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Invited review: Annatto usage and bleaching in dairy foods

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ABSTRACT

Annatto is a yellow/orange colorant that is widely used in the food industry, particularly in the dairy industry. Annatto, consisting of the carotenoids bixin and norbixin, is most commonly added to produce orange cheese, such as Cheddar, to achieve a consistent color over seasonal changes. This colorant is not all retained in the cheese, and thus a percentage remains in the whey, which is highly undesirable. As a result, whey is often bleached. Hydrogen peroxide and benzoyl peroxide are the 2 bleaching agents currently approved for bleaching whey in the United States. Recent studies have highlighted the negative effect of bleaching on whey flavor while concurrently there is a dearth of current studies on bleaching conditions and efficacy. Recent international mandates have placed additional concern on the use of benzoyl peroxide as a bleaching agent. This review discusses the advantages, disadvantages, regulatory concerns, flavor implications, and optimal usage conditions of 2 widely used bleaching agents, hydrogen peroxide and benzoyl peroxide, as well as a few alternative methods including lipoxygenase, peroxidase, and lactoperoxidase systems.
Key words: annatto, bleach, flavor, whey

INTRODUCTION

Annatto is a yellow/orange carotenoid that is widely used in the food industry. Annatto comes from seeds of the tropical tree fruit *Bixa orellana*, which was named after Francisco de Orellana, a scientist and explorer of the upper Amazon (Giuliano et al., 2003). Clusters of the fruit, which is capsular shaped and covered in burrs, grow on this tree and there are about 10 to 50 small seeds inside the fruit (Ames and Hofmann, 2001). The seeds are covered in a bright red pulp and this pulp contains the annatto pigment (Giuliano et al., 2003). Latin America produces about 60% of the world's annatto, followed by Africa (27%) and Asia (12%) (Giuliano et al., 2003). Prices for annatto seeds depend on production and are proportional to the content of bixin, the major pigment (Giuliano et al., 2003). The United States and Europe are the 2 largest importers of annatto seeds, but the Japanese market has grown rapidly since the introduction of the colorant in 1963 (Ames and Hofmann, 2001).

Annatto as a colorant is not a new concept. The Aztecs used annatto extract as a dye for textiles, body dye (such as in lipsticks), and as a food colorant in the drink *cacahuatl* (Giuliano et al., 2003). In addition to being used to impart color in various items, annatto has been used as a spice. Native Americans commonly used ground bixa seeds in their cacao beverages to impart a slight red color and to add a musky flavor comparable to that of paprika or saffron (Miksicek et al., 1981; Norton, 2006). Today, annatto is still used as a spice, especially in Latin American dishes such as *cochinita pibil*, a pork dish with ground bixa seeds and bitter orange juice (Gerlach and Gerlach, 2002). It is also used in sausages, fish, margarine, snacks, dressings, sauces, and confections, but usage varies from country to country due to different food customs and legislations (Ames and Hofmann, 2001; Scotter, 2009). The major application of annatto in the United States is within the dairy industry, where it is used to color cheeses and other dairy products (Emerton, 2008).

Extraction of Annatto

Three commercial processes are 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 a colorant that is mainly bixin (Mortenson et al., 2008). Extraction with aqueous alkali (the form used in the dairy industry, e.g., for cheese color) saponifies bixin's methyl group on bixin, yielding norbixin as the principal colorant (Giuliano et al., 2003; Mortenson et al., 2008). Both bixin and norbixin naturally occur in the *cis* form but can be converted to the *trans* form by light and heat (McKeown 1963, 1965). The *trans* forms of both

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bixin and norbixin are more red than their *cis* forms (Preston and Rickard, 1980; Scotter et al., 1994).

Volatile Compounds

Although annatto has been used in foods as a spice (flavoring), there is little information about how volatile compounds of annatto contribute to flavor. Galindo-Cuspinera et al. (2002) evaluated volatile profiles of oil and water-soluble annatto extracts and reported that β-humulene was the major volatile present. β-Humulene is described as having a woody, spicy aroma and a slightly bitter taste (Galindo-Cuspinera et al., 2002). Other important volatiles found in annatto extracts that might contribute to flavor were p-xylene, toluene, α- and β-pinene, γ-elemene, and spathulenol. Annatto extraction uses heat (Scotter et al., 2002), which means that different commercial products may experience varying degrees of thermal degradation. Further studies also suggest that heat treatment of annatto in foods may result in additional flavor-contributing volatile compounds. Scotter (1995, 1998, 2000, 2001) studied the thermal degradation products of both bixin and norbixin and found that heat released the aromatic compounds m-xylene and toluene. As mentioned previously, annatto is a major colorant used in the dairy industry, often in cheese and in processed cheese that involves heat treatment. Volatiles from thermal degradation of annatto in actual dairy products (e.g., cheese) have yet to be studied. Studies conducted by Scotter et al. (2002) and Galindo-Cuspinera et al. (2002) indicate that there are numerous odorants in annatto, such as β-humulene, p-xylene, toluene, α- and β-pinene, γ-elemene, and sapathulenol, with potential to influence food aroma; however, more studies are needed in this area.

Discoloration – Oxidation

Annatto has been used in dairy products since the 1800s to standardize the color of cheese, which varies due to seasonal feed variations in the milk. Chr. Hansen Inc., a major, modern-day supplier of annatto to the dairy industry, opened their first factory in 1874 (Kristin Schneider, Chr. Hansen Inc., Milwaukee, WI; personal communication). Over the years, an orange color has become expected in many cheeses, requiring addition of annatto to cheesemilk. According to 21CFR73.30 (US FDA, 2009a), annatto extract may be used for coloring foods as long as good manufacturing practices (**GMP**) are followed. Therefore, in the United States, there is no "maximum level" of usage for annatto; however, Scotter et al. (2002) reported that the maximum level of annatto addition in commercial Red Leicester cheese in the United Kingdom was 50 mg of norbixin/kg of cheese and the estimated range of annatto in Red Leicester was 23.7 to 37.5 mg of norbixin/kg of cheese. For ripened orange, yellow, and broken white cheese, and flavored processed cheese in the United Kingdom, the maximum level of annatto addition is 15 mg/kg. The estimated range of annatto is 0.2 to 9.6 mg/kg ($n = 16$) and 0.2 to 21.4 mg/kg ($n = 8$) for ripened orange, yellow, and broken white cheese, and for flavored processed cheese, respectively. Currently in the United States, no study has been conducted to determine the amounts of annatto typically found in cheese. In the United States, annatto is approved as a color additive in foods at GMP, meaning that the "Annatto extract may be safely used for coloring foods generally, in amounts consistent with good manufacturing practice..." (Hallagan et al., 1995; Giuliano et al., 2003; US FDA, 2009a). Although annatto is used to standardize the color of cheese, some studies have reported the usage of annatto leading to pinking or discoloration of cheeses (Hood and White, 1929; Moir, 1933; Morgan, 1933; Barnicoat, 1937, 1950; Govindarajan and Morris, 1973).

Bixin and norbixin have highly conjugated structures making these compounds susceptible to both oxidation and reduction. Oxidation is important to the fluid whey industry because oxidation leads to color loss, which is the primary goal of bleaching and will be discussed later in this review. Also important, a study suggested that norbixin might be able to bind with proteins such as β-casein or β-lactoglobulin and form a stable complex that can help prevent oxidation and color loss (Govindarajan and Morris, 1973). This reaction has not been proven; it may be desirable in some products such as cheese but reduce color removal during bleaching of whey.

CAROTENOIDS IN MILK

Carotenoids are lipophilic molecules that are found in milk fat. The diet of the cow can influence the color of cheese (Carpino et al., 2004). A wide variety of carotenoids and their degradation products are present in the forage that cows eat, but only a small number of different carotenoids can be identified in milk. This is because carotenoid transfer from diet to milk is low and carotenoids can be broken down to colorless compounds in the gastrointestinal tract (Noziere et al., 2006). Some of these colorless compounds may be transferred to the milk and have effects on the sensory profile even though they do not impart any color to the final product. Carotenoids identified in milk include lutein, violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, all-trans β-carotene, α-carotene, and 13-cis β-carotene. Among those, lutein and β-carotene are the most quantitatively

important (Noziere et al., 2006) with β-carotene comprising about 90% of total carotenoids present in cow's milk (Hulshof et al., 2005).

Carotenoids can influence the sensory properties of milk either indirectly through their antioxidant properties by stabilizing oxidizable compounds or directly through their yellowing properties. β-Carotene can be converted to vitamin A, but incomplete conversion leaves a yellow color in the milk (Patton et al., 1980). The main components in milk are riboflavin (vitamin B_2), a green compound present in the aqueous phase, and β-carotene in the lipid phase (Noziere et al., 2006). Riboflavin and carotenoid contents vary widely in milk and are influenced by diet, breed, and season (Schröder, 2003; Noziere et al., 2006). Croissant et al. (2007) reported that milk from pasture-fed Holstein and Jersey cows exhibited more yellow color than that from their similar counterparts fed a conventional, TMR diet. These findings were expected because pasture-fed cows ingested fresh forage, containing carotenoids, thus raising the concentration of carotenoids in milk fat. Carotenoids found in milk also carry over into fluid whey during cheese production, where they may increase the color of spray-dried whey and require a bleaching process.

Carotenoid Measurement in Foods

Carotenoid content can be difficult to measure because carotenoids isomerize rapidly due to their conjugated double bonds (de Oliveira and Rodriguez-Amaya, 2007). Isomerization can be promoted by acid, light, and heat (de Oliveira and Rodriguez-Amaya, 2007). As such, precautions must be taken to minimize these reactions during extraction and measurement. These precautions include, but are not limited to, completion of analysis within the shortest time possible, protection from light, avoiding high temperatures or contact with acid, and the use of high-purity solvents (de Oliveira and Rodriguez-Amaya, 2007). Once carotenoids are isolated, qualification and quantification are typically performed using the same methods.

Before quantification, the carotenoid must be positively identified. This is done using chromatography—open column, thin layer, or HPLC (Bushway, 1985). The best of these 3 methods, HPLC, saves time and can distinguish between stereoisomers (Bushway, 1985). The carotenoid can be identified using retention times, co-chromatography with carotenoid standards, or UV-visible absorption spectra (de Oliveira and Rodriguez-Amaya, 2007). After the carotenoid is positively identified, it is then quantified by using an external standard curve (de Oliveira and Rodriguez-Amaya, 2007).

Annatto Measurement in Foods

Annatto can be found in a wide array of foods ranging from dairy products to other naturally colored products. A colorimetric method for measuring the amount of annatto in commercial bleached and unbleached dry whey powder by extracting the annatto using ammonia, ethanol, and phosphate solution was reported by Hammond et al. (1975). Lancaster and Lawrence (1995) extracted annatto from high-fat dairy products, with good repeatability, although methods were labor intensive. Annatto was confirmed and components were identified via HPLC (Lancaster and Lawrence, 1995). Scotter et al. (2002) extracted and analyzed annatto in margarine, ice cream, custard powder, breakfast cereals, cakes, fish, and jellies using various extraction methods tailored to each food matrix followed by HPLC coupled with spectral confirmation. Bareth et al. (2002) described a rapid, simple extraction and detection method specific for annatto in cheese and milk products using HPLC and a spectrophotometric method. Very recently, Croissant et al. (2009) reported a successful method to extract and quantify norbixin (the primary annatto constituent in whey) from liquid whey, liquid retentate, and spray-dried whey protein concentrate. Analytical methods for annatto and analysis in food have been reviewed by Scotter (2009).

BLEACHING

Food is bleached not only to improve color, but in some cases to improve its quality such as improving gluten development in flour. The bleaching process can be categorized into 3 groups depending on the mechanism: oxidizing bleach, reducing bleach, and biochemical bleach (Balls et al., 1943; Bottomley et al., 1989; Roos et al., 2006).

Oxidizing Bleach

The portion of a molecule that emits color is called a chromophore and it is usually the most fragile part of the molecule. Destroying the chromophore will often render a colored molecule colorless. Oxidizing agents (bleach) destroy (oxidize) the double bond(s) within a chromophore. This reaction changes the molecule into a different substance in which the chromophore does not exist or a shorter chromophore exists. A shorter chromophore will absorb light of a shorter wavelength than visible light (UV light) and therefore will not appear colored (Winter et al., 2008). Many oxidizing bleaches are used in industry including chlorine dioxide, which is used for the bleaching of wood pulp, fats and oils,

cellulose, flour, textiles, and beeswax. Organic peroxides, such as hydrogen peroxide and benzoyl peroxide and bromates, are oxidizing bleaches used for whey and flour bleaching and as maturing agents. Another application is in the wood and pulp industry to produce chlorine-free paper for which the industry uses peracetic acid, ozone, hydrogen peroxide, and oxygen in bleaching sequences.

Reducing Bleach

A reducing bleach works by converting double bonds in a chromophore into single bonds. This eliminates the ability of the chromophore to absorb visible light. Sunlight releases high-energy photons, often in the violet or UV range, that can disrupt bonds in a chromophore, rendering the substance colorless. Many colored molecules are relatively fragile and are damaged by photons of UV light. Traditionally, exposure to sunlight was the way to bleach fabrics and make them white (Bloomfield, 2007). Reducing bleaches commonly used in foods include lemon juice (in combination with sunlight) and sulfur dioxide.

Biochemical Bleach

Enzymes such as lipoxygenase (EC 1.13.11.12; lipoxydase) can fall in the third category of bleaching agents: biochemical bleaches. Lipoxygenase was first discovered in 1928 by Bohn and Hass (1953) as a carotene-destroying enzyme ("carotene oxidase") in soybeans, and later designated as lipoxygenase. Acceleration of the oxidation of xanthophylls to colorless products has long been recognized as a property of soybean lipoxygenase used in the bleaching of bread dough (Balls et al., 1943). A patent on biochemical bleaching of dairy products was introduced in the United States recently (Roos et al., 2006). Roos et al. (2006) disclosed the use of lipoxygenases to bleach dairy products, including whey. Only small amounts of lipoxygenases have a whitening effect on dairy products such as milk, cheese, butter oil, cream, and whey products. The mechanism of bleaching by lipoxygenase is based on the oxidative transition of double bonds in carotenoids by radicals produced in the reaction of lipoxygenase and linole(n)ic acid. Soy lipoxygenase has been used for bleaching purposes in wheat and maize flour; however, it has not been previously applied to bleach whey. Because a more specific enzymatic reaction is occurring rather than a general oxidation treatment, as is the case with oxidizing bleaches, enzymatic bleaching may reduce off-flavors. Recently (November 2009), an external enzymatic (biochemical) bleach was launched for bleaching of fluid whey. MaxiBright (DSM Food Specialties, Delft, the Netherlands) is a fungal peroxidase that can purportedly be used in place of an oxidizing bleach to bleach fluid whey. The enzyme is specific for carotenoids (Zorn et al., 2003), thus yielding whiter whey without the use of traditional bleaching agents, and possibly reducing unwanted side effects of traditional bleaching. Like all peroxidases, MaxiBright requires activation with 0.5 to 1 m*M* hydrogen peroxide, but the hydrogen peroxide is consumed and catalase addition is not required. There are currently no available published studies on the use of MaxiBright with whey or in comparison to traditional oxidizing bleaches. Food and Drug Administration's "generally regarded as safe" (**GRAS**) status has been filed for MaxiBright, and approval is anticipated in September 2010.

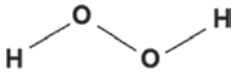
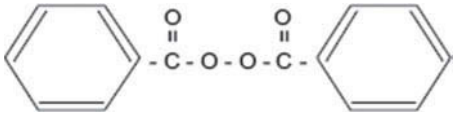
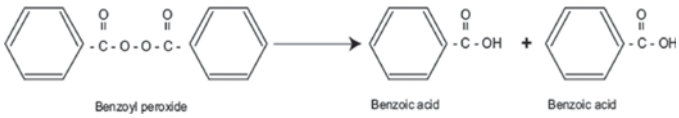
BLEACHING WHEY

Annatto is used by the dairy industry to color cheese. The specific amount of annatto that partitions into cheese and whey has not, to our knowledge, been directly studied. However, approximately 20% of the annatto added to cheese milk is estimated to pass into whey (Barnicoat, 1950). The color from annatto is highly unfavorable in dried whey products and thus a decolorizing process must be performed and has been in place in the dairy industry for more than 40 yr (McDonough et al., 1968). This process involves bleaching of liquid whey or liquid retentate with hydrogen or benzoyl peroxide. Hydrogen peroxide and benzoyl peroxide are the only bleaching agents legally allowed for treatment of whey in the United States and there are several restrictions to their use.

Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a clear, colorless liquid with a slightly pungent odor. Hydrogen peroxide decomposes to oxygen and water during bleaching (Table 1). Residual hydrogen peroxide must be removed from whey and cheese milk physically or by the addition of catalase according to FDA regulations 184.1366 and 133.113 (US FDA, 2009l,e). Catalase converts hydrogen peroxide into oxygen and water (Table 1). Catalase use must not exceed 20 ppm and must be sufficient to remove any residual hydrogen peroxide (US FDA, 2009e). As hydrogen peroxide is a GRAS substance, the maximum treatment level for bleaching annatto-colored whey using hydrogen peroxide is 0.05% (<500 ppm) of the whey (US FDA, 2009l). There are no specific provisions in European Union regulations regarding the use of hydrogen peroxide as a bleaching agent for dairy products. When no national provisions on processing aids exist, their use is controlled by general safety pro-

Table 1. Summary of hydrogen peroxide and benzoyl peroxide for whey bleaching

Item	Bleaching agent: Hydrogen peroxide	Bleaching agent: Benzoyl peroxide
Structure	 (National Center for Biotechnology Information, 2008b)	 (National Center for Biotechnology Information, 2008a)
Breakdown	$2H_2O_2 \rightarrow O_2 + 2H_2O$ (hydrogen peroxide → oxygen + water) (Smith, 2004)	 Benzoyl peroxide; Benzoic acid; Benzoic acid (Smith, 2004)
Regulation	21CFR184.1366 (US FDA, 2009l)	21CFR184.1157 (US FDA, 2009k)
Pros	• little to no effect on the nutrients present • more acceptable for usage in other countries	• effective at lower usage levels than hydrogen peroxide • does not require a catalase addition to remove residues • does not pit stainless steel; therefore, is less corrosive to equipment • effective across a wide range of temperatures
Cons	• must be inactivated with catalase • could possibly cause oxidized flavors • corrosive to equipment • less economical to use because it requires much more peroxide for satisfactory bleaching	• possible formation of oxidized flavors • possibility that the carrier used may be considered an allergen • concerns from other countries because it has just been recently approved by Codex

visions. Therefore, it can be understood that European Union regulations are in agreement with FDA regulations for the use of hydrogen peroxide as a bleaching agent for whey.

In addition to being used as a bleaching agent, hydrogen peroxide can be used as an antimicrobial agent in milk, intended for cheese making at 0.05% (wt/wt) level (US FDA, 2009l). Likewise, hydrogen peroxide can be used as an antimicrobial agent during the preparation of modified whey by electrodialysis methods at 0.04% (wt/wt) level (US FDA, 2009l). Hydrogen peroxide can be used as a microbial agent in cheese milk and a side effect will be bleaching milk. However, federal food and drug regulations do not support the use of hydrogen peroxide solely for the use of bleaching milk for cheese and related cheese products (US FDA, 2009l).

Benzoyl Peroxide

Benzoyl peroxide ($C_{14}H_{10}O_4$) is a colorless, crystalline solid permitted for use in removing color in whey products that are not used for infant formula. Like hydrogen peroxide, benzoyl peroxide (or a mixture of benzoyl peroxide with potassium alum, calcium sulfate, and magnesium carbonate) is also a GRAS substance and can be used to bleach dairy ingredients. Unlike hydrogen peroxide, benzoyl peroxide has no limitation on usage rates in foods other than current GMP rules (US FDA, 2009k). Benzoyl peroxide is GRAS as a direct human food ingredient upon the following current GMP conditions of use: (1) the ingredient is used as a bleaching agent in food, (2) the ingredient is used in the following foods, milk used for production of Asiago fresh and Asiago soft cheese (US FDA, 2009b), Blue cheese (US FDA, 2009c), Caciocavallo Siciliano cheese (US FDA, 2009d), Gorgonzola cheese (US FDA, 2009f), Parmesan and Reggiano cheese (US FDA, 2009g), Provolone cheese (US FDA, 2009h), Romano cheese (US FDA, 2009i), and Swiss and Emmentaler cheese (US FDA, 2009j), annatto-colored whey, such that the final bleached product conforms to the descriptions and specifications for whey, concentrated whey, or dried whey, at levels not to exceed current GMP. In contrast to hydrogen peroxide, benzoyl peroxide may be used to bleach milk for certain cheese productions as listed above, whereas hydrogen peroxide may not be used to bleach milk for cheese make in any cheese, but hydrogen peroxide is permitted to be used at 0.05% in milk for antimicrobial purposes. The current GMP is as follows: the weight of the benzoyl peroxide is not more than 0.002% (20 ppm) of the weight of the milk being bleached, and the weight of the potassium alum, calcium sulfate, and magnesium carbonate, singly or combined, should not be more than 6 times the weight of the benzoyl peroxide used (US FDA, 2009b,c,d,f,g,h,i,j). Whey is considered a GRAS substance and thus has no standard of identity. This means that there is no federal regulation for usage rates of benzoyl peroxide in whey for bleaching but using

benzoyl peroxide as a preservative is not acceptable. Should benzoyl peroxide be used at levels >1% (wt/wt) in liquid whey, the manufacturer would need to justify the usage rate. Most additives are used at a rate of 1 to 3% (wt/wt), but any usage rate over 1% (wt/wt) raises many red flags (N. Ratzlaff, USDA, Lisle, IL; personal communication).

There are concerns about benzoyl peroxide bleaching as it has only been recently approved by Codex Alimentarius, with the maximum level (100 mg/kg) for liquid whey and whey products (excluding infant applications) being adopted in 2007, and the maximum level (100 mg/kg) for dried whey and whey products (excluding infant applications) being adopted in 2005 (Codex, 2008). The European Commission (EC) allows both hydrogen peroxide and benzoyl peroxide to be used as whey bleaching agents (designated as processing aids, not food additives), but individual country provisions may be stricter (Bianca Herr, Leatherhead Food Institute, Leatherhead, UK; personal communication). Many Asian and European government regulators do not like the use of benzoyl peroxide and consider benzoic acid and other breakdown products of benzoyl peroxide harmful. There is a possibility that the carrier used for benzoyl peroxide may be considered an allergen. In addition, certain carriers may pose problems with membrane fouling or leave residues of insoluble carrier on equipment. Benzoyl peroxide reacts with oxidizable compounds and is converted into water-soluble benzoic acid following bleaching (Table 1). A petition was submitted by the US Dairy Export Council (USDEC) to the Taiwan Department of Health, Food Sanitation, and Safety for the use of benzoyl peroxide in whey powder and was approved on December 20, 1999 (Johnson, 2006). Benzoyl peroxide is not an approved bleaching agent for whey in China or Japan and neither country's regulations allow for residual benzoic acid in dried whey products.

Because of stricter regulations in China with their new food safety law (June 1, 2009), the Chinese government is now routinely testing for the presence of benzoic acid (Anonymous, 2009). The safety of benzoic acid and the derivative benzoates have been studied extensively (Sharratt et al., 1964; Nair, 2001; Qi et al., 2009). Benzoic acid is found naturally in milk at low concentrations, 0 to 1 mg/kg, and in fermented dairy products at higher concentrations, typically about 20 mg/kg but up to 50 mg/kg (Sieber et al., 1995). During the fermentation process, lactic acid bacteria convert hippuric acid, a naturally occurring component of milk, to benzoic acid (Sieber et al., 1995). Benzoic acid can also be added as a preservative to cheese rennet and thus be found in cheeses from that route as well as from lactic acid fermentations. Benzoic acid is found in cheeses, but at lower concentrations than in fermented dairy products (Sieber et al., 1995). Subsequently, benzoic acid occurs naturally in whey and whey protein powders. Adverse reactions to benzoic acid-related compounds are rare, and life-threatening reactions are extremely rare (JECFA, 2004). Chang et al. (1977) found that when benzoyl peroxide was added to Edam cheese whey, after it was heated to 69°C for 6 h and cooled, 91.7% of the benzoyl peroxide used was recovered as benzoic acid, and minor amounts of hydroxybenzoic acids, phenylbenzoate, phenol, and benzoyl peroxide were found.

Lactoperoxidase

When the lactoperoxidase system is used for antimicrobiological purposes, external addition of thiocyanate or peroxide is required. However, when bleaching whey with hydrogen peroxide, companies often take advantage of the naturally existing lactoperoxidase system in milk in combination with externally added hydrogen peroxide to obtain maximum bleaching effectiveness of fluid whey. No additional enzyme or thiocyanate needs to be added for bleaching purposes (E. Bastian; unpublished data). Lactoperoxidase (**LP**; EC 1.11.1.7) is a natural enzyme found in milk and constitutes about 0.5% of the total whey proteins in bovine milk. Lactoperoxidase is the second most abundant enzyme in raw milk (Kussendrager and van Hooijdonk, 2000; Fox and Kelly, 2006). The LP system consists of 3 components: lactoperoxidase, thiocyanate (SCN^-), and hydrogen peroxide, and the system is only active in the presence of these 3 components (Seifu et al., 2005). The LP system is a potent bacteriocidal system that has been used to preserve raw milk without refrigeration. Depending on the concentrations of SCN^- and hydrogen peroxide used to activate the LP system, preservation can vary from 24 h at 35°C to 48 h at 20 to 22°C (Fweja et al., 2007). This system is 50 to 100 times more effective than hydrogen peroxide alone (Fox and Kelly, 2006). Lactoperoxidase is relatively heat stable with denaturation starting at about 70°C (Kussendrager and van Hooijdonk, 2000). Heat stability studies were conducted in milk, whey, permeate, and buffer, and the enzyme was reported to be more stable to heat in whey and milk possibly because of their higher calcium ion concentration (Kussendrager and van Hooijdonk, 2000). Below pH 5.3, LP is less heat stable (Kussendrager and van Hooijdonk, 2000) and thus the optimal pH is 5.5 to 6.3 (Bottomley et al., 1989), which includes the pH range of whey. As such, sufficient amounts of the active enzyme are present in pasteurized milk and whey.

This system can be applied to bleach fluid whey by the addition of low concentrations of hydrogen peroxide

to activate they system. In 1989, a patent was issued for decolorizing whey products using the LP system (Bottomley et al., 1989). Lactoperoxidase reacts in the presence of hydrogen peroxide to convert SCN^- to hypothiocyanite ($OSCN^-$). Hypothiocyanite is a strong oxidizing agent that reacts with carotenoids such as norbixin and oxidizes the double bonds, removing the conjugation and thus removing the color of the compound. It should be noted that no additional enzyme or thiocyanate needs to be added, nor does catalase need to be added because the added hydrogen peroxide is consumed. The time required can be variable but is generally 20 to 45 min at 40°C. The recommended time and temperature is 30 min at 40°C; 40°C is acceptable from a regulatory perspective as long as the whey is not held at that temperature for more than 2 h. The bleaching reaction occurs within 30 min (Bottomley et al., 1989).

Lactoperoxidase catalyzes the oxidation of annatto but is inactivated by high concentrations of hydrogen peroxide (Bottomley et al., 1989). As such, hydrogen peroxide concentrations should be kept at or near 10 ppm (Bottomley et al., 1989). Additionally, the inactivation effect depends on the concentration of hydrogen peroxide and on the length of time the enzyme is exposed. If the concentration of hydrogen peroxide present in the whey falls below the inactivating concentration before complete inactivation of the enzyme has occurred, decolorization will still occur but at a slower rate (Bottomley et al., 1989).

ADVANTAGES AND DISADVANTAGES OF BLEACHING

Hydrogen Peroxide

Higher temperatures are generally more effective than lower temperatures. Reaction temperatures above 74°C increased neither the rate nor the extent of color removal but only caused protein denaturation (McDonough et al., 1968). The oxidized flavors that were apparent immediately after bleaching purportedly disappear after evaporation and drying (McDonough et al., 1968), although a recent study demonstrated that this is not the case (Croissant et al., 2009). In addition, hydrogen peroxide has little to no effect on the nutrients present (Teply et al., 1958). Teply et al. (1958) analyzed milks and subsequent cheese and whey when the milk was bleached using 5, 10, and 25 times the normal amount of hydrogen peroxide and found that a strong treatment may alter proteins and amino acids in milk but in general there was no effect on the composition or nutritional value of the milk, cheese, or whey. The disadvantages to using hydrogen peroxide are that it must be inactivated with catalase, it could cause oxidized flavors, it is corrosive to equipment (Gilliland, 1969), and it is less economical to use because it requires more peroxide for satisfactory bleaching (Chang et al., 1977). A current review of prices for hydrogen peroxide and benzoyl peroxide suggests that the previous statement may be dependent on the bleaching conditions and usage rate. Hydrogen peroxide is sold in 53-gal drums for $200/drum; the liquid is 34% solids. Benzoyl peroxide is sold as a powder and costs $4.62 per kg.

Depending on the concentration, temperature, reaction time, and pH, bleaching with hydrogen peroxide may alter the functionality of total and individual whey proteins (Cooney and Morr, 1972; Munyua, 1975). Unfortunately, the 2 prominent, albeit dated, studies (Cooney and Morr, 1972; Munyua, 1975) on the functional properties of whey protein bleached with hydrogen peroxide dealt with concentrations above the legal limit. Because these studies used a level above the legal maximum, the effects documented in those studies may not be representative of the effects of hydrogen peroxide used at lower levels. According to Munyua (1975), hydrogen peroxide concentrations greater than 0.1% in fluid whey or milk caused a 5 to 8% decrease in the nonpolar amino acids such as aspartic acid, threonine, glutamic acid, methionine, trypsin, phenylalanine, histidine, lysine, tryptophan (25% decrease), and arginine. In contrast, free sulfhydryl groups increased as the hydrogen peroxide concentration increased. Increasing exposure time increased the number of free sulfhydryl groups up to 24 h. The researchers postulated that hydrogen peroxide reacted first with readily oxidized amino acids such as methionine. Higher concentrations of hydrogen peroxide, increased temperatures, and longer holding times all increased the amount of whey protein denaturation.

Reaction temperature during bleaching can affect whey protein denaturation. Cooney and Morr (1972) demonstrated 4% protein denaturation when whey was treated with 1% (10,000 ppm) hydrogen peroxide for 24 h at 25°C, whereas 28% protein denaturation was achieved by treating whey for 6 h at 50°C. It should be noted that the hydrogen peroxide concentration range used in this study was above the legal limit for hydrogen peroxide usage. More studies are needed in this area to determine if these effects are observed at lower concentrations. Although the effect of pH was minor compared with other variables, the pH resulting in the greatest amount of protein denaturation depended on the specific whey protein. For instance, immunoglobulins and bovine serum albumin were more readily denatured at lower pH, whereas β-lactoglobulin

denaturation was enhanced at pH closer to neutral. α-Lactalbumin exhibited much less denaturation than β-lactoglobulin under the same conditions (Law and Leaver, 2000).

Benzoyl Peroxide

The effectiveness of benzoyl peroxide for removing color in whey depends on the amount used, how it is applied, the whey components present, and the exposure time and temperature. McDonough et al. (1968) reported that both benzoyl peroxide and hydrogen peroxide were effective bleaching agents, but that benzoyl peroxide was more effective at all temperatures. The rate and extent of decolorization by hydrogen peroxide and benzoyl peroxide for annatto in Cheddar cheese whey increased as the temperature was increased from 32.2 to 63°C. However, no additional increase with either agent was seen at 74°C. The advantages to using benzoyl peroxide are that it is effective at lower usage levels than hydrogen peroxide, it does not require addition of catalase to remove residues, and it does not pit stainless steel, and therefore is less corrosive to equipment (Chang et al., 1977). As with reports of off-flavors from hydrogen peroxide, oxidized flavors in Cheddar cheese whey were strong immediately after treatment with benzoyl peroxide; however, off-flavors purportedly dissipated following evaporation and drying (McDonough et al., 1968), an effect not observed in a more recent study (Croissant et al., 2009).

Contrary to the findings of McDonough et al. (1968), our recent unpublished research has reported that hydrogen peroxide is more temperature-dependent than benzoyl peroxide. McDonough et al. (1968) recommended bleaching temperatures in the range of 55 to 65°C. Benzoyl peroxide reacts quickly to remove color and additional time will not increase color removal. At 30°C, benzoyl peroxide may never completely bleach whey (Roos et al., 2006).

Peroxide bleaching (hydrogen or benzoyl) may have an effect on flavor in whey proteins, although few studies have been conducted. Mortenson et al. (2008) studied the flavor of whey protein concentrates (**WPC**) and whey protein isolates. Contrary to expectations, they found that flavor of WPC34 (WPC with 34–36% protein) and whey protein isolate were not affected by instantizing, ion exchange, or bleaching. However, lack of strict experimental controls and other processing variables suggest confounding factors in this study. Other recent studies have unequivocally established sensory and volatile compound differences associated with whey sourced from different cheeses, agglomeration and instantization (Carunchia Whetstine et al., 2005; Gallardo-Escamilla et al., 2005; Drake et al., 2009; Wright et al., 2009). Kuramoto and Jezeski (1954) studied the bleaching effects of benzoyl peroxide in cream (30% fat) for blue cheese manufacture at temperatures ranging from 52 to 85°C for periods of up to 4 h with concentrations of benzoyl peroxide of 4.5, 9, and 18 mg/kg. They found that flavor problems, such as tallow or oxidized flavors, were more apparent with increasing temperature, contact time, and benzoyl peroxide concentration. McDonough et al. (1968) reported that oxidized flavors present in fluid whey bleached with benzoyl peroxide at 20 and 10 mg/kg at 52°C and 63°C for 1.5 h or hydrogen peroxide at 500 and 300 mg/kg at 52°C and 63°C for 1.5 h were not detected in the dried whey powder. However, Croissant et al. (2009) conducted a controlled study with hot bleaching of liquid whey, with either hydrogen peroxide at 250 or 500 mg/kg at 60°C for 90 min or benzoyl peroxide at 10 or 20 mg/kg at 60°C for 90 min, and then manufactured WPC from those wheys. They demonstrated sensory effects and volatile compound changes in WPC from hydrogen peroxide or benzoyl peroxide bleached whey compared with unbleached whey.

Whey with higher total solids, such as condensed whey, needs greater amounts of peroxide to remove color (JECFA, 2004). The most effective conditions are 60°C for 15 min at pH 6 to 7 (El-Samragy, 2004). Longer holding times are required if lower temperatures are used (McDonough et al., 1968; El-Samragy, 2004). Once whey has been dried, the annatto becomes highly resistant to bleaching. It is important to note that much of the above discussion regarding the use of hydrogen peroxide and benzoyl peroxide for bleaching whey originates from old literature or unpublished data and has not recently been thoroughly evaluated.

REGULATORY CONCERNS

Regulatory concerns focus on the use of either hydrogen or benzoyl peroxide for preservation of whey rather than bleaching. That is, these agents are approved for bleaching, not for maintenance of membrane flux during processing or for microbial control. The use of peroxide for preservation of whey during any process other than electrodialysis is prohibited. When bleaching is applied, the agent, concentration, time, and temperature vary widely within the industry depending on the existing facility and its specific process regimen. Regulatory agencies typically use the point of peroxide addition in the process to determine if the purpose of peroxide is bleaching or preservation (USDA/AMS/Dairy Division, 2008). Bleaching whey is usually applied at 1 of 2 possible steps during the whey production process. Per-

oxide can be added to fluid whey after pasteurization, before or after fat separation, as it is pumped into a storage tank, or when whey retentate is in the hot well of the evaporator. Bleaching is also conducted under a wide range of temperatures from 5 to 70°C. The USDA cites 2 specific cases where use of peroxide would be assumed to be for preservation purposes (Hammond et al., 1975; USDA/AMS/Dairy Division, 2008). The first case is addition of peroxide before the separator or any point in the process before preheating for the evaporator. Fluid whey can legally be bleached following pasteurization if the preheated whey goes into a storage tank for bleaching followed by fat separation. In this situation, the plant typically alternates between 2 tanks. Alternating between 2 tanks for bleaching must be completed within 4 h for microbiological reasons. In addition, legal bleaching may also be carried out if the bleach is added to the hot well before condensing. The second situation generating concern is addition of peroxide before holding the whey for more than 2 h at temperatures between 7 and 63°C (USDA/AMS/Dairy Division, 2008).

Legally, whey can be bleached at any temperature so long as the whey is not held between 7 and 63°C for more than 2 h. To determine the legality of bleaching during ultrafiltration (**UF**) of whey, the point of application at which the agent is added and how long the whey is being held before UF is considered. If the bleaching agent is immediately added and flow begins, this is well within legal limits, regardless of the temperature at which flow occurs. In addition, should the bleaching agent be added to a balance tank before UF, the feed is considered fast enough that these bleaching conditions are considered acceptable. It should be noted that UF units have maximum temperatures at which the membranes can operate and this should be considered when deciding bleaching conditions. Hot bleaching temperatures during UF may not lend themselves to long membrane life in the case of spiral wound membranes. In contrast, should the bleaching agent be added to a silo tank before UF and held for more than 2 h between 7 and 63°C, this would be considered illegal because the bleaching agent would now be considered a preservation agent (N. Ratzlaff, USDA, Lisle, IL; personal communication).

CONCLUSIONS

Bleaching can create a more desirable color in whey proteins but it may also alter functionality and flavor. The majority of published literature dealing with bleaching of whey is quite dated (>25 yr). Since these studies have been conducted, milk quality, cheese making practices, and whey protein processing have all greatly evolved, emphasizing a need to scientifically evaluate bleaching and its effect on whey protein. Only two agents, hydrogen peroxide and benzoyl peroxide, are currently approved for bleaching. Of these two, the latter is viewed negatively in many countries and some regulations prevent its use. Both bleaching agents can negatively affect whey protein flavor. More precise application of currently approved bleach agents (e.g., minimum concentrations, optimal time/temperature exposure) or development of bleaching alternatives may facilitate enhanced whey protein flavor.

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