J. Dairy Sci. 98:2982–2991
http://dx.doi.org/10.3168/jds.2014-9159

Decolorization of Cheddar cheese whey by activated carbon

Yue Zhang,* Rachel Campbell,† MaryAnne Drake,† and Qixin Zhong*[1]
*Department of Food Science and Technology, University of Tennessee, Knoxville 37996
†Southeast Dairy Foods Research Center, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh 27695

ABSTRACT

Colored Cheddar whey is a source for whey protein recovery and is decolorized conventionally by bleaching, which affects whey protein quality. Two activated carbons were studied in the present work as physical means of removing annatto (norbixin) in Cheddar cheese whey. The color and residual norbixin content of Cheddar whey were reduced by a higher level of activated carbon at a higher temperature between 25 and 55°C and a longer time. Activated carbon applied at 40 g/L for 2 h at 30°C was more effective than bleaching by 500 mg/L of hydrogen peroxide at 68°C. The lowered temperature in activated-carbon treatments had less effect on protein structure as investigated for fluorescence spectroscopy and volatile compounds, particularly oxidation products, based on gas chromatography-mass spectrometry. Activated carbon was also reusable, removing more than 50% norbixin even after 10 times of regeneration, which showed great potential for decolorizing cheese whey.
Key words: activated carbon, norbixin (annatto) removal, Cheddar cheese whey, bleaching, volatile analysis

INTRODUCTION

Cheese whey is a by-product of cheese manufacturing and has been recently used as a major source of producing nutritional and functional whey protein ingredients (Tejayadi and Cheryan, 1995; Sinha et al., 2007). The high demand of whey protein ingredients warrants the use of both colored and uncolored cheese whey. To produce ingredients with light colors from colored Cheddar cheese whey, bleaching agents such as hydrogen peroxide (H_2O_2) and benzoyl peroxide are used to oxidize the colorant annatto (norbixin) in liquid whey (Croissant et al., 2009). However, decolorization by the oxidation process may affect the flavor and functionality of whey protein ingredients (Croissant et al., 2009; Jervis et al., 2012). Enzymatic bleaching has also been studied, but the cost and, similar to chemical bleaching, oxidation products are possible constraints in the practical application (Kang et al., 2010; Campbell et al., 2012; Campbell and Drake, 2013).

Physical methods such as effective adsorbents may be used to remove norbixin without the oxidation reactions. Granular activated carbon has been recognized as "the best available technology for removing synthetic organic compounds" from drinking-water sources in the 1986 amendments to the United States Safe Drinking Water Act (Magnuson and Speth, 2005). Activated carbon has been widely used in the food, beverage, pharmaceutical, and chemical industries to purify water (Hamdaoui and Naffrechoux, 2007). The feasibility of decolorizing Japanese soy sauce by activated carbon has been recently demonstrated (Miyagi et al., 2013). In our previous study, the adsorption of annatto by activated carbon was evaluated in buffers at various solution conditions, and the results showed the potential of activated carbon in decolorizing cheese whey (Zhang et al., 2013).

In the present study, the first objective was to evaluate the effectiveness of 2 activated-carbon products on decolorizing colored Cheddar cheese whey, with comparison to bleaching by H_2O_2. The effectiveness was evaluated for the reduction of both color and residual norbixin content in Cheddar whey. The second objective was to characterize effects of treatments on protein structure and composition using SDS-PAGE, fluorescence spectroscopy, and the proximate compositions and volatile profiles of the lyophilized whey powders. The last objective was to characterize the possibility of regenerating activated carbon for repeated use.

MATERIALS AND METHODS

Materials

Activated carbon CA (SGL 8 × 30) and CB (CAL 12 × 40) were from Calgon Carbon Corp. (Pittsburgh, PA). Characteristics of these 2 adsorbents have been presented elsewhere (Zhang et al., 2013). The carbon agents were washed several times with distilled water

Received November 25, 2014.
Accepted January 7, 2015.
[1]Corresponding author: qzhong@utk.edu

and dried in an oven at 150°C for 24 h before use. Cheddar cheese whey was provided by a local dairy farm in Sweetwater, Tennessee. Unless stated otherwise, other chemicals were purchased from either Sigma-Aldrich Corp. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

Decolorization of Cheddar Cheese Whey by Adsorbents

The adsorbent was applied in 20 mL of cheese whey in amber vials. After incubation in a C76 shaking water bath (New Brunswick Scientific, Edison, NJ) operating at 25, 30, 40, or 55°C and 130 rpm for up to 240 min, the mixture was filtered through a shark-skin filter paper into vials that were wrapped with aluminum foil to minimize degradation and stored at 4°C before the next measurement. The treatments without adsorbent were similarly conducted and used as controls for comparison.

Bleaching Cheddar Cheese Whey

Bleaching-agent H_2O_2 was used at 500 mg/L, which was the maximum level in a previous study (Listiyani et al., 2012). The cheese whey was preheated to 68°C, mixed with H_2O_2, and incubated for different durations at 68°C. After cooling to room temperature (21°C), 20 mg/L of catalase was added to the whey to remove any residual H_2O_2 (Listiyani et al., 2012).

Measurement of Norbixin in Liquid Samples

A previously developed method (Croissant et al., 2009; Li et al., 2012) was used to extract and quantify norbixin. In a 50-mL centrifuge tube with 6 mL of liquid whey, 3 mL of ethanol, 3 mL of chloroform, and 1 mL of 1% acetic acid were added sequentially and vortexed for 30 s in each step. The sample was centrifuged at 4,564 × *g* for 20 min at 4°C. The bottom (chloroform) layer was collected, and the volume was measured before solid-phase extraction. To perform solid-phase extraction, 1 mL of the chloroform extract was transferred onto the Strata-NH_2 solid-phase extraction column (model 500 mg/3 mL, Phenomenex Inc., Torrance, CA) previously conditioned with 7 mL of *n*-hexane. The column was washed sequentially with 5 mL of a binary solvent mixture with equal volumes of *n*-hexane and diethyl ether and 1 mL of acetone to remove the fat and β-carotene. The elution of norbixin was enabled with 3 mL of a 7:3 (vol/vol) methanol:glacial acetic acid mixture. The final norbixin extract (eluent) was measured immediately for absorbance at 460 nm using a UV–vis spectrophotometer (model 201, Thermo Scientific, Waltham, MA). A standard curve was created within the concentration range from 62.5 μg/kg to 1 mg/kg norbixin. The norbixin standard was a WS28 annatto product containing 2.8% norbixin (DD Williamson LLC, Port Washington, WI) and was diluted in a methanol-glacial acetic acid (7:3, vol/vol) mixture to obtain the absolute norbixin concentrations in the standard curve. The measured norbixin concentration was used to calculate the norbixin mass extracted from cheese whey by multiplying the volume of eluent. All treatments were analyzed in duplicate.

SDS-PAGE

Reducing SDS-PAGE was performed to investigate the effects of adsorbent treatment on protein compositions. Whey samples were diluted 5 times in an SDS-PAGE sample buffer (GenScript Corp., Piscataway, NJ) and then heated at 95°C for 5 min. Ten microliters of the sample was loaded onto a precast 4 to 20% gradient polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA) for electrophoresis at 150 V. After fixing, the gel was stained with Coomassie brilliant blue G-250. The destained wet gel was scanned, and the composition of the bands was compared by ImageJ software (National Institutes of Health, Bethesda, MD) using the gel-analysis function. Apparent molecular weights were determined by using a kaleidoscope prestained standard (Bio-Rad Laboratories).

Fluorescence Spectroscopy

The fluorescence spectra were recorded using an RF-1501 spectrofluorometer (Shimadzu Corp., Tokyo, Japan). The excitation wavelength was 285 nm. Both the excitation and emission slit widths were set at 10 nm. The emission spectra were recorded between 300 and 450 nm, with the background fluorescence calibrated using distilled water.

Cheese Whey Powder Production

After treatment by 40 g/L adsorbents at room temperature (21°C) for 2 h or 500 mg/L H_2O_2 at 68°C for 2 h, cheese whey samples were filtered through a 0.45-μm Durapore membrane filter (Millipore, Billerica, MA). Following centrifugation at 4,564 × *g* for 10 min at 4°C to remove the fat and residual carbon agents, the middle transparent portion was collected and freeze-dried (model 12 EL freeze drier, VirTis Company Inc., Gardiner, NY). The control sample was prepared by filtration and centrifugation of cheese whey directly, followed by freeze-drying.