ELSEVIER

International Dairy Journal 10 (2000) 213–220

INTERNATIONAL DAIRY JOURNAL

www.elsevier.com/locate/idairyj

Characteristics of carbonated fermented milk and survival of probiotic bacteria

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Received 30 November 1999; accepted 13 April 2000

Abstract

The carbonation of pasteurised milk was evaluated as a method for improving bacterial viability in fermented milk added with probiotic bacteria (*Lactobacillus acidophilus* and/or *Bifidobacterium bifidum*). The behaviour of microorganisms during fermentation and cold storage, and the biochemical and sensory properties of the products were assessed. In AT (*Streptococcus thermophilus*/*L. acidophilus*) and ABT (*S. thermophilus*/*L. acidophilus*/*B. bifidum*) products, the fermentation times to decrease the pH to 5 were significantly lowered when CO_2 or lactic acid was added to milk. The higher acidity levels of carbonated (as a result of production of carbonic acid) and lactic acidified samples enhanced growth and metabolic activity of the starter during fermentation and was the reason for this reduction in incubation time. Cell counts of *S. thermophilus*, *L. acidophilus* and *B. bifidum* gradually decreased through the cold storage of carbonated and non-acidified fermented milks, although the counts were always higher than 10^6 viable cells g^{-1}. The CO_2 did not exert any influence on the viability of *S. thermophilus* and *L. acidophilus* in AT fermented milks stored at 4°C but the presence of *B. bifidum* and CO_2 in ABT-type products was associated with lower viability of *L. acidophilus* during the refrigerated storage. The higher acetate concentrations of ABT products made with non-acidified milk as compared with the carbonated products could have contributed to major survival of *L. acidophilus* in the former. The use of milk acidified with CO_2 had no detrimental effects on the sensory properties of ABT fermented milks. Therefore, we concluded that the carbonation of pasteurised milk prior to the starter addition could be satisfactorily used to reduce the manufacture time of fermented milk.

Keywords: CO_2; Fermented milks; Probiotic bacteria

1. Introduction

Fermented dairy products have been consumed for nutrition and maintenance of good health for a very long time (Wood Brian, 1992). In recent years, there has been an increasing interest in the addition of *Lactobacillus acidophilus* and bifidobacteria to fermented milks and a great variety of products containing them have been formulated world-wide (Kneifel & Pacher, 1993). After ingestion, these cultures must overcome biological barriers including acid in the stomach and bile in the intestine (Gilliland, 1978; Kanbe, 1992; Lankaputhra & Shah, 1995), implant in the intestinal tract and exert health-promoting effects (Kailasapathy & Rybka, 1997; Klaver, Kingma & Weerkamp, 1993). In order to produce therapeutic benefits, a suggested minimum level for probiotic bacteria in fermented milk is from 10^5 to 10^6 CFU mL^{-1} (Samona & Robinson, 1994). However, these organisms often show poor viability in market preparations (Dave & Shah, 1997; Klaver et al., 1993; Ravula & Shah, 1998). Thus, efforts to increase growth of probiotics in milk and their viability in various dairy products have drawn the attention of researchers in recent years.

Lactic acid bacteria seem to be relatively tolerant to CO_2 (Enfors & Molin, 1980; Louaileche, Bracquart, Saulnier & Desmazeaud, 1993). Growth, acidification capacity and production of volatile compounds in mesophilic starters are not affected by the presence of CO_2 during prolonged incubation times (Ruas-Madiedo,

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0958-6946/00/$ - see front matter
PII: S0958-6946(00)00031-5

Alonso, González de Llano & G. de los Reyes-Gavilán, 1998a). However, at early stages of incubation, growth and production of L-lactic acid can be slightly retarded in both mesophilic and thermophilic cultures (Calvo, Montilla & Olano, 1993; Champagne, St-Gelais & de Candolle, 1998; Montilla, Calvo & Olano, 1995). Furthermore, there is a reason to suspect that the lactobacilli might react positively to CO_2 injection in milk. It is known that CO_2 produced by *Streptococcus thermophilus* stimulated *Lactobacillus delbrueckii* ssp. *bulgaricus* (Driessen, Kingma & Stadhouders, 1982; Tinson, Broome, Hillier & Jago, 1982). On the other hand, by incorporating *S. thermophilus* having high oxygen utilisation ability, the level of dissolved oxygen in fermented milk can be reduced and the viability of *Bifidobacterium* improved (Ishibashi & Shimamura, 1993). Furthermore, milk stored at refrigeration temperatures under carbonation was successfully used for cheese-making (Ruas-Madiedo, Bada-Gancedo, Alonso & G. de los Reyes-Gavilán, 1998b) demonstrating that no significant chemical changes were induced by CO_2 injection.

The present work was undertaken to evaluate the carbonation of pasteurised milk as a method for improving fermentation conditions and/or bacterial viability in fermented milk added with probiotic bacteria (*L. acidophilus* and/or *Bifidobacterium bifidum*) as adjunct starter culture. To the best of our knowledge, this is the first report on the manufacture of fermented milk using pasteurised and carbonated milk.

2. Material and methods

2.1. Strains and culture conditions

S. thermophilus strain St73 (from PROLAIN collection-Programa de Lactología Industrial, National University of Litoral, Argentina) and a commercial strain of *L. acidophilus* (LaA3) (Williner S.A., Argentina) were used as lactic starters for fermented milk preparations. A commercial strain of *B. bifidum* (BBI) (from Centro Sperimentale del Latte, Italy) was used as probiotic adjunct. *S. thermophilus* St73 and *L. acidophilus* LaA3 were propagated in 10% sterilised reconstituted skim milk at 37°C for 12 h and then mixed (1 : 1 v/v) to inoculate pasteurised whole milk (non-acidified control and carbonated) as further indicated. Cultures of *B. bifidum* BBI were grown in MRS broth (Biokar, Beauvais, France) for 24 h at 37°C using the Anaerocult A system (Merck, Darmstadt, Germany). These cultures were then centrifuged (10 min at $4340 \times g$) in a Sorvall RC-5B centrifuge (Du Pont Company, Wilmington, Delaware, USA), suspended in an 8% (w/v) solution of sucrose and frozen at −80°C. The preparation was then lyophilised at 0.16 mmHg for 18 h in an Alpha 1–4 freeze-dryer (B. Braun Biotech International, Melsungen, Germany). The lyophilised culture of *B. bifidum* was used to inoculate pasteurised whole milk together with *S. thermophilus* and *L. acidophilus* strains.

2.2. Production of fermented milk

Raw milk (15 L) collected from one farm in Asturias (northern Spain) was supplemented with 2% skim milk powder. The mix was pasteurised at 85°C for 30 min, cooled to 4°C, divided into 3 lots of 5 L each and held overnight with constant stirring (14 rev min^{-1}). During this time one lot was carbonated with food-grade CO_2 (Carburos Metálicos, Barcelona, Spain) to pH 6.3 as previously described (Ruas-Madiedo, Bada-Gancedo, Fernández-García, González de Llano & G. de los Reyes-Gavilán, 1996) and another one was kept as a non-acidified control. To determine whether results obtained during the fermentation process in carbonated samples could be attributed to an increase of acidity caused by carbonic acid, an additional control lot was also performed using milk acidified with lactic acid to pH 6.3. The three lots were then inoculated (2% v/v) with a mixture of *S. thermophilus* St73 and *L. acidophilus* LaA3 to produce AT fermented milk. For the production of ABT fermented milk, in addition to the AT inoculum a lyophilised culture of *B. bifidum* BBI was added in order to give an initial cell count of *B. bifidum* ranging from 10^6 to 10^7 CFU mL^{-1}. Inoculated carbonated and control milks (both non-acidified and acidified with lactic acid) were each distributed in sterile glass bottles (200 mL) and incubated without stirring at 42°C. After a pH of 5 was reached, the fermented milk were stored at 4°C to avoid an excessive post-acidification during the first few days of refrigerated storage that could lead to a reduction of the viability of probiotic bacteria. Three trials of each type of fermented milk (AT and ABT) were carried out. For microbial counts and biochemical analyses, samples were taken during manufacture in all lots of fermented milks and during refrigerated storage in carbonated and non-acidified samples.

2.3. Microbiological analysis

Serial dilutions of milk were made in a quarter-strength Ringer's solution (Oxoid, Unipath, Basingstore, Hampshire, UK) and spread plated in duplicate on several media. *S. thermophilus* was counted in M17 agar (Biokar) whereas MRS agar (Biokar) was used for *L. acidophilus*. Both cultures were incubated aerobically at 37°C for 3 days, since the same counts for *L. acidophilus* in MRS are obtained in aerobic and anaerobic conditions (data not shown). Viable cell numbers of *B. bifidum* were counted on MRS-LP agar (Vinderola & Reinheimer, 1999) after anaerobic (Anaerocult A system, Merck) incubation for 3 days at 37°C. This medium

allows the selective colony count of *B. bifidum* in the presence of *L. acidophilus* and *S. thermophilus*.

2.4. Chemical analysis

Titratable acidity was measured according to Bradley et al. (1992) and the pH was determined by direct measurement with a MicropH 2001 pH meter (Crison Instruments S.A., Barcelona, Spain). Total solids and fat contents were determined according to Fontecha et al. (1990). The protein content was derived from total nitrogen using the Kjeldahl method (IDF standard 20B, 1993).

2.5. HPLC analysis of organic acids and lactose

The method described by González de Llano, Rodríguez and Cuesta (1996) was modified for the simultaneous determination of lactose and organic acids. Separations were performed isocratically at 0.7 mL min^{-1} and 65°C on a 300×7.8 mm ion-exchange column (HPX-87H Aminex) protected by a cation H + Microguard cartridge (Bio-Rad Laboratories, Richmond, CA, USA) using 3 mM H_2SO_4 as the mobile phase. Two detectors were connected in series and controlled by a software system Millenium 2010 (Waters Corporation, Milford, CA, USA): a Photodiode Array (Waters) 996 for the determination of organic acids, and a 410 Differential Refractometer (Waters) for quantification of lactose. Detection wavelength was set at 210 and 280 nm.

2.6. Sensory evaluation

Sensory evaluation was carried out after 24 days of cold storage by a panel of 20 trained members on fermented milk served at approximately 10°C. The odour, mouth-feel, taste, acidity and overall acceptability of samples were scored on a hedonic scale of 1–5.

2.7. Statistical analysis

Statistical analysis was performed by using the SPSS-PC + 4.0 software (SPSS Inc., Chicago, IL, USA). Data of microbiological counts, pH, acidity and organic acids content during manufacture [time 0, 2 h and end of manufacture (time taken to reach a pH 5.0)] and cold storage (fermented milk of 2, 7, 14, 21, 28, 35, 42 and 49 d) were subjected to ANOVA using milk treatment as factor with two or three categories according to the controls used: product made from carbonated milk, from non-acidified milk and from milk acidified with lactic acid. The LSD test ('less significant difference') was applied for means comparison when appropriate (Snedecor & Cochran, 1980). Sensory scores were also subjected to ANOVA test with CO_2 treatment as factor with two categories: carbonated and non-acidified samples.

3. Results and discussion

3.1. Milk composition

Raw milk used for the manufacture of fermented milk had an initial average protein content of 2.9%, fat content of 4.3% and solid content of 12.36%. The initial pH of milk (6.84 ± 0.03) decreased to 6.63 ± 0.05 and the acidity (0.144 ± 0.004 g acid 100 mL^{-1}) increased to 0.181 ± 0.009 g acid 100 mL^{-1} after pasteurisation. Just before the inoculation, carbonated and lactic acidified milks had pH values of 6.31 ± 0.04 and 6.28 ± 0.02 and an acidity of 0.289 ± 0.029 and 0.22 ± 0.04 g acid 100 mL^{-1}, respectively. For the non-acidified control milk the values of pH and acidity were 6.71 ± 0.03 and 0.180 ± 0.011 g acid 100 mL^{-1}, respectively. This slight increase of pH in non-acidified milk samples could probably be attributed to a partial solubilisation of the calcium phosphate initially precipitated by pasteurisation during the overnight storage of milk before the inoculation.

3.2. Chemical and microbiological evolution

Fig. 1 shows the microbiological profiles of carbonated and non-acidified AT and ABT fermented milk. The behaviour of microorganisms in our study was similar to that previously reported by others (Dave & Shah, 1997; Kneifel, Jaros & Erhard, 1993; Shah, Lankaputhra, Britz & Kyle, 1995). As expected, *B. bifidum* did not increase in number during manufacture of ABT fermented milk although the counts of this microorganism slightly increased between the end of fermentation and the second day of refrigerated storage (Fig. 1b). However, the population of *S. thermophilus* and *L. acidophilus* increased in AT and ABT products during manufacture (Fig. 1a and b). Between the end of this period and the 7th day of refrigerated storage, counts of *L. acidophilus* slightly increased or remained stable, reaching a level of around 7-log CFU mL^{-1}. Generally, after several days of refrigeration the counts of *S. thermophilus*, *L. acidophilus* and *B. bifidum* decreased slowly throughout the remaining storage period. With respect to the CO_2 treatment, no differences were obtained during the manufacture of ABT fermented milks ($P > 0.05$) for *B. bifidum* among non-acidified, carbonated and lactic acidified samples. There were also no differences in microbial counts of *B. bifidum* during cold storage found between carbonated and non-acidified milk ($P > 0.05$). It is interesting to note that for both AT and ABT products, the levels of *L. acidophilus* and *S. thermophilus* in carbonated and lactic acidified milk were similar (data not shown) and slightly higher than in non-acidified samples after two hours of incubation although no significant differences ($P > 0.05$) were found during the manufacture. These results indicated that the acidification of milk (either by carbonic or lactic

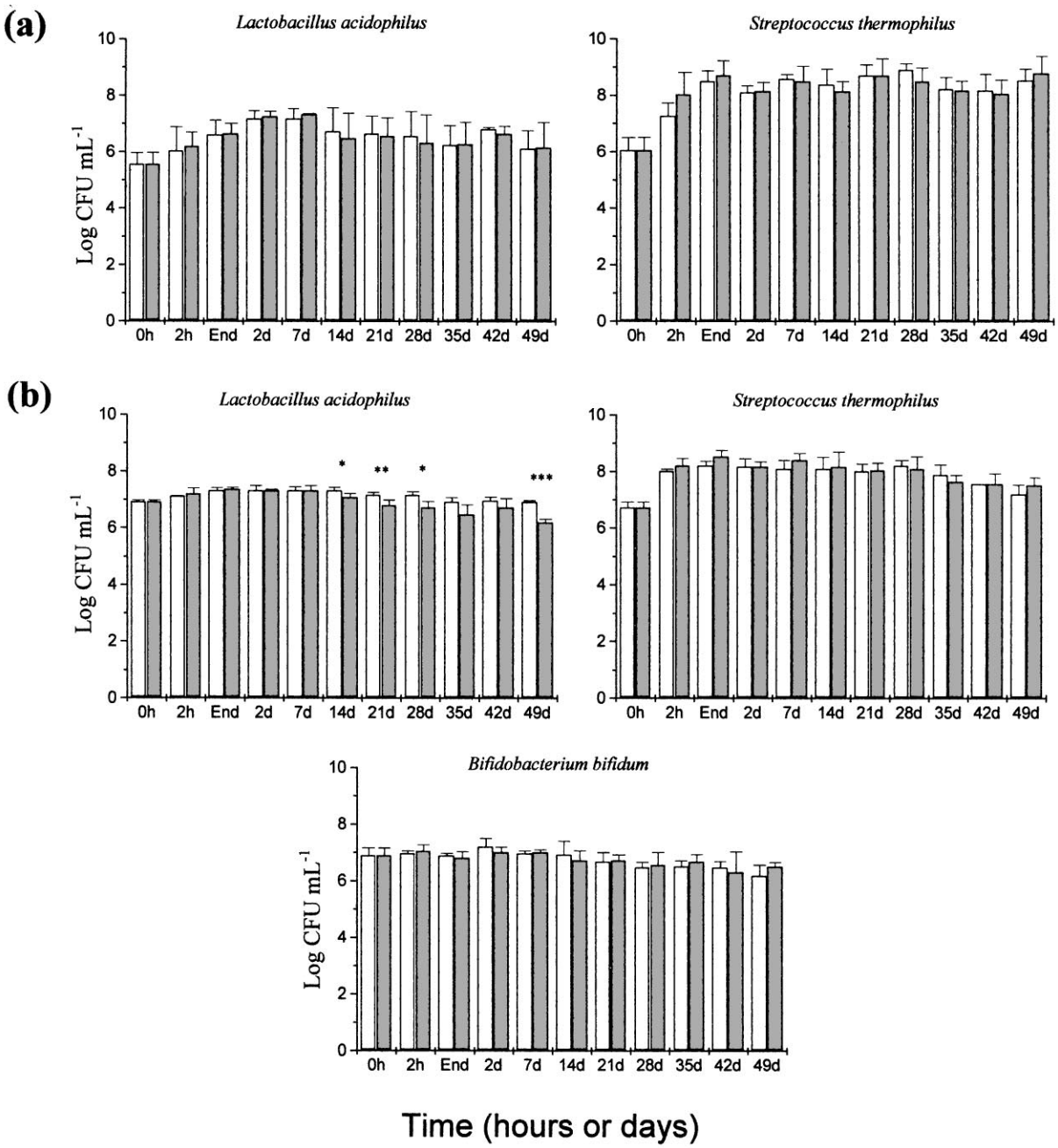

Fig. 1. Evolution of cell counts of *L. acidophilus* LaA3, *S. thermophilus* St73 and *B. bifidum* BBI during manufacture (hours) and cold storage (days) in non-acidified control (□) and carbonated (■) samples of AT (a) and ABT (b) fermented milk (*, ** and *** mean significant differences at levels of $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively). Vertical lines on the bars represent standard deviations.

acid) exerted a slight growth stimulation on *L. acidophilus* and *S. thermophilus*. On the other hand, similar counts for *L. acidophilus* and *S. thermophilus* were obtained through the cold storage in AT products made from carbonated and non-acidified milk (Fig. 1a). Conversely, in ABT products made with carbonated milk the counts of *L. acidophilus* from the 14th day of refrigeration were significantly lower ($P < 0.05$) than in the corresponding non-acidified controls although the recommended level of 10^6 viable cells g^{-1} for this species was maintained throughout the storage at 4°C. These results indicated that the CO_2 did not have any influence on the viability during refrigeration of *S. thermophilus* and *L. acidophilus* in AT fermented milk types but the presence of this gas was associated to a lower viability of *L. acidophilus* in ABT products. This fact will be further analysed.

During the manufacture of AT and ABT products, as a result of the activity of microorganisms, the pH

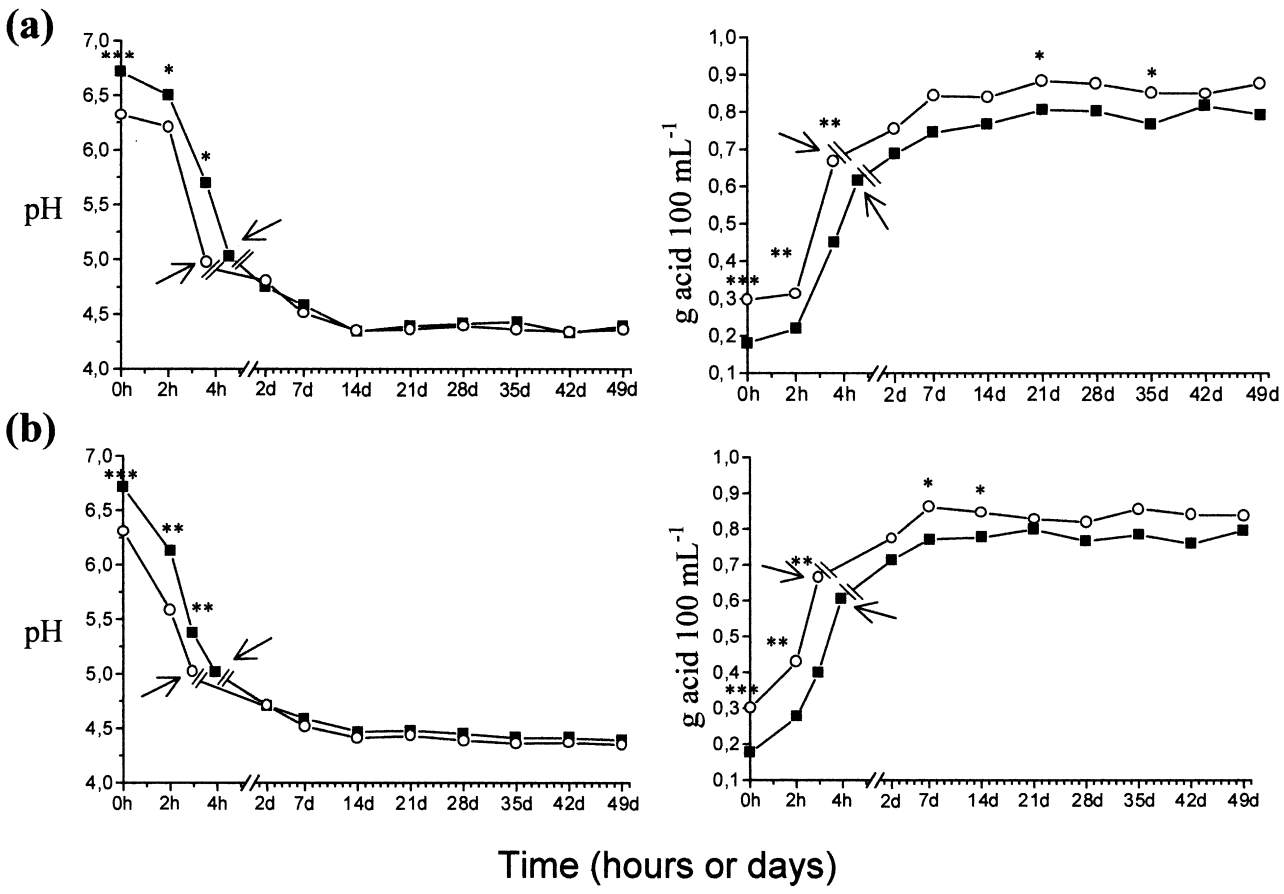

Fig. 2. Evolution of pH and acidity during manufacture (hours) and cold storage (days) in non-acidified control (■) and carbonated (○) samples of AT (a) and ABT (b) fermented milk (*, ** and *** mean significant differences at levels of $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively). Arrows indicate the end of fermentation (time taken to reach a pH 5.0).

dropped and the acidity rose in both non-acidified and carbonated milk (Fig. 2). The decrease in pH and increase in acidity slowed down during the first few days of cold storage of the product and these two parameters remained stable afterwards. Regarding the CO_2 treatment, at 2 h of fermentation the pH was lower ($P < 0.05$) and the acidity higher ($P < 0.01$) in AT and ABT products made from carbonated and lactic acidified milk (mean pH values 5.58–6.21 and 5.70–6.11 and mean acidity values 0.43–0.31 and 0.39–0.30 g acid 100 mL^{-1} for carbonated and lactic acidified milk, respectively) than in those made from non-acidified milk (mean pH values 6.13–6.50 and mean acidity values 0.20–0.22 g acid 100 mL^{-1}). Thus, carbonic or lactic acid added to milk promoted greater acidity and lowered pH at early stages of manufacture in fermented milk. On the other hand, no differences of pH values were found ($P > 0.05$) through the cold storage between non-acidified and carbonated samples although the acidity was greater in both types of fermented milk when carbonated milk rather than non-acidified milk was used.

3.3. Organic acids and lactose

The evolution of organic acids and lactose contents of raw milk to the end of refrigeration of carbonated and non-acidified fermented milks are shown in Table 1. The increase of lactose content in pasteurised milk with respect to the initial levels in raw milk was due to the supplementation with skim milk powder before the pasteurisation to improve the firmness of the final product. A 5.15–5.30% of residual lactose was present in fermented milk just after manufacture. During the first two days of cold storage lactose content decreased to 4.80–4.90 and 4.56–4.60% in AT and ABT products, respectively, due to the residual metabolic activity of the starter. This amount slowly decreased to values comprised between 4.00 and 4.30% through the remaining storage period and no differences were detected due to the CO_2 treatment.

In AT- and ABT-fermented milk minor variations were found from the start of fermentation to the end of cold storage on the content of orotic (mean values between 8.73–10.11 and 6.76–8.71 mg 100 mL^{-1}, respectively),

Table 1
Concentrations of lactose and several organic acids (mean ± SD, $n = 3$) in fermented milk type AT (*Streptococcus thermophilus*/*Lactobacillus acidophilus*) and ABT (*Streptococcus thermophilus*/*Lactobacillus acidophilus*/*Bifidobacterium bifidum*) during the manufacture and cold storage

Type	Time	Treatment	Lactose (g 100 mL^{-1})	Organic acids (mg 100 mL^{-1})		
				Pyruvic	Lactic	Acetic
AT	Raw milk		5.57 ± 0.27	1.09 ± 0.29	[a]	1.45 ± 0.48
	Supplemented milk	Non-acidified	6.44 ± 0.11	1.21 ± 0.28	[a]	1.66 ± 0.41
		Carbonated	6.53 ± 0.13	1.15 ± 0.23	[a]	1.63 ± 0.66
	End of fermentation	Non-acidified	5.30 ± 0.03	6.66 ± 2.08	745.48 ± 0.60	6.61 ± 1.70
		Carbonated	5.17 ± 0.24	7.32 ± 1.08	826.85 ± 82.04	5.31 ± 0.65
	Cold storage 2 d	Non-acidified	4.80 ± 0.28	7.05 ± 0.21	814.25 ± 68.09	7.25 ± 2.05
		Carbonated	4.90 ± 0.14	7.00 ± 0.14	892.75 ± 67.17	6.20 ± 1.84
	Cold storage 7 d	Non-acidified	4.43 ± 0.07	7.45 ± 0.64	875.72 ± 10.05	6.65 ± 1.80
		Carbonated	4.23 ± 0.51	6.63 ± 2.06	900.82 ± 103.59	6.51 ± 1.54
	Cold storage 28 d	Non-acidified	4.21 ± 0.37	5.49 ± 1.04	837.67 ± 95.39	7.29 ± 2.36
		Carbonated	4.15 ± 0.23	4.56 ± 1.30	841.00 ± 56.23	6.38 ± 1.79
	Cold storage 49 d	Non-acidified	4.00 ± 0.08	3.48 ± 0.56	721.75 ± 22.09	6.01 ± 4.13
		Carbonated	4.28 ± 0.06	3.40 ± 0.35	867.55 ± 122.12	6.49 ± 3.03
ABT	Raw milk		5.25 ± 0.17	0.68 ± 0.06	[a]	0.90 ± 0.21
	Supplemented milk	Non-acidified	6.34 ± 0.24	0.99 ± 0.27	[a]	0.96 ± 0.02
		Carbonated	6.33 ± 0.22	1.00 ± 0.24	[a]	1.11 ± 0.06
	End of fermentation	Non-acidified	5.11 ± 0.24	7.39 ± 0.31	700.58 ± 60.48	8.29 ± 0.56
		Carbonated	5.16 ± 0.17	8.11 ± 0.78	723.26 ± 65.57	6.91 ± 0.26[b]
	Cold storage 2 d	Non-acidified	4.56 ± 0.32	6.73 ± 0.47	861.93 ± 34.43	9.36 ± 0.55
		Carbonated	4.60 ± 0.10	7.36 ± 0.06	905.90 ± 29.29	7.77 ± 0.57[b]
	Cold storage 7 d	Non-acidified	4.44 ± 0.23	7.22 ± 0.36	876.27 ± 64.94	10.18 ± 0.76
		Carbonated	4.32 ± 0.17	7.79 ± 0.16	914.18 ± 20.57	7.96 ± 0.98[b]
	Cold storage 28 d	Non-acidified	4.33 ± 0.15	5.37 ± 0.84	798.92 ± 54.03	10.22 ± 0.72
		Carbonated	4.18 ± 0.21	4.52 ± 0.36	763.11 ± 31.68	8.32 ± 0.57[b]
	Cold storage 49 d	Non-acidified	4.11 ± 0.15	3.07 ± 1.89	724.86 ± 15.90	10.42 ± 1.48
		Carbonated	4.03 ± 0.08	2.50 ± 1.21	703.08 ± 56.03	8.20 ± 1.42

[a] Not detected.
[b] $P < 0.05$.

citric (mean values between 255.94–265.17 and 232.10–248.45 mg 100 mL^{-1}, respectively), uric (mean values between 3.29–4.63 and 4.06–5.09 mg 100 mL^{-1}, respectively) and hippuric acids (mean values between 2.18–2.31 and 1.75–1.94 mg 100 mL^{-1}, respectively). No significant differences ($P > 0.05$) in the content of these acids were found between products made from carbonated and non-acidified milk (data not shown). On the other hand, pyruvic, acetic and lactic acid contents (Table 1) increased during fermentation and until the 7th day of refrigeration and modified slightly afterwards. The decrease of lactic acid upon storage, especially in the ABT products, should be attributed to its conversion by lactic acid bacteria to other compounds such as acetic acid. Interestingly, during fermentation and refrigeration of AT fermented milk and until the 7th day of storage of ABT products the lactic acid levels in carbonated samples were slightly higher than in the non-acidified controls, although no significant differences were obtained ($P > 0.05$). This indicates that the CO_2 slightly enhanced the metabolic activity and production of lactic acid by the starters probably as a consequence of the increased acidity and lowered pH produced by the carbonic acid formed. Contrary to that, it has been previously reported that growth and acid production of mesophilic (Calvo et al., 1993; Montilla et al., 1995) and certain thermophilic starters (Champagne et al., 1998) were slightly retarded by the presence of CO_2. However, at prolonged incubation times the acidification capacity of mesophilic starters did not seem to be affected by the presence of CO_2 (Ruas-Madiedo et al., 1998a).

Smaller amounts of acetic acid were detected in ABT products manufactured with carbonated milk with respect to those made from non-acidified milk. Taking into account that *B. bifidum* is the main acetic acid producer in ABT fermented milk, lower levels of this acid in

Table 2
Manufacture time (measured from the inoculum addition until a pH 5.0 was reached) for AT and ABT fermented milk elaborated with non-acidified, acidified with lactic acid and carbonated milk[a]

Fermented milk	Treatment	Time (min)[a]
AT	Non-acidified	275.00 ± 39.50^{b}
	Carbonated	217.50 ± 10.61^{a}
	Acidified with lactic acid	220.00 ± 7.40^{a}
ABT	Non-acidified	235.00 ± 17.79^{b}
	Carbonated	177.50 ± 26.30^{a}
	Acidified with lactic acid	189.80 ± 16.85^{a}

[a]Means with different letters in the same group differ significantly ($P < 0.05$)

products made from carbonated milk could probably indicate an inhibitory effect of this gas on the metabolic activity of this microorganism. On other hand, Marshall (1991) indicated that the growth of *L. acidophilus* is enhanced by acetate. Thus, the higher acetate concentrations in ABT non-acidified milk with respect to the carbonated ones could have been the reason for a major survival of *L. acidophilus* in the former products.

3.4. Manufacture time

The manufacture time significantly lowered in AT ($P < 0.05$) and ABT ($P < 0.01$) products elaborated with carbonated milk and with milk acidified with lactic acid with respect to those made from non-acidified milk (Table 2). The initial lowered pH of carbonated and lactic acidified samples together with a slightly enhanced growth and metabolic activity of the starter caused by this low pH could be the reasons for this time reduction. Thus, the more rapid fermentation in carbonated samples could be attributed to the lowered pH caused by carbonic acid in milk and not to a specific effect of the CO_2.

3.5. Sensory analyses

In order to elucidate whether the presence of CO_2 and the differences in *L. acidophilus* and acetic acid levels present through the refrigerated storage could adversely modify the sensory properties of ABT fermented milk, sensory evaluation of these products was carried out at the end of the legal period of cold storage (24 days) (Table 3). Panellists did not detect any significant difference between the products made from carbonated and non-acidified milk, thus indicating that the use of milk acidified with CO_2 had no detrimental effects on the sensory properties of ABT fermented milk. Moreover, mean scores for mouth-feel, taste, acid acceptability and overall acceptability were slightly higher in samples manufactured from carbonated milk than in the corresponding controls.

4. Conclusions

Fermentation times were significantly lowered when CO_2 or lactic acid were added to milk for the manufacture of AT (*S. thermophilus*/*L. acidophilus*) and ABT (*S. thermophilus*/*L. acidophilus*/*B. bifidum*) fermented milk. The more rapid fermentation in carbonated fermented milks could be attributed to the lowered pH caused by carbonic acid formed from the CO_2 and not to a specific effect of this gas. The CO_2 did not exert any influence on the viability of *S. thermophilus* and *L. acidophilus* in AT products stored at 4°C but its presence was associated to a lower viability of *L. acidophilus* during the refrigeration of ABT products. In spite of that, during refrigeration the levels of *S. thermophilus*, *L. acidophilus* and *B. bifidum* remained higher than the minimum suggested levels and the use of CO_2 had no detrimental effects on the sensory properties of ABT fermented milk. Therefore, the carbonation of pasteurised milk can be satisfactorily used before the manufacture of fermented milk to reduce the fermentation time.

Acknowledgements

This work was financially supported by the European Union FEDER founds and Plan Nacional de I + D (project IFD97-0346) and by a Contract with the S.E. de Carburos Metálicos S.A. C.G. Vinderola was a short-time postgraduate fellow from the AECI (Agencia Española de Cooperación Internacional), M. Gueimonde was funded by a shared grant from CSIC and S.E. de Carburos Metálicos (Beca para Formación y Especial-

Table 3
Grading scores (Mean ± SD, $n = 3$) of ABT fermented milk (*Streptococcus thermophilus*/*Lactobacillus acidophilus*/*Bifidobacterium bifidum*) after 24 days of cold storage

Treatment	Odour	Mouth-feel	Taste	Acid acceptability	Overall acceptability
Non-acidified	2.95 ± 0.76	3.00 ± 0.86	2.85 ± 0.99	2.90 ± 0.79	3.00 ± 0.86
Carbonated	2.80 ± 1.06	3.35 ± 0.81	2.95 ± 1.05	3.15 ± 1.09	3.10 ± 1.12

ización en Líneas de Investigación de Interés para el Sector Industrial) and T. Delgado was the recipient of a contract of the CSIC (Contrato de Incorporación de Doctores a Equipos de Investigación en España).

We acknowledge J. Carlos Bada for his help and valuable comments during this work. We thank the members of the taste panel for their sensory assessment of fermented milk, as well as Manuel Matilla and M. José González for their excellent technical assistance.

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