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# The use of lactoperoxidase for the bleaching of fluid whey

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## ABSTRACT

Lactoperoxidase (LP) is the second most abundant enzyme in bovine milk and has been used in conjunction with hydrogen peroxide ($H_2O_2$) and thiocyanate ($SCN^-$) to work as an antimicrobial in raw milk where pasteurization is not feasible. Thiocyanate is naturally present and the lactoperoxidase system purportedly can be used to bleach dairy products, such as whey, with the addition of very little $H_2O_2$ to the system. This study had 3 objectives: 1) to quantify the amount of $H_2O_2$ necessary for bleaching of fluid whey using the LP system, 2) to monitor LP activity from raw milk through manufacture of liquid whey, and 3) to compare the flavor of whey protein concentrate 80% (WPC80) bleached by the LP system to that bleached by traditional $H_2O_2$ bleaching. Cheddar cheese whey with annatto (15 mL of annatto/454 kg of milk, annatto with 3% wt/vol norbixin content) was manufactured using a standard Cheddar cheesemaking procedure. Various levels of $H_2O_2$ (5–100 mg/kg) were added to fluid whey to determine the optimum concentration of $H_2O_2$ for LP activity, which was measured using an established colorimetric method. In subsequent experiments, fat-separated whey was bleached for 1 h with 250 mg of $H_2O_2$/kg (traditional) or 20 mg of $H_2O_2$/kg (LP system). The WPC80 was manufactured from whey bleached with 250 mg of $H_2O_2$/kg or 20 mg of $H_2O_2$/kg. All samples were subjected to color analysis (Hunter color values and norbixin extraction) and proximate analysis (fat, protein, and moisture). Sensory and instrumental volatile analyses were conducted on WPC80. Optimal LP bleaching in fluid whey occurred with the addition of 20 mg of $H_2O_2$/kg. Bleaching of fluid whey at either 35 or 50°C for 1 h with LP resulted in >99% norbixin destruction compared with 32 or 47% destruction from bleaching with 250 mg of $H_2O_2$/kg, at 35 or 50°C for 1 h, respectively. Higher aroma intensity and increased lipid oxidation compounds were documented in WPC80 from bleached whey compared with WPC80 from unbleached whey. Monitoring of LP activity throughout cheese and whey manufacture showed that LP activity sharply decreased after 30 min of bleaching (17.01 ± 1.4 to <1 U/mL), suggesting that sufficient bleaching takes place in a very short amount of time. Lactoperoxidase averaged 13.01 ± 0.7 U/mL in unpasteurized, fat-separated liquid whey and 138.6 ± 11.9 U/mL in concentrated retentate (11% solids). Lactoperoxidase may be a viable alternative for chemical whey bleaching.
**Key words:** whey, flavor, bleaching, lactoperoxidase

## INTRODUCTION

Lactoperoxidase (**LP**) is an oxidoreductase enzyme belonging to the peroxidase family and is found in a wide range of mammalian milks, including humans (Seifu et al., 2005). This enzyme is heat stable and is inactivated after 15 s at 78°C (de Wit and van Hooijdonk, 1996). Historically, the LP system has been used to inhibit microbial growth in bovine milk. The LP system consists of 3 components: LP, thiocyanate ($SCN^-$), and hydrogen peroxide ($H_2O_2$). The system is not active unless all 3 components are present in sufficient amounts (Seifu et al., 2005). The major intermediate oxidation product of the LP-catalyzed oxidation of $SCN^-$ is the hypothiocyanate ion ($OSCN^-$), which is bactericidal (Seifu et al., 2005). Hydrogen peroxide is sometimes added to activate the system if no $H_2O_2$ is naturally present. Catalase-negative organisms (such as lactic acid bacteria) can generate $H_2O_2$ under aerobic conditions and, thus, can also activate the LP system. Many lactobacilli, lactococci, and streptococci produce sufficient $H_2O_2$ under aerobic conditions to activate the LP system (Seifu et al., 2005). Exogenous $H_2O_2$ must be added to activate the system (Reiter and Harnulv, 1982) if it is not supplied by catalase-negative organisms. Gram-negative, catalase-positive organisms (such as *Pseudomonas* spp., coliforms, salmonellae, and shigellae) are not only inhibited by the LP system, but may be killed, provided that $H_2O_2$ is supplied exogenously (Seifu et al., 2005). Gram-positive, catalase-negative bacteria (such as streptococci and lactococci) are generally inhibited but not killed by the LP system

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(Seifu et al., 2005). If raw milk is stored at ≤15°C, the LP system can effectively preserve raw milk for 24 to 26 h (Reiter and Harnulv, 1982).

Measurement of LP can be done using a variety of methods and, as such, LP activity values vary widely in the literature. In 1994, a method was established to quantify LP activity (Pruitt and Kamau, 1994). This assay uses 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (**ABTS**) as a chromophore and the measurement is carried out at 412 nm. This method has been widely accepted (Pruitt and Kamau, 1994). Bovine milk contains 1.2 to 19.4 U of LP/mL (Seifu et al., 2005), although levels in liquid whey are reported to be 30 mg/L or about 0.5% (wt/wt) of whey proteins (de Wit and van Hooijdonk, 1996). Levels of LP can vary depending on the lactation cycle of the cow, season, feeding regimen, and breed (Kussendrager and van Hooijdonk, 2000). Like LP, thiocyanate can vary widely due to feeding regimen (Seifu et al., 2005). The third component of the LP system, $H_2O_2$, 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 become the limiting factor.

In addition to inhibiting microbial growth, $OSCN^-$ (produced when LP reacts with $SCN^-$), also has bleaching capabilities. The strong oxidizing capacity allows $OSCN^-$ to react with carotenoids, leading to destruction of conjugation and subsequent color loss of norbixin. Very few papers have investigated the use of the LP system for decolorization of whey, and no scholarly journal articles to our knowledge have been published. Bottomley et al. (1989) published a patent describing the decolorizing of whey and products derived from whey using the LP system. Though some process details were provided, quantitative norbixin destruction was not addressed, LP activity was not monitored throughout processing, and the flavor of the finished product was not addressed. Subsequently, all of these items were a focus of the current study. The United States is a major consumer of yellow Cheddar cheese, in which the natural colorant, annatto, comprising the carotenoids bixin and norbixin, is added. Some of the color remains in the liquid whey following curd separation and it is necessary to bleach the whey to achieve a desired lack of color in dried whey ingredients (Kang et al., 2010). The objectives of this study were to quantify the amount of $H_2O_2$ necessary for optimal bleaching using the LP system in fluid whey, to monitor LP activity throughout cheesemaking and whey processing, and to compare the flavor of whey protein concentrate 80% (**WPC80**) bleached by the LP system to that bleached by high levels of $H_2O_2$ (traditional chemical bleaching). The overall goal of this study was to determine if whey bleaching via the LP system would be a viable alternative to chemical $H_2O_2$ bleaching for the dairy industry.

## MATERIALS AND METHODS

### Experimental Design Overview

Two experimental components were involved: liquid whey trials and the production of WPC80. Optimum $H_2O_2$ levels to activate the LP system were first determined. Liquid whey trials were then conducted as a 2 by 3 factorial design with temperature (35 or 50°C) and bleach treatment [control, 20 mg of $H_2O_2$/kg (LP), or 250 mg of $H_2O_2$/kg (**HP**)]. The samples with the highest bleaching efficacy were then selected for WPC80 manufacture. All samples within each trial were made from the same lot of milk. Lactoperoxidase and $SCN^-$ were monitored throughout the entire process of cheese and whey manufacture. All experiments were conducted in triplicate.

### Production of Liquid Whey

Cheddar whey was manufactured from vat pasteurized whole bovine milk (195 kg) as described by Campbell et al. (2011). Double strength annatto color (3% norbixin wt/vol; Danisco USA Inc., New Century, KS) was added at 15 mL/454 kg of milk and diluted 20 times in deionized water before addition to pasteurized milk. The whey was drained from the curds at pH 6.35 and a sieve was used to remove any remaining particles. The whey was immediately processed with a hot bowl cream separator (model SI600E; Agri-Lac Tecnologia Lactea, Miami, FL) to decrease the fat content.

### Activation of the LP System

The optimum level of $H_2O_2$ to activate the LP system was determined by adding 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 mg of $H_2O_2$/kg to unpasteurized, fat-separated liquid Cheddar whey. Bleaching was then carried out as described below. The concentration of $H_2O_2$ that resulted in the most bleaching [20 mg/kg, according to percent destruction via the degree of yellowness or blueness (**b***) reflectance values] was selected for further trials.

### LP and HP Bleaching

Small aliquots (50 mL) of liquid whey were placed in amber glass jars to prevent light degradation. The