J. Dairy Sci. 90:90–98
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Characteristics of Reduced Fat Milks As Influenced By the Incorporation of Folic Acid

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ABSTRACT

Folic acid plays an important role in the prevention of neural tube defects (e.g., spina bifida and anencephaly), heart defects, facial clefts, urinary abnormalities, and limb deficiencies. Milk and milk products serve as a potential source for folic acid fortification because of the presence of folate-binding proteins that seem to be involved in folate bioavailability. Although milk is not a good source of folic acid, fortification could help in the prevention of the above-mentioned defects. The objective of this study was to examine the physicochemical characteristics of reduced fat milks fortified with folic acid. Reduced fat milks were prepared using 25, 50, 75, and 100% of the recommended dietary allowance of 400 μg of folic acid. Treatments included addition of folic acid at these levels before and after pasteurization. Color, pH, fat, protein, viscosity, folic acid concentration, folate-binding protein concentration, folate-binding protein profile, standard plate count, and coliform counts were determined on d 1, 7, 14, and 21. A consumer acceptability test was conducted on d 7. Data from the consumer panel were analyzed using ANOVA (PROC GLM) with means separation to determine the differences among treatments. Data obtained from the color, pH, fat, protein, viscosity, folic acid concentration, folate-binding protein concentration, standard plate count, and coliform counts were analyzed using the GLM with a repeated measure in time. Significant differences were determined at $P < 0.05$ using Tukey's Studentized Range Test. There were no differences in the electrophoretic mobility of folate-binding protein in the samples. The concentration of folic acid was significantly higher in reduced fat milks fortified with folic acid after pasteurization compared with the treatments in which folic acid was added before pasteurization. The consumer panelists did not find any significant differences in flavor, appearance, or texture of folic acid fortified reduced fat milks compared with that of the control. Fortification of reduced fat milks with folic acid can be accomplished without adversely affecting the product characteristics.
Key words: folic acid, reduced fat milk, pasteurization

INTRODUCTION

Consumer demand exists for healthier products, which is evidenced by the growth of the functional food industry from $31.6 billion in 1999 (Anonymous, 2000) to $55 billion in 2001 (Nutrition Business Journal, 2002). Competition in the dairy industry is driving the fluid milk sector to improve the nutritional value and decrease the fat content of dairy products to enable them to compete with innovative products. As the production and consumption of low fat but nutritionally rich products increases, industry focuses on the importance of improving these products. Consumers expect fluid milk products to be healthy and nutritious. Low fat and nonfat dairy products are targeted toward people who are trying to lose weight and those with cardiovascular problems. There has been a tremendous increase in the sales of reduced and low fat milk from 15,918 million pounds in 1980 to 23,559 million pounds in 2003 (Milk Facts, 2004). Of the total fluid milk product sales in 2003, reduced and low fat milk accounted for 43.5% compared with 32.9% for whole milk and 14.41% for nonfat milk (Milk Facts, 2004).

Vitamins are essential for human health. Folic acid is one of the important vitamins that can cause severe negative health effects when deficient. Folic acid deficiency is responsible for neural tube defects such as anencephaly and spina bifida in humans (Czeizel, 1995). Folic acid is important in the nervous system at all ages, but in elderly people, deficiency leads to aging brain processes, increases the risk of Alzheimer's disease and vascular dementia and, if critically severe, can lead to reversible dementia (Reynolds, 2002).

Folic acid is a water-soluble vitamin and is known by several different names including folate, folacin, folacid, folbal, pteroylglutamic acid (PGA), pterol-L-glutamic acid, pterol-L-monoglutamic acid, and vitamin B_9 (American Chemical Society, 2002). Folic acid is found in broccoli, spinach, romaine lettuce, dried beans, and liver (Wardlaw, 1999). Milk and milk products are not

Received June 16, 2006.
Accepted August 14, 2006.
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a good source of folic acid; cow's milk contains 5 to 7 μg of folic acid (Renner, 1983; Scott, 1989).

The US Centers for Disease Control (CDC) recommends that women of childbearing age consume 400 μg of folic acid daily to prevent neural tube defects (CDC, 1993). The current recommended daily allowance (**RDA**) for children 1 to 3 yr of age is 150 μg/d; for children 4 to 13 yr of age is 300 μg/d; and for ages 14 to >70 yr is 400 μg/d (Food and Nutrition Board, 2002).

Folate-binding protein (**FBP**) exists as a minor whey protein in milk (Salter et al., 1981) that is crucial to the assimilation, distribution, and retention of folic acid (Davis and Nichol, 1988). Almost all naturally occurring folate in milk is bound to FBP (Ghitis et al., 1969). The FBP in bovine milk exists at concentrations of about 10 mg/L (Salter et al., 1972), and binds approximately 1 mole of folate per mole of protein at pH 7.2 (Salter et al., 1981). The FBP from cow's milk has a molecular weight of 35,000 ± 1,500 daltons with a high proportion of half-cystine (18 residues/molecule) and 10.3% of carbohydrate (fucose, mannose, and galactose; Salter et al., 1981). Folate-binding protein is temperature stable; FBP isolated from whey powder obtained from milk vacuum evaporated at 68°C and spray dried at 180°C was able to bind 0.5 mol of folate/mol (Salter et al., 1981). Pasteurization of milk (at 72°C for 15 s) is thought to result in heat-induced alteration of the FBP molecule (Gregory, 1982).

Folate-binding proteins in milk are interesting because they seem to be involved in bioavailability of folate (Verwei et al., 2003). Folate-binding proteins may protect folate from bacterial uptake and degradation (Ford, 1974). Adding folic acid to reduced fat milk may have additional health benefits. It is common practice to add vitamins A and D during fluid milk processing. The effects of direct addition of a water-soluble vitamin, folic acid, to reduced fat milk on the physicochemical and sensory characteristics are not known.

The objectives of this study were 1) to determine the effect of different concentrations of folic acid on the physicochemical and consumer acceptability of reduced fat milks over a 3-wk storage period; and 2) to elucidate the effect of the stage of addition of folic acid on the physicochemical and sensory characteristics of reduced fat milks over a 3-wk storage period.

MATERIALS AND METHODS

Experimental Design

Reduced fat milks were fortified with folic acid at 25, 50, 75, or 100% of the RDA of 400 μg of folic acid per 236-mL serving. Folic acid was added before or after HTST pasteurization. Color (L*, a*, b*), pH, fat, protein, viscosity, folic acid concentration, FBP concentration, FBP profile, SPC, and coliform counts of fortified milks were determined on d 1, 7, 14, and 21. A consumer acceptability test was conducted on d 7. The experiment was conducted and analyzed as a randomized complete block with repeated measures. The replications were the blocks and 3 replications were conducted.

Preparation of Reduced Fat Milks

Raw whole milk (approximately 360 L) was obtained from the Louisiana State University (**LSU**) dairy farm and held at 4°C until use (<1 h). Cream and skim milk at 4°C were separated using a DeLaval 392 Airtight Cream separator (Alfa Laval Inc., Richmond, VA). Reduced fat milk (2% total fat) was prepared by adding an appropriate amount of cream to skim milk. Before pasteurization, pharmaceutical grade folic acid (Ampak Co., New York, NY) at 25, 50, 75, or 100% of the RDA of 400 μg was added separately to 4 equal volumes of the reduced fat milk. Milks were then homogenized using a 2-stage homogenizer (APV Americas, Lake Mills, WI) at 12.4 and 3.4 MPa on the first and second stages, respectively, followed by pasteurization for 16 s at 72.5°C (163°F). Folic acid at 25, 50, 75, or 100% of the RDA of 400 μg was also added separately to 4 equal volumes of unfortified reduced fat milks after homogenization and pasteurization. Control was homogenized and pasteurized reduced fat milk.

Analytical Procedures

Folic Acid Concentration. Folic acid concentration was determined by using HPLC with methods modified from Albala-Hurtado et al. (1997). The HPLC system consisted of a Waters 501 pump, Waters 717 Plus autosampler, and Waters 486 tunable UV detector set at 282 nm (Waters Corp., Milford, MA). Peak areas were calculated using the Waters Millennium software. The separation was carried out isocratically using a Waters Spherisorb 5 μm ODS2 4.6- × 250-mm column with guard cartridge. Samples were prepared as follows: 10.5 g of the sample was weighed into a 50-mL centrifuge tube with a screw-on cap, 1 g of crystalline TCA was added, and the mixture was shaken for 10 min on a mechanical shaker. The mixture was centrifuged at 1,250 × g for 10 min. The supernatant was decanted to a 10-mL volumetric flask and 3 mL of 4% (wt/vol) TCA was added to the solid phase. The mixture was shaken for 10 min and centrifuged again at 1,250 × g for 10 min. The supernatant was then added to the 10-mL volumetric flask, and the flask wrapped in aluminum foil to protect it from light. Samples were filtered through a 45-μm filter (Sigma Aldrich, St. Louis, MO) and placed in clear glass HPLC vials protected from

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