ABSTRACT

LI, XIAOMENG. The Influence of Storage, Heat Treatment and Solids Composition on the Bleaching of Cheddar Whey with Hydrogen Peroxide. (Under the direction of Dr. MaryAnne Drake).

The residual annatto colorant in liquid whey is removed by bleaching to provide a desired neutral color in dried whey ingredients. The objective of this study was to evaluate if starter culture, whey pasteurization with fat separation, liquid whey and retentate storage, solids composition, or spray drying affected the bleaching efficacy of liquid whey and retentate. Cheddar cheese whey with annatto was manufactured using a mesophilic lactic starter culture or by addition of lactic acid and rennet (rennet-set). Pasteurized fat-separated whey was ultrafiltered to 9% solids (w/w) and spray dried to 34% whey protein concentrate (WPC34). Aliquots of liquid whey were bleached at 60°C for 1 h (hydrogen peroxide, 250 ppm), before pasteurization and after fat separation, after pasteurization and fat separation, after storage at 3°C for 24 h, 48 h, and after freezing at -20°C for 10 d. Aliquots of retentate were bleached analogously immediately and after storage at 3 or -20°C. Freshly spray dried WPC34 was rehydrated to 9% (w/w) solids and bleached. In a final experiment, pasteurized fat-separated whey was ultrafiltered to 9% solids (w/w) and continued to 12% solids (w/w) with diafiltration and spray dried to WPC34 and WPC80. The WPC34 retentate was diluted to 7% solids (w/w) and WPC80 retentate was diluted to 9% or 7% solids (w/w) respectively. Samples were bleached at 50°C for 1 h. Freshly spray-dried WPC34 and WPC80 were also rehydrated to 9% solids and 12% solids and bleached. Bleaching efficacy was measured by extraction and quantification of norbixin. Proximate analyses and color analyses (Hunter Lab) were conducted. Each experiment was replicated 3 times. Starter culture, fat separation or pasteurization did not impact bleaching efficacy (p>0.05). Cold or frozen storage
decreased bleaching efficacy of liquid whey compared with immediate bleaching (p<0.05) while spray drying increased that of 34% retentate, but decreased that of 80% retentate (p<0.05). Bleaching efficacy of 80% (w/w) protein liquid retentate was higher than liquid whey or 34% (w/w) protein liquid retentate (p<0.05). These results confirm that processing steps, particularly hold times, and solids composition can influence bleaching efficacy of whey.

**Key words:** whey, retentate, norbixin, bleach
The Influence of Storage, Heat Treatment and Solids Composition on the Bleaching of Cheddar Whey with Hydrogen Peroxide

by
Xiaomeng Li

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

2011

APPROVED BY:

Dr. Brian E. Farkas

Dr. Timothy H. Sanders

Dr. MaryAnne Drake
Chair of Advisory Committee
DEDICATION

To Mom and Dad, who are always so supportive even though they miss me so much while I am studying on the other side of the world.
BIOGRAPHY

Xiaomeng Li was born in Sichuan, China on November 19th, 1987. Her parents, Xinjian Li, who is a government employee; and Xuedong Zhang, who is an ophthalmologist and professor of Chongqing Medical University, reside in Chongqing, China. Xiaomeng moved to Chongqing at the age of eight, graduated from Chongqing Foreign Languages School in 2005 and entered Zhejiang University in Hangzhou, China with an exemption of Chinese College Entrance Exam to study Biomedical Engineering. She joined a conjoint NCSU-ZJU program in 2009 and come to North Carolina State University to pursue her non-thesis master degree in Food Science one year before graduating from undergraduate. She got her bachelor degree in 2010. She found her love on research and started to work as a thesis student under the direction of Dr. MaryAnne Drake in August 2010.
ACKNOWLEDGMENTS

Two years ago this week, I was not sure if I could survive the conjoint program and become a graduate student in NCSU. I would like to really thank my parents who supported me so much during this journey. They comfort me when I had doubts and gave me great advices. To Dr. MaryAnne Drake for giving me the opportunity and taking me as a graduate student and being a great advisor. To Evan Miracle for spending time with me when I had concerns on analytical methods. To every member of MAD lab, thank you so much for helping me with my project, staying with me in the pilot plant for long long hours, forgiving my mistakes, and giving me wonderful advices both academically and in life. To NCSU dairy staff who were always so kind for helping me with the equipment in pilot plant. I would also like to thank all my friends for cheering me up when I was upset and didn’t have the confidence to move forward.
# TABLE OF CONTENTS

**LIST OF TABLES** ......................................................................................... vi

**LIST OF FIGURES** .................................................................................. vii

Chapter 1. Literature Review ........................................................................ 1

  - Introduction ........................................................................................................ 2
  - Whey processing and products ........................................................................... 4
  - Whey protein properties ..................................................................................... 8
  - Flavors of whey protein ...................................................................................... 11
    - Flavors in Liquid Whey .................................................................................. 12
    - Flavors in Dried Whey Protein Concentrate and isolates .................................. 14
    - Flavors of Additive - Annatto ........................................................................ 17
  - Analytical methods of flavor of liquid whey and dried whey ingredients .......... 19
    - Descriptive Sensory Analyses ......................................................................... 19
    - Instrumental Analyses ..................................................................................... 20
  - Bleaching of whey ............................................................................................. 26
    - The Color of Whey .......................................................................................... 26
    - Bleaching Agents ............................................................................................ 27
    - Effect of Bleaching on Flavor .......................................................................... 30
  - Research objective ............................................................................................ 31
  - References ......................................................................................................... 33

Chapter 2. The influence of storage, heat treatment and solids composition on the bleaching of Cheddar whey with hydrogen peroxide ......................................................... 44

  - Abstract .............................................................................................................. 44
  - Introduction ........................................................................................................ 48
  - Materials and methods ....................................................................................... 49
  - Results and discussion ....................................................................................... 56
  - Conclusions ......................................................................................................... 60
  - Acknowledgements ............................................................................................. 61
  - References ......................................................................................................... 62
LIST OF TABLES

Chapter 1.

Table 1.1  Typical composition of liquid and dry whey…………………………..  3

Chapter 2.

Table 2.1  Least squares mean values of percent norbixin destruction (250 ppm HP at 50°C) of liquid whey, 34% (w/w) protein retentate and 80% (w/w) protein retentate at different solids (experiment III)……………………………………………………………………………………..  65

Table 2.2  Least squares mean values of L* of liquid whey, 34% (w/w) protein retentate and 80% (w/w) protein retentate (experiment III)……….. 66

Table 2.3  Least squares mean values of b* of 34% (w/w) protein retentate (experiment II)………………………………………………………………………………..  67
LIST OF FIGURES

Chapter 1.

Figure 1.1   Liquid whey processing ................................................................. 6
Figure 1.2   Structures of bixin and norbixin ...................................................... 18
Figure 1.3   SPME Extraction Procedure .............................................................. 23
Figure 1.4   Chemical Structure of Hydrogen Peroxide ......................................... 27
Figure 1.5   Chemical Structure of Benzoyl Peroxide ........................................... 27
Figure 1.6   Reaction of Hydrogen Peroxide Breakdown ........................................ 28
Figure 1.7   Reaction of Benzoyl Peroxide Breakdown ........................................... 28

Chapter 2.

Figure 2.1a  Experimental design experiment I. The influence of starter culture,
unit operations and storage on bleaching efficacy .......................................... 68
Figure 2.1b  Experimental design experiment II. The influence of retentate
storage and spray drying on bleaching efficacy .............................................. 69
Figure 2.1c  Experimental design experiment III. The influence of solids and
solids composition on bleaching efficacy ....................................................... 70
Figure 2.2   Percent norbixin destruction in hydrogen peroxide bleached liquid
whey (60ºC for 1 h) from rennet or culture (experiment I) ............................. 72
Figure 2.3   Percent norbixin destruction in hydrogen peroxide bleached liquid
whey (60ºC for 1 h) and 34% (w/w) protein retentate (experiment II) .................. 73
Figure 2.4   b* values of control (Con) and hydrogen peroxide (HP) liquid whey
by cheese type and bleaching treatment (experiment I) ................................... 74
Figure 2.5   b* values of control (Con) and hydrogen peroxide (HP) liquid whey,
34% protein retentate, and 80% protein retentate (experiment I) ...................... 75
CHAPTER 1: LITERATURE REVIEW
INTRODUCTION

Cheese is a very popular dairy product worldwide. It was “invented” perhaps as early as 6,000 B.C.E (Anonymous, 2005). It was made from cows’ or goats’ milk and stored in jars by fermenting the milk. Nowadays we know that cheese is produced by milk protein casein’s coagulation. Cheese is a good source of protein, calcium and vitamins vital to good health. The nutritional value, great taste and various cooking methods of cheese as additives to food have resulted in the increased production of cheese to meet increasing demand. In 2009, total cheese production (excluding cottage cheese) in the United States was 10.1 billion pounds (USDA, 2010).

Whey is the separated watery portion of milk usually obtained by acid, heat, or rennet coagulation. It is opaque and greenish-yellow with a total solids of 6.0 to 6.5 percent and a biological oxygen demand (BOD) of 3,200 ppm or higher (Kosikowski and Mistry, 1997). In the 1970s, whey protein as a value-added by-product of the manufacture of cheese and casein started to show its usefulness in the food industry; and many technical and nutritional applications were discovered for whey or whey components (Kuehler and Stine, 1974). The production of whey products expanded quickly. One billion pounds of dry whey, 723 million pounds of lactose (human and animal), and 415 million pounds of whey protein concentrate (WPC) were produced in 2009 (USDA, 2010).

Cows’ milk contains 87.3% water and 12.7% solids. The solids are comprised of 3.7% fat, 3.4% protein (2.8% casein, 0.6% whey protein), 4.7% lactose, and 0.7% ash (Teubner, 1998). The protein of milk is from the six major gene products of the mammary gland: αs1-caseins, αs2-caseins, β-caseins, κ-caseins, β-lactoglobulins, and α-lactalbumins (Swaisgood et al., 2008). Milk proteins are classified as either caseins or whey proteins.
Agglomeration of casein micelles during cheese manufacture forms the cheese curd and retains most of the total milk protein, while the other proteins pass into the cheese whey. In Cheddar cheese manufacture, chymosin cleaves the κ-caseins at Phe<sub>105</sub> - Met<sub>106</sub>, and releases a part of the casein called glycomacropeptide (GMP) into solution with other water-soluble proteins that are separated in the whey. This protein and whey protein (mainly β-lactoglobulins and α-lactalbumins) are the primary proteins in liquid cheese whey. The remaining casein (κ-casein, para-κ-casein), stay in the casein micelle to form the cheese curd. The typical compositional ranges are shown in Table 1.

<table>
<thead>
<tr>
<th>Product</th>
<th>Protein (g/L whey)</th>
<th>Lactose (g/L whey)</th>
<th>Minerals (g/100g powder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet whey</td>
<td>6-10</td>
<td>46-52</td>
<td>2.5-4.7</td>
</tr>
<tr>
<td>Acid whey</td>
<td>6-8</td>
<td>44-46</td>
<td>4.3-7.2</td>
</tr>
<tr>
<td>WPC-34</td>
<td>34</td>
<td>50</td>
<td>7.2</td>
</tr>
<tr>
<td>WPC</td>
<td>65-80</td>
<td>4-21</td>
<td>3-5</td>
</tr>
<tr>
<td>WPI</td>
<td>88-92</td>
<td>&lt;1</td>
<td>2-3</td>
</tr>
</tbody>
</table>

The composition of liquid whey varies with different methods of manufacture of individual cheese and casein products, and also varies during the milk production season (Mawson, 1994). Besides 93% water (Foegeding et al., 2002), the major components of liquid whey are β-lactoglobulin, α-lactalbumin, proteosepeptones, blood proteins, lactose, and minerals (Walzem et al., 2002). Before cheese making, these proteins are called serum proteins, they have also been referred to as “native” whey proteins (Evans et al., 2010).

The applications of dried whey ingredients in the food industry are numerous. They are used in bakery goods, ice cream mixes, sherbets, candies, breakfast foods, beverages, and cheese (Kosikowski and Mistry, 1997). However, the yellow color of whey is not desirable when dried whey ingredients are used in other foods. Milk has natural carotenoids which are
affected by the diet of cow (Noziere et al., 2006), and these may be present in whey (Kang et al., 2010). However, the color of many cheeses is enhanced by the addition of annatto which will be further discussed later in this review. The natural (feed-derived) carotenoid content of milk mostly consists of lipophilic molecules: carotenoids, including lutein, violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, all-trans β-carotene, α-carotene, and 13-cis β-carotene (Noziere et al., 2006).

**WHEY PROCESSING AND PRODUCTS**

In order to obtain the final products of whey that can be used directly in food and beverage production, many processing steps need to be done. First the milk needs to be made into cheese and the whey drained from the cheese curd. The standard cheese make process of Cheddar cheese includes pasteurization of milk, addition of calcium chloride and starter/acidification, addition of color, addition of rennet for milk coagulation, cutting cheese curd/drainning whey, salting and finally pressing (Farkye, 2004). Drained liquid whey at the pH of 6.35 (Croissant et al., 2009) is then pasteurized followed by fat content reduction, which is normally done by centrifugal separation. Nowadays the basic categories of whey are sweet-type dried whey with a pH of at least 5.8, which accounts for the majority of whey production; and acid-type dried whey with a pH below 5.0, which is derived from cottage and cream cheese manufacture (Gallardo-Escamilla et al., 2005). Standard whey powders are rich in lactose, total protein and minerals. Less lactose, lecithin (21-32 mg/kg in acid whey powder, 46-85 mg/kg in sweet whey powder), and more lactic acid, phosphorus, calcium, zinc, iron and copper, exist in acid whey powder compared with the sweet product (Kosikowski and Mistry, 1997; Mavropoulou and Kosikowski, 1973). Reduced-lactose whey, whey protein concentrates (WPC), and isolates (WPI) are prepared from these initial
products (Walzem et al., 2002). Since liquid whey is rarely used in the industry, it is generally further processed into whey protein concentrates. WPC and WPI can be processed by various steps including pasteurization, vacuum evaporation (VE), ultrafiltration (UF), reverse osmosis, ion exchange, gel filtration, electro-dialysis, crystallization and spray-drying (SD) (Ji and Haque, 2003).

The general whey processing from liquid whey to multiple whey protein products is summarized (Tunick, 2008) as Figure 1. Whetstine et al. (2005) demonstrated compositional differences between commercial WPC80 (80-89% protein) and whey protein isolate (WPI). Primary differences noted were mineral content and these differences are likely due to different ways of concentrating and processing the liquid whey by different manufacturing facilities. Different filtration and processing procedures may selectively filter out different minerals and salts.
This review will mainly focus on WPC and WPI since they are the most value-added products and as such, the most widely researched.

**Whey Protein Concentrates**

Whey protein concentrate contains 34 to 89% protein. Various technologies are now available to produce whey protein concentrates, and functional properties of the various WPC’s vary widely with the technology used (Tunick, 2008). In the 1970s manufacture of a WPC was normally preceded or followed by a lactose recovery operation (Jelen, 1979). Ultrafiltration is a promising technique for recovering the solids from whey, and vacuum evaporation is one of the most practical methods of recovering the solids without affecting the nutritive and functional properties of whey proteins (Haque and Ji, 2003). Ultrafiltration is frequently used in current whey protein manufacture (Muthukumarana et al., 2004).
Ultrafiltration allows permeate (low-molecular-weigh soluble compounds) to flow through the membrane while the retentate (protein and other materials) is retained (Tunick, 2008).

Whey retentate after separation by UF consists of protein, fat, and insoluble salts. Diafiltration (DF), the addition of water to the retentate followed by a second UF, has been developed for the further removal of salts and lactose which are primaries in the permeate. A combination of UF and DF removes minerals and lactose from the retentate, allowing for production of WPC with >50% protein (Tunick, 2008). After ultrafiltration to a desired solids and protein concentration, whey concentrates are dried on drums or in spray driers. Drum drying of whey is no longer widely used in North America (Kosikowski and Mistry, 1997).

**Whey Protein Isolates**

Whey protein which contains at least 90% protein and all the lactose is removed is called whey protein isolate. To produce WPI, there are two ways including an ion-exchange tower and additional steps to WPC production. To manufacture a whey protein with greater than 90% protein, microfiltration or ion exchange is needed in addition to UF and DF to further remove fat and lactose (Huffman, 1996). The other process method, ion exchange, separates components by ionic charge instead of molecular size, and can be used in conjunction with membrane filtration (Tunick, 2008). Whey protein will be positively charged when the pH of whey is decreased. By pumping through negative $e_g$ charged beads in a tank, the whey protein will be retained while other components such as fat, lactose and minerals will be removed (Huffman, 1996). GMP is not present in WPI manufactured by ion exchange.

**Lactose**
Lactose is a disaccharide formed from glucose and galactose that is naturally present in milk. Lactose is often recovered by crystallization from, or by spray drying after substantial purification of the liquid whey material (Jelen, 1979). Crystallization of lactose is a critical step in the manufacture of lactose from whey, and either sweet or acid whey or permeate may be used (Siso, 1996). Deproteination of whey by ultrafiltration or high heating and removal by centrifugation helps improve lactose yield (Kosikowski and Mistry, 1997).

Lactose is the major contributor to the BOD of whey and can potentially be used in many bioconversion processes to produce biomass or extracellular products (Mawson, 1994). As shown in Figure 1, lactose can be used as a browning agent, sweet syrups, and as a fermentation substrate, but hydrolysis of lactose to glucose and galactose significantly increases the by-product yield from liquid whey, and also increases the sweetness and solubility of lactose (Siso, 1996). Organic acids such as lactic, acetic and propionic acids can be obtained by fermentation of whey permeate where lactose is a major component (Yang et al., 1993).

**WHEY PROTEIN PROPERTIES**

*Functional Properties*

The functional properties of whey proteins supported the development of its industrial value. The composition of whey and its functional properties can be affected by processing method and whey source (Morr et al., 1973). WPC has a long history of commercial production since WPC can be used to improve the protein quality and content of many food products. WPC are valuable commercially as sources of protein, flavor enhancers, egg white substitutes, and food binders for conventional food use (Mawson, 1994). Permeate is used as
a specialized food ingredient and important biotechnological reagent, such as a source for the production of methane gas by mixed anaerobic bacteria (Mawson, 1994).

The high solubility over a wide pH range (Walzem et al., 2002) and the ability to aggregate and provide desirable structure to foods of whey protein concentrates and whey protein isolates defines their essential role as food ingredients. The maximum region of WPC solubility is between pH 6.0 and 6.6 (Fachin and Viotto, 2005). Although the solubility of WPC at pH 4.6 is lower than pH 7, WPC is still used widely for beverages at low pH (Lim et al., 2008). The relatively wide pH range solubility enables whey protein to fulfill the requirements of sports beverages applications. Whey protein isolate can be a good functional supplement for a various beverages including juices.

Whey protein products can also be used in baked products and processed meats due to their ability to bind water and gelation properties (Foegeding et al., 2002). Whey protein concentrates are widely used in salad dressings, artificial coffee creamers, creamed soups and functional foods as emulsifiers (Tunick, 2008).

**Emulsifier, Films and Coatings**

The major components of whey protein, β-lactoglobulin and α-lactalbumin, are amphiphilic molecules which give them the ability of facilitating formation and stability of emulsions (McClements, 2008). The interfacial tension of oil droplets is lowered by proteins that adsorb to surfaces and form a protective membrane around it. This ability plays an important role in stabilizing the droplets during long-term storage (McClements, 2008). Many beverages are oil in water emulsions which require high stabilization to maintain product quality. Using whey proteins as emulsifiers facilitates the stability of emulsifiers due to their amphiphilic property (Chanamai and McClements, 2002).
To prevent loss of moisture and to maintain quality and texture during storage, edible films and coatings have been used for centuries. The most important characteristics of edible films are mechanical, barrier and appearance properties. Whey proteins films have good tensile properties, they can also prevent permeability of oxygen, water vapor, and aroma and oil (Dangaran and Krochta, 2008). Since whey protein forms transparent films, it has good appearance properties which will improve consumer acceptability (Dangaran and Krochta, 2008). Whey protein films formed as coatings are used for nuts, eggs, and meat products to prevent lipid oxidation, or to carry antimicrobials that extend shelf life (Dangaran and Krochta, 2008).

**Nutritional Properties**

In addition to their rich functional properties, whey proteins have also been used for many years as highly nutritious food supplements. The main components of whey proteins, \(\alpha\)-lactalbumin and \(\beta\)-lactoglobulin, are used as nutritional and specialized physically functional food ingredients. Many people who pursue physical well being seek benefits from diet and nutritional supplements, hence whey proteins and amino acid supplements and their suggested role in increasing muscle mass in conjunction with appropriate training are well noticed (Ha and Zemel, 2003). Studies show that whey proteins and their constituent amino acids efficiently promote protein synthesis (Bos et al., 1999; Fouillet et al., 2002). Whey proteins or essential amino acids might promote recovery from exercise and theoretically enhance performance (Ha and Zemel, 2003). In this case, since whey protein isolate contains little to no fat, lactose or cholesterol, it is an ideal supplement.

The pathway of whey proteins in the human body system is quite different from caseins since whey protein is more rapidly absorbed than casein due to their different
physical properties (Ha and Zemel, 2003). Whey proteins have higher biological values compared with other dietary proteins because of the content of essential amino acids (Walzem et al., 2002). The dose and composition of the amino acids mixture or protein primarily influences the protein synthesis efficiency provided by amino acids (Wolfe, 2000), and the ingestion of only essential amino acids is sufficient for the acute stimulation of muscle protein synthesis (Ha and Zemel, 2003). Whey proteins have a high protein quality and relatively contain a high proportion of branched-chain amino acids (BCAAs) which have been proved to help strengthen the immune system, muscle building, and provide high energy (Burke, 2003). Whey protein can also enhance the production of the natural body antioxidant, glutathione, which can help prevent damage from free radicals (Burke, 2003).

**FLAVORS OF WHEY PROTEIN**

Whey and dried whey ingredients are expected to not have strong or predominant flavor for use as food ingredients. In reality, whey protein products are complex with many different chemical compounds contributing to a variety of flavor characteristics. WPC and WPI can have typical dairy flavors, such as sweet aromatic and cooked/milky, although heat-generated and lipid oxidation compounds also contribute to nondairy flavors such as cardboard and cabbage flavors (Whetstine et al., 2005). Flavor quality of whey and dried whey ingredients depends on the following aspects: the quality of the milk from which the cheese was made; the type of cheese manufactured; the method of whey handling immediately after curd draining; and the elapsed time between draining and pasteurization (Karagul-Yuceer et al., 2003a); liquid whey processing time (Tomaino et al., 2004); bleaching of liquid whey and spray drying (Croissant et al., 2009). Some of the flavors formed during whey protein production are not desirable, and they may affect the application
of whey protein since off-flavors may carry through into ingredient applications (Evans et al., 2010).

**Flavors in Liquid Whey**

The huge variability of flavors in liquid whey may be associated with the liquid whey itself, or may be an outcome of processing techniques of liquid whey production (Whetstine et al., 2003; Karagul-Yuceer et al., 2003a; Croissant et al., 2009; Liaw et al., 2010). Whetstine et al. (2003) demonstrated that milk source, processing and handling, and starter culture blend contributed to flavor differences in liquid whey. The flavor of liquid cheddar cheese whey is variable and impacted by milk source and starter culture rotation (Whetstine et al., 2003). Lipolysis, proteolysis and lipid oxidation are the major contributors to off-flavors in liquid whey, while lipid oxidation products are the primary contributes to off-flavors in dried whey products (Whetstine et al., 2003; Whetstine et al., 2005).

Due to the undesirable flavor of whey, many products such as protein drinks which use whey proteins as food ingredient turn out to be heavily flavored. This limits the usage of whey proteins since sports beverages and baking mixes normally don’t have such a strong and heavy flavor. As mentioned previously, studies (Tomaino et al., 2004; Tomaino et al., 2001) have been conducted to have a better understanding of the flavor variability in liquid whey, and to minimize flavor variability in dried whey ingredients. Cheddar-type liquid whey from different starter culture strains have differences in flavor (Whetstine et al., 2003).

For commercially produced liquid whey, two distinctions of liquid whey flavor observed by Whetstine et al. (2003) were differences in flavor between plants and differences within plants. Milk source, handling, processing and starter culture may contribute to the differences between plants while starter culture rotation may attribute to differences within
plants since other factors are the same. This research also established that there were two different classes of volatile compounds in liquid wheys: aroma-active compounds which have a discernable aroma (alcohols, ketones, aldehydes) and aliphatic hydrocarbons. In the aroma-active category, Karagul-Yuceer et al. (2003a) identified 2,3-butanedione, 2-butanol, hexanal, 2-acetyl-1-pyrroline, methional, (E,E)-2,4-non-adienal, (E,E)-2,4-decadienal, and various short-chain volatile acids that may contribute to the formation of liquid whey flavor. Aliphatic hydrocarbons do not contribute directly to flavor since they do not have an odor.

Lipid oxidation products are the main contributors to off-flavors in whey ingredients (Wright et al., 2009; Drake and Civille, 2003). Whetstine et al. (2003) reviewed starter culture ability to form lipid oxidation products during fermentation and the binding ability of whey proteins to lipid oxidation products (Meyers et al., 1996). The oxidation flavors were related to the breakdown of unsaturated fatty acids. Since milk lipase was destroyed during pasteurization, starter culture was the only source of lipolytic enzymes in whey and cheese (Whetstine et al., 2003). The main off-flavor “cardboard” in liquid whey was due to lipid oxidation products (Drake and Civille, 2003; Liaw et al., 2010), and Tomaino et al. (2001) also indicated the relationship between off-flavor in liquid whey and quantity of free fatty acids. Off-flavors associated with dried whey ingredients such as WPC and WPI will be further addressed later in this review.

Milk source also played a role in the variability of whey flavor since compounds found in milks were also found in wheys (Croissant et al., 2007). Feed source and milk processing temperature/time will impact flavor of milk and the resulting wheys, and dimethyl sulfide is often found in milk that has been pasteurized at higher temperatures (Contarini et al., 1997).
Since the influence of starter culture on whey lipid oxidation has been established, differences between starter cultures are another contributing factor in whey flavor variability. The differences of lipase activity, proteolytic activity and differences in type and amount of volatile compound production of Lactococcus species and strains were evaluated, and Whetstine et al. (2003) reported that wheys from the same manufacturing facility were differentiated indicating that starter culture rotation for the same cheese type impacted whey flavor. Gallardo-Escamilla et al. (2005) demonstrated that Cheddar, Gouda, and rennet wheys (“sweet” cheese and rennet wheys) provided a bland, “sweet”/“milky” flavor base, adequate for producing liquid dairy beverages. Liaw et al. (2010) compared the sensory profiles of Cheddar liquid whey and Mozzarella liquid whey, Mozzarella liquid whey had lower intensities of sweet aromatic flavor and sour aromatic flavor was only found in Mozzarella liquid whey. Cheddar liquid whey had more lipid oxidation products than Mozzarella liquid whey, and this was part of the attributed reason for their distinct flavor profiles (Liaw et al., 2010).

Liaw et al. (2010) also demonstrated the impact of antioxidant addition to Cheddar whey on flavor of WPC80. Addition of ascorbic acid or whey protein hydrolysate to liquid whey decreased off-flavor (mainly cardboard), which confirmed the significance of lowering lipid oxidation during processing of liquid whey. If flavor variability in liquid whey can be diminished, this may lead to higher quality of dried whey ingredients, and the utilization of whey and whey ingredients then could be increased.

**Flavors in Dried Whey Protein Concentrate and isolates**

Since fresh liquid whey is associated with high transportation costs and susceptibility to deterioration during storage, dried whey and dried whey ingredients such as WPC and
WPI, are widely used in industry because of their exceptional functional characteristics and storage stability. The majority of published research on flavor of dried whey has focused on whey protein concentrates and isolates.

Dried whey ingredients have been associated with oxidized, unpleasant flavors that are not appealing to customers. Morr and Ha (1991) first reviewed that factors that may impact the development of off-flavors in WPC during storage including processing treatments which affect the composition of the whey and WPC; drying conditions which control moisture content of the WPC and initiate lipid oxidation and non-enzymatic browning reactions; and moisture and temperature conditions which affect the kinetics of lipid oxidation and browning reactions during WPC storage. Many other studies have been subsequently conducted and demonstrated that processing from whey to WPC and WPI contains many potential sources of flavor formation (Whetstine et al., 2005; Wright et al., 2006; Wright et al., 2009; Evans et al., 2010). Although dried whey only contains a small amount lipid, volatile lipid oxidation products are still the main sources of off-flavors (Whetstine et al., 2003; Liaw et al., 2010). Tomaino et al. (2004) identified the existence of lipid oxidation in fresh liquid whey and that off-flavors increased during storage. Subsequent studies confirmed that off-flavors in liquid whey were also present in WPC and WPI (Whetstine et al., 2005; Evans et al., 2009; Wright et al., 2009). Proteolysis is also an important source of flavor and reactions in WPC and WPI (Whetstine et al., 2003).

Gallardo-Escamilla et al. (2005) suggested that starter cultures might also influence flavor of dried whey to different degrees, and absence of a starter culture in rennet whey led to a sweet-tasting product with a subtle odor suitable as a neutral base for production of dairy type beverages. Campbell et al. (2011) also reported that aldehydes were higher in culture set
WPC than rennet set WPC, and culture set WPC also had higher concentrations of hexanal and 1-octen-3-ol and 2-nonanone.

Whetstine et al. (2005) reported that commercial WPI had similar flavor profiles to WPC80, but also contained soapy, animal/wet dog, cucumber flavors, and bitter taste (all nondairy flavors) and a lack of desirable dairy flavors, sweet aromatic and cooked/milky. This study showed the possibility of fat content differences influencing flavor stability with increased storage time, but no effect on flavor in the fresh product. Whetstine et al. (2005) also reported the presence of animal/wet dog flavor in several WPI, and hypothesized that this protein degradation flavor may be related to or similar to the wet dog flavor reported in caseins and caseinates (Karagul-Yuceer et al., 2003b). Bitterness and astringency have been associated with proteolysis (Whetstine et al., 2005). Wright et al. (2006) characterized cabbage off-flavor in WPI and demonstrated that a sulfur compound, dimethyl trisulfide, was the source of the off-flavor.

The role of Maillard browning in the formation of stale off-flavors in WPC was reviewed (Morr and Ha, 1991). Maillard browning involves the interaction of protein and lactose as its initial step, and the factors affecting browning reaction rate in dried whey include temperature, water activity, pH and availability of reactant. Mahajan et al. (2004) suggested that Maillard browning could partly explain the generation of many aroma compounds in sweet whey powder, however, Sithole et al. (2005) reported that there were no significant differences due to Maillard browning were found in odor and flavor in long-term stored sweet whey powder samples. Whetstine et al. (2005) reported thermally generated compounds pyrrolines, pyrazines, and furanones were found in WPC80 and WPI due to maillard browning reactions during spray drying, and Wright et al. (2009) reported that
Maillard browning might account for the presence of Cabbage/brothy flavor due to dimethyl trisulfide in WPC80 and WPI.

Wright et al. (2009) reported that the flavor profiles of dried whey proteins changed with storage time. Cardboard flavors increased, sweet aromatic flavors decreased and cucumber, fatty, and raisin flavors were detected as products aged. Potato/brothy flavor was detected initially in some products while in others, it was initiated by storage (Wright et al., 2009). Lipid oxidation compounds increased with storage and these products are highly reactive and may react with other compounds, flavor degradation of whey protein occurred during storage (Wright et al., 2009).

Above all, precise control of the temperature of fluid whey or inactivation of starter cultures, particularly fast-acidification or fast fermenting commercial strains, and the storage temperature and moisture content of dried products are important for controlling off-flavor of liquid whey and dried whey ingredients such as WPC and WPI in order to provide bland whey protein ingredients.

**Flavors of Additive - Annatto**

Annatto is a yellow/orange carotenoid that is widely used in the food industry as a colorant. It is extracted from the seeds of the fruit of tropical tree *Bixa orellana* (Giuliano et al., 2003). The two main processed products of annatto are norbixin (water-soluble) and the ester bixin (oil-soluble) (Giuliano et al., 2003) (Figure 2). In order to give cheese a desirable yellow/orange color, norbixin is added into milk during cheese making and some of the annatto is also present in whey.
Figure 2 Structures of bixin and norbixin

There is little information on the volatile compounds of annatto that contribute to its flavor although it also has been used in food as a spice. Galindo-Cuspinera et al. (2002) reported that β-humulene was the major volatile present in both oil and water-soluble annatto extracts. This compound is described as woody/spicy in aroma and has a slightly bitter taste. Other important volatiles found in annatto extracts that might contribute to flavor were p-xylene, toluene, α- and β-pinene, γ-elemene, and spathulenol (Scotter et al., 2002).

Annatto will produce additional volatile compounds that contribute to flavors during the heat treatment which is required during the cheese making process, and different commercial products will have various degrees of thermal degradation (Scotter, 1995; Scotter, 1998; Scotter, 2000). Galindo-cuspinera et al. (2002) indicated that there were
numerous odorants in annatto, such as β-humulene, p-xylene, toluene, α- and β-pinene, γ-elemene, and sapathulenol, with potential to influence food flavor.

**ANALYTICAL METHODS OF FLAVOR OF LIQUID WHEY AND DRIED WHEY INGREDIENTS**

Knowledge and understanding of how the flavors of liquid whey and dried whey protein ingredients are affected by technological variables is important. It may help: 1) to develop products such as carbonated beverages, whey-based fruit drinks, dairy type products, and alcoholic beverages, 2) to understand the mechanism of chemical reactions responsible for the flavor of liquid whey, WPC and WPI and to control their flavor, and 3) to allow control of the shelf life of such products (Karagul-Yuceer et al., 2003; Whetstine et al., 2005; Gallardo-Escamilla et al., 2005). Research on sensory evaluation of liquid whey is also necessary to improve the flavor of dried whey ingredients. Sensory evaluation and instrumental analyses have been applied to determine which components are responsible for desirable and undesirable flavors.

**Descriptive Sensory Analyses**

Descriptive analysis is an analytical sensory test that provides both qualitative and quantitative aspects of a product by trained panelists (Meilgaard et al., 2007). A descriptive panel generally consist of 6-12 individuals, each panelist has to be trained in order to provide valid and reliable data and the training time is dependent on the complexity of attributes of the food that need to be evaluated (Drake, 2007). Each panelist operates as an individual sensor of an instrument, and the results generated by the panel should be analogous to instrumental data (Drake, 2007). To make sure the panel generates reliable and consistent data, the sensory attributes need to be clearly defined (Drake and Civille, 2003). The
commonly used descriptive analysis methods include Flavor Profile, Texture Profile, Quantitative Descriptive Analysis (QDA), Spectrum Descriptive Analysis, and Time-Intensity Descriptive Analysis (Meilgaard et al., 2007).

Sensory profiles of rehydrated whey proteins (WPC34, WPC80, WPI) have been established (Whetstine et al., 2005; Evans et al., 2009; Evans et al., 2010). Sensory profiling is usually conducted with a trained sensory panel (n=7-10 panelists) (Whetstine et al., 2005; Gallardo-Escamilla et al., 2005; Drake, 2007; Evans et al., 2010). The panel is used to evaluate the flavor attributes of the rehydrated whey proteins and commercial powders using an established lexicon for dried dairy ingredients (Drake and Civille, 2003) or by defining a list of descriptors after discussion (Gallardo-Escamilla et al., 2005). In some studies (Whetstine et al., 2003; Whetstine et al., 2005; Croissant et al., 2009; Liaw et al., 2010), the Spectrum descriptive analysis method was used to train panelists by presenting panelists solutions of sweet, sour, salty, and bitter tastes to learn to use the 0 to 15-point universal intensity scale followed by more extensive training on other attributes (Drake and Civille, 2003). Other studies (Gallardo-Escamilla et al., 2005) used different scales such as 100-mm unstructured line scales. The data of descriptive analysis is often presented by principal component analysis (PCA), which is a multivariate data compression technique that graphically displays the differences on multiple variables of multiple treatments (Drake, 2007).

**Instrumental Analyses**

Establishing the relationship between descriptive analysis data and instrumental data is very important to associate off-flavors with volatile compounds. In general, there are two steps of instrumental analyses to analyze the volatile compounds in foods: 1) extraction of
volatile compounds, and 2) volatile compound separation and detection (commonly used instruments are gas chromatography and gas chromatography/mass spectrometry) (Ferhat et al., 2007).

**Volatile Extraction Methods**

One way of aroma isolation is solvent extraction. The commonly used approaches of solvent extraction include Direct Solvent Extraction (DSE), Simultaneous Distillation-Extraction (SDE) and Solvent Assisted Flavor Evaporation (SAFE). DSE is most useful on foods which do not contain any lipids, which gives DSE a huge limitation. SDE is a very popular method that uses steam distillation, however, the elevated temperatures applied during distillation may lead to artifact formation (Engel et al., 1999). SAFE is essentially DSE with an additional vacuum distillation step which can be applied to food extracts containing fat.

SAFE is a standard method for preparing volatile extracts of liquid whey and dried whey ingredients (Karagul-Yuceer et al., 2001, Whetstine et al., 2005, Evans et al., 2010). Liquid whey was extracted directly while WPC and WPI were reconstituted to 10% solids prior to extraction (Whetstine et al., 2005). Diethyl ether with internal standard (2-methyl-3-heptanone and 2-methyl pentanoic acid in methanol) and sodium chloride were combined and equally distributed. The mixtures were mixed and then centrifuged to separate the non-polar solvent phase from the mixture. The solvent phases contain the aroma-active volatiles. A high vacuum distillation technique (Karagul-Yuceer et al., 2001) is often used to separate volatiles from nonvolatile residues in solvent extraction. After distillation, the volatile extract was recovered and concentrated under a gentle nitrogen gas stream. The dried and concentrated ether extract is then ready for gas chromatography (GC).
Another way of extracting volatile compounds is by headspace methods, which includes static headspace (SHS) extraction and dynamic headspace (DHS) extraction. SHS allows equilibration between the food and the sample headspace for 30-60 min and then incorporates direct injection onto a GC while DHS uses an inert gas to purge the sample and to strip aroma constituents, then traps them by a cryogenic, Tenax, charcoal or other trapping system (Reineccius, 2006). Solid phase microextraction (SPME) is often used with SHS. The advantage of SPME is to avoid complicated solvents and apparatus for concentrating volatile or non-volatile compounds (SUPELCO, Bulletin 923). The SPME extraction process is shown in Figure 3. A polymer coated fused silica fiber is exposed to sample headspace to adsorb the organic analytes. The fiber, containing adsorbed volatile compounds, is then injected onto a GC for separation. To analyze whey protein, rehydrated WPC and WPI (10% solids) with internal standards in SPME vials were heated to 48°C and stirred for 30 minutes before SPME fiber exposed to the headspace for 30 minutes and desorption at 250°C for 5 minutes (Wright et al., 2006; Whitson et al., 2010).
Figure 3 SPME Extraction Procedure (SUPELCO, Bulletin 923)

**Analysis of Volatile Extracts**

1. Separation of volatile compounds

   Chromatography is a physical method that separates sample components in which these components distribute themselves between two phases, one stationary (solid or liquid) and the other mobile (Grob, 2004). Gas chromatography (GC) is a chromatography in which the mobile phase is gas. GC is widely used for volatile compounds from separation for whey products (Whetstine et al., 2005, Evans et al., 2010). Volatile compounds are separated by GC due to various physical properties. Chemical compounds have different interactions with the column which results in a distribution of the sample components between the solid/liquid adsorbent and carrier gas, which leads to separation of the sample components. The separation is often achieved by three techniques: frontal analysis, displacement development, or elution development (Grob, 2004), and in which case elution development has the most
advantages and is the most widely applied. Sample components exit separately at the end of the column at a different time, components with lower boiling points pass through the column first. The actual length of time required for a compound to pass through the column is called the retention time (RT) of that compound. Each component is detected when it elutes off the column and a peak is recorded. The size of the peak and the amount of the compound that was put on the column are proportional (Pavia et al., 2005). Retention index is a number that is relating the adjusted retention volume (which is the product of RT of the sample component and the volumetric flow rate of the carrier gas) of a sample compound to the adjusted retention volume of normal paraffins, which is an index of 100 times its carbon number (Grob, 2004).

2. Detection of volatile compounds

Flame ionization (FID) is a detector used frequently in GC, it works by burning carbon compounds to produce ions when they are eluted from the GC column into the hydrogen flame of the detector. A recordable signal is generated by an electrical current that passes between electrodes placed near the flame and held at a suitable potential (Holm, 1999). The advantages of FID are its high sensitivity and it has a linear response over a very wide range, and the response of the FID is predictable (Holm, 1999).

Gas chromatography/olfactometry (GC/O) is a method that combines olfactometry (human detectors) to assess odor activity in volatiles separated by GC. In order to differentiate the odor-active volatiles from the much larger number of odorless volatiles present in food, GC/O is widely used (Friedrich and Acree, 1998). The extracts of whey protein were injected into a GC and detected by a sniffer or human detector and recorded. The data of GC/O is qualitative as the description of aromas and their retention times are
recorded by sniffers. Aroma extract dilution analysis (AEDA) (flavor dilution values) and CharmAnalysis\textsuperscript{TM} (Charm values) are the two ways of evaluation of the data which express the degrees of dilutions when an odor is no longer detectable (Friedrich and Acree, 1998). GC/O has some weaknesses since it uses humans as detectors. GC/O is time intensive and typically only uses 1-2 pre-screened or trained sniffers who have good sensitivity, and the monitoring of sample preparation, room and sample temperature, time of day, duration of analysis, repetition of analysis, repeated standardization of sniffers and use of a standard lexicon is the key to reduce experimental error and variation (Risch and Ho, 2000). Also, the aroma detected by sniffers does not always indicate the flavor it contributes to food and the effect of the food matrix on the flavor will be missed (Wright et al., 2006), meaning that descriptive sensory analysis of a food is always required.

The use of GC and mass spectrometry (MS) for detection and identification of the components of a mixture of compounds is rapidly increasing (McMaster and McMaster, 1998). The mass spectrometer has three essential functions (Pavia et al., 2005): 1) molecules are bombarded by a stream of high-energy which converts or fragments molecules into positive ions then are accelerated in an electric field; 2) the accelerated ions are separated according to their mass-to-charge ratio in a magnetic or electric field. 3) the ions with a particular mass-to-charge ratio are detected by a device that is able to count the number of ions that strike it. The substance can be identified by comparing its mass spectrum with the mass spectrum of a known substance which makes MS more specific than other detection methods (Pavia et al., 2005). Since whey protein contains so many volatile compounds, GC/MS can be very useful in the flavor analysis of different whey proteins.
BLEACHING OF WHEY

The Color of Whey

Annatto is widely used in the food industry as a colorant and is carried into the by-product of the cheese making process, whey. It is added into cheese milk during the ripening time of milk. The original reason for addition of annatto into dairy products was because of standardization of cheese color which varied due to seasonal feed variations in the milk. However, the orange color has become expected in many cheeses, and hence drives the addition of annatto to cheese milk. Currently in the United States, no study has been conducted to determine the amounts of annatto typically found in cheese, however in Europe, Bareth et al. (2002) used HPLC and spectrophotometry to determine concentrations of annatto in cheese and they reported that the concentration of norbixin in the rinds of several commercial cheeses (Butter cheese, Edamer, Camembert etc.) was between 5.6 and 11.9 mg/kg.

Annatto is a carotenoid and is very unstable to oxidation (heat, light, oxygen) except in the presence of ascorbic acid (Scotter, 2009). There are three commercial processes used to extract the carotenoid pigment from dried annatto seeds: direct extraction into oil, direct extraction into aqueous alkali, or indirect extraction with solvents (Preston and Rickard, 1980). Extraction with oil or solvent yields the colorant bixin (Mortenson et al., 2008), while extraction with aqueous alkali saponifies the methyl group of bixin, yielding norbixin as the principal colorant.

Another carotenoid, xanthophylls, also affect the color of whey. It is present due to cow forage and results in a yellow color in milk, and is also carried through into whey (Croissant et al., 2007; Smith, 2004). Maillard browning also contributes to the color of
whey. The lysine residues in whey protein make a huge contribution to Maillard browning reaction which results in color development (Rich and Foegeding, 2000).

**Bleaching Agents**

Approximately 20% of the annatto used to color cheese will be carried through into liquid whey (Barnicoat, 1950). The yellow color is undesirable (Chang et al., 1977). Because of the highly conjugated structures of bixin and norbixin (Figure 2), they are very susceptible to oxidation. The oxidation of both bixin and norbixin destroys double bond(s) and changes the light absorption which makes them colorless. Currently in the United States, hydrogen peroxide (Figure 5) and benzoyl peroxide (Figure 6) are the only two compounds that are permitted to bleach whey.

![Figure 4 Chemical Structure of Hydrogen Peroxide (USGS, 2008)](image)

![Figure 5 Chemical Structure of Benzoyl Peroxide (Smith, 2004)](image)

Hydrogen peroxide (H₂O₂) is a clear, colorless liquid with a slightly pungent odor. Hydrogen peroxide used for bleaching of whey typically has a concentration of 30 to 35% (v/v). The way hydrogen peroxide works is to decompose to oxygen and water while oxidizing annatto. The reaction of hydrogen peroxide breakdown is shown in Figure 7.
The bleaching efficacy of hydrogen peroxide on annatto in Cheddar cheese whey is often affected by temperature (McDonough et al., 1968). Higher temperatures are generally more effective than lower temperatures during bleaching, however reaction temperatures above 74°C increased neither the rate nor the extent of color removal but only caused protein denaturation (McDonough et al., 1968). Hydrogen peroxide use as a bleaching agent is regulated under 21CFR 184.1366 and may be used at a rate less than 0.05% (500 ppm) (US FDA, 2009b). According to US FDA regulation 133.113, hydrogen peroxide needs to be removed by adding catalase, which must not exceed 20 ppm to remove any residual hydrogen peroxide (US FDA, 2009c).

Benzoyl peroxide (C\textsubscript{14}H\textsubscript{10}O\textsubscript{4}) is a colorless, crystalline solid and its use as a bleaching agent for whey protein is regulated under 21 CFR 184.1157. There is no rate limitation of benzoyl peroxide usage (US FDA, 2009a). Benzoyl peroxide converts into water-soluble benzoic acid during bleaching (Figure 8).

Compared to hydrogen peroxide, benzoyl peroxide is more effective at all temperatures for bleaching liquid whey (McDonough et al., 1968). The advantage of benzoyl peroxide is that it does not need to be deactivated by catalase, which saves processing time and is better.
for equipment maintenance (Chang et al., 1977). Bleaching whey is usually applied during the whey production process after pasteurization, before or after fat separation (Kang et al., 2010).

Generally, the bleaching process involves bleaching of liquid whey or liquid retentate with hydrogen or benzoyl peroxide. Bleaching is generally conducted immediately following the production of liquid whey or liquid retentate (Kang et al., 2010). Hydrogen peroxide and benzoyl peroxide are approved with generally recognized as safe status (GRAS) for use in the production of annatto-colored whey in the United States (US FDA, 2008). Hydrogen peroxide and benzoyl peroxide cannot be used as preservatives; therefore if the bleaching agent is immediately added before the ultrafiltration of liquid whey, there is no legal concern (Kang et al., 2010).

The usage of benzoyl peroxide for bleaching is not permitted in China as benzoic acid with other breakdown products are considered harmful (Kang et al., 2010). The normal way of detecting benzoic acid in milk and dairy products in China is high performance liquid chromatography with UV detection (Liu et al., 2007). Currently there is no published regulation about the legal maximum level of benzoic acid in dairy products in China. The usage of hydrogen peroxide and benzoyl peroxide as bleaching agents for whey in the European Union is regulated by the European Commission (Kang et al., 2010).

The way of detecting the level of decolorization of whey is annatto extraction. Croissant et al. (2009) extracted norbixin, the primary carotenoid compound in water-soluble annatto (Preston and Rickard, 1980), from both liquid and dried whey products. Croissant et al. (2009) used chloroform to extract norbixin and solid-phase extraction (SPE) was applied to purify norbixin. HPLC can be used to quantify the annatto content in combination with
spectrophotometric detection after using SPE (Bareth et al., 2002). Bareth et al. (2002) used this method to extract annatto from cheese, butter and ice cream.

There is little information about the color difference of different bleaching treatments of whey. Croissant et al. (2009) stated that norbixin concentration was lower in hydrogen peroxide (500 ppm) liquid whey compared with benzoyl peroxide (20 ppm) liquid whey and in liquid retentate, but differences were only in liquid whey. WPC bleached by either bleaching treatment showed no significant difference in annatto content. The color of whey protein concentrates measured by L*a*b* color scale shows that hydrogen peroxide resulted in a brighter and more yellow product compared to benzoyl peroxide-bleached WPC (Croissant et al., 2009).

**Effect of Bleaching on Flavor**

There is little information on the effect of bleaching on the flavor of dried whey. McDonough et al. (1968) stated that oxidized flavors found in bleached liquid whey were not present after spray drying. However, a recent study (Croissant et al., 2009) demonstrated that flavors relating to bleaching and type of bleach were also identified in WPC. Off-flavors in liquid whey subjected to bleaching were identified by sensory and instrumental analysis, and differences between WPC from different bleaching treatments and the control were reported (Croissant et al., 2009). McDonough et al. (1968) also concluded that bleaching negatively affected the flavor of liquid whey.

Hydrogen peroxide may produce free radicals ·OOH and ·OH and benzoyl peroxide produces benzoyl radicals. The initiation of lipid oxidation starts from an alkyl radical (L·). Then during propagation, an alkyl radical reacts with triplet oxygen to form a peroxyl radical (LOO·), which then reacts with another carbon-hydrogen bond to form a second radical and a
hydro peroxide (LOOH). In the case of whey protein, the alkyl radical often comes from a lipid or carotenoid. The hydro peroxide (LOOH) formed may decompose by heat, reaction with UV and visible light, or reaction with transition metals (Croissant et al., 2009). Hydro peroxide decomposition generally leads to the formation of volatile oxidation products since alkoxy radical forms an aldehyde and a radical by cleaving an adjacent carbon-carbon bond. Croissant et al. (2009) reported that hydrogen peroxide bleached liquid whey has significantly higher levels of aldehydes, which suggested that hydrogen peroxide was a more active oxidation initiator than benzoyl peroxide in liquid whey.

Volatile oxidation products attributed to whey protein and dried ingredients flavor now are linked to bleaching. Hundreds of volatile compounds, including aldehydes, may be produced by the oxidation of lipids and the subsequent decomposition of hydro peroxides. Increased aldehyde concentrations were correlated with increased fatty and cardboard flavors in bleached WPC compared with control WPC (Croissant et al., 2009).

**RESEARCH OBJECTIVE**

Whey proteins and whey components are useful as functional and nutritional supplements, and their important role in health maintenance and healing is noticed. Hence new processes for purifying and modifying whey products will be developed which lead to increasing numbers of products.

Bleaching is used to create a more appealing color of whey protein products which contain undesirable color carried from the cheese making process. However, bleaching affects the function and flavor of whey proteins. There are few published studies on the influence of bleaching on flavor, or the impact of different heat and time treatments on bleaching efficacy. More studies need to be conducted in this area in order to provide a
further understanding of the bleaching process in order to improve the techniques of whey protein production.

The objective of research is to study the effect of processing steps on bleaching efficacy of liquid whey and retentate. Generally, liquid whey and retentate are bleached by different processors at varying steps which may include storage time. This study will identify if starter culture, pasteurization, fat separation, holding time, and spray drying affect the bleaching efficacy of liquid whey and retentate.
REFERENCES


Rautureau, and D. Tome. 1999. Assessment of net postprandial protein utilization of


Chanamai, R. and D. J. McClements. 2002. Comparison of Gum Arabic, Modified Starch,
and Whey Protein Isolate as Emulsifiers: Influence of pH, CaCl2 and Temperature. J.
Food Sci. 67:120-125.

with whey. J. Dairy Sci. 60:40-44.


of bleaching agent on the flavor of liquid whey and whey protein concentrate. J.
Dairy Sci. 92:5917-5927.

and consumer perception of fluid milk from conventional and pasture-based


Smith, K. 2004. Whey processing CDR technical review: bleaching. Wisconsin Center for Dairy Research, Madison, WI.


US FDA. 2009c. 21 CFR 133.113: Cheddar cheese.

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=133.113


CHAPTER 2: THE INFLUENCE OF STORAGE, HEAT TREATMENT AND SOLIDS COMPOSITION ON THE BLEACHING OF CHEDDAR WHEY WITH HYDROGEN PEROXIDE
The influence of storage, heat treatment and solids composition on the bleaching of Cheddar whey with hydrogen peroxide

X. E. Li*, R. E. Campbell*, A.J. Fox*, P. D. Gerard†, and M. A. Drake*

*Southeast Dairy Foods Research Center, Department of Food, Bioprocessing & Nutrition Sciences, North Carolina State University, Raleigh, NC 27695
†Department of Applied Economics and Statistics, Clemson University, Clemson, SC 29634

*Corresponding author:
MaryAnne Drake
Email: mdrake@unity.ncsu.edu
Phone: (919) 513-4598
Fax: (919) 515-7124
ABSTRACT

The residual annatto colorant in liquid whey is removed by bleaching to provide a desired neutral color in dried whey ingredients. The objective of this study was to evaluate if starter culture, whey pasteurization with fat separation, liquid whey and retentate storage, solids composition, or spray drying affected the bleaching efficacy of liquid whey and retentate. Cheddar cheese whey with annatto was manufactured using a mesophilic lactic starter culture or by addition of lactic acid and rennet (rennet-set). Pasteurized fat-separated whey was ultrafiltered to 9% solids (w/w) and spray dried to 34% whey protein concentrate (WPC34). Aliquots of liquid whey were bleached at 60°C for 1 h (hydrogen peroxide, 250 ppm), before pasteurization and after fat separation, after pasteurization and fat separation, after storage at 3°C for 24 h, 48 h, and after freezing at −20°C for 10 d. Aliquots of retentate were bleached analogously immediately and after storage at 3 or −20°C. Freshly spray dried WPC34 was rehydrated to 9% (w/w) solids and bleached. In a final experiment, pasteurized fat-separated whey was ultrafiltered to 9% solids (w/w) and continued to 12% solids (w/w) with diafiltration and spray dried to WPC34 and WPC80. The WPC34 retentate was diluted to 7% solids (w/w) and WPC80 retentate was diluted to 9% or 7% solids (w/w) respectively. Samples were bleached at 50°C for 1 h. Freshly spray-dried WPC34 and WPC80 were also rehydrated to 9% solids and 12% solids and bleached. Bleaching efficacy was measured by extraction and quantification of norbixin. Proximate analyses and color analyses (Hunter Lab) were conducted. Each experiment was replicated 3 times. Starter culture, fat separation or pasteurization did not impact bleaching efficacy (p>0.05). Cold or frozen storage decreased bleaching efficacy of liquid whey compared with immediate bleaching (p<0.05) while spray drying increased that of 34% retentate, but decreased that of 80% retentate.
(p<0.05). Bleaching efficacy of 80% (w/w) protein liquid retentate was higher than liquid whey or 34% (w/w) protein liquid retentate (p<0.05). These results confirm that processing steps, particularly hold times, and solids composition can influence bleaching efficacy of whey.

**Key words:** whey, retentate, norbixin, bleach
INTRODUCTION

The demand for whey protein as a value-added by-product of the manufacture of cheese and casein has been, and continues to steadily increase in the world market (Tunick, 2008). More than 454.4 million kilograms of dry whey, 332.7 million kilograms of lactose (human and animal), and 188.6 million kilograms of whey protein concentrate (WPC) were produced in 2009 (USDA, 2010). Colorless whey ingredients are desirable in many food applications. Therefore, bleaching is a common and necessary whey processing step (Kang et al., 2010). Currently in the United States, benzoyl peroxide (BP) (US FDA, 2009a) and hydrogen peroxide (used at a rate less than 500 mg/kg) (HP) (US FDA, 2009b) are the two approved chemical bleaching agents. In the United States, there is no regulation for the usage rate of BP in whey bleaching, however, the use of BP for whey bleaching is not permitted for products exported to China (Kang et al., 2010), making HP bleaching the primary chemical bleaching agent of interest.

Bleaching is applied by different processors at different steps during whey processing: before or after fat separation of liquid whey, or to retentate. Additionally, liquid whey or retentate may be stored or transported prior to further processing and bleaching (Whitson et al., 2011). Croissant et al. (2009) recently established that WPC70 from liquid whey that was hot bleached with HP or BP had higher off flavor intensities and higher relative abundances of lipid oxidation products compared to unbleached products. Listiyani et al. (2011) confirmed these effects with WPC34 from whey hot bleached with HP or BP. Clearly, bleaching and bleaching agent can negatively impact the flavor of dried whey ingredients, emphasizing that careful selection of bleaching agent and bleach conditions are warranted. Knowledge on the influence of common whey processing steps on bleaching
efficacy is needed in order to select optimal bleaching conditions. To our knowledge, there are no published studies on the impact of specific whey processing steps (heating, storage, solids content) on bleaching efficacy. Studies are needed to determine if the cheesemake procedure or processing factors influence bleaching efficacy in order to optimize bleaching conditions. The objectives of this study were to evaluate if starter culture, whey pasteurization with fat separation, liquid storage, solids composition, or spray drying affected the bleaching efficacy of liquid whey and retentate by hydrogen peroxide. HP was selected as the bleaching agent since current international concerns with benzoic acid residues suggest that BP may not be a commercially viable bleaching agent.

**MATERIALS AND METHODS**

*Experimental design overview*

Three experiments (experiment I, II, III (Figure 1a, 1b, 1c)) were conducted to achieve the objectives of this study. In experiment I, our objective was to determine if unit operation processing steps or storage influenced bleaching efficacy. Cheddar cheese whey was manufactured using a mesophilic lactic starter culture or by addition of lactic acid and rennet. Aliquots of liquid whey were bleached immediately (no fat separation or pasteurization), after fat separation, after storage at 3°C for 24 h, 48 h and after freezing at -20°C for 10 d. In experiment II, the role of increased solids and storage was investigated. Pasteurized fat-separated Cheddar cheese whey manufactured using a mesophilic lactic starter was bleached following pasteurization and fat separation or ultrafiltered to 9% solids (w/w) and bleached or bleached following storage at 3 or -20°C. Freshly spray-dried whey protein concentrate (WPC) from unbleached retentate was rehydrated to 9% solids and bleached. In experiment III, the role of solids and solids composition on bleaching efficacy
was investigated. Pasteurized fat-separated Cheddar cheese whey was ultrafiltered to 34% (w/w) protein retentate and continued to 80% (w/w) protein retentate with diafiltration. Undiluted 34% (w/w) retentate (9% solids w/w), diluted 34% (w/w) retentate (7% solids w/w), undiluted 80% (w/w) retentate (12% solids w/w), diluted 80% (w/w) retentate (9%, 7% solids w/w) were bleached to compare bleaching efficacy at different solids composition. Freshly spray-dried WPC34 and WPC80 were also rehydrated to 9% and 12% (w/w) solids respectively and bleached. Experiments I, II, and III were replicated three times. Color analysis (Hunter Lab and norbixin) were performed on liquid whey, retentate and rehydrated WPC.

**Liquid Whey Production**

Whole raw bovine milk (195 kg) was obtained from the North Carolina State University Dairy Education Unit, Raleigh, NC. The milk was vat-pasteurized (Model MPD1050, Micro Process Design, D & F Equipment Co, McLeansville, NC) at 63°C for 30 min. Pasteurized milk was separated to make two batches of cheese (with culture and without culture) in a cheese vat (model MX4, Kusel Equipment Company, Watertown, WI). Cheese whey with or without culture was manufactured as described by Campbell et al. (2011). For the treatment with culture, the pasteurized milk was inoculated with a freeze-dried mesophilic starter culture (50 DCU/454 kg, Choozit MA 11, Danisco, New Century, NJ) at 31°C followed by the addition of calcium chloride solution (50% w/v, Dairy Connection Inc., Madison, WI) at the rate of 0.39 mL/kg of milk. The milk was ripened for 60 min. After 30 min of ripening, double strength annatto color (3% w/v norbixin, Dairy Connection Inc., Madison, WI) was added at 15 mL/454 kg milk and diluted 20 times in deionized (DI) water. Double strength recombinant rennet (Dairy Connection Inc., Madison, WI) (0.09 mL/kg of milk diluted 80 times in DI water) was added after 60 min of ripening then coagulated for 30
min. For treatment without culture, the pH was adjusted to 6.40 using a 1:4 dilution of lactic acid (85% w/v, Fisher Scientific, Pittsburgh, PA) then coagulated the same way. Liquid whey was drained at pH 6.4. The whey were pasteurized at 63°C for 30 min, then immediately processed to reduce the fat content using a hot bowl separator (Westfalia Separator, C.A. De Fehr & Sons Ltd., Winnipeg, Manitoba, Canada).

**Retentate and WPC production**

In the production of 34% (w/w) protein retentate and spray dried WPC in experiment II, 39 kg pasteurized, fat-separated whey was placed into a stainless steel fermenter (F3-14, Tri-clamp models, Blichmann Engineering, LLC) and bleached by hydrogen peroxide (HP) (250 ppm, 30% v/v, Columbus Chemical Industries, Inc., Columbus, WI) at 60°C for 1 h followed by deactivation by 20 ppm catalase (FoodPro CAT, Danisco, New Century, NJ). Liquid whey was then concentrated using an ultrafiltration (UF) system (Model Pellicon 2, Millipore Inc., Billerica, MA) equipped with five polyethersulfone cartridge membrane filters (Model P2B010V05, nominal separation cutoff: 10 kDa, surface area: 0.5 m²) to reach 34% (w/w) protein at 50°C. The rest of the liquid whey was weighed and placed in a stainless steel fermenter (F3-27), then directly concentrated using UF to reach the same protein percentage. Temperature was maintained using a stainless steel heating coil (PAC Stainless LTD, Seattle, WA) with hot water (82°C). A variable speed peristaltic pump (Model 77410-10) equipped with Model 77601-00 pumphpnels with silicone tubing (Model 96440-73) was used to circulate the product. Pumps, pumphpnels, and tubing were obtained from Cole-Palmer (Vernon Hills, IL). The 34% (w/w) protein retentate was spray dried at 200°C (Model Lab 1, Anhydro Inc., Soeberg, Denmark) and stored in mylar bags at 21 or -80°C.

In the production of experiment III, pasteurized fat-separated Cheddar cheese whey was ultrafiltered using the same UF system in experiment II to 34% (w/w) protein retentate
and then to 80% (w/w) protein retentate with diafiltration. 34% (w/w) retentate (9% solids w/w) were diluted to 7% solids (w/w), and 80% (w/w) protein retentate (12% solids w/w) were diluted to 9%, or 7% solids (w/w). The rest of the undiluted 34% and 80% (w/w) retentate were spray dried to WPC34 and WPC80 and stored in mylar bags at 21 or -80°C.

**Bleaching Methods**

In experiment I, for both culture set and rennet set, whey aliquots were placed in 2 L glass bottles (VWR International, West Chester, PA) and placed into a 60°C water bath (VWR International) for bleaching. Additional aliquots of whey were stored in brown nalgene bottles (VWR International) at 3°C or -20°C. For bleaching, after 60°C was reached, 250 ppm hydrogen peroxide was added. An aliquot of heated whey without added bleach was the control. The samples were maintained at 60°C for 1 h and were shaken manually at 10 min intervals. After 1 h, wheys were placed into an ice bath for 10 min, catalase (20 ppm FoodPro CAT, Danisco, New Century, NJ) was added to the HP bleached sample to deactivate the HP. The samples were shaken manually at 5 min intervals until the concentration of HP was below 2 ppm (confirmed by EM Quant peroxide testing strips, EMD Chemicals Inc., Gibbstown, NJ). The process took around 30 min. For stored samples, samples were bleached analogously with the same concentration of HP (250 ppm). For experiment II, 34% (w/w) protein retentate samples were bleached analogously as fat-separated liquid whey. Spray-dried WPC34 was rehydrated to 9% (w/w) solids. The powders were allowed to rehydrate with constant stirring for 6 h at 21°C (Campbell et al., 2011). Then, the rehydrated whey was subjected to the same bleaching process as for liquid wheys.

In experiment III, each aliquot of liquid whey, 34% and 80% (w/w) protein retentate were placed into two 500 mL glass bottles, and then into a 50°C water bath. After the
samples reached 50°C, 250 ppm HP (30% v/v) was added to one sample of each treatment and followed by deactivation with catalase (20 ppm). The other heated sample served as the control. Spray-dried WPC34 and WPC80 were rehydrated to 9% and 12% (w/w) respectively and bleached analogously. After 1 mo storage at 21°C, spray-dried WPC34 and WPC80 were rehydrated and bleached analogously.

**Proximate analysis**

Total solids and fat content of liquid milk were measured using a Smart System 5 moisture/solids analyzer with SmartTrac rapid fat analysis (CEM, Matthews, NC). The solids of liquid whey and retentate were determined by performing forced-air oven drying (AOAC, 2000; method number 990.20; 33.2.44). Total nitrogen was determined by the NCSU Analytical Services Laboratory (Raleigh, NC) using the Kjeldahl method (AOAC, 2000; method number 991.20; 33.2.11); crude protein was calculated by multiplying the TN by 6.38. The fat content of liquid whey was determined by the Pennsylvania modified Babcock method (AOAC 2000; method number 989.04) and the fat content of retentate and WPC were determined by Modified Mojonnier ether extraction (AOAC, 2000; method number 989.05; 33.2.26 and AOAC, 2000; method number 932.06; 33.5.08).

**Norbixin Extraction and Measurement**

For experiments I and II, norbixin extraction methods were those described by Campbell et al. (2011), solvent extraction was followed by UV-VIS spectrophotometry. Each sample was extracted in duplicate. The norbixin extract was measured immediately by spectrophotometry (UV-1700 PharmaSpec, Shimadzu Scientific Instruments, Columbia, MD) at 458 nm, which was standardized by a standard curve that was generated from 0 to 10 mg/kg norbixin (45% w/w, Chr. Hansen, Milwaukee, WI). Norbixin was first dissolved in
2.5% potassium hydroxide (J.T.Baker) and then in 7:3 methanol:glacial acetic acid. The concentration recovery was calculated based on the absorbance and the standard curve.

In experiment III, the norbixin extraction method was modified for high performance liquid chromatography (HPLC). Six mL liquid whey (for 34 and 80% (w/w) protein retentates, 5 mL of 7% solids, 4 mL of 9% solid, 2 mL of 12% solids) were weighed into a 50 mL centrifuge tube. Three mL ethanol (99.5% v/v, EMD Chemicals, Gibbstown, NJ), 3 mL chloroform (EMD Chemicals) and 1mL 1% acetic acid (99.5% v/v, J.T.Baker, Phillipsburg, NJ) were added and vortexed sequentially. The sample was centrifuged at 11,952xg for 10 min at 4°C. The bottom layer was collected and the volume was measured prior to solid phase extraction (SPE) (Strata-NH2 SPE column, 500 mg/3 mL, Phenomenex, Torrance, CA). Four mL n-hexane (EMD Chemicals) 2.5 mL n-hexane:diethyl ether (1:1 v/v) (EMD Chemicals) and 1 mL acetone (EMD Chemicals) were used for conditioning the SPE column. Then the norbixin was eluted with 2 mL methanol:glacial acetic acid (7:3 v/v). The final product volume was measured and was then placed on the HPLC (Waters 1525 Binary Pump, Waters, Milford, MA). An isocratic mobile phase (70% v/v acetonitrile (99.9% w/v, ACROS, Fair Lawn, NJ), 29.9% v/v HPLC grade water (Honeywell, Burdick & Jackson, Muskegon, MI) and 0.1% v/v formic acid (98-100% w/v Sigma-Aldrich, St. Louis, MO)) was used at a flow rate of 1mL/min. Fifteen µL of the sample was injected (Waters 2707 Autosampler) onto the column (Phenomenex Kinetex 2.6µ 100x4.6mm, 40°C). The injector temperature was at 4°C and a photodiode array detector (Waters 2998) at 460 nm was used. The standard curve and maxima were the same as for experiments I and II. The norbixin recovery of liquid control and HP bleached samples were measured and calculated as
norbixin percentage destruction of liquid HP bleached samples compared to liquid unbleached control samples.

**Hunter L*a*b***

The colors of liquid whey and retentate were measured using a Minolta Chroma meter (CR-410, Ramsey, NJ). Ten mL of each sample were placed into a 60 mm x 15 mm polystyrene petri dish in duplicate (Beckton Dickinson, Franklin Lakes, NJ), each petri dish was measured in duplicate as well. Spray-dried samples were not measured by this method due to insufficient volume.

**Statistical Analyses**

All data was analyzed by 2-way and 1-way analysis of variance (ANOVA) using PROC GLMMIX in SAS (SAS Statistical Analysis Software, version 9.2, SAS Institute, Cary, NC). Both main effects (bleaching time point) for experiments I and II and interaction effects (with or without culture) for experiment I, and main effects (solids content and protein content) for experiment III were analyzed. Replication and all interactions with replication effects were designated random effects.

**RESULTS AND DISCUSSION**

**Proximate analysis**

The milk used for experiments I-III were consistent in composition: 3.90 ± 0.04% protein, 12.46 ± 0.05% total solids and 3.85 ± 0.10% fat. In experiment I, there was no difference in protein content of rennet set liquid wheys and culture set liquid wheys before pasteurization (BPA) or after fat separation (AFS) (p>0.05) (0.98 ± 0.07%). There were differences in fat content for BPA and AFS as expected (p<0.05): BPA 0.16 ± 0.02% and AFS 0.01 ± 0.01%. There was no difference in solids between rennet set liquid whey BPA
and AFS (6.52 ± 0.26%) (p>0.05), however, culture set liquid whey BPA was higher in solids than AFS (6.71 ± 0.12 vs 6.61 ± 0.18%) (p<0.05). In experiment II, the 34% (w/w) protein retentates were not different (p>0.05) (34.3 ± 0.54% protein (dry weight), 8.43 ± 0.03% total solids, 0.15 ± 0.07% fat). Spray dried WPC34 were consistent in compositions: 34.0 ± 1.63% protein, 4.20 ± 0.94% moisture and 1.85 ± 0.16% fat.

In experiment III, liquid wheys were not distinct in composition with 0.86 ± 0.03% protein, 6.43 ± 0.07% total solids, 0.01 ± 0.01% fat. The 34% (w/w) protein retentates were not different (p>0.05) (32.4 ± 3.39% protein (dry weight), 8.18 ± 0.16% total solids, 0.14 ± 0.08% fat) and the 80% (w/w) protein retentates were not distinct in composition (76.7 ± 4.30% protein (dry weight), 12.33 ± 0.15% total solids, 0.49 ± 0.08% fat). Spray dried WPC34 were consistent in compositions: 31.6 ± 0.36% protein, 3.82 ± 0.98% moisture, 1.55 ± 0.12% fat as well as WPC80 with 79.6 ± 1.36% protein, 3.00 ± 0.67% moisture, 4.72 ± 0.53% fat.

**Norbixin percentage destruction**

In experiment I, there was an interaction between cheese type and bleaching treatment (p<0.05) (Figure 2), but these differences were small from a practical perspective. Bleaching before pasteurization and after fat separation were not distinct from each other in norbixin destruction, indicating that fermentation, pasteurization or fat separation did not affect bleaching efficacy of liquid whey. Listiyani et al. (2011) also reported that fat separation of liquid Cheddar whey had no effect on bleaching efficacy of liquid whey.

Significant differences in norbixin destruction were noted between wheys that were immediately bleached compared to wheys bleached following cold or frozen storage (p<0.05) (Figure 2). Norbixin destruction decreased from 47.8% (control whey, immediate bleaching)
to an average of 33.1% (bleaching after storage) indicating that storage decreased bleaching efficacy. Govindarajan and Morris (1973) hypothesized that norbixin was bound with β-casein or β-lactoglobulin and was resistant to decolorization or bleaching. It is possible that if the previous statement is correct, minor protein changes during storage might further alter bleaching efficacy. Lipid oxidation and volatile compounds from protein degradation, presumably due in part to free radicals from lipid oxidation, increase in relative abundance during storage of liquid whey and liquid retentate and might contribute to conformational changes in proteins (Whitson et al., 2011; Campbell et al., 2011).

Consistent results were observed in experiment II (Figure 3). There were no differences between liquid whey or WPC34 retentate that was immediately bleached, but liquid WPC34 retentate stored at 3ºC or frozen at -20ºC did not bleach as well as fresh liquid whey (p<0.05). However, bleaching efficacy of rehydrated spray-dried WPC34 was not different from that of liquid whey (p>0.05). The spray drying of WPC34 retentate increased bleaching efficacy compared to bleaching of liquid WPC34 retentate (p<0.05) and resulted in the same level of bleaching efficacy compared to liquid whey. The effect of spray drying on bleaching will be further discussed in the results of experiment III.

Our objective in experiment III was to determine if composition impacted bleaching efficacy. Norbixin destruction of 34% (w/w) protein retentate at 9% (undiluted) and 7% total solids (w/w) were not different (p>0.05) (Table 1). Similarly, norbixin destruction of 80% protein retentate adjusted to different solids (undiluted 12%, 9% and 7% (w/w)) were not different (p>0.05) (Table 1). These results indicated that solids level did not directly impact bleaching efficacy of liquid whey or retentate. However, the norbixin destruction of 80% (w/w) protein retentate was significantly higher than that of 34% (w/w) protein retentate and
liquid whey which suggests that the solids composition directly impacted bleaching efficacy (p<0.05). Seifu et al. (2005) stated that the natural enzyme lactoperoxidase found in milk can form a lactoperoxidase (LP) system with thiocyanate (SCN-) and HP for antimicrobial purpose. Similarly, addition of low concentrations of HP (ca 40 ppm) into liquid whey can activate lactoperoxidase then the oxidation of norbixin is catalyzed by enzymatic bleaching, but LP is inactivated by high concentrations of HP (>100 ppm) (Bottomley et al., 1989). Under typical liquid whey bleaching with HP, concentrations of HP are such that any inherent LP is inactivated (200-500 ppm HP). It is possible that our higher bleaching efficacy of liquid WPC80 retentate was due to concentrated protein levels which included higher concentrations of lactoperoxidase which could activate LP in the presence of 250 ppm HP. Additional experiments would be required to confirm this hypothesis.

In the case of spray drying, norbixin destruction was increased in rehydrated spray-dried WPC34 to a level equivalent to immediate bleaching of liquid whey (Figure 3, Table 1). However, bleaching rehydrated WPC80 resulted in decreased norbixin destruction compared to rehydrated spray-dried WPC34 or liquid 80% (w/w) protein retentate. In the case of WPC80, the high temperature of spray drying (200°C) will inactivate LP, and the combination of the higher protein compare to WPC34 and spray drying might results in different interactions between norbixin and protein due to conformational changes. For rehydrated spray-dried WPC34, although the same high temperature of spray drying is applied, the protein content of WPC34 is not high enough to negatively impact bleaching which results in higher bleaching efficacy compared to fresh liquid WPC34 retentate.

*Hunter L* *a* *b*
L* increased after HP bleaching in 34% and 80% (w/w) protein retentates, which indicated that HP bleaching increased brightness (Table 2). Similar results were observed in experiments I and II (results not shown). L* value is associated with white and black color and the higher positive value represents whiter color. Croissant et al. (2009) stated that HP bleaching of liquid whey resulted in a whiter whey protein product (WPC70) compared to control unbleached WPC70. However, Listiyani et al. (2011) reported that L* values of HP bleached WPC34 were not different from unbleached WPC34. The whey protein products in Croissant et al. (2009) (WPC70 bleached at liquid whey) and Listiyani et al. (2011) (WPC34 bleached during ultrafiltration) had different protein content and bleaching conditions, which may cause the different results. b* value is associated with blue and yellow, a higher positive value represents a more yellow color. b* values of liquid HP bleached samples decreased compared to liquid unbleached controls (Figure 4, 5, Table 3). Listiyani et al. (2011) also demonstrated decreased b* values of HP bleached liquid wheys compared to control liquid wheys. The b* values of HP bleached rehydrated WPC80 were not different from control WPC80. Miao and Roos (2004) reported that Maillard browning occurred during the spray drying of dairy products. In addition to the low bleaching efficacy of rehydrated spray-dried WPC80, this may be the reason why differences in b* values were not observed. It is possible that Maillard browning also occurred in the spray drying of WPC34, but b* value differences were still observed because of the higher bleaching efficacy of rehydrated spray-dried WPC34. Hunter values are a rapid method to measure color differences but direct measurement of norbixin destruction is more sensitive for quantifying differences in bleaching efficacy (Listiyani et al., 2011).
CONCLUSIONS

A light color of whey protein products is desired by the food industry but bleaching efficacy of whey is influenced by many factors during processing. This study investigated the effects of starter culture, pasteurization with fat separation, liquid storage, spray drying, solids level and solids composition on bleaching efficacy of liquid whey and retentate by hydrogen peroxide. Fermentation, whey pasteurization with fat separation had no effect on bleaching efficacy of liquid whey (p>0.05) but cold and frozen storage of liquid whey products decreased efficacy (p<0.05). Bleaching efficacy of 80% (w/w) protein retentate was higher than that of liquid whey or 34% (w/w) protein retentate (p<0.05) while dilution of retentates demonstrated that solids content had no direct effect on bleaching. Bleaching efficacy after spray-drying was distinct between WPC34 and WPC80 (p<0.05) and further confirmed the role of composition on bleaching. Future studies need to be conducted to determine if norbixin binds to whey components to further elucidate the role of processing conditions on bleaching efficacy.

ACKNOWLEDGEMENTS

Funding provided in part by the Dairy Research Institute (DRI) (Rosemont, IL). The use of tradenames does not imply endorsement nor lack of endorsement by those not mentioned.
REFERENCES


Table 1 Least squares mean values of percent norbixin destruction (250 ppm HP at 50ºC) of liquid whey, 34% (w/w) protein retentate and 80% (w/w) protein retentate at different solids (experiment III).

<table>
<thead>
<tr>
<th>Solids (% w/w)</th>
<th>Protein (% w/w)</th>
<th>Bleaching point</th>
<th>% Destruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.98</td>
<td>immediately</td>
<td>25.7d</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>immediately</td>
<td>28.1cd</td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>immediately</td>
<td>31.7cd</td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>after rehydration of spray-dried WPC</td>
<td>49.5b</td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>after rehydration of 1 mo stored spray-dried WPC at 21°C</td>
<td>43.3b</td>
</tr>
<tr>
<td>7</td>
<td>80</td>
<td>immediately</td>
<td>92.5a</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>immediately</td>
<td>92.4a</td>
</tr>
<tr>
<td>12</td>
<td>80</td>
<td>immediately</td>
<td>93.3a</td>
</tr>
<tr>
<td>12</td>
<td>80</td>
<td>after rehydration of spray-dried WPC</td>
<td>34.2c</td>
</tr>
<tr>
<td>12</td>
<td>80</td>
<td>after rehydration of 1 mo stored spray-dried WPC at 21°C</td>
<td>29.5cd</td>
</tr>
</tbody>
</table>

a-d Means in the same column that do not share a common superscript are different (p<0.05)
Table 2. Least squares mean values of L* of liquid whey, 34% (w/w) protein retentate and 80% (w/w) protein retentate (experiment III).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con(^1)-liquid whey 7% (w/w) solids</td>
<td>75.25(^a)</td>
</tr>
<tr>
<td>HP(^2)-liquid whey 7% (w/w) solids</td>
<td>75.59(^a)</td>
</tr>
<tr>
<td>Con-34% protein retentate 9% (w/w) solids</td>
<td>70.09(^e)</td>
</tr>
<tr>
<td>HP-34% protein retentate 9% (w/w) solids</td>
<td>70.95(^b)</td>
</tr>
<tr>
<td>Con-80% protein retentate 12% (w/w) solids</td>
<td>61.88(^c)</td>
</tr>
<tr>
<td>HP-80% protein retentate 12% (w/w) solids</td>
<td>67.44(^d)</td>
</tr>
</tbody>
</table>

\(^a\) means in the same column that do not share a common superscript are different (p<0.05)

\(^1\) Con = control

\(^2\) HP = hydrogen peroxide
Table 3. Least squares mean values of $b^*$ of 34% (w/w) protein retentate (experiment II).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$b^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con(^1) - Directly bleach 34% (w/w) protein retentate (9% w/w solids)</td>
<td>26.40(^a)</td>
</tr>
<tr>
<td>immediately</td>
<td></td>
</tr>
<tr>
<td>Con-After 24 h at 3°C</td>
<td>26.43(^a)</td>
</tr>
<tr>
<td>Con-After 48 h at 3°C</td>
<td>26.33(^a)</td>
</tr>
<tr>
<td>Con-After 10 d at -20°C</td>
<td>26.28(^a)</td>
</tr>
<tr>
<td>Con-Spray-dried WPC34, rehydrated to 9% solids</td>
<td>28.22(^a)</td>
</tr>
<tr>
<td>Bleached liquid whey then concentrated to 34% (w/w) protein retentate</td>
<td>19.88(^b)</td>
</tr>
<tr>
<td>(9% w/w solids)</td>
<td></td>
</tr>
<tr>
<td>HP(^2) - Directly bleach 34% (w/w) protein retentate (9% w/w solids)</td>
<td>21.29(^b)</td>
</tr>
<tr>
<td>(without bleaching of liquid whey) immediately</td>
<td></td>
</tr>
<tr>
<td>HP-After 24 h at 3°C</td>
<td>21.91(^b)</td>
</tr>
<tr>
<td>HP-After 48 h at 3°C</td>
<td>21.74(^b)</td>
</tr>
<tr>
<td>HP-After 10 d at -20°C</td>
<td>20.81(^b)</td>
</tr>
<tr>
<td>HP- Spray-dried WPC34, rehydrated to 9% solids</td>
<td>21.62(^b)</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Means in the same column that do not share a common superscript are different (p<0.05)

\(^1\) Con = control

\(^2\) HP = hydrogen peroxide
Figure 1a. Experimental design experiment I. The influence of starter culture, unit operations and storage on bleaching efficacy.

1Bleach = hydrogen peroxide bleached liquid samples
2Control = nonbleached liquid control samples
Figure 1b. Experimental design experiment II. The influence of retentate storage and spray drying on bleaching efficacy.

1Bleach = hydrogen peroxide bleached liquid samples
2Control = nonbleached liquid control samples
Figure 1c. Experimental design experiment III. The influence of solids and solids composition on bleaching efficacy.

1Bleach = hydrogen peroxide bleached liquid samples

2Control = nonbleached liquid control samples
Spray dry

WPC80

rehydrated

1 mo at 21°C

rehydrated

Retentate
12% solids
80% protein

Bleach and Control

Retentate
12% solids
80% protein

Bleach and Control
Figure 2. Percent norbixin destruction in hydrogen peroxide bleached liquid whey (60ºC for 1 h) from rennet or culture (experiment I).

BPA = before pasteurization; AFS = after fat separation; 24h = after 24 h at 3ºC holding (after fat separation); 48h = after 48 h at 3ºC holding (after fat separation); 10dAFS = after 10 d at -20ºC freezing (after fat separation); 10dBPA = after 10 d at -20ºC freezing (before pasteurization)

Means in the same column that do not share a common superscript are different (p<0.05)
Figure 3. Percent norbixin destruction in hydrogen peroxide bleached liquid whey (60°C for 1 h) and 34% (w/w) protein retentate (experiment II).

1liquid whey = Bleached liquid whey then concentrated to 34% (w/w) protein retentate (9% w/w solids); 34% retentate = Directly bleach 34% (w/w) protein retentate (9% w/w solids) (without bleaching of liquid whey) immediately; 24h = after 24 h at 3°C holding (after fat separation); 48h = after 48 h at 3°C holding (after fat separation); 10d = after 10 d at -20°C freezing

 Means in the same column that do not share a common superscript are different (p<0.05)
Figure 4. b* values of control (Con) and hydrogen peroxide (HP) liquid whey by cheese type and bleaching treatment (experiment I).

BPA = before pasteurization; AFS = after fat separation; 24h = after 24 h at 3°C holding (after fat separation); 48h = after 48 h at 3°C holding (after fat separation); 10dAFS = after 10 d at -20°C freezing (after fat separation); 10dBPA = after 10 d at -20°C freezing (before pasteurization)
Figure 5. b* values of control (Con) and hydrogen peroxide (HP) liquid whey, 34% protein retentate, and 80% protein retentate (experiment I).

1whey = liquid whey 7% solids; spray dried WPC34 = spray-dried WPC34, rehydrated to 9% solids; spray dried WPC80 = spray-dried WPC34, rehydrated to 12% solids