Ingredient Supplementation Effects on Viability of Probiotic Bacteria in Yogurt

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ABSTRACT

The present investigation studied the effects of cysteine, whey powder, whey protein concentrate, acid casein hydrolysates, or tryptone on the viability of *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and bifidobacteria. Changes in pH, titratable acidity, redox potential, and viability of bacteria were monitored during 24 h of fermentation and refrigerated storage (4°C) of yogurt for 35 d. The incubation time that was needed to reach pH 4.5 was considerably affected by the added ingredients. Also, the drop in pH or the increase in acidity and redox potential was dependent on the added ingredients. The addition of cysteine, whey protein concentrate, acid casein hydrolysates, or tryptone improved the viability of bifidobacteria to a variable extent, but whey powder failed to improve their viability. The morphology of *S. thermophilus*, as shown by electron microscopy, was affected by cysteine at 500 mg/L, possibly as a result of reduced redox potential. Sodium dodecyl sulfate-PAGE and amino acid analyses suggested that the nitrogen source in the form of peptides and amino acids improved the viability of bifidobacteria in yogurt made with a commercial ABT (*Lactobacillus acidophilus*, bifidobacteria, and *Streptococcus thermophilus*) starter culture, which showed a dramatic decline in the counts of this organism in previous studies.

(**Key words**: viability, probiotic bacteria, redox potential, electron microscopy)

Abbreviation key: **ACH** = acid casein hydrolysate, **TA** = titratable acidity, **WP** = whey powder, **WPC** = whey protein concentrate.

INTRODUCTION

Fermented dairy products have been a major part of the diet of people around the world. Numerous scientific papers and review articles (8, 15, 20) have been published on the health benefits associated with the consumption of fermented dairy products. Over the past decade, considerable interest has developed in the use of probiotic organisms (*Lactobacillus acidophilus* and *Bifidobacterium* spp.) in food, pharmaceutical, and feed products. The consumption of probiotic products has increased dramatically in most European, Asia-Pacific, and American countries, and >90 products containing *L. acidophilus*, or bifidobacteria, or both are available in the market worldwide (9, 10). Some of the proposed health benefits are thought to be conferred by live bacteria contained in the products. Suggested minimum numbers of probiotic bacteria at consumption are 10^5 to 10^6 cfu/g (15, 25). However, recent market surveys have revealed that the viability of probiotic organisms in commercial preparations has often been low (1, 27).

Probiotic bacteria grow slowly in milk because of a lack of proteolytic activity (14), and the usual practice is to add yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) to reduce the fermentation time. *Lactobacillus delbrueckii* ssp. *bulgaricus* produces essential amino acids owing to its proteolytic nature, and the symbiotic relationship of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* is well established; the former organism produces amino nitrogen for the latter organism. However, *L. delbrueckii* ssp. *bulgaricus* also produces lactic acid during refrigerated storage, known as postacidification, which is claimed to affect the viability of probiotic bacteria. To overcome the problem of postacidification, the present trend is to use starter cultures that are devoid of *L. delbrueckii* ssp. *bulgaricus* such as ABT (*L. acidophilus*, bifidobacteria, and *S. thermophilus*). Such starter cultures may necessitate the incorporation of micronutrients (peptides and amino acids) through whey powder (**WP**), whey protein concentrate (**WPC**), acid casein hydrolysate (**ACH**), or tryptone for reducing the fermentation time and for improving the viability of probiotic bacteria. *Streptococcus thermophilus*, which is less pro-

Received November 24, 1997.
Accepted June 29, 1998.
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teolytic than *L. delbrueckii* ssp. *bulgaricus*, is the main organism responsible for fermentation in ABT cultures. The ACH is a product prepared by the acid hydrolysis of casein, and tryptone is a tryptic digest of casein; both of these substances are rich in the peptides and essential amino acids required by *S. thermophilus*. Both WP and WPC are the by-products of the cheese industry and are used to replace part of the skim milk powder in order to increase the solid contents of the yogurt mix.

Several factors affect the viability of probiotic bacteria (13, 14, 17, 18, 23). An increase in the acidity of the product during storage adversely affects the viability of probiotic bacteria. Hydrogen peroxide, produced by some lactobacilli, is known for its antimicrobial effects. Bifidobacteria are anaerobic in nature, and, therefore, higher oxygen content may affect their growth and viability. The composition of product, the presence of preservatives as a result of added fruits and nuts, and the availability of growth factors are also reported to affect the growth and viability of yogurt and probiotic bacteria. Antagonism among the bacteria used in the starter culture caused by the production of antimicrobial substances such as bacteriocins may decrease the numbers of sensitive organisms that may be present in a product or starter culture. In earlier studies, low viability of bifidobacteria was reported (4, 5, 6) in yogurt made with one of the four commercial starter cultures. In those studies, a dramatic decline in the numbers of bifidobacteria in the same starter culture was observed, and higher concentrations of inoculum (5) or use of ascorbic acid as an oxygen scavenger (6) did not improve the viability of bifidobacteria to a satisfactory level. No antagonism was observed between the yogurt bacteria and the probiotic bacteria used in this starter culture (11).

The objective of this study was to examine the effects of L-cysteine, WP, WPC, ACH, or tryptone on the growth and viability of yogurt and probiotic bacteria in yogurt made with a commercial starter. Changes in pH, titratable acidity (**TA**), redox potential, and viable counts of *S. thermophilus*, *L. acidophilus*, and bifidobacteria were monitored during 24 h of incubation and during storage of yogurts for 35 d at 4°C. Changes in morphology of *S. thermophilus* were observed by electron microscopy. The SDS-PAGE of filtrates of yogurt samples and amino acid analyses of the ingredients that improved the viability of bifidobacteria were also performed.

MATERIALS AND METHODS

Starter Cultures

A commercial ABT starter culture (Chr. Hansen Pty. Ltd., Bayswater, Australia) that produces polysaccharides during fermentation was used in this study. The organisms were characterized, and their identities were verified using Gram staining and biochemical tests such as a catalase test, oxidase test, growth at various temperatures, and sugar fermentation patterns. The starter culture was in freeze-dried direct-to-vat set form. After procurement, the starter cultures were stored at –18°C in the absence of atmospheric air.

Yogurt Preparation

A commercial homogenized and pasteurized milk (20 L) containing 3.8% fat and 13.4% total solids was tempered to 45°C and fortified with 2% (wt/vol) skim milk powder, WP, or WPC 312 or WPC 392 (New Zealand Dairy Board, Melbourne, Australia) which will be referred as WPC 1 and WPC 2, respectively. The mix was heated to 85°C for 30 min and then cooled to 40 to 43°C, and starter culture (0.1 g/L of yogurt mix) was added. The mix supplemented with SMP was divided into eight lots, and 5% L-cysteine·HCl (Sigma Chemical Co., St. Louis, MO) was added to four lots to achieve a final concentration of 0, 50, 250, or 500 mg of cysteine/L. The ACH (Sigma Chemical Co.) and tryptone (Oxoid, Hampshire, England) were added at 250 or 500 g/L to the remaining four lots of inoculated mix. The concentrations used in this study for each of the ingredients were based on the preliminary results, which compared a broader range of these ingredients. The mix was distributed in 100-ml plastic cups. Incubation was carried out at 37 ± 0.5°C, and fermentation was terminated at pH 4.5. The time taken to reach pH 4.5 was recorded for each sample. After fermentation, some yogurt samples were removed and stored at 4°C. The remaining samples were incubated for 24 h to study the changes in pH, redox potential, and viable counts of starter bacteria during prolonged fermentation of yogurt mix.

Time Interval Specifications

The 0-h time represents the observations taken immediately after the addition of starter cultures into the heat-treated and cooled (40 to 43°C) yogurt

mixes that were supplemented with various ingredients. The 0-d period represents analyses carried out after overnight cold storage of yogurt samples, and periods 10 to 35 d represent analyses of yogurt samples after 10, 25, and 35 d of storage, respectively.

Sample Preparation

The measurements for redox potential were taken from at least three 100-ml cups from each batch. After this analysis, the contents from the cups were uniformly mixed in a sterile glass beaker; a sample was taken aseptically for microbiological, pH, TA, total solids, protein, redox potential, and gel electrophoresis analyses.

Chemical Analyses

pH values of the yogurt and inoculated yogurt mix samples were measured at 17 to 20°C using a pH meter (model 410A; Orion, Boston, MA) after calibration with fresh pH 4.0 and 7.0 standard buffers. The TA was determined after mixing the yogurt sample with 10 ml of hot distilled water (~90°C) and titration with 0.1*N* NaOH using a 0.5% phenolphthalein indicator to an end point of faint pink color. Redox potential was measured with a platinum electrode [model P14805-SC-DPAS-K8S/325; Ingold (now Mettler Toledo), Urdorf, Switzerland] connected to a pH meter (model H 18418; Hanna Instruments, Padova, Italy). Protein content was analyzed by the Kjeldahl method using a Kjeltec digestion system and distillation unit (Tecato ag, Hoganas, Sweden). A multiplication factor of 6.37 was used to convert percentage nitrogen to percentage protein. Total solids were determined by drying duplicate samples at 110°C for 2 h.

Microbiological Analyses

A 1-g yogurt sample was diluted with 9 ml of 0.15% sterile peptone and water diluent (Oxoid). Subsequent serial dilutions were prepared, and viable numbers were enumerated using the pour plate technique. Counts of *S. thermophilus*, *L. acidophilus*, and bifidobacteria were enumerated on ST agar, MRS-salicin agar, and MRS agar (Oxoid) added with neomycin sulfate, nalidixic acid, lithium chloride, and paromomycin sulfate (all from Sigma Chemical Co.). The enumeration protocols were the same as those reported by Dave and Shah (3). The colony-forming units were converted to $\log_{10}$ and the results are reported.

Electron Microscopic Examination of Cells of *S. thermophilus*

Electron microscopic examination of *S. thermophilus* in the yogurt samples was performed. For the preparation of sample for electron microscopy, yogurt was mixed with an equal volume of peptone water (0.15%) containing 0.9% saline, and the slurry was passed through Whatman number 42 filter paper (Whatman Corp., Clifton, NJ) to remove the curd particles. The filtrate was cooled to 4°C and centrifuged (model Microspin 24; Sorvall® Instruments, Melbourne, Australia) at 12,043 × *g* for 2 min in Eppendorf microcentrifuge tubes. The cells that were collected as pellets were fixed in 2.5% gluteraldehyde in 0.1 *M* sodium cacodylate buffer (pH 7.4) for 2 h at room temperature (~20°C). After samples were washed four times in sodium cacodylate buffer, they were postfixed with osmium tetroxide in the same buffer for 2 h at room temperature (~20°C) using a vertical rotator. The samples were then washed twice in distilled water, dehydrated in a series of graded acetone solutions (30, 50, 70, 95, and 100%), and embedded in Araldite-Epon resin (Probing and Structure, Melbourne, Australia). The blocks were polymerized at 60°C for 48 h.

Semithin (1μ) and ultrathin (~80 nm) sections showing gold and silver interference colors were cut using an ultramicrotome (model OmU2; Reichert Microtome, Vienna, Austria). Semithin sections were mounted on glass slides and stained with a solution of 1% methylene blue and 1% sodium tetraborate. Ultrathin sections were collected on 200-mesh uncoated copper grids that were cleaned with acetone. These sections were stained with a 5% aqueous solution of uranyl acetate for 10 min and Reynold's lead citrate for 10 min. Sections so obtained were examined for cells of *S. thermophilus* with a transmission electron microscope (model 300; Philips, Einghoven, The Netherlands) at 60 kV and 55,000× magnification.

SDS-PAGE of Filtrate Collected from Various Yogurts

The SDS-PAGE analysis was carried out on a 15% separating gel containing acrylamide and bisacrylamide (26). Standards in the range of 14,400 to 97,400 Da (Bio-Rad Laboratories, Hercules, CA) were used for identification. Yogurt samples (~20 g) were mixed with an equal volume of phosphate buffer, and the content was filtered (number 42 filter paper; Whatman Corp.) to remove casein. The casein-free filtrate was filtered through a 0.45-μm membrane, and the filtrate was mixed with Laemmli buffer (16) contain-

TABLE 1. Protein and total solids contents of yogurt supplemented with various ingredients.

Composition	Control[1]		Cys_{50}		Cys_{250}		Cys_{500}		WP		WPC 1		WPC 2		ACH_{250}		Try_{250}	
	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE
Protein, %	3.85	0.08	3.85	0.06	3.87	0.05	3.90	0.09	3.75	0.10	4.55	0.12	4.50	0.13	3.85	0.06	3.87	0.05
Total solids, %	15.55	0.12	15.50	0.15	15.50	0.18	15.45	0.26	15.69	0.23	15.75	0.29	15.70	0.35	15.49	0.11	15.40	0.15

[1]Control = Yogurt with 2% skim milk powder; Cys_{50}, Cys_{250}, and Cys_{500} = yogurt containing 50, 250, and 500 mg of cysteine/L of yogurt mix, respectively; WP = yogurt containing 2% whey powder; WPC 1 and WPC 2 = yogurt supplemented with 2% whey protein concentrates 1 and 2, respectively; ACH_{250} = yogurt supplemented with 250 mg of acid casein hydrolysate/L of yogurt mix; and Try_{250} = yogurt supplemented with 250 mg of tryptone/L of yogurt mix.

ing SDS. This mixture was heated in a boiling water bath for ~2 min. Samples were loaded in the wells of SDS gels, and electrophoresis was carried out at 30 mA for 2.5 to 3.0 h until the bromophenol blue dye reached the bottom of the gel. The gels were fixed in 10% TCA and silver stained to study the concentration and molecular mass of peptides present in the yogurt filtrate. The relative quantification of various bands was carried out using an enhanced laser densitometer (Ultroscan XL; Amrad Pharmacia Biotech Ltd., Boronia, Australia).

Amino Acid Analyses

A complete amino acid profile of the two WPC samples, ACH, and tryptone was carried out. Quantification of various amino acids required three separate steps. The majority of amino acids were estimated via hydrolysis with 6*N* HCl, followed by separation on strong cation-exchange column in sodium form (part number 80002; Waters Associates, Milford, MA) and detection using ninhydrin (24). The sulfur-containing amino acids (methionine, cysteine, and cystine) are partially or completely destroyed by acid hydrolysis. Hence, these amino acids were oxidized with performic acid to form stable products prior to hydrolysis with 6*N* HCl and analyzed as per the method described by Mason et al. (19). Tryptophan is also destroyed by acid hydrolysis; therefore, a basic hydrolysis with barium hydroxide was performed, followed by acidification and quantification with an HPLC using a C18 column and UV detector (7, 21, 22). The gradient mobile phases were used for HPLC analyses. For the normal hydrolysis run, the gradient mobile phase was obtained with buffer A (0.2 *M* Na; pH 3.1) and buffer B (1.2 *M* Na; pH 6.4). For detection of cysteine and methionine, buffer A (0.2 *M* Na; pH 3.6) and buffer B (1.2 *M* Na; pH 6.4) were used. For detection of tryptophan, the mobile phase was 0.07 *M* acetate buffer and methanol (80:20 vol/vol) as suggested by Delhaye and Landry (7).

Unless otherwise indicated, all analyses were carried out in duplicate, and all the experiments were repeated at least twice. The results shown are the averages of all data. The standard error of at least four observations was calculated using an Excel 5.0 Microsoft computer package (Microsoft Corp. Pty. Ltd., Redmond, WA) and is presented as plus or minus of the mean or as an error bar in the figures.

RESULTS AND DISCUSSION

Composition

The mean values for protein and total solid contents of each sample are given in Table 1. The protein content was 0.7% higher in yogurt supplemented with WPC and 0.1% lower in yogurt supplemented with WP. The total solids contents were in the range of 15.40 to 15.75% for all products.

Changes During Fermentation of Yogurt Mix for 24 h

Changes in pH and redox potential. Changes in pH during the 24-h fermentation of yogurt mix are presented in Figure 1. The decrease in pH was faster in yogurt containing WP, WPC, ACH, or tryptone than that of the control yogurt. Samples with added cysteine at a concentration of 50 mg/L showed a drop in pH during the first 24 h of fermentation that was similar to that of the control yogurt; however, an increase in the concentration of cysteine >50 mg/L adversely affected the rate of acid production. Overall, the time taken to reach a pH 4.5 was ~12 h for the control yogurt and for the yogurt supplemented with 50 mg of cysteine/L. The incubation time increased to ~18 and 20 h for yogurt supplemented with 250 or 500 mg of cysteine/L, respectively. Conversely, the fermentation time decreased to ~9 h for yogurt supplemented with WP, ACH, or tryptone and ~7.5 and 8.5 h for that supplemented with WPC 1 or WPC 2, respectively. At 24 h of fermentation, the pH of the

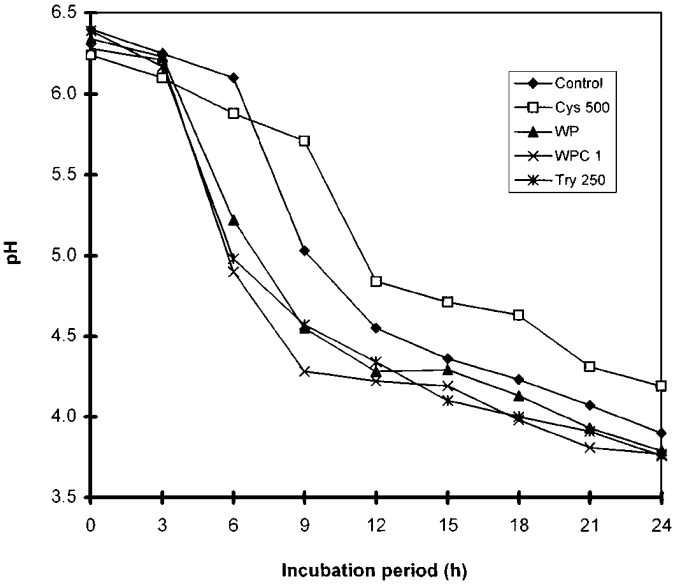

Figure 1. Changes in pH during a 24-h fermentation of yogurt mix with ABT (*Lactobacillus acidophilus*, bifidobacteria, and *Streptococcus thermophilus*) starter culture. Control = Yogurt with 2% skim milk powder (♦), Cys_{500} = yogurt supplemented with 500 mg of cysteine/L of yogurt mix, WP = yogurt supplemented with 2% whey powder, WPC 1 = yogurt supplemented with 2% whey protein concentrate, and Try_{250} = yogurt supplemented with 250 mg of tryptone/L of yogurt mix.

yogurt supplemented with 250 or 500 mg of cysteine/L was 4.16 and 4.19, respectively; the pH dropped to <4.0 in the rest of the yogurts. Changes in the pH of yogurt supplemented with 50 mg/L of cysteine were similar to that of control yogurt. Also, the changes in pH of yogurt supplemented with 250 mg of cysteine/L and WPC 1 were similar to that of yogurt supplemented with 500 mg of cysteine/L and WPC 2, respectively. Yogurt supplemented with 500 mg of ACH or tryptone/L gave results that were similar to those for yogurt supplemented with 250 mg of ACH or tryptone/L, respectively. Champagne et al. (2) tested the suitability of WPC as growth medium for the production of starter cultures compared with milk and commercial media. They (2) concluded that WPC could be successfully used to prepare starter cultures because it gave higher populations of bacteria than when milk was used as the medium. Supplementation of WPC with milk protein hydrolysate also stimulated starter growth.

The redox potential of control yogurt mix after heat treatment was approximately –70 mV. The redox potential decreased to about –130, –180, and –217 mV on addition of 50, 250, and 500 mg of cysteine/L, respectively. The redox potential dropped to –121, –169, and –143 mV in yogurt supplemented with WP, WPC 1, or WPC 2, respectively, and to –80 mV on addition of ACH and tryptone (data not shown). Cysteine is a strong reducing agent and is known for lowering redox potential. Yogurt supplemented with WP and WPC is expected to have higher whey protein content (which is rich in sulfur-containing amino acids) than control yogurt. The whey proteins are known to liberate these sulfur-containing amino acids during heat treatment, which could be the reason for the low redox potential in the yogurt mix containing cysteine, WP, WPC 1, or WPC 2. Redox potential remained negative throughout the 24-h fermentation period upon addition of cysteine, for 21 h in control yogurt, but only for 12 h in yogurt supplemented with ACH or tryptone. The redox potential fluctuated in yogurt supplemented with WP or WPC, and no uniform pattern of increase or decrease in redox potential was observed in these samples (data not shown). Changes in the redox potential of yogurt mix supplemented with 500 mg of ACH or tryptone/L were similar to that of yogurt supplemented with 250 mg of ACH or tryptone/L, respectively. Overall, the redox potential remained negative in all the yogurts until the pH reached 4.5. Thus, the loss of viability of bifidobacteria in yogurt made with the ABT starter culture did not appear to be due to dissolved oxygen in the yogurt mixes.

Changes in counts of S. thermophilus, L. acidophilus, and bifidobacteria. Changes in pH during the 24-h fermentation of yogurt mix with ABT starter culture are shown in Figure 1. Changes in the counts of *S. thermophilus* during fermentation of yogurt mixes are presented in Table 2. As shown in Figure 1, the time taken to reach pH 4.5 was affected by the addition of various ingredients. Cysteine at 500 mg/L adversely affected the growth of *S. thermophilus* (Table 2) when the counts of this organism from the time of inoculation to that required to reach pH 4.5 are taken into consideration (Figure 1), and counts of *S. thermophilus* remained lower in yogurts supplemented with 250 or 500 mg of cysteine/L. Conversely, WP, WPC, ACH, or tryptone supported the growth of *S. thermophilus*, and multiplication of this organism was faster in yogurts supplemented with these ingredients, which could have been the reason for the shorter incubation time needed to reach pH of 4.5 for these samples.

When the time to reach pH 4.5 is taken into consideration (Figure 1) and the counts of *L. acidophilus* (Table 3) are compared, the *L. acidophilus* counts increased in the yogurt that was supplemented with 500 mg of cysteine/L. Thus, 500 mg of cysteine/L

TABLE 2. Changes in counts of *Streptococcus thermophilus* during manufacture of yogurt supplemented with various ingredients.

Incubation period	Control[1]		Cys_{50}		Cys_{250}		Cys_{500}		WP		WPC 1		WPC 2		ACH_{250}		Try_{250}	
	(log_{10} cfu/ml)																	
	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE
0 h	6.34	0.05	6.34	0.05	6.34	0.05	6.34	0.05	6.30	0.09	6.32	0.08	6.30	0.05	6.36	0.03	6.36	0.05
3 h	7.76	0.04	7.50	0.03	7.08	0.06	6.90	0.09	8.12	0.05	8.85	0.11	8.62	0.09	8.17	0.05	8.20	0.03
6 h	8.68	0.05	7.81	0.05	7.59	0.03	7.58	0.01	9.23	0.03	9.15	0.05	9.13	0.06	8.95	0.03	9.00	0.05
9 h	9.05	0.03	8.74	0.02	7.86	0.05	7.65	0.03	9.27	0.05	9.15	0.06	9.15	0.05	9.15	0.05	9.30	0.08
12 h	8.89	0.06	9.10	0.08	8.67	0.04	8.43	0.05	8.98	0.06	9.05	0.07	9.29	0.05	9.13	0.06	9.28	0.05
15 h	8.92	0.03	8.96	0.06	8.66	0.06	8.40	0.06	8.84	0.04	9.02	0.05	9.17	0.03	9.08	0.04	9.20	0.03
18 h	8.88	0.02	8.95	0.05	8.65	0.03	8.16	0.03	8.81	0.05	8.98	0.03	9.21	0.06	9.01	0.03	9.15	0.06
21 h	8.78	0.05	8.83	0.06	8.56	0.05	8.10	0.06	8.83	0.02	8.79	0.02	9.06	0.03	8.98	0.02	8.93	0.03
24 h	8.66	0.03	8.65	0.04	8.41	0.03	8.20	0.05	8.78	0.06	8.56	0.04	8.98	0.05	8.85	0.06	8.72	0.04

[1]Control = Yogurt with 2% skim milk powder; Cys_{50}, Cys_{250}, and Cys_{500} = yogurt containing 50, 250, and 500 mg of cysteine/L of yogurt mix, respectively; WP = yogurt containing 2% whey powder; WPC 1 and WPC 2 = yogurt supplemented with 2% whey protein concentrates 1 and 2, respectively; ACH_{250} = yogurt supplemented with 250 mg of acid casein hydrolysate/L of yogurt mix; and Try_{250} = yogurt supplemented with 250 mg of tryptone/L of yogurt mix.

promoted the growth of *L. acidophilus* but not *S. thermophilus* (Table 2); other cysteine concentrations had no effects. Overall, multiplication of *L. acidophilus* was faster in yogurt that had been supplemented with ACH and tryptone.

Bifidobacteria in the control yogurt increased for ≤6 h and then declined (Table 4). During a 9-h period, their numbers were reduced by 1 log cycle followed by ~3-log reduction over the 12-h period. The pH of control yogurt also reached 4.5 at this period; hence, the counts of bifidobacteria in the control yogurt were much lower at 0 d than their initial numbers at 0 h (Tables 4 and 5). A similar trend of decreases was observed during the 12-h period for yogurt supplemented with WP. The time taken to reach pH 4.5 was considerably shorter in yogurt supplemented with WPC, ACH, or tryptone. Therefore, the bifidobacteria counts declined by <1 log cycle in samples other than control yogurt and that supplemented with WP. Also, bifidobacteria counts never dropped $<10^5$ cfu/g throughout the 24-h incubation in yogurt supplemented with 500 mg of cysteine/L, WPC 1, ACH, or tryptone. This result indicated that an incubation period of >12 h (similar to that required for control yogurt and that supplemented with WP) could maintain the viability of bifidobacteria, provided that similar peptides and amino acids as present in WPC 1 or tryptone were available in the yogurt mix during incubation. It was also observed that bifidobacteria started to multiply in some

TABLE 3. Changes in counts of *Lactobacillus acidophilus* during manufacture of yogurt supplemented with various ingredients.

Incubation period	Control[1]		Cys_{50}		Cys_{250}		Cys_{500}		WP		WPC 1		WPC 2		ACH_{250}		Try_{250}	
	(log_{10} cfu/ml)																	
	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE
0 h	6.41	0.10	6.45	0.10	6.45	0.10	6.45	0.10	6.40	0.15	6.40	0.12	6.40	0.13	6.40	0.08	6.40	0.03
3 h	6.53	0.06	6.60	0.03	6.57	0.03	6.58	0.10	6.58	0.06	6.53	0.06	6.56	0.10	6.60	0.05	6.56	0.05
6 h	7.11	0.08	7.09	0.05	7.16	0.05	7.26	0.11	6.93	0.04	7.28	0.05	7.18	0.05	7.28	0.08	7.23	0.04
9 h	7.34	0.06	7.46	0.07	7.50	0.06	7.57	0.08	7.04	0.06	7.66	0.03	7.45	0.05	7.98	0.06	8.08	0.08
12 h	7.81	0.09	7.78	0.05	7.76	0.05	7.88	0.04	7.49	0.03	7.95	0.08	7.79	0.11	8.15	0.05	8.43	0.09
15 h	7.90	0.05	7.78	0.03	7.79	0.03	7.98	0.07	7.64	0.08	8.05	0.06	7.90	0.06	8.30	0.04	8.48	0.05
18 h	8.10	0.04	7.95	0.09	7.99	0.02	8.00	0.09	7.95	0.01	8.07	0.05	8.02	0.05	8.45	0.07	8.54	0.06
21 h	8.22	0.03	8.00	0.05	8.05	0.04	8.32	0.11	8.14	0.11	8.18	0.05	8.06	0.04	8.41	0.06	8.54	0.03
24 h	8.35	0.05	8.15	0.08	8.24	0.06	8.59	0.05	8.22	0.06	8.27	0.03	8.23	0.06	8.43	0.06	8.46	0.03

[1]Control = Yogurt with 2% skim milk powder; Cys_{50}, Cys_{250}, and Cys_{500} = yogurt containing 50, 250, and 500 mg of cysteine/L of yogurt mix, respectively; WP = yogurt containing 2% whey powder; WPC 1 and WPC 2 = yogurt supplemented with 2% whey protein concentrates 1 and 2, respectively; ACH_{250} = yogurt supplemented with 250 mg of acid casein hydrolysate/L of yogurt mix; and Try_{250} = yogurt supplemented with 250 mg of tryptone/L of yogurt mix.

TABLE 4. Changes in counts of bifidobacteria during manufacture of yogurt supplemented with various ingredients.

Incubation period	Control[1]		Cys_{50}		Cys_{250}		Cys_{500}		WP		WPC 1		WPC 2		ACH_{250}		Try_{250}	
	(log_{10} cfu/ml)																	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
0 h	6.65	0.09	6.65	0.09	6.65	0.09	6.65	0.09	6.65	0.10	6.65	0.06	6.65	0.03	6.69	0.03	6.70	0.05
3 h	6.68	0.06	7.14	0.06	6.93	0.05	6.99	0.09	6.74	0.09	6.78	0.05	6.69	0.05	6.65	0.06	6.65	0.07
6 h	6.92	0.19	6.95	0.04	6.85	0.06	6.97	0.10	5.30	0.14	6.56	0.05	6.48	0.04	6.59	0.05	6.63	0.05
9 h	5.78	0.14	6.65	0.08	6.69	0.08	6.72	0.06	5.15	0.26	6.51	0.03	6.15	0.04	6.28	0.04	6.41	0.03
12 h	3.72	0.23	5.30	0.09	5.65	0.04	6.30	0.08	4.00	0.15	6.18	0.05	6.11	0.03	5.95	0.06	6.43	0.07
15 h	2.63	0.12	5.00	0.10	5.52	0.06	6.15	0.09	4.05	0.11	6.23	0.06	6.08	0.04	5.70	0.05	6.30	0.06
18 h	3.70	0.06	4.60	0.16	5.32	0.05	6.11	0.12	3.85	0.30	6.18	0.04	5.08	0.09	5.70	0.05	6.18	0.08
21 h	3.97	0.10	5.20	0.15	5.04	0.07	5.90	0.09	3.30	0.11	5.75	0.10	4.90	0.11	5.78	0.04	6.04	0.11
24 h	4.10	0.09	5.34	0.14	4.86	0.08	6.25	0.17	3.39	0.05	5.80	0.05	4.28	0.06	5.65	0.06	6.41	0.12

[1]Control = Yogurt with 2% skim milk powder; Cys_{50}, Cys_{250}, and Cys_{500} = yogurt containing 50, 250, and 500 mg of cysteine/L of yogurt mix, respectively; WP = yogurt containing 2% whey powder; WPC 1 and WPC 2 = yogurt supplemented with 2% whey protein concentrates 1 and 2, respectively; ACH_{250} = yogurt supplemented with 250 mg of acid casein hydrolysate/L of yogurt mix; and Try_{250} = yogurt supplemented with 250 mg of tryptone/L of yogurt mix.

products (Table 4) after 15 to 18 h of incubation. During fermentation, changes in counts of *S. thermophilus*, *L. acidophilus*, and bifidobacteria in yogurt mixes supplemented with 500 mg of ACH or tryptone/L were similar to those of yogurt supplemented with 250 mg of ACH or tryptone/L, respectively. Lower redox potential was not solely responsible for improving the viability of bifidobacteria, but the additional nitrogen source in the form of peptides or amino acids was required to keep bifidobacteria viable in the product during manufacture of the yogurt. The pH levels of all samples were kept almost identical during fermentation because low pH or higher acidity has been reported to affect the viability of probiotic bacteria (12, 25, 27). In a study by Klaver et al. (13), no growth of *Bifidobacterium bifidum* occurred in the absence of other lactic acid bacteria (*L. acidophilus* or *S. thermophilus*) or at higher oxygen concentrations. Further, 15 of 17 strains of bifidobacteria did not grow in milk because their growth re-

TABLE 5. Changes in counts of *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and bifidobacteria during storage of yogurt supplemented with various ingredients.

Storage period	Control[1]		Cys_{50}		Cys_{250}		Cys_{500}		WP		WPC 1		WPC 2		ACH_{250}		Try_{250}	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
	S. thermophilus (log_{10} cfu/g)																	
0 d	8.81	0.06	8.86	0.05	8.15	0.02	7.91	0.05	8.90	0.05	8.92	0.03	8.91	0.05	9.00	0.06	9.00	0.02
10 d	8.84	0.05	9.00	0.03	8.02	0.04	7.86	0.05	8.83	0.04	8.95	0.05	8.98	0.03	9.21	0.04	9.25	0.05
25 d	8.73	0.03	9.03	0.04	8.15	0.03	7.68	0.03	8.76	0.03	9.02	0.07	9.00	0.06	9.17	0.06	9.18	0.03
35 d	8.69	0.04	9.03	0.04	8.11	0.03	6.59	0.04	8.72	0.02	9.00	0.06	8.98	0.05	9.18	0.05	9.08	0.04
	L. acidophilus (log_{10} cfu/g)																	
0 d	7.65	0.05	7.58	0.04	8.33	0.05	8.22	0.06	7.19	0.05	7.38	0.08	7.36	0.07	7.41	0.05	7.45	0.06
10 d	7.41	0.06	7.40	0.05	8.10	0.07	8.00	0.05	7.08	0.03	7.20	0.05	7.09	0.07	7.13	0.03	7.11	0.07
25 d	6.68	0.10	6.88	0.07	7.74	0.09	7.69	0.08	6.77	0.05	6.43	0.10	6.64	0.12	6.72	0.04	6.70	0.08
35 d	5.71	0.13	6.11	0.05	6.77	0.10	7.00	0.09	5.45	0.04	5.48	0.05	5.30	0.10	5.78	0.05	5.95	0.09
	Bifidobacteria (log_{10} cfu/g)																	
0 d	2.85	0.23	5.60	0.12	5.40	0.09	5.60	0.05	3.18	0.12	6.52	0.05	5.90	0.07	4.93	0.05	5.95	0.05
10 d	2.70	0.25	5.58	0.15	5.32	0.05	5.46	0.10	3.08	0.13	6.34	0.06	5.72	0.08	4.62	0.04	5.78	0.06
25 d	2.48	0.21	4.95	0.13	5.10	0.06	5.26	0.11	2.69	0.14	6.08	0.07	5.54	0.06	4.79	0.09	5.69	0.05
35 d	2.30	0.12	4.76	0.10	5.04	0.08	5.18	0.05	2.70	0.05	6.20	0.03	5.34	0.05	5.00	0.12	5.60	0.03

[1]Control = Yogurt with 2% skim milk powder; Cys_{50}, Cys_{250}, and Cys_{500} = yogurt containing 50, 250, and 500 mg of cysteine/L of yogurt mix, respectively; WP = yogurt containing 2% whey powder; WPC 1 and WPC 2 = yogurt supplemented with 2% whey protein concentrates 1 and 2, respectively; ACH_{250} = yogurt supplemented with 250 mg of acid casein hydrolysate/L of yogurt mix; and Try_{250} = yogurt supplemented with 250 mg of tryptone/L of yogurt mix.

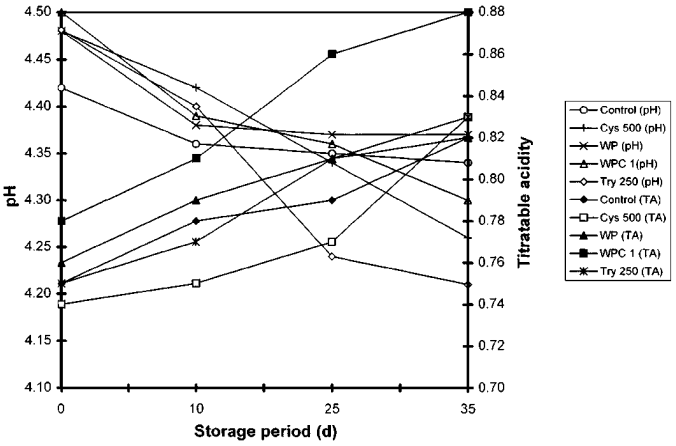

Figure 2. Changes in pH and titratable acidity (TA; percentage lactic acid) during the refrigerated storage of yogurt supplemented with various ingredients. For pH, control = yogurt with 2% skim milk powder, Cys_{500} = yogurt supplemented with 500 mg of cysteine/L of yogurt mix, WP = yogurt supplemented with 2% whey powder, WPC 1 = yogurt supplemented with 2% whey protein concentrate 1, and Try_{250} = yogurt supplemented with 250 of mg tryptone/L of yogurt mix. For TA, control = yogurt with 2% skim milk powder, Cys_{500} = yogurt supplemented with 500 mg of cysteine/L of yogurt mix, WP = yogurt supplemented with 2% whey powder, WPC 1 = yogurt supplemented with 2% whey protein concentrate 1, and Try_{250} = yogurt supplemented with 250 of mg tryptone/L of yogurt mix.

quired peptides or amino acids derived from casein degradation (14). It was concluded that bifidobacteria lacked proteolytic activity, and the organism could be grown by adding casein hydrolysates or by coculturing bifidobacteria with proteolytic species such as *L. acidophilus.*

Changes During Refrigerated Storage of Yogurts for 35 d

Changes in pH, TA, and redox potential. Changes in pH and TA during the refrigerated storage of yogurts are shown in Figure 2. Approximately a 0.08-unit drop in pH and a 0.07% increase in lactic acid were observed in the control yogurt. The drop in pH or rise in TA in yogurt supplemented with cysteine, WPC, ACH, or tryptone was more than double that observed in the control yogurt. Yogurt supplemented with WP showed a similar trend of decrease in pH or increase in TA to that of control yogurt. Overall, maxima of ~0.3-unit drop in pH and ~0.1% increase in TA were observed during 35 d of storage for yogurt supplemented with ACH or tryptone, possibly because of the availability of an amino nitrogen source through the added ingredients. Thus, the pH or TA of all yogurt samples stabilized within this range, and the drop in pH or increase in TA did not seem to be factors that affected the viability of the bifidobacteria during refrigerated storage of yogurt supplemented with these ingredients (Table 5). During refrigerated storage, changes in the pH and TA of yogurt supplemented with 50 mg of cysteine/L were similar to that of control yogurt. Also, the changes in pH of yogurt supplemented with 250 mg of cysteine/L or WPC 1 were similar to that of yogurt supplemented with 500 mg of cysteine/L or WPC 2, respectively. Yogurt supplemented with 500 mg of ACH or tryptone/L gave results similar to yogurt supplemented with 250 mg of ACH or tryptone/L, respectively.

The redox potential in yogurt supplemented with 50 or 250 mg of cysteine/L remained negative for up to 10 and 25 d, respectively (Figure 3). At 500 mg of cysteine/L, the redox potential remained negative throughout the 35-d storage period. In control and other yogurts, the redox potential ranged between +81 to +95 mV at 0 d, which increased to +160 mV in the control and between +114 to +128 mV in yogurts supplemented with WP, WPC, ACH, or tryptone during storage.

Changes in Counts of S. thermophilus, L. acidophilus, and bifidobacteria. At 0 d, counts of *S. thermophilus* were highest in yogurt supplemented with ACH or tryptone and lowest in yogurt supplemented with 500 mg of cysteine/L (Table 5). During further storage, *S. thermophilus* counts declined slightly in control yogurt and in yogurt supplemented with 250 or 500 mg of cysteine/L and WP but increased to some extent in yogurt supplemented with 50 mg of cysteine/L, WPC, ACH, or tryptone. Lower counts of *S. thermophilus,* especially in yogurt supplemented with higher concentrations of cysteine (250 or 500 mg/L), could be due to negative redox potential in the product, as *S. thermophilus* is microaerophilic to aerobic. Counts of *L. acidophilus* showed a constant decline in all products throughout storage. At 0 d, counts of *L. acidophilus* were lower in yogurt supplemented with WP, WPC, ACH, or tryptone than that observed in control yogurt or yogurt supplemented with 50 mg/L of cysteine. Conversely, the counts of *L. acidophilus* were considerably higher in yogurt supplemented with 250 or 500 mg of cysteine/L, unlike those of *S. thermophilus* in these products. This result could be due to the adverse effect of cysteine on *S. thermophilus,* which prolonged the fermentation time and perhaps favored the multiplication of *L. acidophilus* in yogurts supplemented with cysteine. Conversely, for other products, a shorter fermentation time might not have allowed *L. acidophilus* to multiply to a greater extent, resulting

in lower counts of *L. acidophilus* in finished products prepared with WPC, ACH, or tryptone. Overall, the viability of *L. acidophilus* was better in yogurts supplemented with cysteine; however, counts remained $>10^5$/cfu/g in all the products throughout the 35 d of refrigerated storage. Kailasapathy and Supriadi (12) examined the effect of WPC on the survival of *L. acidophilus* and concluded that the partial replacement of dried skim milk by WPC enabled sufficiently high numbers of *L. acidophilus* to remain viable during 21 d of refrigerated storage.

The viability of bifidobacteria was low throughout the storage period for the control yogurt and for the yogurt supplemented with WP. The viability of this organism in yogurt supplemented with cysteine, WPC, ACH, or tryptone was improved by >3 log cycles compared with that of the control yogurt. Improved viability could be due to the amino nitrogen present in WPC, ACH, or tryptone. The counts of bifidobacteria declined considerably between 9 to 12 h of incubation in the control yogurt (Table 4), but fermentation was terminated before 9 h in yogurt supplemented with WPC, ACH, or tryptone as the pH reached 4.5 in these samples. Thus, the reduction in the total fermentation time from the addition of these ingredients and the favorable effects of micronutrients present in these ingredients might have been responsible for the improved viability of bifidobacteria to $>10^5$ cfu/g. The highest viability of bifidobacteria was observed in yogurt supplemented with WPC 1. During refrigerated storage, the changes in counts of *S. thermophilus*, *L. acidophilus*, and bifidobacteria in yogurts supplemented with 500 mg of ACH or tryptone/L were similar to that in yogurt supplemented with 250 mg/L of ACH or tryptone, respectively.

Cell Morphology of *S. thermophilus* as Shown by Electron Micrograph

The cell morphology of *S. thermophilus* was affected with increased concentration of cysteine, as observed by electron microscopy (Figure 4). A low redox potential at 500 mg of cysteine/L seemed to affect the cell wall and cell membrane of *S. thermophilus* cells. In yogurt supplemented with WP or 250 mg of cysteine/L, the morphology of *S. thermophilus* was similar and showed no clear cell membrane or cell wall. This result could be due to the adverse effect on the *S. thermophilus* cells, which confirmed the earlier observations of less drop in pH (Figure 1), the slower the growth of *S. thermophilus* (Table 2) during fermentation of yogurt mix, and the lower the counts of *S. thermophilus* in yogurt supplemented with cysteine. In previous studies (4, 6), counts of *S. thermophilus* were low in yogurt prepared in glass bottles and in yogurt supplemented with ascorbic acid as an oxygen scavenger, possibly because of low redox

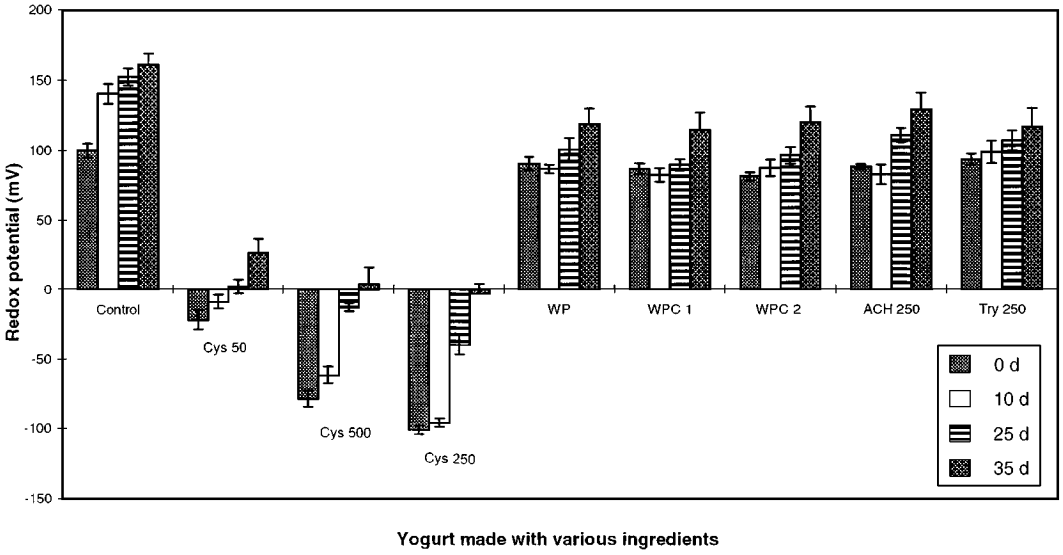

Figure 3. Changes in the redox potential (millivolts) of yogurt during refrigerated storage. Control = Yogurt with 2% skim milk powder; Cys_{50}, Cys_{250}, and Cys_{500} = yogurt supplemented with 50, 250, and 500 mg of cysteine/L of yogurt mix, respectively; WP = yogurt supplemented with 2% whey powder; WPC 1 and WPC 2 = yogurt supplemented with 2% whey protein concentrates 1 and 2, respectively; ACH_{250} = yogurt supplemented with 250 mg of acid casein hydrolysate/L of yogurt mix; and Try_{250} = yogurt supplemented with 250 mg of tryptone/L of yogurt mix.

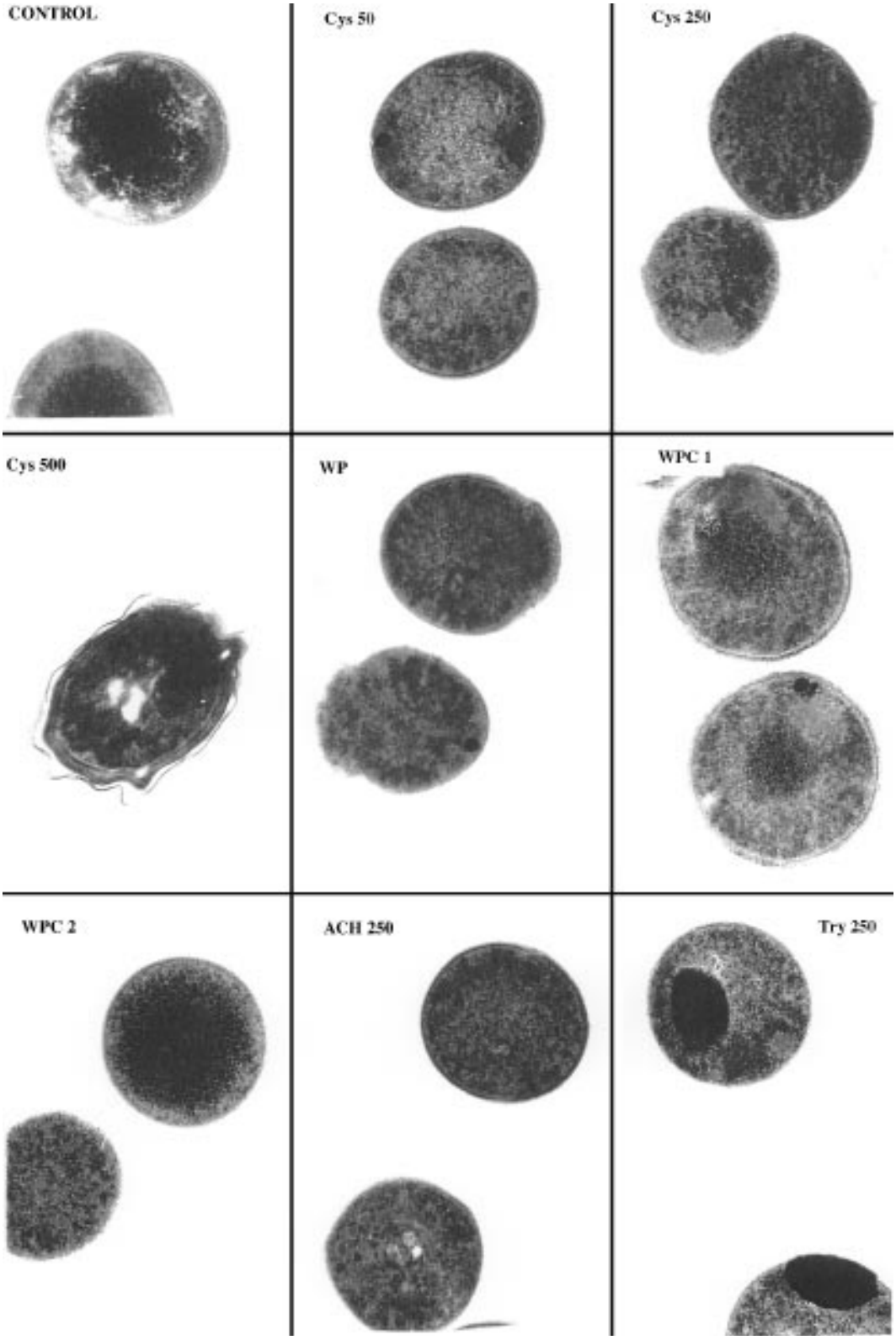

Figure 4. Electron micrographs of *Streptococcus thermophilus* cells in yogurt made with various ingredients. 55,000×. Control = Yogurt with 2% SMP; Cys_{50}, Cys_{250}, and Cys_{500} = yogurt supplemented with 50, 250, and 500 mg of cysteine/L of yogurt mix, respectively; WP = yogurt supplemented with 2% whey powder; WPC 1 and WPC 2 = yogurt supplemented with 2% whey protein concentrates 1 and 2, respectively; ACH_{250} = yogurt supplemented with 250 mg of acid casein hydrolysate/L of yogurt mix; and Try_{250} = yogurt supplemented with 250 mg of tryptone/L of yogurt mix.

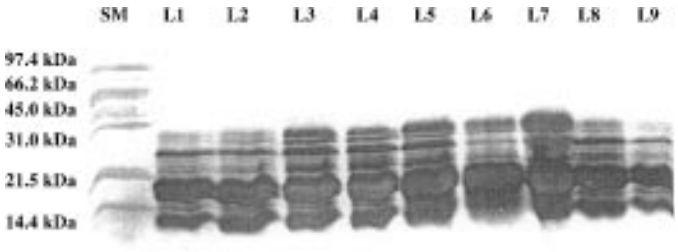

Figure 5. Silver stain of SDS-PAGE of whey collected from various yogurts. SM = Standard molecular mass markers; lane (L) 1 = yogurt with 2% SMP; lanes 2, 3, and 4 = yogurt supplemented with 50, 250, and 500 mg of cysteine/L of yogurt mix, respectively; lane 5 = yogurt supplemented with 2% whey powder; lanes 6 and 7 = yogurt supplemented with 2% whey protein concentrates 1 and 2, respectively; lane 8 = yogurt supplemented with 250 mg of acid casein hydrolysate/L of yogurt mix; and lane 9 = yogurt supplemented with 250 mg of tryptone/L of yogurt mix.

potential. The electron micrographs in this study confirmed that very low redox potential caused damage to the cell wall and cell membrane of *S. thermophilus*. In the control yogurt and the yogurt supplemented with WPC 1, WPC 2, ACH, or tryptone, the cell structure was clearly defined, and the cells of *S. thermophilus* appeared to be healthy without signs of cell damage.

SDS-PAGE and Amino Acids Analyses

The growth of various starter bacteria (*S. thermophilus*, *L. acidophilus*, and bifidobacteria) was affected by the addition of various ingredients. Also, the ingredients that were added were expected to have different intermediate proteins and peptides. Therefore, the SDS-PAGE analysis of the filtrate after the precipitation of casein in different yogurt samples was performed, and the various protein bands obtained were analyzed by a gel densitometer (data not shown). The number of bands and their relative area were different in each product. At higher cysteine concentrations (250 and 500 mg/L), the intermediate peptide products were higher as a total of 12 bands appeared in these yogurts compared with the 8 or 9 bands in other yogurts (Figure 5). The molecular mass of each band, as estimated by a laser densitometer and their relative area, was different in the whey of yogurts supplemented with various ingredients (data not shown). The starter culture used in this study contained three different bacteria, and counts of these organisms varied in the finished products. *Lactobacillus acidophilus* has been reported to be more proteolytic than *S. thermophilus* (13). The higher counts of *L. acidophilus* in yogurts containing higher concentrations of cysteine correlated with the higher numbers of bands in yogurts supplemented with cysteine. The degradation of casein by *L. acidophilus* might have liberated additional peptides during fermentation and might have resulted in a greater number of bands. Yogurt supplemented with WPC 1, WPC 2, ACH, or tryptone also showed differences in the number of peaks and their molecular mass. Yogurt containing WPC 2 showed some high molecular mass bands (data not shown). The rate of acid production (Table 2) was slower, and the viability of bifidobacteria (Table 5) was lower, in yogurt supplemented with WPC 2 than in yogurt supplemented with WPC 1. The change in intermediate peptide products in whey of various yogurts could be due to the change in microbial ecology and to the altered proportion of various starter bacteria. After the SDS-PAGE and viability results were analyzed, we presumed that intermediate peptides (15 to 30 kDa) might have affected the growth of starter bacteria in yogurts during fermentation. Proteins and peptides also seemed to be responsible for the time taken to reach pH 4.5 and for the difference in viability of starter bacteria in yogurts supplemented with various ingredients.

Because the viability of bifidobacteria was improved with WPC 1, WPC 2, ACH, or tryptone to a variable extent, it was desirable to know the protein

TABLE 6. Protein content and amino acid profile of whey protein concentrates, acid casein hydrolysate, and tryptone.

Content	WPC 1[1]	WPC 2	ACH[2]	Tryptone
Protein, %	73.5	72.5	49.6	82.1
Amino acid				
Aspartic acid	8.15	8.46	3.99	6.11
Threonine	4.58	6.25	2.24	3.99
Serine	4.11	4.76	2.65	5.48
Glutamic acid	13.01	13.86	13.07	19.11
Proline	4.32	5.01	6.09	9.39
Glycine	1.56	1.56	1.12	1.62
Alanine	4.19	4.21	2.44	2.74
Valine	4.56	5.09	4.03	6.24
Methionine	1.85	1.79	1.30	2.53
Isoleucine	4.30	5.27	2.94	4.89
Leucine	9.04	8.30	4.93	7.98
Tyrosine	2.83	2.70	2.14	1.73
Phenylalanine	2.92	2.68	2.30	4.15
Lysine	7.43	7.34	4.70	6.98
Histidine	1.50	1.40	1.47	2.42
Ammonia	1.07	1.29	0.33	1.52
Arginine	2.25	1.96	2.02	3.15
Cystine and cysteine	2.16	2.01	0.16	0.27
Tryptophan	1.52	1.41	ND	0.91
Protein recovery	94.9	101.0	96.5	97.4

[1]Whey protein concentrates 1 and 2, respectively.

[2]Acid casein hydrolysate.

and amino acid profiles of these ingredients (Table 6). Although ACH and tryptone are the hydrolysis products of casein, the concentrations of protein and various amino acids were different. The total protein content was fairly similar for WPC 1 and WPC 2, but some differences in their amino acid profiles were found. The viability of bifidobacteria was improved to a lesser extent in yogurt containing ACH. The total protein content and cysteine were less in yogurt containing ACH than in yogurt containing tryptone. Cystine plus cysteine contents were considerably higher in yogurt containing WPC 1 or WPC 2 than in yogurt containing tryptone or ACH (Table 6). Thus, the protein and amino acid contents of WPC 1, WPC 2, ACH, or tryptone might have been responsible for the differences in the viability of probiotic bacteria in yogurt supplemented with these ingredients. This result showed that peptides and amino acids have improved the viability of probiotic bacteria, especially bifidobacteria, which have been reported to be weakly proteolytic (13, 14, 23, 28). *Lactobacillus delbrueckii* ssp. *bulgaricus* is considered to be more proteolytic than *S. thermophilus*. In ABT cultures, no symbiotic relationship exists; as a result, the incubation period to reach pH 4.5 is longer for these cultures. The ACH is a product prepared by the acid hydrolysis of casein, and tryptone is a tryptic digest of casein, both of which are rich in the peptides and essential amino acids required by *S. thermophilus*. Cysteine, WP, and WPC also serve as a source of peptides and amino acids when heat treated in yogurt mix. Whey proteins are rich in sulfur-containing amino acids, which are liberated during heat treatment and lower the redox potential.

Streptococcus thermophilus prefers an aerobic environment to a microaerophilic environment, *L. acidophilus* is microaerophilic to anaerobic, and bifidobacteria are considered to be anaerobes. The addition of cysteine improved the viability of probiotic bacteria during storage. For ABT starter cultures, the acid production mainly is due to *S. thermophilus*, which usually requires an incubation period of 11 to 12 h to reach pH 4.5. The addition of cysteine at >50 mg/L caused damage to the cell wall and the cell membrane of *S. thermophilus*, which increased the incubation time to 18 to 20 h. The results of changes in the pH during fermentation (Figure 1), viable counts (Table 2), and electron micrographs (Figure 4) confirmed this. Thus, addition of cysteine up to 50 mg/L may be sufficient and may be commercially feasible for improving the viability of probiotic bacteria. The addition of WPC 1 and tryptone, however, may be more economical than the addition of cysteine; these ingredients reduced incubation time because of available micronutrients and less drop in redox potential, both of which supported the growth of *S. thermophilus*. Also, WPC 1 and tryptone were equally effective in improving the viability of probiotic bacteria, especially that of bifidobacteria, which in previous studies showed a dramatic decline in their numbers.

CONCLUSIONS

All 11 batches of yogurt made with a commercial ABT starter culture showed different patterns of change in pH, TA, and redox potential during the manufacture and storage of yogurt. Also, the counts of *S. thermophilus*, *L. acidophilus*, and bifidobacteria were noticeably different. The time to reach pH 4.5 increased considerably on addition of 250 and 500 mg of cysteine/L, but the incubation time decreased in yogurt mixes supplemented with WPC, ACH, or tryptone. The redox potential remained negative during refrigerated storage in yogurt supplemented with 250 or 500 mg of cysteine/L.

The viability of *S. thermophilus* was adversely affected, but the viability of *L. acidophilus* was improved on addition of cysteine in yogurt. The counts of *L. acidophilus* remained $>10^5$ cfu/g throughout the storage in all yogurts. A reduction of >3 log cycles in counts of bifidobacteria was observed when the pH reached 4.5 in the control yogurt and in the yogurt supplemented with WP. The viability of bifidobacteria improved to a variable extent in yogurt supplemented with cysteine, WPC, ACH, or tryptone and was highest in yogurt supplemented with WPC 1.

Electron microscopy revealed that incorporation of higher concentrations of cysteine (500 mg/L) in yogurt mix affected the cell membrane and cell wall of *S. thermophilus* cells. The SDS-PAGE and amino acid analyses confirmed that the nitrogen source in the form of peptides and amino acids correlated with improved viability of bifidobacteria in yogurt made with a commercial starter culture, which showed a dramatic decline in the counts of this organism in our previous studies. The nitrogen source in the form of peptides or amino acids might be crucial to improve the viability of bifidobacteria in yogurt made with ABT starter culture.

ACKNOWLEDGMENTS

This study was made possible with the financial assistance of Department of Employment, Education, Training and Youth Affairs, Canberra, Australia. We are grateful to Liliana Tatarczuch of Melbourne

University for her help in electron microscopy work and Maurice Kerr of State Chemistry Laboratory, Werribee, Australia for his help in amino acid analysis. Our thanks are also due to Roy Con Foo, Director, Marketing and Sales, Chr. Hansen, Australia, for his support.

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