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EFFECT OF PRESSURIZED CARBON DIOXIDE ON THE QUALITY CRITERIA OF AYRAN

BASINÇLI KARBONDİOKSİT UYGULAMASININ AYRANIN KALİTE KRİTERLERİ ÜZERİNE ETKİSİ

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ABSTRACT: The effect of pressurized carbon dioxide (CO_2) on the chemical, microbiological and sensory properties of Ayran was investigated. Bulk Ayran was filled into bottles, injected prior to capping with pressurized CO_2 by a pilot injection system at 0.5 (A5), 1.0 (A10) and 1.5 (A15) MPa for one min, respectively, and kept at 5°C for seven days. Results indicated that the CO_2 concentration had no significant effect on the gross composition; however, serum separation was less in A5 and A10 on day 7 than in control and A15 ($P < 0.05$). The number of *Streptococcus thermophilus* decreased significantly with increasing CO_2 concentrations at the end of the storage ($P<0.05$), whereas the number of *L. delbrueckii* ssp. *bulgaricus* did not change. Sensory properties of Ayran were adversely affected by the pressurized CO_2 levels ($P < 0.05$).

Keywords: Ayran, yoghurt beverage, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, carbon dioxide, serum separation

ÖZET: Bu çalışmada basınçlı karbondioksit gazı enjeksiyonunun Ayran'ın kimyasal, mikrobiyolojik ve duyusal kalite kriterleri üzerine etkisi araştırılmıştır. Üretilen Ayran, şişelere doldurulduktan sonra dört gruba ayrılmış ve her bir gruba pilot bir enjeksiyon sistemi yardımıyla 1 dakika sureyle sırasıyla 0 (control), 0,5 (A5), 1.0 (A10) ve 1,5 (A15) MPa basınç altında CO_2 gazı enjekte edilmiştir. Ayran örnekleri daha sonra +5°C de 7 gün süreyle depolanmıştır. Elde edilen sonuçlar basınçlı CO_2 ilavesinin Ayran'ların genel bileşimleri üzerinde bir etkisi olmadığı; ancak, A5 ve A10 nolu örneklerinin serum ayrılmasının kontrol ve A15 nolu örneklerle kıyaslandığında serum ayrılmasını azalttığını göstermiştir. Artan CO_2 konsantrasyonu depolama sonunda *Streptococcus thermophilus*'un sayısını önemli derecede azalttıığı (P < 0.05), *Lactobacillus delbrueckii* ssp. *bulgaricus* sayısını ise değişmediği gözlenmiştir. Duyusal özellikler açısından, CO_2 ilave edilmiş Ayran örnekleri kontrole nazaran daha düşük puanlar almıştır (P <0.05).

Anahtar kelimeler: Ayran, yoğurt içeceği, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, karbondioksit, serum ayrılması

INTRODUCTION

Ayran is a traditional Turkish beverage and basically composed of yoghurt, water and salt. Owing to its acidic and salty taste, it is consumed as a refresher all year long, particularly, during the summer. Twenty to thirty percent of annual yoghurt production in Turkey was estimated to be used for Ayran production (1). Non alcoholic fizzy drinks are increasingly becoming popular in Turkey in that their consumption has increased more than four fold that of Ayran since 1990 (2). However, Ayran has more nutritional value than soft fizzy

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drinks as it contains milk constituents but lacks caffeine, artificial sweeteners or colourings (3, 4, 5). Therefore, developing Ayran as a nutritious carbonated beverage product may benefit consumer health for people of all ages domestically and internationally.

The role of carbon dioxide (CO_2) in yoghurt has been a subject of several studies in recent years as it is produced by yoghurt bacteria as a by-product of their respiration (6, 7). It was used in yoghurt milk as an acidifier with no significant effect on the formation of volatile organic compounds (8), lactic acid production, or rheological properties of the final product (9). Karagül-Yüceer *et al.* (10) supported these findings by demonstrating that carbonation did not alter the sensory properties of flavoured yoghurt after 21 days of storage. In addition, analyses of the microbial, chemical and sensory properties of yoghurt purged with CO_2 revealed that carbonation did not adversely affect the growth of *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium longum, Listeria monocytogenes*, and *Escherichia coli* during storage (11). Similarly, CO_2 did not exert any adverse influence on the viability of probiotic bacteria in AT (*S. thermophilus/L.acidophilus*) and ABT (*S. thermophilus/L. acidophilus/B. bifidum*) milks (12), and was also used as a preservative to increase the shelf life of yoghurt (13). Use of CO_2 in dairy products was reviewed recently (14).

In the above studies, carbonation of yoghurt was carried out in open systems, i.e. at atmospheric pressure. However, in order for Ayran to become a fizzy drink, pressurized CO_2 has to be injected into Ayran. The injection of pressurized CO_2 markedly altered the viability of different microorganisms depending on pressure and exposure time (15, 16). To our knowledge, there is a lack of information as to the effect of pressurized CO_2 on Ayran or yoghurt. This study, therefore, aims at investigating the effect of CO_2 injected at the pressures of 0.5, 1.0 and 1.5 MPa for 1 min on the chemical, microbiological and sensory properties of Ayran during seven days of storage of at 5°C.

MATERIALS and METHODS

Materials

For a standard milk base composition for Ayran production, a blend of whole milk powder and skimmed milk powder was used. The powders were supplied from ENKA Co. (Konya, Turkey) and stored at 2°C (Table 1). Yoghurt culture (YC-380), a blend of *S. thermophilus* and *L. delbrueckii* subsp*. bulgaricus,* was obtained from Peyma-Hansen Ltd. Co (Istanbul, Turkey); table salt from the local market; and food grade CO_2 gas from Müher Ltd. Sti. (Ankara, Turkey).

Table 1. Gross composition of skimmed milk powder and whole milk powder as reported by the supplier

	Total solids (g /100g)	Fat (g/100g)	Acidity (g/100g)	Solubility (ml)
Skimmed milk powder	96.8	0.8	0.14	99.8<
Whole milk powder	94.5	28.7	0.14	99.8<

Preparation of starter culture

The starter culture was designed as a direct-vat-system. For the exact amount for inoculation, a sachet of starter culture (~6-7g) was added into 500 ml of previously reconstituted and heat treated skim-milk (90°C/5 min) with total solids of 110g/kg at 43±2°C. The mixture was stirred continuously for 30 min to dissolve the culture powder completely, and the amount needed for inoculation was taken with a sterile pipette. The required amount of starter culture for Ayran production was determined with preliminary experiments so that the acidity of milk after incubation should reach pH of 4.4-4.6 within 4 to 4.5 h as recommended by the supplier.

Ayran production

Full fat Ayran (8% total solids and 1.5%fat) was produced according to Turkish Food Codex (17). 230 g of whole milk powder and 90 g skimmed milk powder were reconstituted in 3680 ml of deionized water in a stainless steel container (5 L) by a high-speed homogenizer (Ultraturrax, Type T45, Janke and Kunkel, IKA-Werk Labortechnik, Germany). The reconstituted milk was heated at 85°C for 15 min with continuous stirring, followed by immediately cooling to 43±2°C using tap water (18). The treated milk was then inoculated with previously prepared starter culture (16 ml) and incubated at 43±2°C for about 4.5 h until a gel was formed, and pH of 4.40±0.05 was reached. The gel was cooled down to 20°C in an ice bath by stirring and mixed with a high speed homogenizer (90 s) during which 20 g of salt was added. The Ayran was further cooled to 4°C and filled into sterile wine bottles of 750 ml. The bottles were divided into four groups: the control group; CO_2 injection at 0.5 MPa (A5); (3) at 1.0 MPa (A10); and (4) at 1.5 MPa (A15) for one min. Injection systems consisted of a gas cylinder with pressurized CO_2, linked to the injection head by a valve. All samples were stored at 5°C for seven days. The experiment was repeated three times.

Methods of analyses

The Ayran samples were analyzed for total solids, fat, titratable acidity (TA), salt and density (19). Titratable acidity was expressed as °SH. The contents of acetaldehyde (20) and lactic acid (21) were determined as the major taste and flavour components. pH was measured using a combined electrode connected to a pH-metre (Orion 420, Model 250, Orion Research Inc., USA). Whey separation was measured volumetrically and expressed as whey ml/100 ml Ayran (22). CO_2 concentration was determined by a titrimetric method (23). Among the above analyses, total solids, fat, salt contents and density of the Ayran samples were determined only on day 1.

Enumeration of yoghurt starter bacteria:

Yoghurt bacteria were enumerated according to Bracquart (24). The yoghurt bacteria was differentiated based on the morphology of their colonies. The colonies of *S. thermophilus* were opaque and spherical, while those of *L. delbrueckii* ssp. *bulgaricus* appeared to be larger and of irregular shape.

Sensory analysis

A consumer preference test was carried out using a 9-point hedonic scale ranging from 1 = dislike extremely to 9 = like extremely (25). Seventy-five students of the Agricultural Faculty of Ankara University participated in sensory evaluations.

Statistical analyses

Statistical analyses were done using Minitab 13.32 (Minitab, Inc., State College, PA). Based on a completely randomized design, two-way analysis of variance (ANOVA) was used to determine the interaction effects of the CO_2 treatments and storage time on the mean chemical, microbiological and sensory properties of the Ayran samples. Duncan's multiple comparison tests was used to test differences in means at the significance level of 0.05. Log_{10} transformations were performed on microbial data.

RESULTS and DISCUSSION

Effect of pressurized CO_2 injection on gross composition

As can be seen in Table 2, CO_2 concentrations increased significantly with increasing injection pressures of A5, A10 and A15 ($P<0.05$). The presence of CO_2 in the control samples can be attributed to the metabolic activity of yoghurt bacteria (6, 7). Neither did the CO_2 concentrations of the samples change during the storage nor the CO_2 injection change the total solids, fat, salt content and density of the Ayran samples ($P > 0.05$).

Table 2. Effect of pressurized CO_2 injection at 0 (control), 0.5 (A5), 1.0 (A10) and 1.5 (A15) MPa for one min on mean chemical properties of Ayran stored at 5°C for seven days (*n* = 3)[a]

Composition	Control		A5		A10		A15	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
Total solids (g)	8.3^{A}	-	8.2^{A}	-	8.3^{A}	-	8.2^{A}	-
Fat (g/100 g)	1.55^{A}	-	1.52^{A}	-	1.53^{A}	-	1.53^{A}	-
Density (g/ml)	1.025^{A}	-	1.026^{A}	-	1.026^{A}	-	1.026^{A}	-
Salt (g/100 g)	0.65^{A}	-	0.64^{A}	-	0.65^{A}	-	0.64^{A}	-
CO2 (mg/100 ml)	9.9^{D}	11.7^{D}	71.5^{C}	75.9^{C}	136.7^{B}	117.9^{B}	183.3^{A}	199.0^{A}
pH	4.15^{C}	4.05^{D}	4.28^{A}	4.17^{C}	$4.27^{A,B}$	$4.19^{B,C}$	$4.26^{A,B}$	$4.19^{B,C}$
°SH	31.9^{F}	34.9^{E}	35.5^{E}	$37^{D,E}$	$39.5^{C,D}$	$40.8^{B,C}$	$43.4^{A,B}$	45.0^{A}
Lactic acid (g/100 g)	0.56^{A}	0.61^{A}	0.55^{A}	0.55^{A}	0.55^{A}	0.59^{A}	0.56^{A}	0.57^{A}
Acetaldehyde (mg/kg)	10.6^{A}	10.8^{A}	14.3^{A}	14.1^{A}	11.7^{A}	13.8^{A}	13.2^{A}	14.2^{A}
Whey separation (ml/100ml)	3.2^{C}	24.8^{A}	3.1^{C}	14.1^{B}	4.3^{C}	15.2^{B}	4.1^{C}	27.6^{A}

[a] Means in the same row without a common superscript differ significantly ($P < 0.05$).

Similarly, the CO_2-injection or the storage did not influence the acetaldehyde and lactic acid contents of the samples ($P > 0.05$). It is well known that yoghurt bacteria, particularly *L. delbrueckii* ssp. *bulgaricus,* are mainly responsible for acetaldehyde production (26) and that most of acetaldehyde and lactic acid in yoghurt is produced during incubation (8). Therefore, it is likely that the injection of CO_2 after the incubation did not affect the production of these compounds since their production must have been completed during incubation, and also the growth of *L. delbrueckii* ssp. *bulgaricus* remained unaffected during storage (Table 3). In yoghurts produced from milk acidified with CO_2, no effect of the CO_2 treatment was reported on acetaldehyde and lactic acid contents (8, 9). Note that the acetaldehyde contents of Ayran samples were found to be within the range of those reported for yoghurt and yoghurt-related products (2 to 40 µg g^{-1}) (18).

Table 3. Effect of pressurized CO_2 injection at 0 (control), 0.5 (A5), 1.0 (A10) and 1.5 (A15) MPa for one min on mean numbers of *Streptecoccus thermopilus* and *Lactobacillus delbureckii* subsp. *bulgaricus* in Ayran stared at 5°C for seven days (Log_{10} colony forming units per gram) (*n*=3)[a]

	S. thermophilus		*L. delbrueckii* subsp. *bulgaricus*	
	Day 1	Day 7	Day 1	Day7
Control	8.6^{A}	8.6^{A}	$8.3^{A,B}$	8.6^{A}
A5	6.5^{A}	3.4^{B}	8.4^{A}	8.5^{A}
A10	6.6^{A}	3.2^{B}	$8.3^{A,B}$	$8.3^{A,B}$
A15	6.0^{A}	2.6^{C}	$8.3^{A,B}$	8.0^{B}

[a]Means in the same column, row and species without a common superscript differ significantly ($P < 0.05$)

TA values increased with the increasing CO_2 concentrations due to the formation of carbonic acids ($P < 0.05$). The storage time did not change TA values of the CO_2-injected, thus indicating the cessation of bacterial activity ($P > 0.05$). Unlike TA values of the CO_2-injected samples, those of the control samples significantly increased during storage as an indication of ongoing bacterial activity ($P < 0.05$). The CO_2-injected samples had slightly higher pH than did the control samples ($P < 0.05$). The results were consistent with those of Karagül-Yüceer *et al.* (11). Changes in pH observed during the storage were notable for the control samples and A5 as an indication of possible bacterial activity ($P < 0.05$).

When whey separation was monitored, A5 and A10 showed considerably lower whey syneresis at the end of the storage than the control samples and A15 ($P < 0.05$). The microscopic examination of the CO_2-injected samples showed that CO_2 was entrapped in the matrix as uniformly distributed minute bubbles (no picture shown). Serum separation in fermented products is well known to stem from the differences between the density of serum and protein (25). Therefore, CO_2 bubbles in these samples most likely acted as a physical barrier to a certain extent, thus preventing downward movement of coagulated milk proteins which would result in phase separation. It could also be possible that denatured milk proteins were adsorbed onto the gas/serum interface (27), which reduced the amount of dry matter present in the serum phase. Currently, the reason(s) why a higher CO_2 content in A15 did not improve the serum separation further remains to be explored and accounted for. However, the results clearly indicated that the CO_2 concentration in A5 and A10 reduced whey separation by about 50%.

Effect of pressurized CO_2 injection on the survival of yoghurt bacteria

As was shown in Table 3, the number of *S. thermophilus* in the CO_2-injected samples tended to decrease after 1 day of storage. The effect of the injection of pressurized CO_2 was more pronounced at the end of the storage. The number of cocci in the CO_2-injected samples decreased more than 3 logs ($P < 0.05$), with the highest reduction in A15. As for the control samples, the survival of *S. thermophilus* was not affected by storage ($P>0.05$). Unlike *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus* was not influenced by the CO_2 treatment and the storage ($P > 0.05$). Note that *L. delbrueckii* ssp. *bulgaricus* requires more than 32 mg CO_2/kg milk for optimal growth (6).

Several mechanisms explaining the inhibitory action of CO_2 on bacteria have been suggested, such as displacement of oxygen, influence of pH, disruption of cell membrane, and metabolic interference (28). The fact that *S. thermophilus* as opposed to *L. delbrueckii* ssp. *bulgaricus* is not an acid tolerant bacterium (18) suggests that the increased acidity in the cytoplasm of *S. thermophilus* due to the formation of carbonic acid with the addition of CO_2 may have inhibited its metabolic activity (27). However, this factor alone may not be sufficient to elucidate the results since the changes in pH and titratable acidity of the samples due to CO_2 were small. The destructive effect of the pressurized CO_2 levels on the cell membrane should also be taken into consideration (15, 29).

The discrepancy of our microbiological findings on *S. thermophilus* in the CO_2-injected samples from those by Karagül-Yüceer *et al.* (11) and Vinderola *et al.* (12) may result from the differences in the CO_2 concentrations of the products between this study and the above studies. Consistent with the findings of the above studies, van Hekken *et al*. (16) observed less than 1 log reduction in the number of *S. thermophilus* when milk was sparged with CO_2 to a pressure of 5.52 MPa, held for 5 min at 38°C and yielded cheese curd. In this study, however, the pH of the curds was higher, and the duration during which the microorganisms were subjected to high pressure CO_2 was much shorter than used in our study. It should be kept in mind that the effect of pressurized CO_2 on viability of different microorganisms hinges on several factors such as pressure, exposure time (15, 16), the type of microorganisms, the phase of growth, and the suspension medium (31).

Finally, owing to the above changes, the ratio of cocci to bacilli in the CO_2-injected samples changed in favour of bacilli from approximately 1:1.3 to 1:2.7. In yoghurt, this ratio is expected to be 1:1 (18), as was determined in the control samples.

Effect of pressurized CO_2 injection on the sensory properties

The CO_2 injection adversely affected the sensory properties of Ayran (Table 4). In general, increasing CO_2 concentrations decreased the flavour, body/texture and overall acceptance scores ($P < 0.05$). The panellists remarked that the CO_2-injected samples were too sour to consume. Although the samples did not differ in their acetaldehyde contents, a major aroma compound of yoghurt, most panellists pointed out that the CO_2-injected samples lacked yoghurt flavour. This may suggest that the injection of CO_2 masked the yoghurt aroma to some extent although the acetaldehyde values in our study were found to be well above the odour threshold value of acetaldehyde (30). Recent studies showed that the main factor responsible for the intensity of flavour perception of yoghurt is the acidity not the aroma compounds such as acetaldehyde (32). In addition, the CO_2-injected samples were perceived to be more watery and less viscous than the control samples by the panellists with the increasing CO_2 concentrations. Eventually, the panellists' preferences were reflected in the overall lower scores of the CO_2-injected samples than in the control samples ($P < 0.05$).

Table 4. Effect of pressurized CO_2 injection at 0 (control), 0.5 (A5), 1.0 (A10) and 1.5 (A15) MPa for one min on mean sensory properties of Ayran stored at 5°C for seven days (*n* = 75).[a]

	Flavour		Body texture		Overall acceptance	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
Control	6.8^{A}	6.5^{A}	6.1^{A}	6.2^{A}	6.6^{A}	6.5^{A}
A5	$3.6^{B\text{-}C}$	4.0^{B}	$4.4^{B,C}$	$5.4^{A,B}$	$4.0^{B,C}$	4.9^{B}
A10	$2.7^{C,D,E}$	$3.2^{C,D}$	4.0^{C}	$4.5^{B,C}$	$3.0^{C,D}$	3.5^{C}
A15	2.0^{E}	$2.6^{D,E}$	3.7^{C}	4.1^{C}	2.5^{D}	$3.1^{C,D}$

[a] Means in the same column and row for the same properties without a common superscript differ significantly (P < 0.05)

CONCLUSIONS

This study showed that injection of pressurized CO_2 into Ayran did not affect the chemical composition of Ayran considerably but did affect the survival of *S. thermophilus* as well as sensory properties. Injection of CO_2 in the range of 71 to 135 mg/100 ml can be used to prevent serum separation in fermented drinks. With regard to sensory properties, Ayran with the current CO_2 concentrations were not favoured by young Turkish consumers who are apparently enjoying the traditional form. Therefore, producing carbonated Ayran necessitates CO_2 concentrations lower than those used in this study, which should be explored.

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