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Cold enzymatic bleaching of fluid whey

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ABSTRACT

Chemical bleaching of fluid whey and retentate with hydrogen peroxide (HP) alone requires high concentrations (100–500 mg of HP/kg) and recent studies have demonstrated that off-flavors are generated during chemical bleaching that carry through to spray-dried whey proteins. Bleaching of fluid whey and retentate with enzymes such as naturally present lactoperoxidase or an exogenous commercial peroxidase (EP) at cold temperatures (4°C) may be a viable alternative to traditional chemical bleaching of whey. The objective of this study was to determine the optimum level of HP for enzymatic bleaching (both lactoperoxidase and EP) at 4°C and to compare bleaching efficacy and sensory characteristics to HP chemical bleaching at 4°C. Selected treatments were subsequently applied for whey protein concentrate with 80% protein (WPC80) manufacture. Fluid Cheddar whey and retentate (80% protein) were manufactured in triplicate from pasteurized whole milk. The optimum concentration of HP (0 to 250 mg/kg) to activate enzymatic bleaching at 4°C was determined by quantifying the loss of norbixin. In subsequent experiments, bleaching efficacy, descriptive sensory analysis, and volatile compounds were monitored at selected time points. A control with no bleaching was also evaluated. Enzymatic bleaching of fluid whey and retentate at 4°C resulted in faster bleaching and higher bleaching efficacy (color loss) than bleaching with HP alone at 250 mg/kg. Due to concentrated levels of naturally present lactoperoxidase, retentate bleached to completion (>80% norbixin destruction in 30 min) faster than fluid whey at 4°C (>80% norbixin destruction in 12 h). In fluid whey, the addition of EP decreased bleaching time. Spray-dried WPC80 from bleached wheys, regardless of bleaching treatment, were characterized by a lack of sweet aromatic and buttery flavors, and the presence of cardboard flavor concurrent with higher relative abundance of 1-octen-3-ol and 1-octen-3-one. Among enzymatically bleached WPC80, lactoperoxidase-bleached WPC80 contained higher relative abundance of 2,3-octadienone, 2-pentyl furan, and hexanal than those bleached with added EP. Bleach times, bleaching efficacy, and flavor results suggest that enzymatic bleaching may be a viable and desirable alternative to HP bleaching of fluid whey or retentate.

Key words: whey, lactoperoxidase, flavor, bleach

INTRODUCTION

Whey is a by-product of cheese manufacture and is often further processed into value-added products, such as whey protein concentrate 34 or 80% or whey protein isolate (>90% protein). Typical whey processing steps include fat separation, pasteurization, bleaching, UF, diafiltration, and spray drying. The flavor of fluid whey carries through into the final spray-dried products (Croissant et al., 2009), and consumers and product manufacturers demand that dried whey ingredients be colorless with a bland flavor (Kang et al., 2010).

The manufacture of Cheddar cheese has continued to increase and Cheddar whey is one of the main sources of cheese whey. Norbixin, a natural orange-colored carotenoid, is added to Cheddar cheese milk to impart the desired orange color and a portion of the norbixin is retained in the fluid whey (Kang et al., 2010) and must be bleached. Off-flavors in dried whey proteins associated with bleaching, either with benzoyl peroxide or hydrogen peroxide (**HP**), have been well documented in the literature (Croissant et al., 2009; Listiyani et al., 2011, 2012; Jervis et al., 2012). Due to the increased demand for bland, colorless whey ingredients and international concerns with the use of benzoyl peroxide and increasing concerns with HP, chemical bleaching alternatives are desirable (Campbell et al., 2012; Kang et al., 2012). Campbell et al. (2012) recently demonstrated that as little as 10 mg of HP/kg was sufficient for greater than 80% norbixin destruction by lactoperoxidase (**LP**) in fluid whey at 35°C. Enzymatic bleaching, either using the native LP system or by adding an exogenous peroxidase (**EP**) has yet to be fully explored.

Lactoperoxidase, a native enzyme found in milk, is often used to increase storage stability and reduce the loss of fresh milk quality due to microbial spoilage. Lactoperoxidase is a member of the peroxidase family and

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when its activators thiocyanate and HP are present, hypothiocyanate, a potent antimicrobial, is produced (Reiter and Harnulv, 1982). In addition to milk preservation, the LP system can be used to bleach whey (Bottomley et al., 1989; Campbell et al., 2012). The strong oxidizing capacity of hypothiocyanate results in the destruction of carotenoid conjugation and subsequent color loss of norbixin in cheese whey. Using the LP system to bleach whey can be highly variable, as levels of LP can vary depending on the lactation cycle of the cow, season, feeding regimen, and breed (Kussendrager and van Hooijdonk, 2000). Similar to LP, thiocyanate concentration in milk and whey can vary widely due to feeding regimen (Seifu et al., 2005). The third component of the LP system, HP, is not normally detected in raw milk and is typically added exogenously. Hydrogen peroxide can be generated endogenously by bacteria, although amounts sufficient to activate the LP system may not be generated (Seifu et al., 2005). Depending on the milk, any 1 of the 3 components that make up the LP system could limit LP activity.

To facilitate enzymatic whey bleaching, a commercial EP is available and can be added to fluid whey product in small quantities to help achieve desired and consistent bleaching efficacy. This enzyme, MaxiBright (**MB**), is derived from a mushroom, *Marasmius scorodonius* (Zorn et al., 2003). Very little is known about the enzyme mechanism compared with that of LP; however, it is known that both of these enzymes require similar amounts of HP to activate their respective systems (Bottomley et al., 1989; Zorn et al., 2003). Since the original patent was filed in 2006, several studies have addressed the bleaching capacity of MB on β-carotene in model systems (Scheibner et al., 2008; Pühse et al., 2009; Zelena et al., 2009); however, the bleaching efficacy and subsequent effects on the flavor of MB in conjunction with the natural LP system in fluid whey has yet to be investigated. Studies have demonstrated that chemical bleaching at colder temperatures (<10°C) results in less lipid oxidation (Listiyani et al., 2011). Additionally, colder temperatures enhance membrane stability, microbial quality, and protein integrity. As such, cold bleaching is an attractive process. The objective of this study was to optimize enzymatic bleaching of whey and retentates with both LP and EP at 4°C and to evaluate their subsequent effects on the flavor of whey protein concentrate with 80% protein (**WPC80**).

MATERIALS AND METHODS

Experimental Design Overview

The study had 2 experimental components: liquid whey and retentate trials and the manufacture of WPC80. Optimum HP levels to activate the LP and EP systems were first determined. Liquid whey and retentate trials were then conducted to determine optimum bleach times at 4°C. The liquid whey treatments with the most bleaching and the fastest bleaching times were then selected for WPC80 manufacture. All treatments within each trial were made from the same lot of milk. All experiments were conducted in triplicate.

Production of Liquid Whey

Cheddar whey was manufactured from HTST (15 s at 72°C) pasteurized whole milk (720 kg/h; model T4 RGS-16/2; SPX Flow Technology, Greensboro, NC). The milk was then cooled to 31°C and transferred to a cheese vat (Kusel Equipment Co., Watertown, WI). Colored Cheddar whey manufacture proceeded as described by Campbell et al. (2011). The whey was drained from the curds at pH 6.3 and a sieve was used to remove any remaining particles. The whey was immediately processed with a hot bowl cream separator (model SI600E; Agrilac, Miami, FL) to reduce the fat content. Fat-separated, fluid whey was then HTST pasteurized as described previously. Whey was cooled to 4°C before bleaching experiments.

Production of Retentate

Fat-separated, pasteurized fluid whey was transferred into a 102-L stainless vat (Fermenator; Blichmann Engineering, Lafayette, IN) equipped with a coil heater (1.3-cm outer diameter; PAC Stainless Ltd., Seattle, WA) and heated to 50°C while recirculating using a peristaltic pump (model 77410-10; Millipore Inc., Billerica, MA). Once the desired temperature was reached, UF commenced. The UF system (model Pellicon 2; Millipore Inc.) was equipped with 10 polyethersulfone cartridge membrane filters (model P2B010V05; 10-kDa nominal separation cutoffs, 0.5 m^2 surface area; Millipore Inc.). Each sample was run through a peristaltic pump (model 77410-10; Millipore Inc.) and the UF assembly using silicone tubing (model 96440-73; Millipore Inc.) that was connected to the vat. Pumps, pump heads, and tubing were all obtained from Cole-Palmer (Vernon Hills, IL). Ultrafiltration and diafiltration continued until the retentate reached 80% protein content on a dry basis (wt/wt), confirmed by a Sprint rapid protein analyzer (CEM Corp., Matthews, NC). Retentates were then collected and cooled to 4°C before bleaching experiments.

Activation of the LP or EP System

The optimum level of HP to activate the LP or EP system was determined by adding 0, 5, 10, 15, 20, 25,