

Effect of Folic Acid Fortification on the Characteristics of Strawberry Yogurt

C.A. Boeneke and K.J. Aryana

Department of Dairy Science, Louisiana Agricultural Experiment Station,
Agricultural Center, Louisiana State University,
111 Dairy Science Bldg, Baton Rouge, LA 70803, USA

Abstract: Development of dairy products with new flavours and health benefits helps the dairy industry increase sales of products as well as provide consumers with products they enjoy. Folic acid is used in the prevention of neural tube defects, heart defects, facial clefts, urinary tract abnormalities and limb deficiencies. The objective of this study, was to determine the effect of different concentrations and stage of addition of folic acid on the physico-chemical and sensory characteristics of strawberry yogurt over a storage period. Strawberry yogurts were manufactured with 0, 25, 50, 75 and 100% of the RDA of 400 µg folic acid per 226 mL cup. Folic acid was added before and after pasteurization of yogurt mix. Moisture, ash, fat and protein concentrations were measured at week 1 only. Folic acid concentration was measured at weeks 1 and 5. Viscosity, pH, TA, syneresis, colour and sensory analysis were measured at weeks 1, 3 and 5. No differences in electrophoretic migration patterns were found over the 5 week storage period. Storage time did not affect product viscosity.

Key words: Folic acid, yogurt, dairy products, strawberry yogurt, protein

INTRODUCTION

Development of dairy products with new flavours and products with health benefits has the potential to increase sales. Dairy products in the market place are targeted to different consumer groups. For example, fat free dairy products are targeted to consumers with cardiovascular problems. Lactose free products are targeted to people with lactose intolerance. Consumers want dairy products that taste good and have increased health benefits. Yogurt is a low calorie dairy product which is consumed as a snack and dessert. There has been a steady increase in per capita sales of yogurt from 1717 million pounds in 1999-2387 million pounds in 2003 (Milk Facts, 2004).

Sources of folic acid include liver, avocado, black beans, spring greens, asparagus and soybean nuts. Dairy products are not a good source of folic acid. Cow's milk contains 5-7 µL⁻¹ 100 g of folic acid (Renner, 1983; Scott, 1989). The major form of folate in milk is 5-methyl-tetrahydrofolate (Gregory, 1996; Forsseen, 2000). Folic acid itself is not reduced and is the most stable form.

Studies have shown that folic acid when taken during initial stages of pregnancy can prevent neural tube defects such as spina bifida and anencephaly, heart

defects, facial clefts, urinary tract abnormalities and limb deficiencies (Hall and Solohdin, 1998).

Folic acid has also been shown to reduce the risk of colorectal and breast cancers (Langenohl *et al.*, 2001). Folic acid can also act as a cofactor for the conversion of homocysteine to methionine (Maxwell, 2000). Epidemiological studies have shown increased concentrations of homocysteine are associated with vascular disease (Maxwell, 2000).

Folic acid fortification of breads and cereals is commonly practiced. Direct addition of vitamins A and D during fluid milk processing is a common practice. Previous research conducted at the Louisiana State University Agricultural Center has shown folic acid can be added successfully in plain yogurt up to 100% of an RDA of 400 µg. The objective of this study, was to determine the effect of different concentrations and stage of addition of folic acid on the physico-chemical and sensory characteristics of strawberry yogurt over a storage period.

MATERIALS AND METHODS

Experimental design: Fat free strawberry flavoured yogurts were manufactured with 0, 25, 50, 75 and 100% of the RDA of 400 µg folic acid per 224 mL cup. Folic acid was added before or after pasteurization of the yogurt mix.

Moisture, ash, fat and protein concentrations were measured at week 1. Folic acid concentration was measured at weeks 1 and 5. Viscosity, pH, syneresis, colour and sensory properties were measured at weeks 1, 3 and 5. The experiment was conducted and analyzed as a randomized complete block with repeated measures. The replications were the blocks. The experiment was replicated three times.

Yogurt manufacture: Fat free strawberry yogurt was manufactured at the Louisiana State University Department of Dairy Science. The dry ingredients (Table 1) were incorporated in the milk in 18.9L stainless steel containers. Mixes were pre heated to 60°C and homogenized at 12.4 and 3.4 MPa on the first and second stages using a Gaulin 300 DJF 4 2PS homogenizer (APV Gaulin, Wilmington, MA). Mixes were then batch pasteurized at 85°C for 30 min. Mixes were cooled to 40°C before addition of a culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Chr. Hansens, Milwaukee, WI) at a rate of 1.3g per 3.78L of mix. Inoculated mixes were poured in 226 mL plastic cups and incubated at 40°C until pH reached 4.5. Samples were cooled immediately and stored at 4°C. Folic acid powder (Lakeshore Tech., Norton Shores, MI) was added with dry ingredients for the pre pasteurization runs and prior to culture addition for post pasteurization runs. Strawberry flavourings were obtained from Target Flavors, Brookfield, CT and added at a manufacture's suggested usage rate of 9.0 and 14.0 mL⁻¹gal respectively. Strawberry puree was obtained from Sensient Flavors, Fenton, MO and was added at a manufacture's suggested usage rate of 20% wt vol⁻¹. Flavourings were added prior to incubation.

Analytical procedures: Protein concentration was determined one week after yogurt was manufactured. Samples were prepared by drying 15 g of sample for 48 h at 100°C in a convection oven (Fisher Scientific, Houston, TX). Dry sample was ground using a mortar and pestle, 0.2 g was loaded into tin cups, folded and loaded into a Leco FP428 (Leco Corp., St Joseph, MI) nitrogen analyzer. Sample was incinerated and results expressed as percent nitrogen. Results were multiplied by a protein correction

factor of 6.38. Fat, moisture and ash contents were also determined one week after production according to Richardson (1985).

Folic acid concentration was determined by using High Performance Liquid Chromatography (HPLC) as modified from Albala'-Hurtado *et al.* (1997). The HPLC system was comprised of a Waters (Waters Corp., Milford, MA) 501 pump, Waters 717 Plus auto-sampler and Waters 486 tunable UV detector set at 282 nm. Peak areas were calculated using the Waters Millennium® software. The separation was conducted isocratically using a Waters Spherisorb 5 µm ODS2 4.6×250 mm column with guard cartridge. Run time for samples was 20 minutes using a flow rate of 1 mL per minute and an injection volume of 10 µL per sample. A standard curve was prepared by dissolving known amounts (1.67, 3.35, 5.03 and 6.70 mg) of folic acid in 3.78 L of HPLC grade double distilled water i.e. the same amount of folic acid that was used in 3.78 L of yogurt mix and analyzed using HPLC-UV. Peak areas results from HPLC analysis were correlated to the standard curve to determine folic acid concentrations.

Viscosity was measured at 21°C using a Brookfield DVII+ viscometer with helipath stand and T-C spindle at 30 rpm. Data points (50 per sample) were collected and averaged using Wingather® software (Brookfield Engineering Lab, Stoughton, MA).

The pH was measured using an Orion model 250 A / 610 pH meter (Fisher Scientific Instruments, Pittsburgh, PA calibrated using pH buffers 7.00 and 4.00).

Syneresis was determined by emptying 295g of yogurt into a cheesecloth-lined funnel placed on top of a graduated cylinder. The mililiter of drained serum was measured at the end of a 2 h period at 21°C.

Colour was determined by L*a*b* values obtained using a handheld Minolta CM 508 d colourimeter (Minolta Labs, Japan). An average of 5 readings per sample was taken.

Sensory scoring was conducted by a highly trained three member panel each with over 12 years experience judging fermented products. The official American Dairy Science Association intercollegiate dairy products evaluation scorecard was used. The scorecard consists of a 1-10 point scale for flavour, 10 indicating no defect; 1-5 point scale for appearance/colour, 5 indicating no defect and 1-5 point scale for body/texture, 5 indicating no defect.

Statistical analysis: Data from strawberry yogurts were analyzed in the following manner: HPLC, viscosity, pH, TA, syneresis, colour and sensory analysis were analyzed by the Statistical Analysis System using the General Linear Model procedure with a repeated measure in time. Data from moisture, ash and protein concentration were

Table 1: Formulae for fat free plain set yogurt

Compositon	Folic acid percentages based an RDA of 400 µg per 226 mL yogurt				
	0%	25%	50%	75%	100%
Skin milk (L)	3.78	3.78	3.78	3.78	3.78
NFDM (g)	114.00	114.00	114.00	114.00	114.00
Starch (g)	23.00	23.00	23.00	23.00	23.00
Aspartame (g)	1.14	1.14	1.14	1.14	1.14
Folic acid (mg)	0.00	1.67	3.35	5.03	6.70
Starter culture (g)	1.30	1.30	1.30	1.30	1.30
Strawberry flavour (mL)	14.00	14.00	14.00	14.00	14.00
Strawberry puree (mL)	73.00	73.00	73.00	73.00	73.00

analyzed using the General Linear Model with Tukey's Studentized Range Test. Significant differences were determined at $p < 0.05$.

RESULTS AND DISCUSSION

Protein contents on dry matter basis ranged from 17.64-24.41%. Fat content for all samples were $< 0.5\%$. Moisture content ranged 82.04-85.58%. Ash content ranged from 0.6647-0.7139%. No significant ($p < 0.05$) differences were found for protein, fat, moisture or ash. Folic acid did not alter the composition of the yogurts.

Folic acid peak area concentrations for strawberry flavoured yogurts are reported in Fig. 1. Mean folate values in strawberry yogurts before and after pasteurization were 2.82 and 2.46 $\text{mg}^{-1} \text{mL}$ for weeks 1 and 5, respectively. Mean folate values in plain yogurt before and after pasteurization were 1.39 and 1.35 $\text{mg}^{-1} \text{mL}$ for weeks 1 and 5, respectively. In a study done by Stralsjo (2003) folate content in strawberries was found to be between 70-90 $\mu\text{g}^{-1} 100 \text{ g}$ based on fresh weight. The study found that almost no folate losses occurred when strawberries were cooked for use in jams or stewed as strawberry desserts from frozen berries and the strawberries had a folate retention of 79-103% with the predominant form of folate in the form of 5 methyltetrahydrofolate (Stralsjo, 2003). This would explain the higher folic acid values in the strawberry yogurts. Pasteurization did not effect folic acid concentrations overall for strawberry yogurt as it did in the plain yogurt. Folic acid peak area values were higher for plain yogurts when folic acid was added to the yogurt mix and then pasteurized. No significant ($p < 0.05$) pasteurization effect was observed in the strawberry flavoured yogurts. Ristow *et al.* (1982) found folic acid to be stable at processing temperatures of 120°C for 20 min. However, Wigertz *et al.* (1996) examined folate and 5 methyl tetrahydrofolate found naturally in milk and

yogurt. They reported significantly reduced levels of both 5 methyl tetrahydrofolate and total folate in pasteurized milk, UHT treated milk and yogurt made from a mix pasteurized at 90°C for 10 min. This discrepancy can possibly be explained by the structure of folic acid. Folic acid has a fully oxidized ring system and has greater stability than other forms. Perhaps this stability protects the folic acid from heat treatments. Its heat stability would allow it to be stable at temperatures that would help it to dissolve within the product and make it more uniform. Plain yogurt was not pre-stirred prior to taking sample for HPLC analysis as was the strawberry yogurt. Plain yogurt folic acid values were higher when added prior to pasteurization vs. addition after pasteurization. Because of this, we can deduce that heating helps to disperse the folic acid in the finished product. Culture addition probably did not affect folic acid concentrations. Rao and Shahani (1987) found skim milk inoculated with *Lactobacillus bulgaricus* lowered folate levels after 36 h incubation. Milk fermented by *Streptococcus thermophilus* and *Lactobacillus acidophilus* increased folate levels significantly. The *Streptococcus thermophilus* and *Lactobacillus bulgaricus* culture used in this experiment probably had little effect on total folate content in the finished product.

Effects of overall level of folic acid addition, pasteurization and storage time on viscosity was found non significant ($p > 0.05$) for strawberry yogurts. Plain yogurt mean viscosity values decreased significantly ($p < 0.05$) as the concentration of folic acid incorporated before pasteurization increased, indicating an effect of folic acid on viscosity. Strawberry yogurt was pre-stirred prior to viscosity testing to equally disperse strawberry puree. This probably diluted any effects of folic acid on viscosity values.

Mean pH values for the yogurts are reported in Fig. 2. There were significant ($p < 0.05$) differences in yogurts containing folic acid added before vs. after

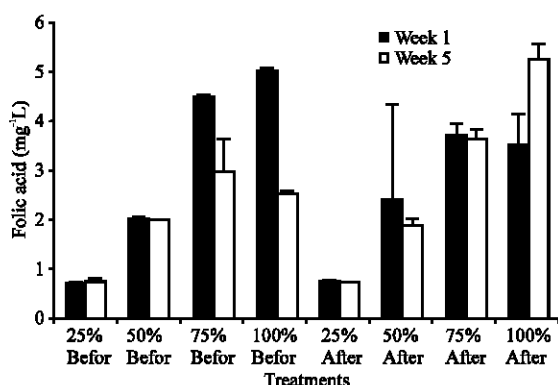


Fig. 1: Mean (+SE) peak areas ($\text{mg}^{-1} \text{mL}$) of folic acid in Fat free strawberry set yogurt at 25, 50, 75 and 100% RDA before and after pasteurization

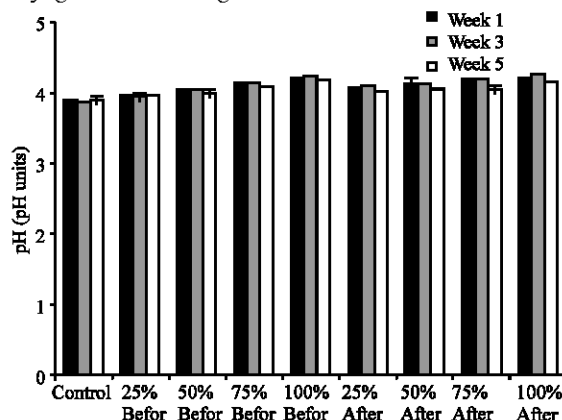


Fig. 2: Mean (+SE) pH of folic acid in fat free strawberry set yogurt at 25, 50, 75 and 100% RDA before and after pasteurization

pasteurization. There were also significant ($p<0.05$) differences in pH values of the strawberry yogurts containing different concentrations of folic acid. No significant ($p<0.05$) differences were found in level of folic acid addition in plain yogurts. The mean pH values for the plain yogurt ranged from 4-4.5. The discrepancy may be due to the strawberries lowering the pH and causing the pasteurization and level of folic acid addition interactions to be significant. Also, mean values were lower for strawberry yogurts containing folic acid added prior to pasteurization. The heat treatment may also have helped folic acid to disperse more in the product and lower the pH.

The mean values for syneresis ranged from 52.33-101.67. There were no significant ($p<0.05$) differences in syneresis with folic acid added before versus after pasteurization. There were also no significant ($p<0.05$) differences in syneresis with different concentrations of folic acid. No significant differences in mean values were found overall for weeks one, three and five.

Folic acid addition before or after pasteurization did not significantly ($p<0.05$) impact the L^* (lightness) values of yogurt. There were no overall significant ($p<0.05$) differences in lightness of yogurts attributable to concentration of folic acid.

Pasteurization did not have any significant ($p<0.05$) effect on a^* (redness) values. There was no significant ($p<0.05$) difference overall in redness due to level of folic acid addition among yogurts. There were, however, significant ($p<0.05$) differences in redness overall in weeks 1, 3 and 5. Values increased by 155% at week 3 and decreased 68% at week 5 indicating that the extent of redness was much more at week 3 compared to week 5.

Level of folic acid addition and time did not have a significant ($p<0.05$) effect on b^* (yellowness) values. Pasteurization did have a significant ($p<0.05$) effect. Mean values were 60% higher in yogurts where folic acid was added prior to pasteurization. The heat treatment helped the folic acid to dissolve in the sample and/or the pH of the yogurt may also have caused changes in the yellow pigment of the folic acid thus affecting a^* and b^* values.

Mean flavour scores are reported in Fig 3. No significant ($p<0.05$) differences in flavour scores were found with folic acid addition before vs. after pasteurization; however, level of folic acid addition did have a significant ($p<0.05$) effect on flavour scores. As levels of folic acid increased mean flavour scores decreased. Mean flavour score was 6.7 compared with 6.11 for plain. Mean flavour scores were higher for strawberry yogurts than for plain yogurt.

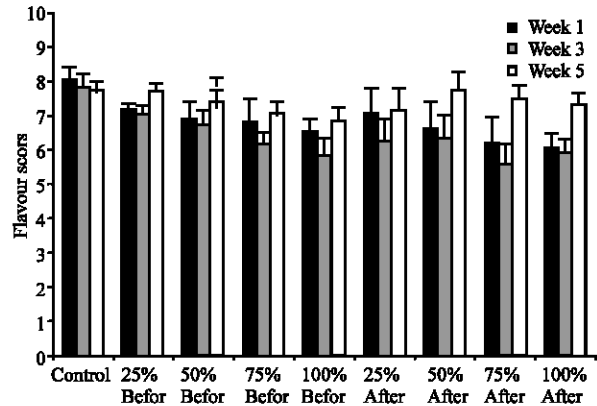


Fig. 3: Mean (+SE) flavour scores of folic acid in Fat free strawberry set yogurt at 25, 50, 75 and 100% RDA before and after pasteurization

Level of folic acid addition did not significantly ($p<0.05$) impact body and texture scores. Pasteurization and overall time were found significant ($p<0.05$) for strawberry yogurts. Mean values were higher where folic acid was added prior to pasteurization. Mean values appeared to decrease over the storage period. Body and texture scores are from 1-5 with 5 being no criticism. A mean value of 5 indicates no defect in body and texture of yogurts. Most values were approximately 4 indicating little defect in body and texture of yogurts. Mean values for plain were similar.

Level of folic acid addition, pasteurization and overall time was found non-significant for appearance and colour. Folic acid addition did not impact appearance and colour of strawberry yogurts. Mean value of strawberry yogurt was 3.36 compared with 3.29 for plain yogurt.

CONCLUSION

Folic acid addition had no effect on protein, fat, moisture, or ash content of strawberry yogurts. Level of folic acid did not impact flavour scores. Body and texture values of strawberry yogurts appeared to decrease over the five week storage period. Strawberry flavoured, folic acid fortified yogurts had high flavour and colour scores which is of use to the dairy industry. Folic acid fortification of flavoured yogurts would give industry another product in which to fill consumer demand for products that taste good and have healthful benefits.

ACKNOWLEDGEMENT

We would like to acknowledge the LSU Agricultural Center for funding and support of this project.

REFERENCES

- Albala'-Hurtado, S., M.T. Vecina-Nogue's, M. Izquierdo-Pulido and A. Marine'-Fone, 1997. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography. *J. Chromatog. A.*, 778: 247-253.
- Forseen, K.M., M.I. Jagerstad, K. Wigertz and C.M. Witthoft, 2000. Folate and dairy products: A critical update. *J. Am. College Nut.*, 19: 100-110.
- Gregory, J.F., 1996. In *Food Chemistry*, (3rd Edn.), (Ed. F. Owen) Marcel Dekker Inc., New York, pp: 590-599.
- Hall, J. and F. Solehdin, 1998. Folic acid for the prevention of congenital anomalies. *Eur. J. Pediat.*, 157: 445-450.
- Langenohl, R., I. Fohr and K. Pietrzik, 2001. Beneficial role for folate in the prevention of colorectal and breast cancer. *Eur. J. Nut.*, 40: 98-105.
- Maxwell, S.R., 2000. Coronary artery disease-free radical damage, antioxidant protection and the role of homocysteine. *J. Basic Res. Cardiol.*, 95: I/65-I/71.
- Milk Facts, 2004. International Dairy Foods Association. Washington, D.C., pp: 33-35.
- Rao, D.R. and K.M. Shahani, 1987. Vitamin content of cultured milk products. *Cultured Dairy Prod. J.*, pp: 6-10.
- Renner, E., 1983. *Milk and Dairy Products in Human Nutrition*. VV Gmbh Volkswirtschaftlicher Verlag, Federal Republic of Germany.
- Richardson, G.H., 1985. *Standard Methods for the Examination of Dairy Products*, (15th Edn.), American Public Health Association, Washington, D.C.
- Ristow, K.A., J.F. Gregory and B.L. Damron, 1982. Thermal processing effects on folacin bioavailability in liquid model systems, liver and cabbage. *J. Agric. Food Chem.*, 30: 801.
- Scott, K.J., 1989. *Micronutrients in Milk and Milk based Products*. Elsevier Applied Sci. London, pp: 71-123.
- Stralsjo Lena, 2003. Doctoral diss. Dept. of Food Science, SLU. Sweedish University of Agric. Sci. *Agraria*, 368: 1-58.
- Wigertz, K., I. Hansen, M. Hoier-Madisen, J. Holm and M. Jagerstad, 1996. Effect of milk processing on the concentration of Folate Binding Protein (FBP), the folate-binding capacity and the retention of 5-methyltetrahydrofolate. *Int. J. Food Sci. Nut.*, 47: 315-322.