor
23	2-Methoxy Phenol	NB	fatty/smoke	3 (2.3)	9 (2.5)	1054		RI, Odor, MS
24	1-nonen-3-one ⁶	NB	mushroom	<1 (1.8)	ND	1072		RI
25	E,Z-2,6-Nonadienal	NB	burnt/cucumber	3 (2)	2 (2)	1080	1499	RI, Odor, MS
26	Nonanal	NB	fatty	3 (2.7)	3 (3)	1096	1406	RI, Odor, MS
27	3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	AC	maple	<1 (3)	<1 (3)	1116	2186	RI, Odor
28	2-Phenethanol	NB	rose	4 (3.5)	4 (2)	1142	1880	RI, Odor
29	unknown	NB	stale/garbage	<1 (2)	ND	1143		
30	E-2-Nonenal	NB	cucumbers	4 (3)	6 (2)	1149	1583	RI, Odor, MS
31	2-Isobutyl-3-methoxypyrazine	NB	bell pepper	3 (3.5)	2 (2)	1162	1403	RI, Odor
32	Ethyl octanoate	NB	sweet/fatty/citrus	<1 (1.8)	<1 (2.75)	1192	1436	RI, Odor
33	Decanal	NB	fatty	6 (1.9)	2 (2)	1214	1484	RI, Odor, MS
34	unknown	NB	cilantro	<1 (2.7)	ND	1261		
35	unknown	NB	oatmeal	6 (3)	2 (1)	1266		
36	γ-Octalactone	NB	coconut	<1 (2.5)	ND	1285	1918	RI, Odor
37	Decanoic Acid	NB	fatty/stale	6 (1.5)	2 (1.75)	1310	2361	RI, Odor
38	γ-Nonalactone	NB	cilantro/waxy	9 (3)	9 (2.25)	1358	2042	RI, Odor
39	4-Methyl Octanoic acid	NB	waxy	4 (3)	<1 (2)	1412	2176	RI, Odor
40	Skatole	NB	mothball	<1 (2)	<1 (1.5)	1459	2486	RI, Odor
41	γ-Decalactone	NB	floral/peach	1 (2)	<1 (1.5)	1483	2228	RI, Odor
42	δ-Decalactone	NB	waxy/coconut	3 (1.5)	2 (1.5)	1532	2103	RI, Odor
43	unknown	NB	sweet/soap	<1 (2.7)	ND	1572	1972	
44	γ-Dodecalactone	NB	peach	1 (3)	<1 (1.25)	1661	2389	RI, Odor
45	(E,E)-2,4-Nonadienal	NB	green	<1 (3.25)	<1 (1.5)		1796	RI, Odor
46	Tetradecanol	AC	bread/coconut	2 (1.5)	ND		2159	RI, Odor

¹ Odor description at the gas chromatograph (GC) sniffing port

² Flavor dilution factors were determined on a DB-5MS column for neutral/basic (NB) compounds and on a DB-WAX column for acidic (AC) compounds.

³ Aroma intensity at the GC sniffing port, on a scale of 1-5.

⁴ Retention indices were calculated from gas chromatography/olfactory data.

⁵ Compounds were identified by comparison with authentic standards on the following criteria; retention index (RI) on DB-WAX and DB_5MS columns, odor property at the GC-sniffing port, and mass spectra in the electron impact mode. Positive identification indicates that mass spectral data compared with authentic standards.

⁶ Compounds were unable to be confirmed by comparison of authentic standards, do to compounds having to be synthesized. Identified by aroma, RI, and literature.

⁷ Compound identified by comparing RI and aroma with literature (Avsar et al., 2004).

Table 3.17. Mean sensory attributes of 6% protein peach beverages manufactured (n=2) from SPC80 and WPC80 manufactured in this study and three commercial WPC80.

Product	Aroma Intensity	Fruity	Sweet Aromatic	Cardboard	Cereal	FFA	Soapy	Bitter	Sweet Taste	Sour taste	astringency
2	2.8 ^{AB}	2.8 ^A	2.1 ^A	1.9 ^A	ND	ND	ND	ND	5.5 ^A	3.5 ^A	3.5
3	3.0 ^A	2.5 ^{AB}	2.2 ^A	0.8 ^B	ND	ND	ND	ND	6.0 ^A	3.5 ^A	3.2
4	1.5 ^B	2.0 ^{ABC}	2.0 ^A	2.4 ^A	ND	ND	ND	ND	5.8 ^A	3.5 ^A	3.7
SPC80	2.3 ^{AB}	1.5 ^C	2.0 ^A	ND	1.5 ^A	1.3 ^A	2.3 ^A	1.8 ^A	5.5 ^A	3.3 ^{AB}	3.6
WPC80	3.0 ^A	1.8 ^{BC}	2.3 ^A	ND	2.0 ^A	ND	1.3 ^B	0.8 ^B	5.8 ^A	3.0 ^B	3.6
R-square	0.75	0.78	0.90	0.98	0.95	0.91	0.98	0.96	0.75	0.81	0.90
SE	0.20	0.15	0.07	0.09	0.12	0.10	0.08	0.09	0.07	0.08	0.28

Means in the same column not sharing a common superscript are different (P<0.05)

Intensities were scored on a 0 to 15-point universal scale where 0 = none and 15 = very high intensity (Meilgaard et al., 1999).

ND = Not Detected. Dried whey ingredient intensities usually fall between 0 and 4 on this scale (Drake et al., 2003; Wright et al., 2009).

Attributes not listed were not detected

FFA = Free Fatty Acid, SE – standard error

Table 3.18. Consumer (n=77) liking scores of 6% protein peach beverages manufactured from SPC80 and WPC80 manufactured in this study and three commercial WPC80.

Product	Aroma liking	Overall liking	Appearance Liking	Flavor	Texture
2	4.58 ^B	5.1 ^B	5.8 ^{AB}	5.2 ^A	5.8 ^A
3	5.55 ^A	5.8 ^A	5.6 ^B	5.6 ^A	5.9 ^A
4	3.58 ^C	3.7 ^D	5.4 ^B	3.6 ^C	5.2 ^B
SPC	5.50 ^A	4.4 ^C	6.2 ^A	4.3 ^B	5.6 ^{AB}
WPC	5.54 ^A	5.2 ^B	6.2 ^A	5.2 ^A	5.8 ^A
R-square	0.19	0.14	0.05	0.13	0.26
SE Comm	0.12	0.13	0.11	0.13	0.11
SE Pilot	0.15	0.15	0.12	0.16	0.13

Means in the same column not sharing a common superscript are different (P<0.05)

Consumers scored attributes using a 9-point hedonic scale with 1 = dislike extremely, and 9 = like extremely

Comm = Commercial. Pilot = Pilot plant products, SE – standard error, NA – not applicable

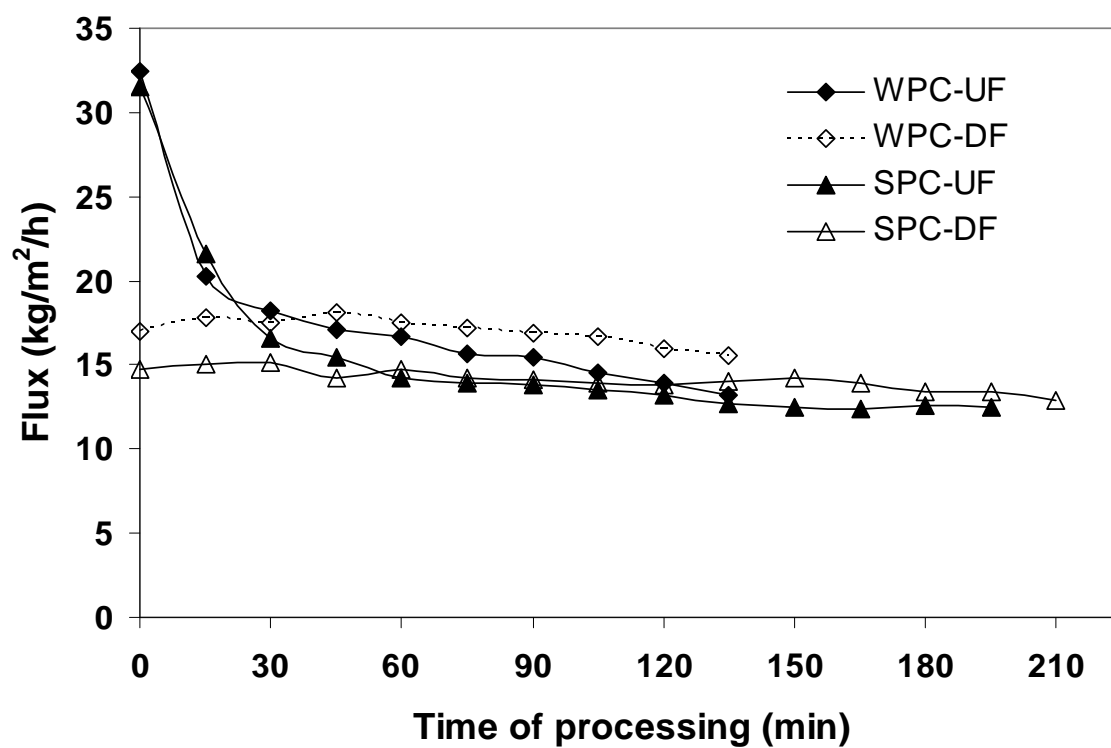


Figure 3. 1. Mean ($n = 3$) ultrafiltration (UF) and diafiltration (DF) flux during the production of 80% WPC and 80% SPC using a spiral wound 10 kDa polyethersulfone membrane.

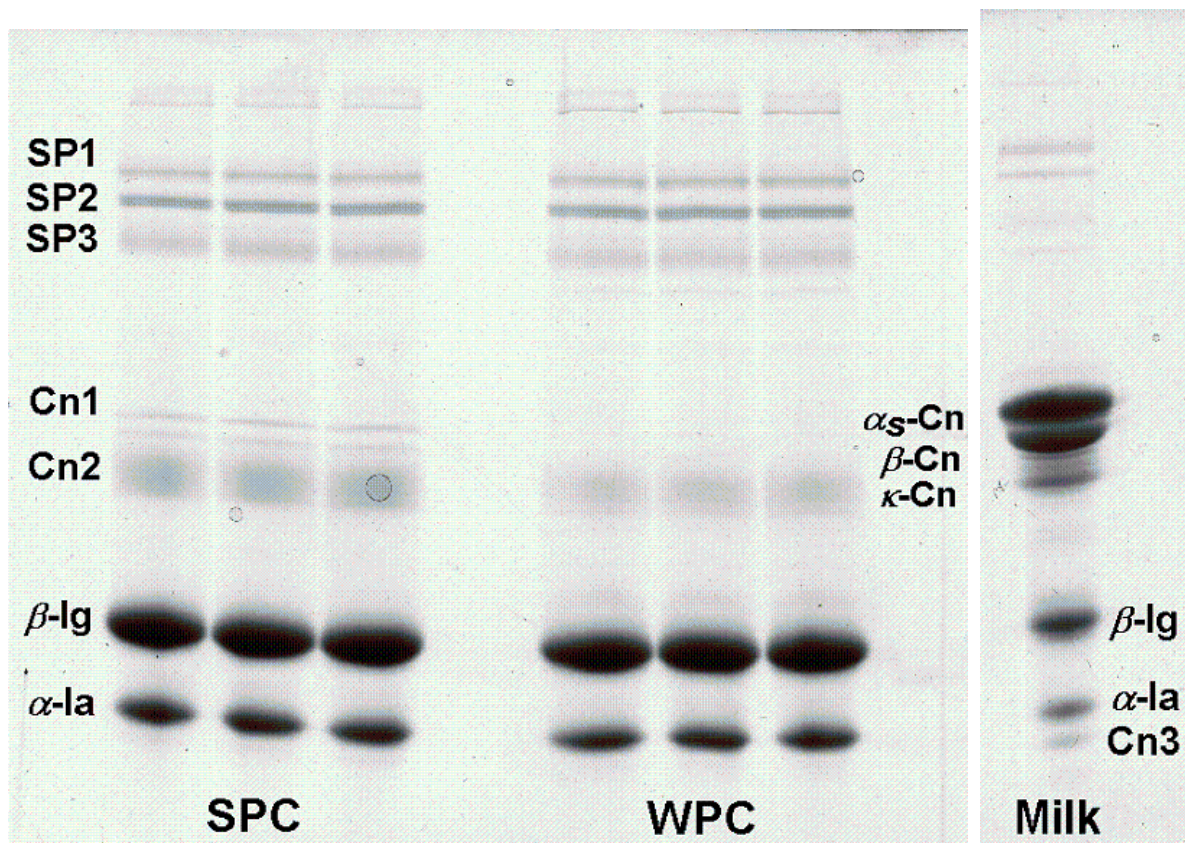
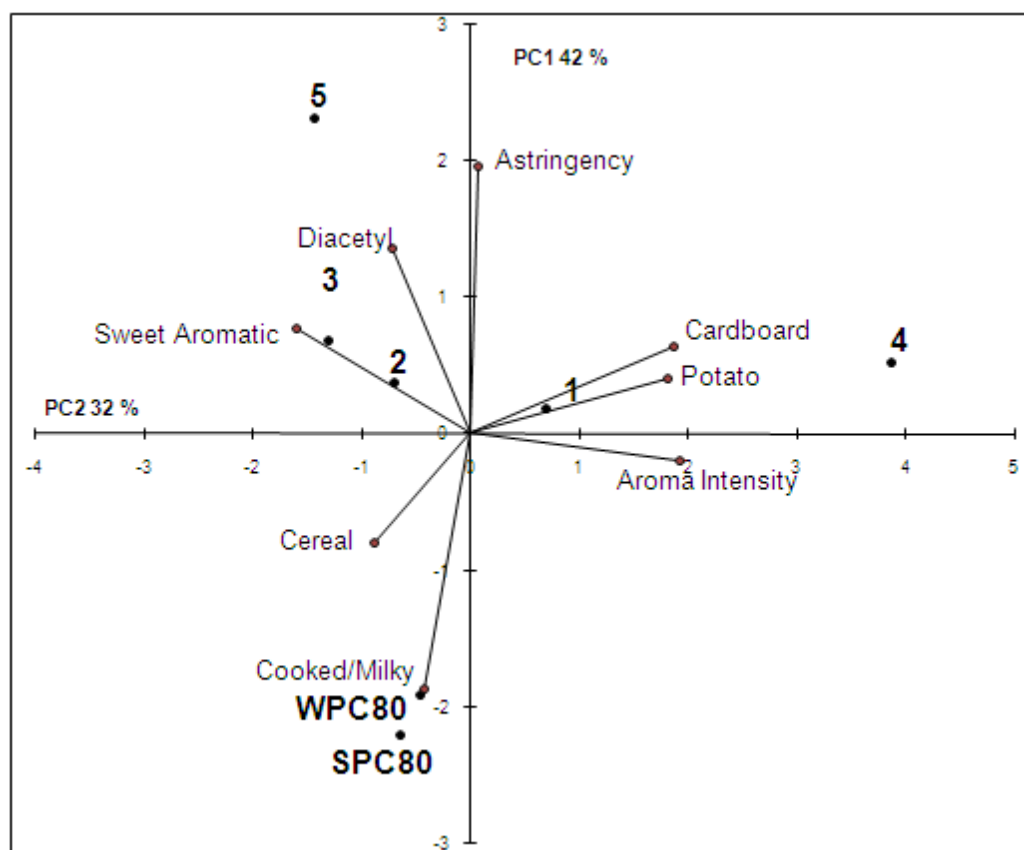


Figure 3. 2. Sodium dodecyl sulfate-PAGE electrophoretogram of proteins in experimental 80% serum protein concentrate (SPC), 80% whey protein concentrate (WPC) and skim milk. Bands are identified on the gel: SP1, SP2, SP3 = high molecular weight serum proteins, Cn1 = α_s -Cn (combination of α_{s1} and α_{s2} -Cn), Cn2 = casein proteolysis products, β -lg (β -lactoglobulin), α -la (α -lactalbumin), β -Cn (β -casein) and κ -Cn (κ -casein) Cn3 = proteolysis products of casein.



Figure 3. 3. Liquid 80% whey protein concentrate (WPC) (on the left) and 80% serum protein concentrate (SPC) (on the right) after diafiltration.



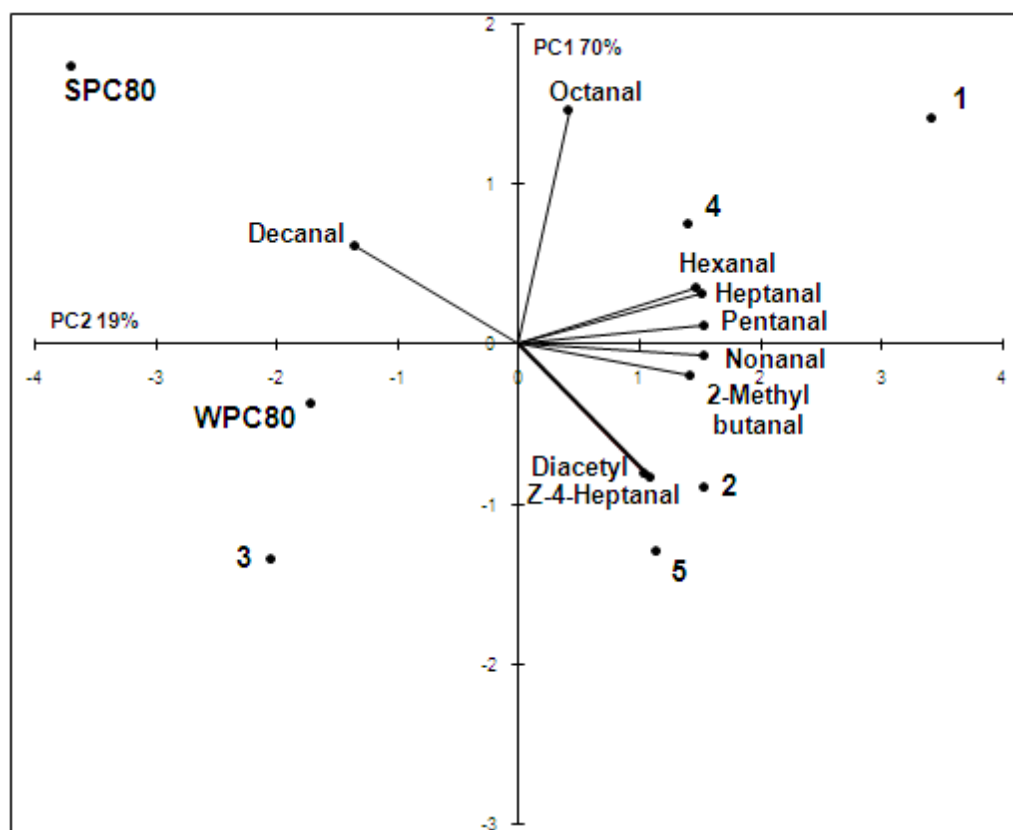
WPC80 – Whey Protein Concentrate 80, SPC80 – Serum Protein Concentrate 80,

PC1 and PC2 – principal components 1 and 2.

Numbers 1-5 represent five commercial WPC80.

WPC80 and SPC80 are the proteins manufactured in this study.

Figure 3. 4. Principal component biplot of sensory attributes of whey and serum protein concentrates manufactured in this study and 5 commercial WPC80.



Commercial whey proteins are designated by numbers 1-5.

WPC80 and SPC80 are the proteins manufactured in this study.

PC1 and PC2 – principal components 1 and 2. WPC80 – Whey Protein Concentrate 80, SPC80 – Serum Protein Concentrate 80.

Figure 3. 5. PCA biplot of SPME volatile compound results for whey and serum protein concentrates manufactured in this study and 5 commercial WPC80.