Lactic acid production and rheological properties of yogurt made from milk acidified with carbon dioxide

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Abstract: The influence of milk acidification up to pH 6.0 with CO_2 on D- and L-lactic acid production and lactose consumption by yogurt starter, changes in the pH, and rheological and sensory properties of yogurt were studied. A slight influence of CO_2 on lactic acid production during yogurt manufacture was detected. No significant changes in lactic acid concentration were observed during storage, although the final concentration was significantly lower in control than in pH 6.2 and 6.0 acidified samples. A great influence of CO_2 on D-lactic acid production was not observed. Yogurt manufactured from milk with lower pH values showed lower final pH values after 7 days of storage. The viscosity was similar in all analysed samples. No significant differences in sensory characteristics between unacidified and acidified yogurts were detected.

Keywords: yogurt; CO_2-acidified milk; lactic acid; rheological properties

INTRODUCTION

The inhibiting effect of CO_2 on the growth of micro-organisms is well recognised.[1] Although it has been much discussed what types of mechanisms are responsible for the growth inhibitory activity of CO_2, it is difficult to explain the mechanism of inhibition.[2]

Inhibition of growth of psychrotrophic bacteria by addition of CO_2 in refrigerated milk has been reported;[1,3–5] this effect could be used to increase the time of storage of raw milk prior to processing in the dairy industry. The method proposed for extending the storage life of milk involves the application of CO_2 until a final concentration of 10–30 mM[1,6] which results in a final milk pH in the range 6.4–6.0.[4,7]

The treatment of milk with CO_2 can affect the sensory properties of milk.[5,8] Previous results showed that sensory properties were affected when milk was acidified to pH 6.2 before heat treatment. However, they were not impaired when CO_2-treated milk was degassed prior to heat treatment.[5]

Since the proposed method of preservation can affect the sensory properties of milk, it would not be adequate for milk destined for liquid consumption. However, this method could be used as a means of preserving the quality of milk destined for the manufacture of dairy products such as cheese or yogurt. McAfee *et al*[9] found that 50% less rennet was required to coagulate CO_2-treated milks, and carbonation did not affect the cheese yield. Montilla *et al*[10] reported that cheese manufactured from CO_2-treated milks with a final pH value of 6.0 showed less proteolysis than the control cheese, but significant differences in sensory characteristics between cheeses were not detected.

CO_2-treated milk could be used in yogurt manufacture as well. However, previous studies[10] showed that acidification reduced the amount of lactic acid produced by a starter containing *Lactococcus lactis* subsp *lactis* and *Lactococcus lactis* subsp *cremoris* (70:30) and that CO_2 acidification could reduce the lactic acid production of the starter used for yogurt. On the other hand, Driessen *et al*[11] have reported that *Lactobacillus delbrueckii* subsp *bulgaricus* needs more than 32 mg $CO_2\,kg^{-1}$ milk for optimal growth. It is also known that *Lactobacillus delbruekii* subsp *bulgaricus* produces virtually only D-lactic acid whereas *Streptococcus thermophilus* produces predominantly L-lactic acid.[12–14]

D(−)-Lactic acid consumption has been considered harmful in human nutrition since it is metabolised much more slowly than the L(+) isomer. Taking this into account, the World Health Organisation recommended that the daily intake of D(−)-lactic acid must be limited to 100 mg kg^{-1} body weight. However, the WHO has now withdrawn its original recommendation. Only for infants in the first 3 months of life is the use of D(−)-lactic acid contraindicated in view of the risk of acidosis.[14] Renner[15] found that a consumption

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of yogurt exceeding 80 g day^{-1} by infants of less than 20 kg body weight may lead to excess intake of D-lactic acid. The FAO/WHO recommended a daily intake for infants of 20 mg D-lactic acid kg^{-1} body weight.[15]

The present work was undertaken to evaluate the manufacture and characteristics of yogurt made from milk acidified with carbon dioxide and whether pH changes of milk used for yogurt manufacture could affect the sensory properties of the final product such as viscosity and flavour properties.

MATERIALS AND METHODS

Samples

Raw milk from herds in the Central Region of Spain was used. The milk was skimmed by centrifugation and filtration through glass fibre pads. Milk samples were acidified to the pH range 6.4–6.0 by bubbling CO_2 through them.

A starter mixture containing *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus* (1:1) was used for yogurt manufacture.

Yogurt preparation

Skim milk (control) or the same milk acidified with CO_2 was used to prepare milk fortified with skim milk powder to give a total non-fat solids content of 13%.

The milk was heated in a water bath in tightly sealed Pyrex glass tubes (30 mm × 200 mm) at 85 °C for 30 min and immediately cooled in an ice water bath at 42 °C. 50 ml of the heated samples kept at 42 °C were inoculated with 0.3% of the starter solution. Incubation was carried out in a 42 °C water bath for 4 h. After that the yogurt was stored at 7 °C for 7 days.

Six different samples of control and CO_2-treated milk were used to manufacture the yogurts.

Starter activity

The starter activity was indirectly determined by measurement of the pH and the concentration of D- and L-lactic acid formed during the 4 h of incubation at 42 °C (elaboration time) and after 1, 3 and 7 days of storage at 7 °C.

In order to determine D- and L-lactic acid production, portions (10 ml) of samples were heated at 80 °C for 15 min to inactivate the starter. Caseins were removed by precipitation at pH 4.6 with 2 M HCl, and D- and L-lactic acid were quantified using an enzymatic test (Boehringer/Mannheim, Germany).

Lactose

One millilitre of yogurt was mixed with 0.5 ml of 50 mg l^{-1} phenyl-β-D-glucoside in 60% methanol. This mixture was diluted to 5 ml with methanol, maintained for 1 h at room temperature and filtered. Then 1 ml of filtrate was evaporated until dryness under vacuum at room temperature and converted to trimethylsilyl derivates using trimethylsilylimidazol as reported by Martínez-Castro and Olano.[16]

Gas chromatographic analysis was performed on a Sigma 3B gas chromatograph (Perkin Elmer) equipped with a 3 m × 1.0 mm id stainless steel column packed with 2% OV-17 in non-silanised 120/140 Volaspher A-2 (Merck). The temperature of the injector and detector was 300 °C. The analysis was performed using temperature programming from 200 to 270 °C at a heating rate of 5 °C min^{-1} with an initial holding time of 2 min.

Dynamic rheological measurement

Samples were analysed after 7 days of storage at 7 °C. Small-amplitude measurement was performed on a Haake Rs rheometer (Haake, Germany) using a cone and plate geometry (35 mm cone diameter and 4° cone angle). A stress sweep test was conducted at a frequency of 0.1 Hz to determine the linear viscosity range.

The complex viscosity (n^*), the storage modulus (G'), the loss modulus (G'') and the complex modulus (G^*) were determined in the frequency range 0.0464–1 Hz at 20 Pa in the linear range. All measurements were carried out at 15 °C.

Sensory evaluation

Sensory analysis of the yogurt stored for 7 days was carried out by a sensory panel of 15 trained members, following the triangle test procedure (ISO Standard 4120). In each experiment, control yogurt was compared with yogurts from CO_2-treated milk. Panellists were presented with randomised samples of three yogurts, distributed so that in each group two samples were the same and the other was different. Panellists were asked to identify the odd sample. Results were subjected to a *t*-test for statistical analysis.

Statistical analysis

Analysis of variance was applied using the BMDP program P2V[17] with a CDC Cyber 180/855 computer in order to evaluate the influence of CO_2 acidification and storage time on D- and L-lactic acid production and on the final pH of yogurt samples. A regression analysis was performed to establish a relationship between the final pH value and the manufacture time.

RESULTS AND DISCUSSION

Influence of acidification on production of D- and L-lactic acid by yogurt starter

The amount of L-lactic acid increased significantly ($P < 0.05$) during manufacture time (4 h); only slight differences were found between samples with different initial pH (Table 1). These results are in disagreement with those reported by Calvo *et al*,[18] who studied CO_2-acidified milk samples by incubation at 35 °C for 140 min with a cheese starter containing *Lactococcus lactis* subsp *lactis* and *Lactococcus lactis* subsp *cremoris* (70:30). The differences could be due to the different influence of CO_2 on the metabolism of the various micro-organisms.

A significant increase was observed after 1 day of

Table 1. L-Lactic acid formation during the elaboration and storage of yogurts manufactured from milk (control) and milk acidified at pH 6.4, 6.2 and 6.0 with carbon dioxide

	L-Lactic acid ($g\,kg^{-1}$)			
Time	Control	pH 6.4	pH 6.2	pH 6.0
1 h	$0.69^{a,A}$ (0.10)*	$0.55^{a,A}$ (0.08)	$0.59^{a,A}$ (0.11)	$0.66^{a,A}$ (0.18)
2 h	$2.32^{a,B}$ (0.33)	$1.74^{b,B}$ (0.11)	$1.77^{b,B}$ (0.13)	$2.17^{a,B}$ (0.43)
3 h	$3.33^{a,C}$ (0.26)	$3.64^{a,b,C}$ (0.45)	$3.54^{a,b,C}$ (0.29)	$4.17^{b,c,C}$ (0.82)
4 h	$4.72^{a,D}$ (0.41)	$5.19^{a,b,D}$ (0.58)	$4.56^{a,c,D}$ (0.36)	$5.17^{a,b,D}$ (0.44)
1 day	$5.37^{a,E}$ (0.35)	$5.63^{a,D,E}$ (0.20)	$6.10^{b,E}$ (0.35)	$6.04^{b,E}$ (0.29)
3 days	$5.44^{a,E}$ (0.62)	$5.93^{a,E,F}$ (0.58)	$5.86^{a,E}$ (0.39)	$6.07^{a,E}$ (0.41)
7 days	$5.07^{a,E}$ (0.27)	$5.41^{a,b,D,F}$ (0.42)	$5.50^{b,c,E}$ (0.23)	$6.02^{c,E}$ (0.30)

* Mean (standard deviation), $n = 6$.
$^{a–c}$ Means in the same row without a common superscript differ ($P < 0.05$).
$^{A–F}$ Means in the same column without a common superscript differ ($P < 0.05$).

Table 2. D-Lactic acid formation during the elaboration and storage of yogurts manufactured from skim milk (control) and skim milk acidified at pH 6.4, 6.2 and 6.0 with carbon dioxide

	D-Lactic acid ($g\,kg^{-1}$)			
Time	Control	pH 6.4	pH 6.2	pH 6.0
1 h	$0.04^{a,A}$ (0.02)*	$0.03^{a,A}$ (0.01)	$0.04^{a,A}$ (0.01)	$0.04^{a,A}$ (0.00)
2 h	$0.26^{a,B}$ (0.03)	$0.27^{a,B}$ (0.03)	$0.18^{a,B}$ (0.08)	$0.23^{a,B}$ (0.09)
3 h	$0.30^{a,B}$ (0.09)	$0.35^{a,B}$ (0.10)	$0.26^{a,b,B}$ (0.12)	$0.43^{a,c,B}$ (0.03)
4 h	$0.31^{a,B}$ (0.07)	$0.58^{b,C}$ (0.10)	$0.66^{b,C}$ (0.15)	$0.69^{b,B,C}$ (0.15)
1 day	$0.54^{a,C}$ (0.12)	$0.56^{a,C}$ (0.08)	$0.79^{b,C,D}$ (0.13)	$0.85^{b,C}$ (0.19)
3 days	$0.60^{a,C}$ (0.15)	$0.62^{a,C}$ (0.15)	$0.83^{a,b,D}$ (0.20)	$0.94^{b,c,C}$ (0.27)
7 days	$0.69^{a,C}$ (0.22)	$0.64^{a,C}$ (0.08)	$0.85^{a,D,E}$ (0.16)	$1.00^{b,C,D}$ (0.23)

* Mean (standard deviation), $n = 6$.
$^{a–c}$ Means in the same row without a common superscript differ ($P < 0.05$).
$^{A–E}$ Means in the same column without a common superscript differ ($P < 0.05$).

Table 3. Final pH values from the elaboration and storage of yogurts manufactured from skim milk (control) and skim milk acidified at pH 6.4, 6.2 and 6.0 with carbon dioxide

	Final pH value			
Time	Control	pH 6.4	pH 6.2	pH 6.0
1 h	$6.26^{a,A}$ (0.04)*	$6.13^{b,A}$ (0.06)	$6.04^{c,A}$ (0.07)	$5.93^{d,A}$ (0.07)
2 h	$5.89^{a,B}$ (0.05)	$5.92^{a,B}$ (0.14)	$5.86^{a,A}$ (0.13)	$5.63^{b,B}$ (0.23)
3 h	$5.24^{a,C}$ (0.16)	$5.23^{b,C}$ (0.24)	$5.12^{a,B}$ (0.29)	$4.88^{a,C}$ (0.07)
4 h	$4.61^{a,D}$ (0.11)	$4.57^{a,b,D}$ (0.17)	$4.49^{a,b,C}$ (0.21)	$4.36^{b,c,D}$ (0.23)
1 day	$4.41^{a,E}$ (0.15)	$4.40^{a,E}$ (0.16)	$4.39^{a,D}$ (0.17)	$4.24^{a,D,F}$ (0.23)
3 days	$4.23^{a,F}$ (0.16)	$4.19^{b,F,G}$ (0.15)	$4.20^{c,D}$ (0.23)	$4.13^{a,D,F}$ (0.07)
7 days	$4.27^{a,G}$ (0.03)	$4.26^{a,E,G}$ (0.03)	$4.20^{b,D}$ (0.02)	$4.08^{c,E,F}$ (0.23)

* Mean (standard deviation), $n = 6$.
$^{a–d}$ Means in the same row without a common superscript differ ($P < 0.05$).
$^{A–G}$ Means in the same column without a common superscript differ ($P < 0.05$).

storage; however, no significant changes were observed during the 7 days of storage under refrigeration. After 7 days of storage the final concentration of L-lactic acid was significantly higher in pH 6.2 and 6.0 acidified (5.5 and $6.0\,g\,kg^{-1}$ respectively) than in control ($5.1\,g\,kg^{-1}$) samples. The final concentration of L-lactic acid was similar to that found by Puhan *et al*[19] ($6.7\,g\,kg^{-1}$) after yogurt incubation for 15–20 days. They also found no influence of storage time on L-lactic acid concentration The final concentration of L-lactic acid was also similar to that found by Blumental and Helbling.[20] Benner[12] observed that L-lactic acid developed rapidly during incubation, reaching a maximum after approximately 4 h and remaining almost constant thereafter.

During manufacture time the D-lactic acid concentration increased significantly ($P < 0.05$) only during the first hour (Table 2). After 3 h the D-lactic acid concentration increased with decreasing pH value of the milk. This could be due to the beneficial influence of CO_2 on *Lactobacillus delbrueckii* subsp *bulgaricus* growth. However, a significant increase in D-lactic acid production was not observed during storage. After 7 days of storage the concentration was significantly ($P < 0.05$) lower in control ($0.69\,g\,kg^{-1}$) than in pH 6.2 and 6.0 acidified (0.85 and $1.0\,g\,kg^{-1}$ respectively) samples.

In general, a low concentration of D-lactic acid was found. This can only be partly attributed to the low recovery of this acid (50–70%) when the Boehringer/Mannheim enzymatic UV test kit is used, as described by Mosso *et al.*[21]

Changes in pH

Changes in the pH of yogurts from control and acidified samples were also studied (Table 3). The difference between the initial pH of the sample and the value of pH after 1 h of manufacture was significantly higher in control than in acidified samples. All samples showed a significant pH decrease during manufacture time.

The final pH after manufacture time was significantly ($P < 0.05$) lower in samples with a lower initial pH value. However, the difference between the initial and final pH values was higher in the yogurt from control milk than in those from acidified milk.

From the results of each initial pH a linear regression analysis was performed to establish a possible relationship between the pH and the incubation time. The slopes of each equation were compared to establish a possible influence of the initial pH on the changes in this parameter during the incubation time. The equations obtained for each initial pH were (with correlation coefficients in parentheses):

- control milk

$$y = 6.76 - 0.514x \quad (R^2 = 0.984)$$

- milk acidified at pH 6.4

$$y = 6.56 - 0.457x \quad (R^2 = 0.946)$$

- milk acidified at pH 6.2

$$y = 6.41 - 0.453x \quad (R^2 = 0.912)$$

- milk acidified at pH 6.0

$$y = 6.22 - 0.432x \quad (R^2 = 0.922)$$

where x is the final pH of the milk and y is the time (in hours) of yogurt manufacture.

A statistical study of the slopes obtained from the different equations showed no differences between them, indicating no influence of the initial pH on the changes in pH during the incubation time.

Slight changes were observed during storage. After 7 days the pH values were 4.27, 4.26, 4.20 and 4.08 for control milk and milk acidified at pH 6.4, 6.2 and 6.0 respectively; a significant ($P < 0.05$) difference in the pH value was found between control and pH 6.2 and 6.0 acidified milk samples. Although acidified samples showed a lower final pH, the difference between the pH of the milk and the final pH of the yogurt was significantly higher in control than in acidified milk yogurts. These results are in disagreement with the lactic acid production; this could be due to the differences in Ca^{2+} activity, taking into account the low solubility of the calcium carbonate formed during CO_2 addition.

Changes in lactose concentration

The remaining lactose concentration was determined after manufacture (4 h) and after 7 days of storage. The concentration of lactose after manufacture was 38.8, 38.3, 38.6 and 37.1 $g l^{-1}$ in the control and in samples acidified to pH 6.4, 6.2 and 6.0 respectively, and for the same initial pH the lactose concentration after

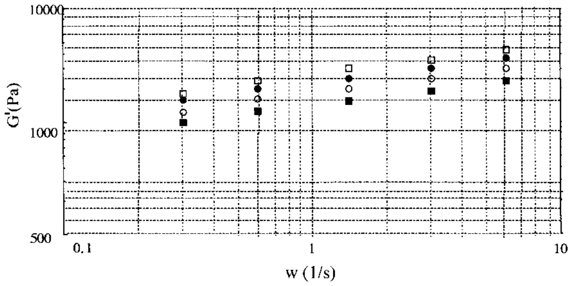

Figure 1. Storage modulus (G') versus frequency for yogurts manufactured from milk (control) (□) and milk acidified at pH 6.4 (●), 6.2 (○) and 6.0 (■) with carbon dioxide.

7 days of storage was 29.7, 29.7, 29.3 and 27.7 ($g l^{-1}$) respectively. In all cases, six different samples were analysed and no significant differences were found between control and acidified samples. However, in all cases a significant decrease in lactose concentration was observed after storage.

Loss of lactose has to be taken into account, since lactic acid bacteria not only produce lactic acid from lactose but also flavour compounds and polysaccharides during storage.

Rheological properties

The viscosity of yogurts was determined after 7 days of storage. Frequency sweep profiles were determined at 20 Pa in the linear range observed with the stress sweep profiles. All samples showed a decrease in the complex viscosity (n^*) while the storage modulus (G') and loss modulus (G'') increased with increasing frequency (Figs 1 and 2). The values of G' were higher than those of G'' at any given frequency; this is the typical behaviour of yogurts.[22]

The storage and loss moduli were higher in control than in acidified samples; the values of G' and G'' showed a slight increase with decreasing pH. Although these parameters are indicative of the firmer characteristics of yogurts, the obtained differences between samples were very low.

Sensory evaluation

Sensory analysis of the yogurt stored for 7 days following the triangle test procedure showed no significant ($P < 0.05$) differences between control and acidified

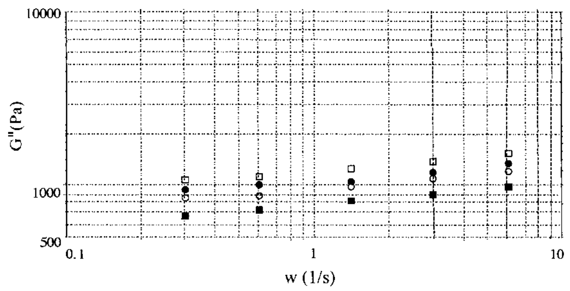

Figure 2. Loss modulus (G'') versus frequency for yogurts manufactured from milk (control) (□) and milk acidified at pH 6.4 (●), 6.2 (○) and 6.0 (■) with carbon dioxide.

yogurts. This indicated that CO_2-treated milk could be used for yogurt manufacture.

CONCLUSIONS

As was indicated in the Introduction, the treatment of milk with CO_2 could be used to increase the time of storage of raw milk prior to processing in the dairy industry; however, the treated milk cannot be used for liquid consumption owing to changes in its sensory properties.

Although previous studies have shown that the CO_2 acidification of milk influences the lactic fermentation of some cheese starters,[18] the results obtained in this paper show that the influence of CO_2 treatment of milk on lactic acid production by *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus* is low. Also D-lactic acid production by the starter is lower than expected.

After 7 days of yogurt incubation, significant ($P < 0.05$) differences were found between those made from unacidified milk and CO_2-acidified milk at pH 6.2 and 6.0. Although acidified samples showed a lower final pH, the difference between the pH of the milk and the final pH of the yogurt was significantly higher in control than in acidified milk yogurts. These results could be due to the differences in Ca^{2+} activity, taking into account the low solubility of the calcium carbonate formed during CO_2 addition.

As has been indicated, significant differences were found between milk samples; however, these differences have no influence on the rheological properties of the yogurt.

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