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The effect of microfiltration on color, flavor, and functionality of 80% whey protein concentrate

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ABSTRACT

The residual annatto colorant in fluid Cheddar cheese whey is bleached to provide a neutral-colored final product. Currently, hydrogen peroxide (HP) and benzoyl peroxide are used for bleaching liquid whey. However, previous studies have shown that chemical bleaching causes off-flavor formation, mainly due to lipid oxidation and protein degradation. The objective of this study was to evaluate the efficacy of microfiltration (MF) on norbixin removal and to compare flavor and functionality of 80% whey protein concentrate (WPC80) from MF whey to WPC80 from whey bleached with HP or lactoperoxidase (LP). Cheddar cheese whey was manufactured from colored, pasteurized milk. The fluid whey was pasteurized and fat separated. Liquid whey was subjected to 4 different treatments: control (no bleaching; 50°C, 1 h), HP (250 mg of HP/kg; 50°C, 1 h), and LP (20 mg of HP/kg; 50°C, 1 h), or MF (microfiltration; 50°C, 1 h). The treated whey was then ultrafiltered, diafiltered, and spray-dried to 80% concentrate. The entire experiment was replicated 3 times. Proximate analyses, color, functionality, descriptive sensory and instrumental volatile analysis were conducted on WPC80. The MF and HP- and LP-bleached WPC80 displayed a 39.5, 40.9, and 92.8% norbixin decrease, respectively. The HP and LP WPC80 had higher cardboard flavors and distinct cabbage flavor compared with the unbleached and MF WPC80. Volatile compound results were consistent with sensory results. The HP and LP WPC80 were higher in lipid oxidation compounds (especially heptanal, hexanal, pentanal, 1-hexen-3-one, 2-pentylfuran, and octanal) compared with unbleached and MF WPC80. All WPC80 had >85% solubility across the pH range of 3 to 7. The microstructure of MF gels determined by confocal laser scanning showed an increased protein particle size in the gel network. MF WPC80 also had larger storage modulus values, indicating higher gel firmness. Based on bleaching efficacy comparable to chemical bleaching with HP, flavor, and functionality results, MF is a viable alternative to chemical or enzymatic bleaching of fluid whey.

Key words: bleaching, whey protein, flavor, functionality

INTRODUCTION

Whey is a by-product of cheesemaking and has become a source for high-value ingredients because of its nutritional and functional attributes (Smithers, 2008). Liquid whey can be dried and further processed into whey protein concentrates (**WPC**; 34–89% protein) and whey protein isolates (>90% protein). Whey proteins are used in many foods and beverages, and bland and colorless whey ingredients are desirable. Therefore, bleaching is a common and necessary whey processing step (Kang et al., 2010). Hydrogen peroxide (maximum usage rate at 500 mg/kg) and benzoyl peroxide (regulated by good manufacturing process, GMP5) are the 2 approved chemical bleaching agents in United States (US FDA, 2013a,b). Chemical bleaching causes lipid oxidation, which results in formation of volatile off-flavors that carry through to final whey protein products (Croissant et al., 2009; Listiyani et al., 2011; Jervis et al., 2012). In addition, it is likely that these oxidants cause oxidation of some AA constituents of protein, which may influence nutritive value and functional properties of whey proteins (Shacter, 2000; Jervis et al., 2012). Thus, alternative bleaching or removal methods are desirable for whey bleaching and were the focus of our study.

Lactoperoxidase (**LP**) is an oxidoreductase enzyme and is found in a wide range of mammalian milks. The LP system consists of 3 components: LP, thiocyanate (SCN^-), and hydrogen peroxide (H_2O_2). The system will not be active unless all 3 components are present in sufficient amounts. The major intermediate oxidation product of the LP-catalyzed oxidation of SCN^- is the hypothiocyanate ion ($OSCN^-$), which inhibits microbial growth (Seifu et al., 2005). In addition, $OSCN^-$ also has bleaching capabilities. The strong oxidizing capacity al-

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lows $OSCN^-$ to react with carotenoids, leading to color loss of norbixin. Campbell et al. (2012) demonstrated that the LP system with an optimal concentration of 20 mg of H_2O_2/kg was more effective than HP alone in norbixin destruction (bleaching >99%), but lipid oxidation products were also higher in concentration in LP-bleached whey compared with unbleached whey (Campbell et al., 2012).

Microfiltration has been used widely in separating fine particles (in the range of 0.1 to 10 μm) from suspension in biological products. In the dairy industry, microfiltration is used for bacteria removal, fat removal, and fractionation of milk proteins and separation of CN micelles and whey proteins (Ye et al., 2011). Zhu and Damodaran (2012) hypothesized that norbixin is more likely in the form of micelles that can be associated with the milk fat globule membrane particles than with globular proteins in Cheddar cheese whey due to its hydrophilic and hydrophobic characteristics. Therefore, fat removal through microfiltration (**MF**) may be an alternative bleaching method for Cheddar cheese whey. The effect of MF bleaching on the flavor and functional properties of 80% whey protein concentrate (**WPC80**) has not been determined. The objective of our study was to characterize and compare the composition, processing, sensory, and functional properties of WPC80 from bleached (HP, LP, and MF) and unbleached Cheddar whey.

MATERIALS AND METHODS

Experimental Design

Two experiments (experiment 1 and 2) were included in our study. The purpose of experiment 1 was to compare the influence of 3 different bleaching methods and norbixin removal (HP, LP, and MF) on color of liquid whey (proof of concept). Cheddar cheese whey with no bleach, HP bleach, LP bleach, and MF were manufactured in triplicate. Norbixin analysis was then conducted to determine the bleaching efficacy of different bleaching efficacy.

The purpose of experiment 2 was to compare the influence of 3 different bleaching and norbixin removal methods on flavor and functional properties of WPC80. Again, Cheddar cheese whey was manufactured. After pasteurization and fat separation of whey, aliquots of liquid whey were taken for bleaching. After bleaching and norbixin removal, liquid whey was ultrafiltered and diafiltered to 12% solids (wt/wt) and 80% protein (wt/wt) and spray dried to produce WPC80. Sensory and volatile analysis, foam stability, protein solubility, microstructure, and small strain rheological properties were conducted to determine the influence of different bleaching and norbixin removal methods on flavor and functionality of WPC80.

Liquid Whey Production

For experiment 1 and 2, whole, raw bovine milk (1,188 kg) was obtained from the North Carolina State University Dairy Research and Education Unit. The milk was HTST pasteurized (720 kg/h) with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC) at 72°C with a hold time of 15 s. The milk was then cooled to 31°C and transferred to a cheese vat (Kusel Equipment, Watertown, WI). Mesophilic starter culture containing *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* (Danisco Choozit MA11 LYO, Dairy Connection Inc., Madison, WI) was added at the rate of 50 DCU (direct culture unit)/454 kg of milk. A calcium chloride solution (50% wt/vol., Dairy Connection Inc.) was also added at the rate of 0.39 mL/kg of milk. The milk was then allowed to ripen for 60 min. After 30 min of ripening, double-strength annatto color (Cheese Color DS Double Strength, Dairy Connection Inc.) was added (0.066 mL/kg of milk). Next, the milk was coagulated with double strength recombinant rennet (Dairy Connection Inc.) for 30 min at a rate of 0.09 mL/kg of milk at the end of the ripening period. The coagulum was then cut with 0.95-cm knives and the curd was allowed to heal for 5 min. The curd was stirred gently for 30 min while the temperature was increased gradually to 39°C. The pH was monitored and liquid whey was drained at pH 6.4. The drained whey was fat separated using a hot bowl centrifugal separator (model SI600E, Agri-Lac, Miami, FL). After fat separation, the whey was HTST pasteurized (750 kg/h) at 72°C for 15 s. The whey was then portioned into batches of 272 kg each. Each portion was transferred into a stainless vat equipped with a coil heater. The whey was heated to 50°C and 1 of 4 treatments was conducted: no bleach for the control whey (50°C, 1 h), 250 mg of hydrogen peroxide/kg (35% wt/vol, VWR International, Westchester, PA) for 1 h, 20 mg of hydrogen peroxide/kg (35% wt/vol, VWR International) for 15 min (LP bleach), hydrogen peroxide was then removed using 20 mL/L of catalase (FoodPro CAT, Danisco, New Century, NJ), followed by 50°C for 45 min. The absence of HP was confirmed by testing with a peroxide strip (EMD Chemicals Inc., VWR International). The HP and LP conditions were selected based on previous studies (Campbell et al., 2012; Jervis et al., 2012) and industry communications. For the fourth treatment in experiment 1, 50°C liquid whey was microfiltered in a pilot scale microfiltration unit (model Lab 46, Filtration Engineering, Champlin, MN) using 2 spiral-wound MF membranes (Synder,