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Short communication: Norbixin and bixin partitioning in Cheddar cheese and whey

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ABSTRACT

The Cheddar cheese colorant annatto is present in whey and must be removed by bleaching. Chemical bleaching negatively affects the flavor of dried whey ingredients, which has established a need for a better understanding of the primary colorant in annatto, norbixin, along with cheese color alternatives. The objective of this study was to determine norbixin partitioning in cheese and whey from full-fat and fat-free Cheddar cheese and to determine the viability of bixin, the nonpolar form of norbixin, as an alternative Cheddar cheese colorant. Full-fat and fat-free Cheddar cheeses and wheys were manufactured from colored pasteurized milk. Three norbixin (4% wt/vol) levels (7.5, 15, and 30 mL of annatto/454 kg of milk) were used for full-fat Cheddar cheese manufacture, and 1 norbixin level was evaluated in fat-free Cheddar cheese (15 mL of annatto/454 kg of milk). For bixin incorporation, pasteurized whole milk was cooled to 55°C, and then 60 mL of bixin/454 kg of milk (3.8% wt/vol bixin) was added and the milk homogenized (single stage, 8 MPa). Milk with no colorant and milk with norbixin at 15 mL/454 kg of milk were processed analogously as controls. No difference was found between the norbixin partition levels of full-fat and fat-free cheese and whey (cheese mean: 79%, whey: 11.2%). In contrast to norbixin recovery (9.3% in whey, 80% in cheese), 1.3% of added bixin to cheese milk was recovered in the homogenized, unseparated cheese whey, concurrent with higher recoveries of bixin in cheese (94.5%). These results indicate that fat content has no effect on norbixin binding or entrapment in Cheddar cheese and that bixin may be a viable alternative colorant to norbixin in the dairy industry.

Key words: annatto, norbixin, bixin, whey, cheese

Short Communication

In the United States, a large majority of dried whey protein is manufactured from Cheddar cheese whey colored with annatto, a coloring agent extracted from the seed of the *Bixa orellana* shrub (Scotter, 2009). The major carotenoids responsible for the yellow color of annatto are norbixin and bixin, although the water-soluble form, norbixin, is the primary carotenoid used in cheese manufacture (Giuliano et al., 2003; Kang et al., 2010), where it is added directly to cheese milk.

Norbixin itself has no direct effect on the flavor of dried whey protein concentrate (**WPC**; Campbell et al., 2010), but it must be removed from fluid whey to produce a desirable white spray-dried product. Currently, benzoyl peroxide and hydrogen peroxide are the only 2 chemical bleaching agents approved by the US Food and Drug Administration (FDA) for bleaching of whey (US FDA, 2011a,b). The use of chemical bleaching agents has many detrimental effects, including off-flavor development, chemical residues, and changes in whey protein functional properties (Listiyani et al., 2011; Jervis et al., 2012).

Off-flavor development in WPC due to lipid oxidation is of special concern when dealing with oxidative chemical bleaching agents. Aldehydes are primarily responsible for off-flavors in dried whey proteins (Carunchia Whetstine et al., 2003; Wright et al., 2009; Whitson et al., 2010). Common off-flavors caused by lipid oxidation products in WPC are cardboard, cabbage, and fatty-oxidized flavors (Whitson et al., 2010; Listiyani et al., 2011). Investigating a method to reduce or eliminate unnecessary and harsh processing steps such as bleaching is important to add overall value to whey ingredients. Many studies have been conducted on the effects of whey processing on whey protein flavor and bleaching efficacy (Croissant et al., 2009; Campbell et al., 2010; Listiyani et al., 2011, 2012; Jervis et al., 2012) but, to our knowledge, a study of norbixin partitioning and partitioning differences between cheeses and wheys with differing fat levels has not been performed. Specific knowledge on norbixin partitioning will enable development of optimal bleaching conditions or alterna-

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tive bleaching approaches. Bixin is the nonpolar form of annatto and is a dicarboxylic monomethyl ester apocarotenoid (Bouvier et al., 2003), and can be found in *cis* (α) and *trans* (β) forms due to geometrical isomerism around the 5,6-carbon atoms (Lancaster and Lawrence, 1995). Because of its nonpolar nature, bixin has been used to color high-fat dairy products such as butter (Lancaster and Lawrence, 1995). To our knowledge, no studies have evaluated bixin as a natural colorant for Cheddar cheese.

Relatively little is known of the partitioning and binding characteristics of norbixin during the cheese making process. Previous studies have postulated that approximately 20% of the norbixin added during the cheese making process partitioned into the whey, but little research has been performed in the past 30 yr that supports this hypothesis (Barnicoat, 1950; Chapman et al., 1980). Although it is often assumed that norbixin is contained within the serum phase of the whey, it is also thought that it may be bound to the retinol-binding site of β-LG or with other whey proteins or whey components (Govindarajan and Morris, 1973; Hammond et al., 1975; Cho et al., 1994; Zhu and Damodaran, 2012). Recently, it has been hypothesized that norbixin exists as a micelle and associates with the milk fat globule membrane portion of whey (Zhu and Damodaran, 2012). The objectives of this study were to investigate norbixin partitioning into cheese and liquid whey using modern methods and instrumentation to better understand norbixin behavior during the cheese making process and to investigate the viability of bixin as an alternative cheese coloring agent.

Experimental Overview

Two sets of experiments were performed in this study. The first was an experiment with both full-fat and fat-free cheeses using norbixin as a colorant. The purpose of this experiment was to determine partitioning of norbixin between cheese and whey when differing amounts of annatto were added to cheese milk and to determine if differences existed between norbixin content in cheese and whey from fat-free and full-fat cheeses. Three sets of Cheddar cheese were produced using whole pasteurized milk with 3 levels of added annatto (7.5, 15, and 30 mL/454 kg of milk). Fat-free Cheddar cheese was manufactured from pasteurized, fat-separated skim milk with 15 mL of annatto/454 kg of milk added (Scotter et al., 2002; Nair et al., 2004; Campbell et al., 2010). Samples taken for norbixin extraction and measurement included milk after addition of annatto, Cheddar cheese, and unseparated, unpasteurized whey. Four trials of all 4 cheeses were conducted.

The second experiment was conducted to establish the incorporation of bixin into cheese milk and then to compare partitioning of bixin and norbixin in cheese and whey. This was a proof-of-concept study to determine if bixin would have a different partition between whey and cheese compared with norbixin. Again, 3 sets of Cheddar cheese (no color, norbixin at 15 mL/454 kg of milk, bixin at 60 mL/454 kg of milk) were produced in quadruplicate. Because of the nonpolar nature of bixin, homogenization of the cheesemilk containing bixin was necessary before cheese manufacture. Samples taken for bixin extraction matched those taken for experiment 1.

Manufacture of Cheddar Cheese and Liquid Whey with Norbixin

Raw whole milk, 98 kg, was obtained from the North Carolina State University Dairy Research and Education Farm (Raleigh). Milk was vat pasteurized (model MPD1050, Micro Process Design, D&F Equipment Co., McLeansville, NC) at 63°C for 30 min. After pasteurization, milk was immediately cooled to 31°C and inoculated with freeze-dried lactic acid starter culture [50 direct culture units (DCU)/454 kg, Choozit MA 11, Danisco, New Century, NJ] and Cheddar cheese manufacture proceeded as described by Campbell et al. (2012). Whey was drained from curds at pH 6.40. The fat-free cheese make procedure was identical except that after pasteurization, hot milk was separated in a hot bowl separator (model JF 125 EAR, Clair, Althofen, Austria) to produce skim milk and cream. The separated skim milk was then immediately cooled to 31°C and inoculated with freeze-dried lactic acid starter culture. Remaining cheese make steps were identical to those performed with the full-fat cheese make.

For all cheeses, once a pH of 6.40 was reached, whey was drained and strained to remove cheese particles, and 1 L was taken for analysis. The cheddaring methods were modified from Nair et al. (2004). During cheddaring, trenched curd loaves were turned every 20 min to a final pH of 5.2 before milling. Curd was milled manually with knives and salted (2.7% salt wt/wt, based on curd weight). Salted milled curds were pressed for 16 h at 2.8 kg/cm^2 (40 psi), vacuum sealed, and stored at 4°C.

Manufacture of Cheddar Cheese and Liquid Whey with Bixin

For experiment 2, to compare partitioning of bixin and norbixin in cheese and whey, the same starting concentrations of bixin and norbixin in homogenized cheese milk were required. The norbixin cheese milk