Manufacture and Quality of Iron-Fortified Yogurt

SHARAREH HEKMAT[1] and DONALD J. McMAHON
Western Dairy Center,
Department of Nutrition and Food Sciences,
Utah State University, Logan 84322-8700

ABSTRACT

Yogurts (nonfat and low fat) were manufactured and fortified with 10, 20, and 40 mg of iron/kg of yogurt. Growth of starter culture bacteria and nonstarter culture bacteria as well as lipid oxidation of the yogurts were monitored over 30 d of storage at 4°C. Sensory characteristics of the yogurts were determined during that time by a trained panel of judges, and consumer panels were used to test acceptability of iron-fortified yogurt.

Counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* after 1 d of storage in iron-fortified skim yogurts were 7.0×10^8 cfu/ml, which were not significantly different from numbers in unfortified yogurts. Counts decreased to 2.5×10^8 and 1.9×10^8 cfu/ml for *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*, respectively, after 30 d of storage. Fortifying yogurt with iron did not support the growth of *Pseudomonas flurescens* or *Escherichia coli*.

No significant increases in chemical oxidation, as measured using the thiobarbituric acid assay, were detected as a consequence of iron fortification. Trained panelists scored all yogurts for oxidized, metallic, bitter, and other off-flavors in the range of "not perceptible" to "very slightly perceptible". Iron-fortified yogurts had slightly higher oxidized flavor scores than did the control yogurt. There was no increase in metallic, bitter, or other off-flavors. The consumer panel did not detect any significant differences in the appearance, mouthfeel, flavor, or overall quality between fortified and unfortified flavored yogurts. All yogurt samples were liked by the consumer panelists, suggesting that yogurt is a suitable vehicle for iron fortification.

(**Key words**: yogurt, iron, fortified, oxidation)

Abbreviation key: $\mathbf{Fe_{CN}}$ = casein-chelated iron, $\mathbf{Fe_{WP}}$ = whey protein-chelated iron, **TBA** = thiobarbituric assay.

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[1]Current address: Department of Home Economics, Brescia College at University of Western Ontario, 1255 Western Road, London, Ontario, Canada, N6G 1H2.

INTRODUCTION

Yogurt has gained widespread consumer acceptance in the US (25), primarily by women, children, and teenagers, who consume yogurt as a luncheon or snack food. These populations have high calcium requirements and are also frequently deficient in iron (7, 9). Yogurt is an excellent source of calcium and protein (30) but, as is typical of all dairy products, contains very little iron (4). Fortification of yogurt with iron would help meet this nutritional need. An advantage of using dairy foods as the vehicle for supplementing the diet with iron is that people who consume diets of low iron density typically consume more dairy products; those with diets high in iron consume the fewest dairy products. Furthermore, iron-fortified dairy foods have a relatively high iron bioavailability (32). However, before any such fortification is undertaken, the effects of iron addition on microbial physiology during manufacture and shelf-life of yogurt, oxidation of milk fat, and the effect of iron on the taste and acceptance of a fortified yogurt must be ascertained.

Lactic acid bacteria do not require iron for growth (24), and iron addition to yogurt may change the balance between lactic acid bacteria and other bacteria that do require iron for growth (17). Many nonlactic acid bacteria possess mechanisms for obtaining the iron needed to sustain growth (19, 32), and additional iron might enhance their growth. *Pseudomonas* spp., the predominant lipolytic psychrotrophs in milk (18), have high affinity systems for iron uptake that use siderophores to capture iron in iron-limited media (1, 14). These siderophores are low in molecular mass, have high affinity, and possess iron-chelating agents that bind iron and return it to the cell. Another widely distributed food spoilage bacteria is *Escherichia coli*, and its virulence is influenced by siderophores (1).

The sensory quality of iron-fortified dairy foods has been shown (27, 33, 35) to be affected by the type of iron source used, the amount of iron added, and the properties of the dairy product being fortified. Two major off-flavors have been associated with fortified dairy products: oxidized flavor resulting from catalysis of lipid oxidation by iron and metallic flavor con-

tributed by iron salts (17). Oxidation of fat occurred in milk that was fortified with ferrous sulfate and ferric or ferrous ammonium sulfate (15, 31). This oxidation was reduced by using a chelated form of iron for milk fortification (16). Similarly, fat oxidation in chocolate milk was not promoted by fortification with a ferripolyphosphate-whey protein complex (12). The fortified chocolate milk had good flavor properties, but skim milk with ferric chloride or ferrous gluconate had oxidized off-flavors. Such oxidation has been detected as high thiobarbituric acid (**TBA**) values in the fortified milk as well as by sensory evaluation. In contrast to fluid milk, ferric ammonium citrate did not promote oxidation in cottage cheese (27).

The objectives of this study were to fortify yogurt with chelated and unchelated forms of iron and to determine the effect of iron on bacterial and sensory qualities of the yogurt.

MATERIALS AND METHODS

Preparation of Protein-Chelated Iron

Ferric chloride (Sigma Chemical Company, St. Louis, MO) was used to prepare protein-chelated iron complexes. Casein-chelated iron ($\mathbf{Fe_{CN}}$) was prepared by adding 50 ml of 0.5 *M* $FeCl_3$ into 600 ml of skim milk and then precipitating the Fe_{CN} at pH 4.6 (33). Whey protein-chelated iron ($\mathbf{Fe_{WP}}$) was made by mixing 50 ml of 0.5 *M* $FeCl_3$ with 600 ml of acid (cottage cheese) whey and adjusting its pH to 3.5 to precipitate the complex (34). The precipitates were centrifuged at $8000 \times g$ for 5 min; washed once with 0.25% lactic acid solution and twice with double-distilled, deionized water; freeze-dried, ground into a very fine powder; and sieved. The iron content of Fe_{CN} was 56.0 mg of Fe/g, and Fe_{WP} was 137 mg of Fe/g as determined by the ferrozine method (8, 29). Prior to yogurt manufacture, aqueous solutions of protein-chelated iron sources were prepared for fortification of the yogurt mix.

Preparation of Cultures

Mother culture. Frozen cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* (Heart to Heart Foods, Inc., Richmond, UT) were inoculated (1.0%) into sterile MRS broth (5, 32) (Difco Laboratories, Detroit, MI) and Elliker broth (13) (Difco Laboratories), respectively. These cultures were then incubated anaerobically (BBL Gas Pak; Becton Dickinson Microbiology Systems, Cockeysville, MD) for 15 h at 41°C. Each culture was then mixed (1:10, vol/vol) at 22°C with a mixture (1:2, vol/vol) of sterile glycerol and autoclaved 11% TS reconstituted NDM and then stored at –70°C.

Starter culture. The day prior to yogurt manufacture, starter culture was made by addition of 1.0% of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* mother cultures into separate containers of autoclaved 11% TS reconstituted NDM for static incubation at 41°C for 15 h.

Nonstarter cultures. *Escherichia coli* DW (isolated from cottage cheese; provided by J. R. Broadbent, Utah State University) was inoculated (0.1%) into sterile *E. coli* broth (2) and incubated aerobically at 37°C for 15 h. *Pseudomonas fluorescens* ATCC 31732 was inoculated (0.1%) into sterile *Pseudomonas* F broth (2) and incubated aerobically at 37°C for 15 h. Both cultures were then centrifuged at $10,000 \times g$ for 10 min, and the cells were resuspended in sterile 0.85% saline to an absorbance (590 nm) of 0.3.

Yogurt Manufacture

Yogurt mix. Yogurt mixes were prepared by adding 6% sugar, 5.8% NDM, and 0.7% stabilizer to either nonfat or 2% milk (Gary H. Richardson Dairy Products Laboratory, Utah State University, Logan). Each mix was then divided into 10 lots: 9 experimental lots that were fortified with iron to either 10, 20, or 40 mg of Fe/kg with either $FeCl_3$, Fe_{CN}, or Fe_{WP} solutions and a control lot that was unfortified. For experiments on growth of nonstarter bacteria, the mixes were divided into an experimental batch, which was iron-fortified to 20 mg of Fe/kg with $FeCl_3$ solution, and an unfortified control batch. These mixes were then heated to 82°C for 30 min. The experimental and control batches of mix that were used during the trials with nonstarter bacteria were then further divided into 5 lots: 4 experimental lots for inoculation with nonstarter bacteria and 1 control lot.

Fermentation. Each lot of mix was cooled to 42°C and inoculated with 1.0% of *L. delbrueckii* ssp. *bulgaricus* starter culture and 1.0% *S. thermophilus* starter culture. Those mixes being used to test the potential growth of nonstarter bacteria were also inoculated with 10^3 or 10^5/g of *E. coli* or *P. fluorescens*. The mixes were then packaged in containers and incubated at 42°C until they reached pH 4.3; the containers were then transferred to a cold room (4°C) for storage.

Microbial Analysis

Viable numbers of each starter culture in yogurt were determined after 1, 15, and 30 d of storage.

Lactobacillus delbrueckii ssp. *bulgaricus* was counted on MRS (Difco Laboratories) spread plates in which the pH had been adjusted to pH 5.4. After incubation at 41°C for 48 h (at pH 5.4, no growth of *S. thermophilus* occurs), *L. delbrueckii* ssp. *bulgaricus* colonies were observed as small star-shaped, white colonies. *Streptococcus thermophilus* was counted on M17-lactose (Difco Laboratories) spread plates after incubation at 41°C for 48 h.

Viable numbers of the added nonstarter bacteria in yogurt were counted at 1, 7, and 14 d of storage. *Escherichia coli* was counted on violet red bile agar (BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD) pour plates after incubation at 37°C for 48 h (21, 26). *Pseudomonas fluorescens* was counted on Pseudomonas F agar (2) spread plates after incubation at 10°C for 10 d.

Chemical Analysis

Iron concentrations of yogurts were determined by the ferrozine method (8, 29). Oxidation products (e.g., malonaldehyde) were analyzed spectrophotometrically using a TBA test (7). One gram of yogurt was weighed into a glass screw-top test tube, and 9 ml of 15% (wt/vol) TCA, 0.375% (wt/vol) 4,6-dihydroxypyrimidine-2-thiol, and 0.25*N* HCl solution were added, mixed well, and heated in a boiling water bath for 15 min. Samples were then cooled to room temperature (20°C) and centrifuged at $7000 \times g$ for 15 min at 20°C; absorbance was measured at 535 nm. Samples were evaluated after 1, 15, and 30 d of storage at 4°C.

Sensory Analysis

The presence of oxidized, metallic, or other off-flavors in nonfat and low fat yogurts that had been fortified with 10, 20, and 40 mg of iron/kg of yogurt was evaluated by a trained panel. A consumer panel was used to evaluate the acceptability of a fruit-flavored yogurt that had been fortified with 20 mg of iron/kg of yogurt.

Trained panel. The trained panel consisted of 11 judges who were selected from a pool of 21 people from the Department of Nutrition and Food Sciences at Utah State University who had been familiarized with oxidized, metallic, and off-flavors in yogurt using yogurt that had been fortified with $FeSO_4$ to 40 and 100 mg of iron/kg of yogurt. The taste panel evaluated the yogurts after 1, 15, and 30 d storage at 4°C. Each judge was given four samples at a time and asked to evaluate each for presence of bitter, oxidized, metallic, and off-flavors on a nine-point rating scale (1 = not perceptible to 9 = extremely strong).

Consumer panel. Seventy-five volunteer lay panelists evaluated appearance, mouthfeel, flavor, and overall quality of strawberry-flavored yogurts on a nine-point hedonic scale (1 = dislike extremely to 9 = like extremely) (1). Panelists were served four samples at a time and asked to rinse their mouths between samples.

Experimental Design

Experiments for testing starter cultures, nonstarter cultures, and chemical oxidation were performed in duplicate using split-plot designs with repeated time measurements. Least significant differences ($P = 0.05$) were used to separate appropriate means of log colony counts and chemical oxidation measurements. Flavor testing by the trained taste panel was analyzed as a repeated-time split-plot design; judges were used as the replicates. An unfortified yogurt was used as a control sample each time the yogurts were evaluated by the panel. To account for variance among judges, the score given for the control sample by each judge was subtracted from that judge's score for the fortified cheeses. Analysis of variance was used to determine differences among iron-fortified yogurts, and least significant difference using a one-tailed t value was used to test differences between corrected sample means and zero (i.e., between iron-fortified yogurts and the nonfortified control yogurts). A completely randomized design was used to analyze the data from the consumer taste panel. Analysis of variance was conducted using Minitab 7.2 software (Minitab Inc., State College, PA). Significance refers to $P \leq 0.05$, and tendencies are $0.05 < P \leq 0.10$, unless otherwise stated.

RESULTS AND DISCUSSION

Both nonfat and low fat iron-fortified yogurts were successfully manufactured at the target iron concentrations (Table 1). A 6-oz (120-g) cup of yogurt fortified with 20 mg of iron/kg of yogurt would provide approximately 15% of the US recommended daily allowance of iron for women, which is sufficient to allow the yogurt to be labeled as an iron-fortified food. In the US, a claim can be made for iron content if the product contains at least 10% of the daily value for iron.

Starter Cultures

Iron fortification had no effect on the incubation time required for the yogurt mixes to reach pH 4.3. All batches reached pH 4.3 ± 0.1 after 5.0 h. Further acid production during storage was also similar; the

TABLE 1. Mean (± SEM) iron concentrations of nonfat and low fat yogurts fortified to target iron concentrations of 10, 20, and 40 mg of iron/kg of yogurt using three different sources of iron: $FeCl_3$, casein-chelated iron (Fe_{CN}), and whey protein-chelated iron (Fe_{WP}).

Yogurt and source	Iron concentration					
	10 mg/kg		20 mg/kg		40 mg/kg	
	(mg of iron/kg of yogurt)					
	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM
Nonfat						
$FeCl_3$	11.3	0.5	20.2	1.0	38.0	3.5
Fe_{CN}	10.8	1.0	20.6	0.8	39.1	1.8
Fe_{WP}	10.6	0.2	20.2	0.8	39.2	1.3
Low fat						
$FeCl_s$	10.3	0.8	18.7	1.4	35.6	2.6
Fe_{CN}	10.7	0.6	19.2	1.0	37.8	1.5
Fe_{WP}	10.9	0.4	21.3	1.9	38.4	1.8

pH values of control and fortified samples reached 4.20 ± 0.05 after 1 d and 4.00 ± 0.07 after 30 d.

At d 1, the mean counts of *L. delbrueckii* ssp. *bulgaricus* for control and iron-fortified yogurts were 6.1×10^8 cfu/ml and 6.9×10^8 cfu/ml, respectively. Similarly, *S. thermophilus* counts were 5.4×10^8 cfu/ml and 7.0×10^8 cfu/ml. By d 15, there was no significant change in either starter culture population. After 30 d of storage, *L. delbrueckii* ssp. *bulgaricus* counts were still $>1 \times 10^8$ cfu/ml. In low fat yogurt, *S. thermophilus* remained at $>3 \times 10^8$ cfu/ml at 30 d; in nonfat yogurt the *S. thermophilus* population decreased to 5×10^7 cfu/ml. In comparison, US commercial yogurts have been shown to contain 2.7×10^8 cfu/ml of *L. delbrueckii* ssp. *bulgaricus* and 6.5×10^8cfu/ml of *S. thermophilus* (20). Similar counts of 2.4×10^8 and 3.3×10^8 cfu/ml were observed in British yogurts (10). Starter culture growth was independent of the type of milk used (2% or skim milk) and whether or not the milk had been fortified with iron (Table 2). The treatments were partitioned into control yogurts versus fortified yogurts, but there was

TABLE 2. Analysis of variance for *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* counts in nonfat and low fat iron-fortified yogurts over 30 d of storage at 4°C.

Source of variation	df	*L. delbrueckii* ssp. *bulgaricus*		*S. thermophilus*	
		MS	*F*	MS	*F*
Replicate	1	0.0826	8.34	0.0024	0.00
Milk (M)	1	0.1029	10.39	2.7008	1.46
Error A	1	0.0099		1.8432	
Treatment (T)	9	0.0336	0.81	0.0094	0.27
Control vs. fortified	1	0.0813	1.97	0.0077	0.02
Among fortified	8	0.0276	0.67	0.0096	0.25
Iron source (I)	2	0.0081	0.20	0.0133	0.39
Level (L)	2	0.0129	0.31	0.0003	0.01
I × L	4	0.0449	1.09	0.0124	0.36
M × T	9	0.0646	1.56	0.0181	0.53
Error B	18	0.0413		0.0345	
Storage time (S)	2	3.1897	121.65**	4.9314	10.00†
Error C	2	0.0262		0.4933	
M × S	2	0.0108	0.38	1.9495	32.33***
T × S	18	0.0207	0.74	0.0134	0.22
M × T × S	18	0.0182	0.65	0.0106	0.18
Error D	38	0.0251		0.0603	
Total	119				

†$P < 0.10$.

**$P < 0.01$.

***$P < 0.001$.

still no significant effect on viable numbers of either starter culture. No significant effects were found when the fortified yogurts were partitioned by source of iron used for fortification ($FeCl_3$, Fe_{CN}, or Fe_{WP}) and level of iron fortification (10, 20, or 40 mg of Fe/kg).

The only significant sources of variation were storage time for *L. delbrueckii* ssp. *bulgaricus* and the interaction between milk type and storage time for *S. thermophilus*. The reduction of *L. delbruekii* ssp. *bulgaricus* was similar in both nonfat yogurt and low fat yogurt. In comparison, viability of *S. thermophilus* was less in nonfat yogurt (Figure 1A) than in low fat yogurt (Figure 1B), which explains the significant interaction between milk and storage time. As is illustrated by the figures, addition of iron did not influence the survival of starter cultures. Furthermore, the same pattern occurred in yogurts fortified with the protein-complexed forms of iron (Fe_{CN} or Fe_{WP}): no significant decrease in starter culture numbers during the first 15 d, followed by significant decrease from 15 d to 30 d. Thus, protein chelation of iron prior to its addition to the yogurt mix had no beneficial effect on the growth of the starter cultures. It is quicker and easier to add the iron as $FeCl_3$.

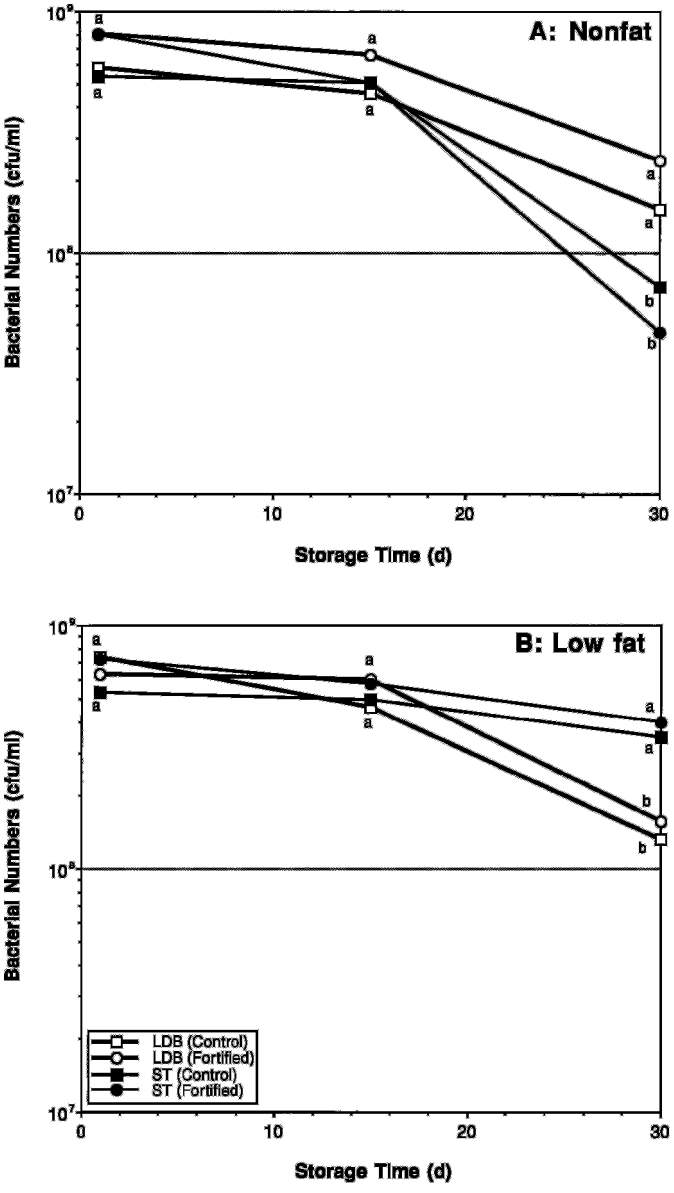

Figure 1. Mean survival of *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (LDB), during 30 d of storage at 4°C, in control yogurts and yogurts fortified using $FeCl_3$ to 20 mg of iron/kg of yogurt. Means within the same line with a common letter (a, b) were not different ($P > 0.05$).

Nonstarter Cultures

No growth of *P. fluorescens* or *E. coli* was found in any of the yogurts (control or iron-fortified) that were made from milk that had been inoculated with 10^3 or 10^5 cfu/ml of these bacteria. Also, addition of these nonstarter bacteria had no effect on the growth of the starter cultures. By d 1, *P. fluorescens* had decreased to <1 cfu/ml. Even though *P. fluorescens* has a highly efficient system of iron uptake that is mediated by siderophores (1, 14), the increased iron contents of the fortified yogurts did not promote survival of this spoilage microorganism. Presumably, the 5-h incubation at 42°C during yogurt manufacture, the produc-

TABLE 3. Analysis of variance for chemical oxidation in nonfat and low fat iron-fortified yogurts over 30 d of storage at 4°C.

Source of variation	df	MS	F
		(×100)	
Replicate	1	0.204	3.00
Milk (M)	1	5.246	77.15†
Error A	1	0.068	
Treatment (T)	9	0.070	1.43
Control vs. fortified	1	0.076	1.55
Among fortified	8	0.069	1.41
Iron source (I)	2	0.031	0.63
Level (L)	2	0.001	0.02
I × L	4	0.122	2.49†
M × T	9	0.039	0.76
Error B	18	0.049	
Storage time (S)	2	0.238	0.77
Error C	2	0.309	
M × S	2	0.536	21.44***
T × S	18	0.037	1.48
M × T × S	18	0.029	1.16
Error D	38	0.025	
Total	119		

†$P < 0.10$.

***$P < 0.001$.

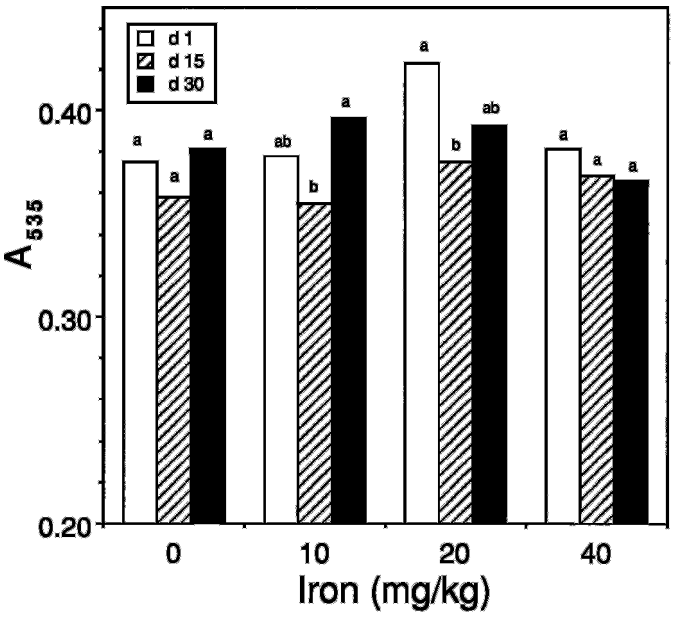

Figure 2. Chemical oxidation, measured as absorbance at 535 nm (A_{535}) using the thiobarbituric acid assay in nonfat yogurt that was fortified with 0, 10, 20, and 40 mg of iron/kg of yogurt using $FeCl_3$ over 30 d of storage at 4°C. Means within the same iron level with a common letter (a, b) were not different ($P > 0.05$). Differences between iron levels were not significant.

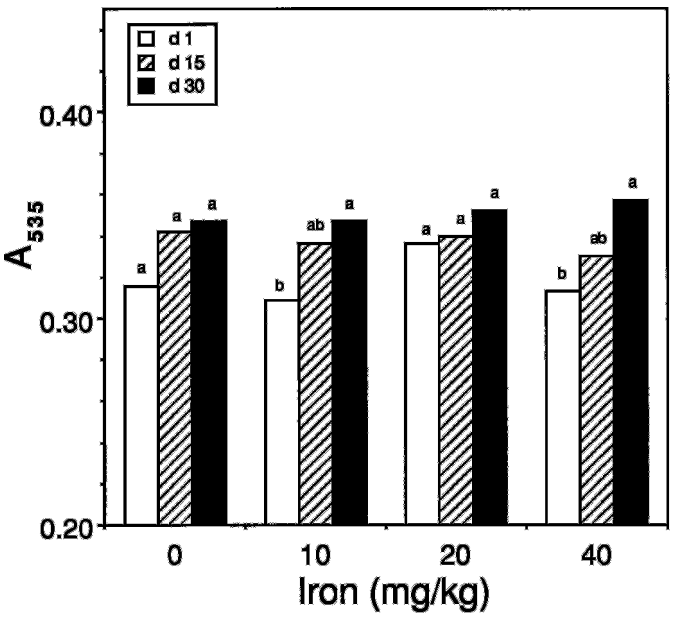

Figure 3. Chemical oxidation, measured as absorbance at 535 nm (A_{535}) using the thiobarbituric acid assay, in low fat yogurt that was fortified using $FeCl_3$ with 0, 10, 20, and 40 mg of iron/kg of yogurt over 30 d of storage at 4°C. Means within the same iron level with a common letter (a, b) were not different ($P > 0.05$). Differences between iron levels were not significant.

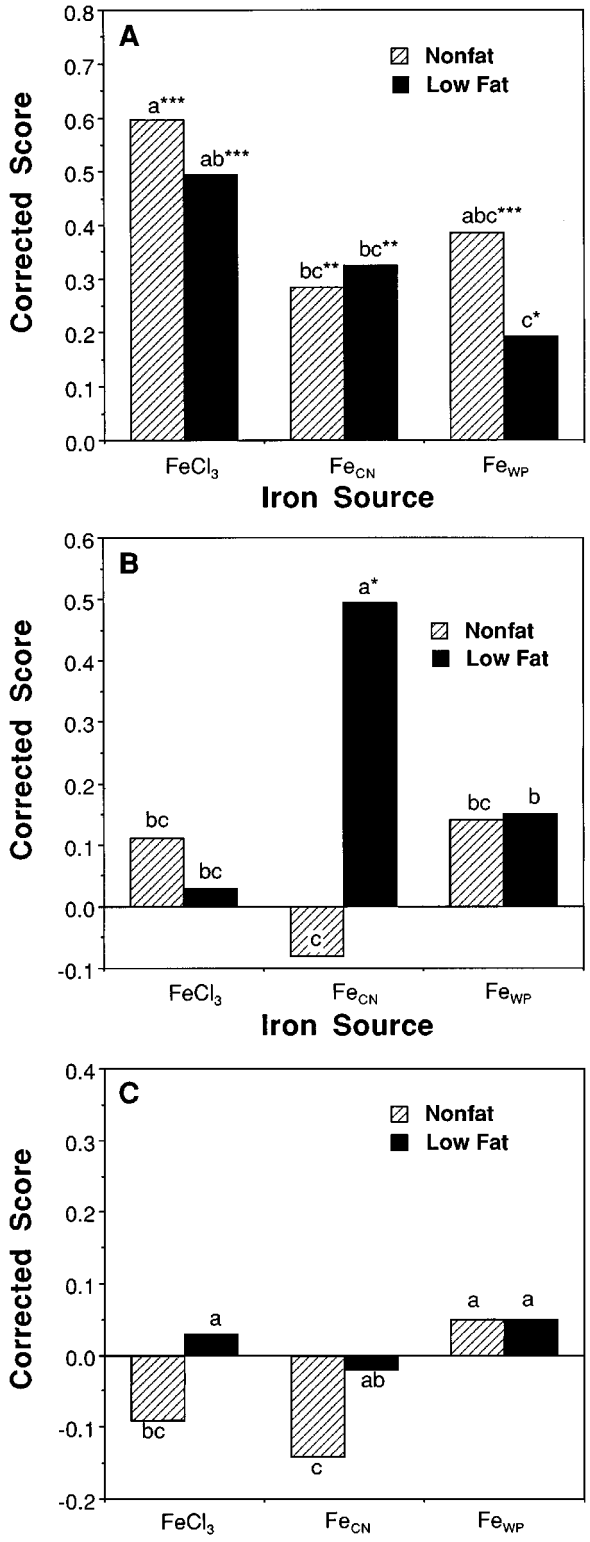

Figure 4. Mean corrected scores (fortified minus control) from the trained panel for oxidized flavor (A), metallic flavor (B), and bitter flavor (C) in nonfat and low fat yogurts fortified using $FeCl_3$, casein-chelated iron (Fe_{CN}), or whey protein-chelated iron (Fe_{WP}). Means with a common letter (a, b, c) were not different ($P > 0.05$). Difference of mean score of iron-fortified yogurt from control is also shown: $^*P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$.

tion of lactic acid by starter culture, and the low pH of the yogurt (pH 4.2) were sufficient to cause the destruction of *P. fluorescens* (28).

The addition of iron to yogurt, however, had a slight effect on survival of *E. coli* when that bacteria was inoculated into yogurt mixes at 10^3 and 10^5 cfu/ml. After 1 d, the fortified yogurts had 3.2×10^3 and 2.5×10^5 cfu/ml, which was significantly higher ($P < 0.01$) than the 1.4×10^3 and 1.8×10^5 cfu/ml of the control yogurts. However, *E. coli* did not survive further storage, and, by d 7, was <1 cfu/ml. Therefore, iron fortification of yogurt poses no additional threat from postpasteurization contamination of yogurt by *P. fluorescens* or *E. coli*.

Yogurt Quality

Chemical oxidation. The overall effect of nonfortification of yogurt on chemical oxidation (as measured by the TBA test) was nonsignificant (Table 3). There were also no significant differences based on the type of iron used ($FeCl_3$, Fe_{CN}, or Fe_{WP}) or the level of iron fortification. The low fat yogurts tended to have slightly lower TBA absorbance scores than did the nonfat yogurts, which was true even when no iron was added (0.318 versus 0.375). The higher fat percentage of the low fat yogurts was expected to produce more oxidation, indicating some unidentified effect of sample composition on the TBA assay.

The overall effect of storage was nonsignificant. There was, however, a significant interaction between milk and days of storage. For the nonfat yogurts, no increase in chemical oxidation occurred during 30 d of storage (Figure 2), but a slight, yet significant increase was observed for the iron-fortified low fat yogurts (Figure 3). This increase in oxidation was much less than had been expected based on the role of iron in promoting lipid peroxidation. The unfortified low fat yogurt also exhibited a tendency toward increased chemical oxidation during storage up to 30 d.

The yogurts that were fortified with protein-chelated iron performed the same as the yogurts fortified with $FeCl_3$ (data not shown), suggesting that the iron added as $FeCl_3$ became chelated to milk proteins after fortification. Lipid peroxidation requires that iron exchange between its oxidation states of Fe^{2+} and Fe^{3+} (6, 22, 23). Milk proteins may reduce the ability of iron to participate in iron-catalyzed hydroxyl radical formation (3, 11) by chelating the iron and inhibiting its involvement in such redox reactions. Furthermore, the high acidity of yogurt may also reduce the formation and oxidation potency of iron hydroxides.

TABLE 4. Analysis of variance of trained panelists' corrected scores (fortified minus control) for oxidized flavor and metallic flavor in nonfat and low fat iron-fortified yogurts fortified to 10, 20, and 40 mg of iron/kg of yogurt using either $FeCl_3$, casein-chelated iron, or whey protein-chelated iron over 30 d of storage at 4°C.

		Oxidized		Metallic	
Source of variation	df	MS	*F*	MS	*F*
Judge	10	15.894	13.06***	17.353	12.58***
Milk (M)	1	1.052	0.86	4.209	3.05†
Iron source (S)	2	4.136	3.40*	0.924	0.67
Iron level (L)	2	1.914	1.57	2.399	1.74
M × S	2	0.678	0.56	6.264	4.54*
M × L	2	0.668	0.55	0.012	0.01
S × L	4	0.982	0.81	0.725	0.53
M × S × L	4	1.711	1.41	1.302	0.94
Error A	170	1.217		1.379	
Day (D)	2	9.651	1.16	0.853	0.12
Error B	20	8.324		7.094	
M × D	2	3.325	2.30†	3.557	2.99†
S × D	4	3.871	2.67*	3.376	2.84*
L × D	4	1.149	0.79	0.063	0.05
M × S × D	4	1.792	1.24	0.514	0.43
M × L × D	4	1.039	0.72	0.171	0.14
S × L × D	8	1.323	0.91	1.071	0.90
M × S × L × D	8	1.537	1.06	1.067	0.90
Error C	340	1.449		1.189	
Total	593				

†$P < 0.10$.

*$P < 0.05$.

***$P < 0.001$.

Trained sensory testing. After 1 d of storage, the mean scores for bitter, oxidized, and metallic flavors in unfortified nonfat yogurt were 1.5, 1.6, and 2.1, respectively; for nonfat yogurt fortified with $FeCl_3$, the mean scores were 1.3, 2.2, and 2.0, respectively. These scores were all in the range of not detectable (score = 1) to very slightly perceptible (score = 3). There were no significant differences based on fat content (nonfat or 2% fat) or level of iron addition (10, 20, or 40 mg of iron/kg of yogurt) for any of the flavors tested by the trained panel (Table 4).

The source of iron used for fortification had a significant effect on oxidized flavor (Table 4) but not on metallic or bitter flavors. Yogurts fortified using protein chelated-iron (either Fe_{CN} or Fe_{WP}) had slightly lower oxidized flavor scores. There was a significant interaction between iron source and milk fat content for metallic flavor, although the differences between scores were minimal, and all were <3. The increase in oxidized, metallic, and bitter flavors in fortified yogurts compared with scores of nonfortified yogurts is shown in Figure 4. Based on the slight increase detected by the trained panel of oxidized flavor in the yogurts fortified with $FeCl_3$, it would be preferable to use a protein-chelated form of iron to manufacture an iron-fortified yogurt.

There was also a significant interaction between iron source and storage time and between milk type and storage time for bitter and metallic flavors (Table 4). These scores were also <3, and only minor differences in these scores were found during 30 d of storage. Because the trained judges were able to differentiate levels of oxidation between some samples indicates that the judges were more sensitive to production of oxidized flavorants in yogurt than was the TBA chemical assay. Perhaps compounds other than malonaldehyde were providing the oxidative flavors, in which case the TBA assay is not a suitable tool for monitoring low levels of oxidation in fermented dairy products.

Consumer sensory testing. The consumer panels did not detect significant differences in the appearance, mouthfeel, flavor, or overall quality among yogurts fortified with $FeCl_3$, Fe_{CN}, or Fe_{WP}. In contrast to the trained panelists, who were trained and selected for their sensitivity to iron-induced oxidation and were aware that the test yogurts contained iron, the consumer panelists were untrained and unaware of the sample treatments. All scores for fortified yogurt were comparable with those of the control unfortified yogurts. All yogurts were rated above average on the hedonic scale and were liked by the panelists, showing that the small increase in oxidized flavor that resulted from iron fortification had a negligible effect on how well the yogurts were liked. The mean appearance, mouthfeel, flavor, and overall scores for unfortified skim yogurt were 7.4, 7.0, 6.5, and 6.8, respectively, and for iron-fortified yogurts were 7.2, 7.1, 6.7, and 6.9, respectively.

CONCLUSIONS

Yogurt, fortified with iron to 10, 20, or 40 mg of Fe/kg, was manufactured without effects on starter culture growth or acid production. Iron addition at these concentrations did not promote the growth of either *P. fluorescens* or *E. coli*, even when these bacteria were added at 10^5 cfu/ml of yogurt mix. Fortification of yogurt with iron is technically feasible; only a very small increase in oxidized flavor was caused by iron fortification, and this increase was still in the range of "not perceptible" to "very slightly perceptible" as determined by trained panelists. When the yogurts were judged by untrained panelists, both unfortified and fortified yogurts were equally liked. Ferric chloride, Fe_{CN}, and Fe_{WP} are all potential iron sources for the fortification of yogurt although use of a protein-chelated iron may help to minimize production of any oxidized flavors.

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