FORTIFICATION OF BANANA STIRRED YOGURT WITH CALCIUM
Yonis. A. A. M.; F. M. Elzamzamy and Shimaa A. Elmorsi
Home Economic Dept., Fac. of Specific Education, Mans. Univ., Egypt

ABSTRACT
Banana yogurt was prepared fortified with calcium gluconate (CaG) and calcium lactate (CaL) at levels 1%, 2% and 3% as a source of soluble calcium, prior to fermentation. The calcium salts were added following pasteurization. Viscosity, pH values, lactic acid bacteria (LAB) counts, rheological properties and sensory scores analysis of the yogurt mix samples were investigated. Results showed an increase in pH values by the increase of calcium levels addition. Also, LAB count of yogurt mix samples increased by the increase of calcium levels fortification. Generally, calcium salts had no influence on the rheological properties (viscosity, hardness, springiness, chewiness and cohesiveness) and sensory properties of yogurt mix up to 2% of CaG and 1% of CaL fortification.

Keywords: Yogurt; Calcium fortification; Rheology.

INTRODUCTION
Yogurt and fermented milk products are among the most popular dairy foods consumed all over the world. Bioavailability of calcium from yogurt is even higher than that from milk. The acidic pH of yogurt ionizes calcium and thus facilitates intestinal calcium absorption (Unal et al., 2005). The low pH of yogurt may also reduce the inhibitory effect of dietary phytic acid on calcium bioavailability (Adolfsson et al., 2004). Various researchers have demonstrated the fortification of yogurt with various calcium salts (Pirkul et al., 1997). Application areas for adding calcium to dairy products include yogurt, yogurt drinks, ice cream, cottage cheese, sour cream, cream cheese preparations and desserts (Gerstner, 2002). Several commercial calcium salts have been used for calcium enrichment of milk/beverages, e.g. calcium carbonate, calcium chloride, calcium phosphate, tribasic calcium phosphate, calcium citrate malate, calcium lactate, calcium gluconate, calcium lactate gluconate and natural milk calcium (Davis, 2002).

In the processing of flavored yogurt, natural fruits could be used. (Nigerian Industrial Standard, 2004) Flavored yoghurt defined as yoghurt to which has been added flavoring food or other flavoring agents (like fruits). Although fruits may be taken in their fresh forms, because of their outstanding perishable and seasonal nature, a lot of fruits are processed traditionally in regions where a food processing culture exists. A part from the use of fresh fruits for natural flavouring, canned, quick frozen and powdered fruits, fruit puree, fruit pulp, jam, fruit juice, chocolate, cocoa, nuts, coffee, spices and other natural flavouring ingredients from temperate fruits (grapes, berries, drupes and pomes) have been utilized.

Fruit mixes improve the nutritional value and the taste of yogurt, and fruit enhancement plays a considerable role in yogurt consumption and sales (Kailasapathy et al., 2008). Banana fruit contains considerable amounts of
starch, dietary fiber, protein, vitamins, and minerals \( (Wall, 2006) \). The addition of banana fruit juice appeared to support the viability of lactobacilli, with higher microorganism numbers observed in fruit yogurts than in plain yogurt throughout the shelf life. Addition of fruit juice significantly increased the syneresis and decreased viscosity and water holding capacity of yogurts, and also enhanced their sensory acceptability \( (Ranadheera et al., 2012) \).

In composition of yogurt mix contributes is the flavor, texture or body, nutritional value and others important attributes of the resulting yogurt. Fortification of the mix with calcium salts alters the sensory properties and general quality of resulting yogurt. Early attempts to fortify plain yogurt mix with calcium gluconate, saccharate and / or citrate produced a yogurt with a week body and the mix required a longer time to coagulate \( (Fligner et al., 1988) \). These processing difficulties may be attributed to added calcium salts, which alter the physico-chemical properties of the milk and the resulting coagulum. However, inhibition of yogurt starter cultures by added salts also may have contributed to these defects. Organic acids may be liberated from calcium salts when pH of milk decreases during yogurt manufacture. Therefore, this study was carried out to investigate whether calcium fortification by addition calcium gluconate \( \text{(CaG)} \) and calcium lactate \( \text{(CaL)} \) inhibits yogurt cultures and to find the effect of \text{CaG} or \text{CaL} addition on the rheological and sensory properties of the produced yogurt.

**MATERIALS AND METHODS**

**Materials:**
Fresh whole buffalos milk was supplied by Faculty of Agriculture Mansoura University, skim milk powder and banana was obtained from the local market in Mansoura. Mixed culture consisted of lactobacillus delbrueckii sub sp. bulgaricus and streptococcus salivarius sub sp. thermophilus \( (1:1) \). Calcium gluconate and calcium lactate, also media used nutrient agar medium \( \text{(Hi Media)} \), MacConkey agar \( \text{(Hi Media)} \) and \( \text{(M.R.S)} \) medium were obtained from El-Goumhouria Co. For trading medicines, chemicals and medical applications. Cairo. Egypt.

**Preparing of banana:**
It was prepared using the method of \( (Bakirci and Kavaz,2008) \) with some modifications as follows; the fruit skin was removed manually and the peeled banana \( (5\%) \) then mixed with yogurt in a blender.

**Yogurt manufacture:**
Seven samples of fresh buffalos milk and skim milk powder were mixed to produce 15.5% total solids in the final mixture and subjected to heat treatment at \( 90^\circ\text{C} \) for 15 min. followed by cooling to \( 42^\circ\text{C} \), then inoculated with 2.5% mixed activated yoghurt starter culture streptococcus salivarious subsp. thermophilus, and lactobacillus delbruekii subsp. bulgaricus. \( (1:1) \). The samples were divided as (control banana), six portions fortified with \text{CaG} and \text{CaL} at levels 1%, 2% and 3%. Finally it was poured into incubation cups and the cups were incubated at \( 42\pm1^\circ\text{C} \) for 3-4h till firm curd was formed at pH 4.6. Then yogurt samples mixed with banana \( (5\%\text{w/w}) \) and stirred at the
mixture. The yogurt mix samples were immediately stored in refrigerator at (6±1°C), then the chemical, microbiological, rheological, and sensory properties were evaluated.

**Analytical Methods:**

The pH values were measured using a laboratory pH meter type HANNA Instrumenting (8417) pH meter, reading was taken as described by Naz, (2012). Viscosity was measured using universal testing machine (Cometech, B type, Taiwan) provided with software. Total LAB count was determined using M.R.S medium according to Deman et al., (1960). The presumptive coliform count was enumerated by plating 1ml of the serially diluted samples on MacConkey agar (Hi Media) and the plates incubated at 37°C for 24h. Dark red non-mucoid colonies are indicative of Coliforms. MacConkey agar was prepared as described by Hartman, (1985). Molds and yeasts counts were determined using potato dextrose agar recommended by the Oxoid Manual, (1962).

Texture profile analysis (TPA) was determined according to Bourne, (2003). From the resulting force–time curve, the values for texture attributes, i.e. Hardness (N), chewiness (N), cohesiveness(N), and springiness(mm) were calculated from the TPA graphic.

Sensory evaluation, fresh yoghurt samples were judged for flavor (45 points), body and texture (40 points), and color and appearance (15 points) by five trained panelists. The score card was designed as described by Bodyfelt et al., (1988).

All the obtained data were statistically analyzed by SPSS computer software. The calculated occurred by analysis of variance ANOVA and follow up test LSD by SPSS ver.16.

**RESULTS AND DISCUSSION**

The effect of calcium salts level on the pH values of fresh banana stirred yogurt was found to be statistically significant (P<0.05) as shown in Table (1). Banana stirred yogurt fortified with CaG and CaL showed an increase in mean pH values with the increase in calcium salts addition. The increase in pH values of control sample with banana was higher than those without banana; this was probably due to the availability of fiber from banana added to the yogurts (Aportela - Palacious et al., 2005).

**Table (1): Effect of using different calcium salts on pH values of calcium fortified stirred banana yoghurt.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH values</th>
<th>Calcium gluconate</th>
<th>Calcium lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>Banana stirred yogurt</td>
<td>4.72 ±0.02</td>
<td>4.52 ±0.03</td>
<td>4.55 ±0.03</td>
</tr>
<tr>
<td></td>
<td>4.64 ±0.02</td>
<td>4.75 ±0.12</td>
<td>4.78 ±0.16</td>
</tr>
</tbody>
</table>

a, b, c, d, and e. Means (±standard deviation) in the same row with different letters are significantly different (P<0.05).

Significant with control * P < 0.05

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Values of viscosity for control and calcium enriched banana yogurt ranged from \((0.499 - 0.491 - 0.333 - 0.292 - 0.664 - 0.367 - 0.323)\), respectively as shown in Figure (1). The results represented that a significant decreased \((P<0.05)\) of viscosity for banana stirred yogurt fortified with CaG in compared with the control. Viscosity of banana yogurt fortified with CaL showed a significant increase \((P<0.05)\) at level 1\% of fortification, but at levels 2\% and 3\% showed a significant decreased\((P<0.05)\) in viscosity in compared with the control. Also, all samples of banana yogurt fortified with CaG and CaL showed a significant decreased in viscosity in compared with us at the increasing of levels of fortification.

![Figure (1): Effect of fortification with CaG and CaL on viscosity of banana stirred yogurt.](image)

Results indicate that count of LAB for banana stirred yogurt fortified with CaG was increased in compared with the control. There were showed a significant differences \((P<0.05)\) at levels 2\% and 3\% of fortification, but at level 1\% showed no significant differences \((P>0.05)\) in compared with the control as shown in Table (2). Count of LAB for banana stirred yogurt fortified with CaL showed no significant differences \((P>0.05)\) in compared with the control.

There were no detection for mold and yeasts for calcium fortified banana stirred yogurt and the control. These results indicate that the manufacture of calcium fortified stirred yogurt was carried out using the proper hygienic practices, resulted in the elimination of the contamination with such undesirable bacteria. These results are in agreement with those of Ammar, (2000). E. coli bacteria were absent (in 0.1g) in all treatments and the control. The absences of coliform bacteria in fresh control and fortified samples were due to pasteurization of premix prior to incubation, which agreed with the report by Younus et al., (2002).

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Table (2): Effect of CaG and CaL on LAB count (cfu×10⁵/ml) of calcium enriched banana stirred yogurt.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>Calcium gluconate</th>
<th>Calcium lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1%</td>
<td>2%</td>
<td>3%</td>
</tr>
<tr>
<td>Banana</td>
<td>10.4 ± 0.21</td>
<td>12.6 ± 0.50</td>
<td>13.6 ± 0.97</td>
</tr>
<tr>
<td>stirred yogurt</td>
<td>a, b, and c</td>
<td>a, b</td>
<td>a*</td>
</tr>
</tbody>
</table>

a, b, and c. Means (±standard deviation) in the same row with different letters are significantly different (P<0.05) Significance with control * P < 0.05 ** P < 0.05

Texture profile analysis

Statistical analysis conducted on tested samples showed a significant difference (P<0.05) for hardness, springiness, chewiness, and cohesiveness. The hardness is defined as force necessary to attain a given deformation (Upir and Mishra, 2004). The tested samples showed a significant difference (P<0.05) for hardness values, which ranged from (0.782 – 0.543 – 0.392 – 0.343 – 1.083 – 0.543 – 0.393) respectively for control CaG and CaL fortified banana stirred yogurt as shown in Figure (2a). Hardness of banana yogurt fortified with CaG showed a significant decrease of hardness in compared with the control. While, banana yogurt fortified with CaL showed a significant increase of hardness at level 1% of fortification, but at level 2% and 3% showed a significant decrease (P<0.05) of hardness in compared with the control.

Springiness is the rate and extent which a deformed material goes back to its un-deformed condition after the deforming force is removed (Kahyaoglu et al., 2005). Values for the springiness of fresh banana yogurt and samples fortified with calcium salts ranged from (0.715 – 0.860 – 0.758 – 0.900 – 0.550 – 0.627 – 0.848) among different treatments as shown in Figure (2b). The results showed that a significant increase (P<0.05) in springiness values of banana yogurt fortified with CaG in compared with the control. While, this result showed a significant decrease (P<0.05) in springiness of banana yogurt fortified with CaL at level 1% and 2%, but showed a significant increase in springiness at level 3% of fortification in compared with the control.

Chewiness is the energy required to masticate a solid food product to make it ready for swallowing (Upir and Mishra, 2004). The chewiness values were reported in Figure (2c). Control and calcium enriched banana yogurt ranged from (0.357 – 0.422 – 0.253 – 0.263 – 0.366 – 0.230 – 0.274), respectively. The results represented that a significant increase (P<0.05) of chewiness of banana stirred yogurt fortified with CaG and CaL at level 1% of fortification, and showed a decrease in chewiness of banana yogurt fortified with CaG and CaL at level 2% and 3% of fortification in compared with the control. This increase of chewiness of yogurt fortified with calcium salts and control may be due to kind of salt used or because the action of microorganisms used S. thermophiles subsp salivarius and L. delbrueckii subsp bulgaricus on the yogurt product matrix. This result may be due the
evidence that starter organisms cause structural changes in the protein matrix (Ganesh, 2006).

Cohesiveness is defined as the extent to which a material can be deformed before its rupture cohesiveness depends upon the strength of the internal bonds (Uprit and Mishra, 2004). Cohesiveness of control and calcium enriched banana yogurt ranged from (0.637 – 0.903 – 0.847 – 0.850 – 0.613 – 0.674 – 0.821), respectively as shown in Figure (2d). The results reveal that banana stirred yogurt fortified with CaG showed a significant increase (P<0.05) in cohesiveness at all levels of fortification compared with the control, while banana yogurt fortified with CaL at level 1% showed a significant decrease (P<0.05) in cohesiveness, and showed an increase in cohesiveness at levels 2% and 3% of fortification compared with that of control. The difference of cohesiveness in all samples may be due to the action of calcium salts used. The protein matrix in yogurt is more responsible for cohesiveness (Tunick, 2000).

![Figure (2a)](image)

![Figure (2b)](image)
Figure (2c)

Figure (2d)

Figure (2): Effect of fortification with CaG and CaL on rheological properties of banana stirred yogurt.

Sensory evaluation
The results of sensory evaluation conducted on the samples fortified with CaG or CaL at levels of 1%, 2%, and 3% are seen in Table (3). Statistical analysis on the original sensory score showed a significantly lower (P<0.05) over all acceptability at 3% addition of CaG and CaL to yogurt. However, the bitterness was not detected at 1% and 2% addition of CaG and CaL. In case of CaL added at 2% and 3% levels scored significantly lower body and texture values (P<0.05), when compared to the control. Whereas, 1% added CaL to yogurt scored no statistically significant difference in body and texture compared to the control. Also, the CaG treatments didn’t affect significantly (P<0.05) the body and texture of yogurt, while the effect of addition 1% CaG
was a significant. CaL may impart some bitter notes in dairy products at high concentration Gerstner, (2002).

The highest mean values for overall acceptability were observed for control sample, banana stirred yogurt fortified with CaG and CaL at level 1% (96.60 – 95 - 93) respectively. The lowest mean value for overall acceptability were observed for banana stirred yogurt fortified with CaG and CaL at level 3% (90.60- 90.20) respectively.

Table (3): Effect of using different calcium salts on sensory properties of fresh fortified Banana stirred yoghurt.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>Calcium gluconate</th>
<th>Calcium lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1%</td>
<td>2%</td>
<td>3%</td>
</tr>
<tr>
<td>Flavor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>43.40</td>
<td>±0.55</td>
<td>42.00</td>
</tr>
<tr>
<td>1%</td>
<td>b</td>
<td>41.40</td>
<td>41.20</td>
</tr>
<tr>
<td>2%</td>
<td>c</td>
<td>40.40</td>
<td>40.20</td>
</tr>
<tr>
<td>3%</td>
<td>d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body and texture</td>
<td>38.60</td>
<td>±0.55</td>
<td>38.40</td>
</tr>
<tr>
<td>1%</td>
<td>a</td>
<td>37.20</td>
<td>37.60</td>
</tr>
<tr>
<td>2%</td>
<td>b</td>
<td>36.60</td>
<td>36.40</td>
</tr>
<tr>
<td>3%</td>
<td>c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color and appearance</td>
<td>14.60</td>
<td>±0.55</td>
<td>14.00</td>
</tr>
<tr>
<td>1%</td>
<td>a</td>
<td>13.00</td>
<td>13.60</td>
</tr>
<tr>
<td>2%</td>
<td>b</td>
<td>13.40</td>
<td>13.00</td>
</tr>
<tr>
<td>3%</td>
<td>c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over all acceptability</td>
<td>96.60</td>
<td>±1.52</td>
<td>95.00</td>
</tr>
<tr>
<td>1%</td>
<td>a</td>
<td>93.40</td>
<td>91.40</td>
</tr>
<tr>
<td>2%</td>
<td>b</td>
<td>90.60</td>
<td>90.20</td>
</tr>
<tr>
<td>3%</td>
<td>c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a, b, c, and d. Means (±standard deviation) in the same row with different letters are significantly different (P<0.05)
Significant with control * P < 0.05 ** P < 0.01 *** P < 0.001

Finally, it could be concluded that calcium in fruit banana stirred yogurt can be increased up to 1% and 2% calcium per 100ml by addition of calcium gluconate and addition of calcium lactate without any negative influence on the sensory properties. Addition of CaG to fresh banana stirred yogurt increase pH value with the increase of calcium addition. Addition of calcium caused an increase in colloidal calcium phosphate cross linking between casein micelles which resulted in more firm structure of fortified fruit yogurt.

REFERENCES


تدعيم مشروب زبادي الموز بالكالسيوم
علي الدين أحمد مرسي يونس ، فاطمة محمد الزمزمى و شيماء علي المرسى
قسم الاقتصاد المنزلي - كلية التربية النوعية - جامعة المنصورة – مصر

تم تدعيم مشروب زبادي الموز بملح جلوكونات الكالسيوم و لاكتات الكالسيوم بنسبة 1% و 2% و 3% كمصادر الكالسيوم الذائبة وذلك قبل عملية التخمر. أضيفت أملاح الكالسيوم للخلط بعد عملية البسترة حتى لا تتأثر بالحرارة. وتتم دراسة كل من رقم الأس الأيدروجيني و اللزوجة وعدد بكتريا حمض اللاكتيك و الخواص الريولوجية و التقييم الحسي لمبيعات زبادي الموز الغني بالكالسيوم. وأظهرت النتائج زيادة في كل من رقم الأس الأيدروجيني وعدد بكتريا حمض اللاكتيك كلما زادت نسبة الأملاح المضافة. وفي العموم تم تحديد أملاح الكالسيوم غير ملحوظ في الصفات الريولوجية (اللزوجة و الصلاحية و الأرتداد و المضغ و التماسك) أو التقييم الحسي لمشروب زبادي الموز حتى نسبة 2% جلوكونات الكالسيوم و 1% لاكتات الكالسيوم.

قام بتحكيم البحث
أ.د / أحمد عبد العزيز الرفاعى
كلية الزراعة – جامعة المنصورة
أ.د / عبد الغنى محمود عبد الغنى
كلية التربية النوعية – جامعة المنصورة