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THE EFFECT OF USING DIFFERENT LEVELS OF CALCIUM CARBONATE ON THE PHYSICAL, CHEMICAL AND SENSORY PROPERTIES OF YOGHURT

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Abstract
In this research, fortification was applied to milk by adding different amounts of $CaCO_3$. Fortification was performed before and after pasteurization of milk with different levels (200, 400, 600 mg/100ml) of calcium carbonate. The effect of adding calcium carbonate to fortified yoghurt samples was determined throughout the storage period (throughout the storage period 21 day at 4 °C). Added calcium carbonate has increased the pH and viscosity values and also decreased the titration acidity and serum separation values of the yoghurt. The results were found to be statistically significant ($P<0.05$). The best results were obtained with the calcium carbonate added before pasteurization. As a result of sensory evaluation, samples in which $CaCO_3$ has been added before pasteurization and control samples have obtained higher scores than the others. While increasing values of added calcium carbonate positively have affected physical and chemical properties of yoghurt, it has negatively affected the sensory properties of yoghurt. Additional concentrations should not exceed 400 mg/100ml.

Keywords: fortified yoghurt, calcium carbonate

FARKLI ORANLARDA KALSİYUM KARBONAT KULLANIMININ YOĞURDUN FİZİKSEL, KİMYASAL VE DUYUSAL ÖZELLİKLERİ ÜZERİNE ETKİSİ

Özet
Bu araştırmada süte farklı oranlarda $CaCO_3$ ilave ederek zenginleştirme işlemi uygulanmıştır. Takviye işlemi kalsiyum karbonatın farklı seviyeleriyle (200, 400, 600 mg/100ml) sütün pastörizasyonundan önce ve sonra uygulanmıştır. İlave edilen kalsiyum karbonatın zenginleştirilmiş yoğurt örneklerine etkisi depolama periyodu boyunca belirlenmiştir (21 gün, 4 °C'de). İlave edilen kalsiyum karbonat yoğurdun pH değerini ve viskozitesini artırmış, titrasyon asitliğini ve serum ayrılması değerini düşürmüştür. Sonuçlar istatistiksel olarak önemli bulunmuştur ($P<0.05$). En iyi sonuçlar kalsiyum karbonatın pastörizasyondan önce ilave edilmesiyle elde edilmiştir. Duyusal değerlendirme sonucunda, pastörizasyon öncesi kalsiyum karbonat ilave edilmiş örnekler ve kontrol örnekleri diğerlerine göre daha yüksek puanlar almıştır. İlave edilen kalsiyum karbonatın artan değerleri yoğurdun fiziksel ve kimyasal özelliklerini pozitif olarak etkilerken, yoğurdun duyusal özelliklerini negatif olarak etkilemiştir. İlave konsantrasyonlar 400mg/100ml'yi aşmamalıdır.

Anahtar kelimeler: Zenginleştirilmiş yoğurt, kalsiyum karbonat.

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INTRODUCTION

Diets which are insufficient in milk and dairy products known as the basic resources for calcium may cause long-term, serious and harmful effects especially on bone health (1). Many medical literatures confirmed that high-calcium intake in diets and its bioavailability decrease the risks for experiencing osteoporosis, colorectal (colon) cancer, high blood pressure, kidney stones, paralysis, lead absorption and pre-menstrual syndrome (2-6). The most important point in selecting nutritive element to be enriched is the fact that the material is keeping its bioavailability during its shelf life. It should be considered that it does not interact with taste, aroma and color of the dairy products and also supply and cost of the additive's form as well as potential disadvantages relating to manufacturing process during the production (7). It is known for many years that yoghurt is a rich source in calcium. People provide 111 mg of calcium when they eat 100 grams of fat yoghurt (8). Daily required amount of calcium is 1000 mg for adults (9); this amount is able to meet 11.1 % of daily calcium proposed. Calcium absorption rate increases due to lactose fermentation in body (10). This situation increases the value of yogurt, in terms of prevention to bone development in children and osteoporosis in adults (11). In the case of low levels of dairy products intake, the amount obtained through diet, has increased the desired levels by also increasing the calcium content in food (12). As a result of insufficiencies of vitamin and mineral seen in recent years, enriching foodstuff with calcium has become prevalent. Purpose of the recent study was to investigate the effects of $CaCO_3$ addition at various concentrations on physical, chemical and sensory properties of yoghurt.

MATERIAL AND METHOD

Material

Materials of the study were prepared by adding $CaCO_3$ before and after pasteurization in a manufacturing area of a private company. K_1 represents control sample without $CaCO_3$ addition before pasteurization and K_2 represents control sample without $CaCO_3$ addition after pasteurization. A_1, A_2 and A_3 represent the samples with $CaCO_3$ addition at the concentrations of 200, 400 and 600 mg/100 ml, respectively and B_1, B_2 and B_3 represent the samples with $CaCO_3$ addition at the concentrations of 200, 400 and 600 mg/100 ml, respectively. Fat content of milk material, which was used in manufacturing of the products, was 3.8% and its non-fatty dry material content was 12.9%. Its density was 46.1, protein was 4.85% and pH was 6.80. Chemical values of the milk used in manufacturing of yogurt sample conform to TS 1330/April 2006 Yoghurt Standard. A reproductive culture consisting of a mixture of *Str. salivarius ssp thermophilus and L. delbruecki ssp. bulgaricus* manufactured by Danisco A/S in Denmark with a trade name and code number of Bulk Set Y-532 and Y-621 was used as starter culture for yoghurt samples. Calcium carbonate which is suitable to be used in foodstuff was supplied by Veser Chemicals SA in Turkey in powder form with a trade name of A-5 (5 Micron) in enrichment of the products.

Method

Yoghurt manufacturing

The milk for yoghurt manufacturing was homogenized at 70-80 °C and 180 bars and varied by keeping a control sample and adding 200, 400 and 600 mg $CaCO_3$ per 100 ml before the pasteurization. The samples were pasteurized at 90 °C for 5 min and 2% of starter culture was added at 45 °C. Automatic filling machinery has completed filling and incubation was completed in incubators at a constant temperature of 45-48 °C between 2 h and 45 min and 3 h and 15 min. Then, samples were stored at 4-6 °C. Same processes applied at second production also but $CaCO_3$ was added after pasteurization.

Physical, chemical and sensory analyses

Raw material milk was analyzed in means of fat, non-fatty dry material, protein, density and pH. pH, titratable acidity, serum separation and viscosity values were have been measured in the enriched samples and control samples on 1st, 7th, 14th and 21st days of storage. Sensory analyses were conducted also on 1st and 14th days. Fat content and non-fatty dry matter were determined according to Anonymous (13). Milk (11 ml) and sulfuric acid (10 ml) was put in butyrometer. Isoamyl alcohol (1 ml) was added and then centrifuged at 1100 rpm. Butyrometer was waited in bain-marie for 5 minute at 60-63 °C. Later, fat content was calculated as% by mass. Protein content was determined and density was measured according to Demirci and Gündüz (14). Protein content was determined through formal titration method. Density was determined using lactodensimeter. pH was determined according to Kosikowski (15) with the help of a digital waterproof hand-held pH/mV/Temperature Meter with 300/310 trade make. Titratable acidity was determined according to Anonymous (13). Yoghurt sample (10g) was mixed with pure water (10g) and phenol ftalein solution was added and titrated with sodium hydroxide solution. Acidity was calculated as % by mass in terms of lactic acid. Serum separation was determined according to the form recommended by Atamer and Sezgin (16) of the method developed by Kessler and Kammerlehner (17). Yoghurt samples (25 g) waited for 2 h at 3 °C. Later, samples were filtered through coarse filter paper.

The amount of separated serum was measured as milliliter (ml). Viscosity was measured by sine-wave vibro viscometer (SV-10; A&D Company, Ltd., Tokyo, Japan) at 20 °C. Sensory analyses were conducted through pointing test method according to TS 1330 by considering sensory evaluation points of 5 panelists about yoghurt samples. These evaluations were conducted on 1st and 14th days of storage. All features were evaluated over 5 points (13).

Statistical analyses

All of the analyses were repeated 3 times. Statistical analyses were conducted according to "Test Plan for Random Parcels" and Statistica 6.0 version (statistical packet program) was used. Variance analysis was conducted for determining whether or not there is a variation between chemical and physical values. "LSD" Multi-comparison test was conducted to determine the variation between the means. Kruskal Wallis test, which is one of non-parametric tests, was performed in evaluation of sensory analyses and the variations between them were found (18-20).

RESULTS AND DISCUSSION

Physicochemical property measurements

pH measurement

It is seen that pH values vary between two different groups, which were enriched by $CaCO_3$ (before and after pasteurization) and pH value decreases during storage (Table 1). According to the results of statistical checks conducted on the enriched yogurts before and after pasteurization, effects of each of storage time and additional $CaCO_3$ ratio on pH values of test samples are considered significant at a level of $P \leq 0.05$. Velez-Ruiz (21) has added calcium lactate and calcium chloride into set and stirred type yoghurts at a concentration of 100, 200 and 300 mg per 100 ml and studied on effects of calcium on physicochemical features of yoghurt. A decrease in pH was observed in yoghurt samples during their storage in this study. Effects of type of yoghurt, type of calcium and ratio of calcium additive on yoghurt's pH were significant according to the statistical analysis. The results of this research are similar to the result obtained Velez-Ruiz. However, their collective effect was considered insignificant in both of the practices ($P>0.05$). As a result, pH values of samples have increased depending on the increase in the amount of additional calcium. Higher pH values of the samples in comparison with their own control samples during storage may suggest that the additional calcium carbonate increases pH of yogurts. In the study of Pirkul et al. (22), milk was mixed with 50mg Ca/100 ml of Ca-lactate and Ca-lactate+Ca-gluconate. The pH of yoghurt fortified by Ca-lactate was determined higher than the pH of control sample. The results of this research indicating that pH has increased effects of calcium carbonate correlate with the results obtained in the study of Pirkul et al.

Lactic acid measurement

As seen on Table 1, lactic acid content of the control samples and those enriched with $CaCO_3$ have increased during storage. According to the results of variance analyses relating to lactic acid values of the samples enriched before and after pasteurization, effects of each of storage time and additional $CaCO_3$ ratio on lactic acid contents of test samples are considered significant at a level of $P \leq 0.05$. However, their collective effect was considered insignificant in both of the practices (P>0.05). As a result, as additional $CaCO_3$ has increased in amount, lactic acid contents of the samples have decreased depending on the increase in the additional amount. This may suggest that the additional calcium carbonate amount affects growth of lactic acid bacteria. Also, this correlates with the view reported by Yousef and Rusli (23) as a result of their study investigating starter culture growth in enriched yoghurt with Ca. Yousef and Rusli have reported that additional Ca concentration should not exceed 400 mg/100 ml according to their study; otherwise, extreme hardening in structure, an increase in intent for serum separation and an extension in incubation period would occur. The reason for extension in incubation period is the fact that the growth of *Streptococcus thermophilus* slows down in presence of calcium. According to this view, growth of *Str. thermophilus* is inhibited at a certain level by the effect of gluconic acid released during yoghurt formation in the media. However, *Lactobacillus delbrueckii* subsp *bulgaricus* was not affected by calcium.

Serum separation measurement

It was observed that serum separation has decreased in all samples, which has been enriched before and after pasteurization, during the 21-day storage (Table 1). According to the results of variance analyses, effects of each of storage time and additional $CaCO_3$ ratio on serum separation of test samples are considered significant at a level of P<0.05. However, their collective effect was considered insignificant in the test samples, which has been enriched before pasteurization (P>0.05) and significant in the test samples, which has been enriched after pasteurization ($P \leq 0.05$). Serum separation has decreased in all samples during storage. Also, as additional $CaCO_3$ has increased in amount, serum separation of the samples has decreased depending on the increase in the additional amount. Omar et al.(24) has determined that calcium addition extends coagulation time in yoghurt production; however, it causes an increase in dry matter content, Ca content and coagulant frequency and yoghurt can be stored for three weeks at 4 °C without serum separation. The results of this research are correlated with the study of Omar et al.

Table 1. The results of pH, lactic acid, serum separated and viscosity measurement, which were conducted on the samples to which $CaCO_3$ was added before and after pasteurization in 1, 7, 14 and 21 days

Yoghurt samples	pH measurement				Lactic acid measurement (% in mass)			
	1	7	14	21	1	7	14	21
K1	4.02^A	3.88^A	3.75^A	3.70^A	0.810^A	0.864^A	0.936^A	1.008^A
A1	4.10^B	4.07^B	3.94^B	3.90^B	0.756^B	0.799^B	0.886^B	0.954^B
A2	4.18^C	4.16^C	1.03^C	4.00^C	0.720^C	0.756^C	0.836^C	0.907^C
A3	4.29^D	4.25^D	4.16^D	4.05^D	0.648^D	0.698^D	0.758^D	0.828^D
K2	4.22^A	3.97^A	3.95^A	3.82^A	0.756^A	0.900^A	0.936^A	0.990^A
B1	4.27^A	4.09^B	4.00^A	3.88^A	0.684^B	0.777^B	0.815^B	0.878^B
B2	4.29^A	4.13^C	4.05^B	4.00^B	0.648^C	0.698^C	0.777^C	0.835^C
B3	4.30^B	4.15^D	4.13^C	4.02^C	0.612^D	0.684^D	0.737^D	0.810^D

Yoghurt samples	Serum separation measurement (ml)				Viscosity measurement (cPs)			
	1	7	14	21	1	7	14	21
K1	6.150^A	6.000^A	5.390^A	5.150^A	700^A	860^A	944^A	780^A
A1	5.750^A	4.850^B	4.790^B	4.680^A	775^B	970^B	1280^B	940^B
A2	5.250^B	4.805^C	4.660^C	4.530^B	880^C	1150^C	1620^C	1140^C
A3	4.950^C	4.640^D	4.550^D	4.470^C	950^D	1254^D	1890^D	1315^D
K2	6.200^A	6.080^A	5.050^A	4.932^A	310^A	797^A	941^A	638^A
B1	5.600^B	5.095^B	4.785^A	4.699^A	371^B	878^B	983^A	792^B
B2	5.150^C	4.840^C	4.705^A	4.601^A	454^C	992^C	1150^B	900^C
B3	4.850^D	4.750^D	4.670^A	4.538^A	310^A	797^A	941^A	638^A

K1, A1, A2, A3 samples, $CaCO_3$ was added before pasteurization. K2, B1, B2, B3 samples, $CaCO_3$ was added after pasteurization. Top to bottom, according to the LSD test indicated by different letters in different groups in terms of statistical samples has taken place ($P \leq 0.05$).

Viscosity measurement

It was observed that viscosity has increased in all samples, which has been enriched before and after pasteurization, until 14th day of storage and started to decrease again on 21st day of storage during the 21-day storage (Table 2). According to the results of variance analyses relating to viscosity values of the samples, effects of each of storage time and additional $CaCO_3$ ratio on viscosity of test samples are considered significant at a level of $P \leq 0.05$. Their collective effect was considered significant in both of the practices ($P \leq 0.05$). It was observed that viscosity values of the test samples, which has been enriched before and after pasteurization, were improved during storage. Due to decreases in serum separation and acidity during storage, protein's capacity for water arresting has increased and coagulant stability was improved. Also, it was seen that improvement in viscosity was higher in the samples with additional $CaCO_3$. This is caused by the fact that the increased $CaCO_3$ content increases formation of calcium phosphate cross-links within casein micelles. In the study of Shing and Muthukumarrapan (25), the calcium enriched mango yogurt was prepared after fortification of pasteurized yoghurt mix with 50mg Ca/100ml of calcium lactate. Measurements performed on slowly stirred samples (flow curves and final apparent viscosity) have showed that calcium-enriched fruit yoghurt has had stronger structures. Firmness of the calcium has fortified fruit yoghurt attributed to the higher extent of colloidal calcium phosphate cross-linking between casein micelles due to increased calcium content by fortification. The results of this research are correlated with the study of Singh and Muthukumarappan.

The results of sensory evaluation tests

The results of sensory evaluation tests, which were conducted on the samples added $CaCO_3$ are seen on Table 3. According to Kruskall Wallis Test conducted on the samples enriched before pasteurization, effects of the additional $CaCO_3$ on the consistency on spoon and taste were considered statistically significant at a level of $P<0.05$. Effects of the additional $CaCO_3$ on the consistency in mouth and odor in the samples enriched before pasteurization was considered as statistically non-significant ($P>0.05$). Effects of storage time on appearance, consistency on spoon and in mouth, odor and taste of the yoghurt samples enriched before pasteurization were considered statistically significant ($P<0.05$).

Table 2. The results of sensory evaluation tests, which were conducted on the samples to which CaCO3 was added before and after pasteurization.

Yoghurt Samples	Days	Appearance	Consistency on spoon	Consistency in mouth	Odor	Taste
K1	7	4.2	4.2	4.2	4.5	4.5
	14	5.0	4.7	4.7	5.0	5.0
A1	7	4.2	4.2	4.4	4.5	4.5
	14	5.0	3.7	4.3	4.3	3.7
A2	7	4.0	4.3	4.5	4.0	4.2
	14	4.7	4.7	4.7	4.7	2.0
A3	7	4.2	4.0	4.0	4.2	3.7
	14	3.3	4.7	4.7	5.0	1.0
K2	7	4.2	4.8	4.6	4.6	4.8
	14	4.6	4.3	4.3	4.2	4.0
B1	7	4.2	4.2	3.8	3.8	3.8
	14	4.3	4.2	4.0	3.6	3.8
B2	7	4.2	4.2	3.8	4.0	3.8
	14	4.3	4.2	4.2	3.7	3.7
B3	7	4.8	4.4	4.2	3.8	3.8
	14	4.0	4.2	3.7	3.7	3.5

K1, A1, A2, A3 samples, $CaCO_3$ was added before pasteurization; K2, B1, B2, B3 samples, $CaCO_3$ was added after pasteurization.

Effects of $CaCO_3$ on the consistency in mouth and on spoon, odor and taste in the samples enriched after pasteurization was considered as statistically significant (*P*<0.05). Effect of $CaCO_3$ on appearance was considered statistically non-significant (*P*>0.05). Effects of storage time on appearance, the consistency in mouth and on spoon and taste in the samples enriched after pasteurization was considered as statistically significant (*P*<0.05). Effect of $CaCO_3$ on appearance was considered statistically non-significant (*P*>0.05). However, its effect on odor was considered statistically significant (*P*<0.05).

Results of the evaluations about appearance are similar to those found by Yousef and Rusli (23). According to the findings of the study and statistical data, it might be said that $CaCO_3$ concentration should not exceed a certain level and as the additional $CaCO_3$ concentration increases, taste is affected negatively depending on it.

CONCLUSIONS

In general, adding CaCO3 has positively affected physical, chemical and sensory properties of yoghurt. This may prevent vitamin/mineral deficiency and the diseases caused by it threatening one third of the world's population and may be used safely by lactose intolerant people. It should be added before pasteurization for better results in means of quality criteria. Additional concentrations should not exceed 400 mg/100 ml in means of sensory properties. The additional $CaCO_3$ at a concentration of 200 mg/100 ml has given minimum quality defects in means of quality criteria in the study and has satisfied calcium demand better compared with traditional yoghurt.

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