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## The effect of bleaching agents on the degradation of vitamins and carotenoids in spray-dried whey protein concentrate

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### ABSTRACT

Previous research has shown that bleaching affects flavor and functionality of whey proteins. The role of different bleaching agents on vitamin and carotenoid degradation is unknown. The objective of this study was to determine the effects of bleaching whey with traditional annatto (norbixin) by hydrogen peroxide (HP), benzoyl peroxide (BP), or native lactoperoxidase (LP) on vitamin and carotenoid degradation in spray-dried whey protein concentrate 80% protein (WPC80). An alternative colorant was also evaluated. Cheddar whey colored with annatto (15 mL/454 L of milk) was manufactured, pasteurized, and fat separated and then assigned to bleaching treatments of 250 mg/kg HP, 50 mg/kg BP, or 20 mg/kg HP (LP system) at 50°C for 1 h. In addition to a control (whey with norbixin, whey from cheese milk with an alternative colorant (AltC) was evaluated. The control and AltC wheys were also heated to 50°C for 1 h. Wheys were concentrated to 80% protein by ultrafiltration and spray dried. The experiment was replicated in triplicate. Samples were taken after initial milk pasteurization, initial whey formation, after fat separation, after whey pasteurization, after bleaching, and after spray drying for vitamin and carotenoid analyses. Concentrations of retinol,  $\alpha$ -tocopherol, water-soluble vitamins, norbixin, and other carotenoids were determined by HPLC, and volatile compounds were measured by gas chromatography-mass spectrometry. Sensory attributes of the rehydrated WPC80 were documented by a trained panel. After chemical or enzymatic bleaching, WPC80 displayed 7.0 to 33.3% reductions in retinol,  $\beta$ -carotene, ascorbic acid, thiamin,  $\alpha$ -carotene, and  $\alpha$ -tocopherol. The WPC80 bleached with BP contained significantly less of these compounds than the HP- or LP-bleached WPC80. Riboflavin, pantothenic acid, pyridoxine, nicotinic acid, and cobalamin concentrations in fluid whey

were not affected by bleaching. Fat-soluble vitamins were reduced in all wheys by more than 90% following curd formation and fat separation. With the exception of cobalamin and ascorbic acid, water-soluble vitamins were reduced by less than 20% throughout processing. Norbixin destruction, volatile compound, and sensory results were consistent with previous studies on bleached WPC80. The WPC80 colored with AltC had a similar sensory profile, volatile compound profile, and vitamin concentration as the control WPC80.

**Key words:** vitamin, degradation, bleaching, whey

### INTRODUCTION

The production of whey protein concentrate (WPC; 25 to 89.9% protein) has grown considerably in recent years. In 2014, approximately 244 million kilograms of WPC were produced in the United States (USDA, 2015), representing a 29.6% increase in WPC sales from 2009 (USDA, 2010). As an ingredient, WPC is often utilized for its unique functional properties (Foegeding et al., 2002; Davis and Foegeding, 2007; Gbassi et al., 2009) and for its high-quality amino acid profile (Coker et al., 2012; Yang et al., 2012; Candow et al., 2006). These characteristics have made WPC a useful ingredient as a protein additive (Varnam and Sutherland, 1994; Graf et al., 2011), an emulsifier (Foegeding et al., 2002), and a thickening agent (Foegeding et al., 2006). However, WPC produced from colored Cheddar cheese retains approximately 10% of the added annatto colorant, which, unless removed, limits its application as a food ingredient (Jervis et al., 2012; Smith et al., 2014). The primary colorant used for Cheddar cheese is annatto (Scotter, 2009), a naturally occurring plant seed that has a dark orange color and is comprised of the carotenoid norbixin (Kang et al., 2010). Because norbixin is soluble in polar solutions, approximately 10% of this compound is not retained in the cheese curd and is present in the whey (Smith et al., 2014).

Oxidizing bleaching agents effectively degrade norbixin by disrupting the double bonds that compose the chromophore of norbixin (Winter et al., 2008; Kang

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et al., 2010). Although effective at removing color, these chemical bleaches are nonspecific and promote lipid oxidation, causing off-flavors in spray-dried whey ingredients (Campbell et al., 2012; Jervis et al., 2012, 2015; Smith et al., 2015). Both chemical (hydrogen peroxide and benzoyl peroxide) and enzymatic (lactoperoxidase) bleaching agents can be applied to remove norbixin with variable results dependent on bleaching agent and bleaching conditions, but all function as oxidizing agents and can contribute lipid oxidation and off-flavors (Campbell et al., 2012, 2014; Jervis et al., 2012; Smith et al., 2015).

One question that has not been addressed is how bleaching agents affect degradation of vitamins. Chemical bleaching reduces vitamin and carotenoid contents in white flour (McCay 1985; Guo et al., 2016). Native peroxidase in vegetables and fruit can degrade ascorbic acid, anthocyanins, fatty acids, and carotenoids (Sivasankar, 2002). However, little work has been done to address how bleaching agents affect vitamins in whey protein ingredients. Although flavor is the primary driver in liking of protein beverages, nutrient composition is an important indicator of consumer acceptance of these products (Childs et al., 2008; Oltman et al., 2015). Riboflavin, pantothenic acid, and cobalamin are above 10% of the nutritional daily value for a typical serving size of whey protein. Other vitamin content is below labeling ranges for a typical serving size of unfortified whey protein, but people that consume several servings of whey protein a day may get a significant amount of vitamins from whey protein. It is therefore important to understand how to minimize the degradation of key nutrients during whey processing. Due to the flavor and functional effects of bleaching, alternative colorants for Cheddar cheese that do not partition into the whey have been proposed (Kang et al., 2012; Smith et al., 2014). The objective of this study was to determine the effects of bleaching whey containing traditional annatto colorant with hydrogen peroxide (**HP**), benzoyl peroxide (**BP**), or native lactoperoxidase (**LP**) on vitamin and carotenoid degradation in WPC80. A control unbleached colored whey and a whey from cheese colored with an alternate  $\beta$ -carotene colorant were also included.

## MATERIALS AND METHODS

### Experimental Design

For each experimental replication, whey was manufactured in the North Carolina State University (**NCSU**) dairy pilot plant from one lot of whole-fat, raw bovine milk from the NCSU dairy enterprise system. Each lot of milk was processed on the same day it was received.

**Table 1.** Sampling points for whey processing

Production point	Stage of processing
Milk following pasteurization	1
Whey drained from vat	2
Liquid whey following fat separation before HTST	3
Liquid whey following pasteurization	4
Liquid whey following bleaching treatment	5
Retentate stream following ultrafiltration	6
Spray-dried powder	7

The milk was divided into 2 portions; one was used to produce Cheddar cheese colored with norbixin, and the other was used to make Cheddar cheese colored with an alternative colorant (**AltC**) at the recommended dosage of 23 mL/454 kg of milk (DairyMax Orange Red 002, a  $\beta$ -carotene-based colorant; Chr. Hansen, Copenhagen, Denmark). The whey from both vats was processed into powdered WPC80. Approximately 120 mL of sample was collected at each of 7 points throughout processing (Table 1). Liquid samples and powdered WPC80 for vitamin and carotenoid testing were stored at  $-80^{\circ}\text{C}$  until evaluation (<30 d). Descriptive sensory analysis and volatile compound analysis were also performed on powdered WPC80. Manufacturing procedures were repeated in triplicate, from 3 different batches of milk.

### WPC Manufacture

The WPC80 was manufactured as described by Park et al. (2014), with raw whole milk received from the NCSU dairy enterprise system. Approximately 68 kg of raw whole milk was HTST pasteurized at  $73^{\circ}\text{C}$  for 17 s (model MPD1050, Micro Process Design, D&F Equipment Co., McLeansville, NC). Milk was separated into 2 cheese vats, heated to  $31^{\circ}\text{C}$ , and inoculated with a mesophilic starter culture (Choozit MA 11, Danisco, New Century, NJ) at a concentration of 50 direct culture units/454 kg of milk. Calcium chloride (50% wt/vol, Dairy Connection Inc., Madison, WI) was then added to both vats at 0.39 mL/kg of milk. The milk was ripened for 30 min under constant agitation before colorants were added based on treatment. One vat received double-strength annatto (**Con**; Cheese Color DS Double Strength, Dairy Connection Inc.) at a rate of 15 mL/454 kg and the other received the alternate colorant (**AltC**; DairyMax Orange Red 002, Chr. Hansen) at a rate of 23 mL/454 kg, before being allowed to ripen for an additional 30 min. Double-strength recombinant rennet (Dairy Connection Inc.) was then added at a rate of 0.09 mL/kg and allowed to set for 30 min. The coagulum was cut into cubes approximately 2.54 cm in length and allowed to rest for 5 min, followed by gradual heating to  $39^{\circ}\text{C}$  over the course of 30 min

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