FORTIFICATION OF STANDARDIZED BUFFALO MILK YOGURT WITH CALCIUM CITRATE AND WHEY PROTEIN CONCENTRATE

SHAMSIA S. M.
Department of Food and Dairy Science & Technology, Faculty of Agriculture, Damanhour University, Egypt.

ABSTRACT

Set Yogurt was prepared from fat-standardized (4%) buffalo milk and fortified with calcium (Ca citrate) to contain 50 and 100% plus calcium and whey protein concentrate (WPC 80% protein) in ratios of 0.2, 0.3 and 0.5%. Total solids, total protein and soluble protein were increased in all treatments proportionally with the amount of fortification while fat content has no significantly (P ≤ 0.05) changed. Acidity values were ranged from 0.85 to 0.89, the ordinary range of acidity in the conventional Yogurt while pH values showed a slight buffering effect of added WPC in all treatments comparing with the control. Sensorial properties showed better appearance, texture, flavor and mouth feel in control sample and samples fortified with low amounts of Ca citrate or WPC. Water holding capacity (WHC) was increased as the amount of fortification from Ca citrate or WPC increased.

Key words: yogurt, Buffalo-milk, Fortification, whey protein concentrate and Calcium Citrate.

INTRODUCTION

Calcium makes up about 2% of the body-weight and about 39% of the total body minerals (Miller and Anderson, 1999). Calcium plays an essential role in building bone mass, preventing osteoporosis, proper
kidney function, regulation of blood pressure and blood cholesterol levels (DiRienzo, 2001). Ionized calcium in blood performs certain regulatory functions such as, contraction of muscle, coagulation of blood, transmission of nerve impulses, activation of enzyme reactions, stimulation of hormone secretions, and integrity of intracellular cement substances (Annon, 1997; Thys-Jacobs et al., 1999; Miller and Anderson, 1999; Heaney, 2000; Miller et al., 2001; McCarron and Heaney, 2004). The World Health Organization (WHO) has defined osteoporosis as the second leading health care problem after cardiovascular disease affecting more than 200 million women worldwide (Wilson, 2004).

Milk is recognized as "the richest natural source of calcium". Even though, cow milk contains only 100-120 mg / 100 ml of Calcium. The Institute of Medicine (1997) recommended 500 mg calcium per day for children 1-3 years; 800 mg calcium per day for children 4-8 years and 1300 mg calcium for 9-18 years old; 1000 mg per day between 19 and 50 years including pregnancy and lactation period and 1200 mg per day over age 51.

Therefore in order to reach significant level of Recommended Daily Allowance (RDA), fortification of some food with calcium is required. Whether set or stirred Yogurt, it is a popular dairy product among all age groups in Egypt. It is being the best choice for calcium enrichment. Yogurt can carry higher calcium density and calcium from yogurt is highly bioavailable, these characteristics make yogurt as ideal vehicle for calcium fortification Gurmeet (2007). It is generally agreed that the bioavailability of organic calcium is much higher than inorganic calcium. From the literature Ramasubramanian et al. (2008) it appears that the bioavailability of organic calcium is 2 to 5 times higher than that of inorganic calcium.

Whey proteins have been used for many years as highly nutritious food supplement (Walzem et al., 2002). The protein efficiency ratio (PER) of a protein source measures the weight gain of young animals per gram of protein eaten over a given time period. Whey proteins have proportionately more sulfur-containing amino acids (cysteine, methionine) than caseins, which contributes to the higher PER of whey proteins (3.2) than of casein (2.6). Any protein with a PER of 2.5 is considered good quality. The high sulfur-containing amino acid content of whey proteins appears important to their
ability to enhance immune function and antioxidant status via modulation of the sulfur-containing tri-peptide glutathione (Bounous and Gold, 1991). Further, among all protein sources, whey proteins contain the highest concentration of the branched-chain amino acids L-isoleucine, L-leucine, and L-valine. Virtually every amino acid present in sweet-type whey exceeds Food and Agriculture Organization/World Health Organization (FAO/WHO) nutritional intake recommendations, both for children aged 2 to 5 years and for adults (Glass and Hedrick, 1976).

The present study has been designed to determine the proper ratios of calcium and whey protein for fortification and supplementation of Yogurt without developing adverse physical or organoleptic characteristics.

MATERIALS AND METHODS

Materials
- Buffalo milk was obtained from a private farm near Damanhour city, Behera Governorate. It was standardized to 4% fat and 13.74% total solids (table 1).
- Calcium citrate ($C_6H_5O_7$)$_2$Ca$_3$.4H$_2$O food additive E333 (21% Ca) was purchased from El-garas com. Alexandria, Egypt.
- Whey protein concentrate 80% protein (WPC 80%) is manufactured by Agri Mark, Inc. (USA), imported and distributed by El-garas com., Alexandria, Egypt. WPC consisting of: Protein 83.7%, lactose 5.5%, minerals 1.2%, moisture 5.0% and milk fat 4.6%.
- Lactic acid culture (MAO 16,20u. Texel, France) containing Streptococcus salivarius ssp. thermophilus and Lactobacillus delbruckii ssp. bulgaricus was supplied from Dairy Pilot Plant, Faculty of Agriculture, Alexandria University, Egypt.

Methods
Fortification with Calcium and whey protein concentrate

Standardized buffalo milk contained 162 mg Ca/100ml (table 1). Ca citrate was added to elevate the Ca content of milk by 50 and 100%. Ca citrate contains 21% calcium, therefore, Ca citrate was added in a ratio of 386mg/100g standardized buffalo milk to make calcium content
150% and 771mg/100g standardized buffalo milk to make calcium content 200%.

Table 1: Chemical composition of native and standardized buffalo milk.

<table>
<thead>
<tr>
<th>Milk</th>
<th>Chemical composition</th>
<th></th>
<th></th>
<th>Ca (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total solids.</td>
<td>Fat</td>
<td>Protein</td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>16.61</td>
<td>6.90</td>
<td>3.75</td>
<td>160</td>
</tr>
<tr>
<td>Standardized</td>
<td>13.74</td>
<td>4.00</td>
<td>3.77</td>
<td>162</td>
</tr>
</tbody>
</table>

WPC 80% was added to standardized buffalo milk in ratios of: 0.2, 0.3 and 0.5% (W/W).

Trials were prepared as follows:
- Control (standardized milk) without addition of neither Ca citrate nor WPC.
- Tr.1: fortified milk with 50% plus Ca + 0.2% WPC.
- Tr.2: fortified milk with 100% plus Ca + 0.2% WPC.
- Tr.3: fortified milk with 50% plus Ca + 0.3% WPC.
- Tr.4: fortified milk with 100% plus Ca + 0.3% WPC.
- Tr.5: fortified milk with 50% plus Ca + 0.5% WPC.
- Tr.6: fortified milk with 100% plus Ca + 0.5% WPC.

Preparation of Yogurt

All treatments were prepared and pasteurized at 85°C/30 min., then cooled to 42°C and inoculated with 2% lactic acid culture (W/W), packaged in PVC containers (120g), then incubated at 42±1°C until coagulation. The samples were immediately stored at 6±2°C until analyzed.

Fresh representative samples were taken from each treatment for chemical analysis and sensory evaluation.

Chemical analysis
- Calcium content was determined by atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT), according to the method of Association of Official Analytical Chemists (1980).
- Total solids content were determined according to the British Standard Institution Bulletin (1952).
- pH values were measured using a pH-meter model HANNA HI9321 microprocessor with a standard, combination glass electrode British Standard Institution Bulletin (1952).
- Titratble acidity was estimated as percentage of lactic acid according to Ling (1963).
- Fat content was determined according to British Standard Institution Bulletin (1955).
- Total and soluble protein content were determined as described in the Association of Official Analytical Chemists (1984).

**Sensory evaluation**
- Sensorial properties were evaluated by ten panelists familiar with the product after 6hr. storage of the samples at 5°C. Sensory characteristics were appearance (5 points), texture (5 points), flavor (10 points) with total score of 20 points Pearce and Heap (1974).

**Water holding capacity (WHC)**
- A sample of about 20 g of native yogurt (NY) was centrifuged for 10 min at 669 xg and 20°C (Remeuf et al., 2003). The whey expelled (WE) was removed and weighed. The WHC expressed in % was defined as WHC (%) = 100 * (NY – WE)/NY.

**Statistical analysis**
- All obtained data were statistically analyzed using SAS software program (2000).

**RESULTS AND DISCUSSION**

Chemical analysis of Yogurt has been shown in table2. Total solids were increased due to addition of calcium citrate and WPC. The amount of increment was relative to the amount of Ca citrate or WPC have been added to each treatment. Total and soluble protein contents were also increased as the added amount of WPC has been increased. Fat content was constant at 4% in control sample and treatments 1, 3 and 5 while 0.05% decrease in fat content has occurred in treatments 2, 4 and 6 where total solids content of Yogurt were significantly (P≤0.005) increased because of addition of excessive amounts of Ca citrate and WPC. Acidity values were slightly differed among treatments and there was no significant (P≤0.005) difference in acidity of control sample and treatments. At the same time, pH values were slightly varied among treatments but control sample had significantly (P≤0.005) lower pH value comparing with those of treatments. It can be attributed to the buffering effect of both Ca citrate and WPC which
added to treatments.

Table 2: Chemical analysis of Yogurt

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chemical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total solids %</td>
</tr>
<tr>
<td>Control</td>
<td>13.75</td>
</tr>
<tr>
<td>Tr. 1</td>
<td>14.34</td>
</tr>
<tr>
<td>Tr. 2</td>
<td>14.72</td>
</tr>
<tr>
<td>Tr. 3</td>
<td>14.48</td>
</tr>
<tr>
<td>Tr. 4</td>
<td>14.88</td>
</tr>
<tr>
<td>Tr. 5</td>
<td>14.60</td>
</tr>
<tr>
<td>Tr. 6</td>
<td>15.03</td>
</tr>
</tbody>
</table>

LSD $P \leq 0.05 = 0.076, 0.057, 0.054, 0.0259, 0.0187$ and $0.0175$ for total solids, fat, total protein, soluble protein, acidity and pH respectively.

Sensory evaluation of Yogurt was represented in table 3. High score for appearance and texture have been gained by most of the prepared Yogurt. Control sample and treatments 1, 2, 3, 4 and 5 gained 80-90% of full score, while treatment 6 had comparatively lower score. These results led to the conclusion that the added amounts of Ca citrate or WPC have no definite effect on appearance or texture as it evaluated organoleptically.

Flavor and mouth feel of prepared Yogurt showed clear differences between treatments. Control sample and treatments 1, 2 and 3 gained 80% or more from the full score of flavor. Then it was gradually decreased from treatments 4 to treatment 6 where it has the minimum score (5.33 points). Treatments 2, 4 and 6 got lower flavor score than those got by treatments 1, 3 and 5. Worthwhile to report here that Ca citrate is odorless and it has a slight sour taste. The added amounts of Ca citrate to treatments 2, 4 and 6 were the duplicate amounts added to treatments 1, 3 and 5. It seems that the sour taste of Ca citrate made the tender acid taste of Yogurt got sharpen, consequently inversely affected the perception, flavor and mouth feel of treatments 2, 4 and 6.

The other factor that strongly affected flavor and mouth feel of prepared Yogurt is the addition of WPC. It is well known that
supplementation of some dairy products such as ice cream, Yogurt and milk-based beverages smoothen the texture and improve body and sensation in the mouth if it has been added in a reasonable amount. On the other hand WPC particularly that contains high protein content such as WPC 80 has a very slight piscine flavor in the aqueous media and may leaves an unpleasant aftertaste feeling if it is added in a significant amount Noha (2008). Table 3 showed that flavor score has gradually decreased with the increase of the amount of WPC added to Yogurt. Treatments 5 and 6 which contained .5% WPC had the lowest flavor score. It can be concluded that there is an integrated inverse effect of both Ca citrate and WPC on the sensorial characteristics when they added in large amounts (treatments 4, 5 and 6). Total score (20 points) of sensory evaluation of Yogurt approved that control sample and treatments 1, 2 and 3 had a good perception. Patel, et al., (2006) substituted skim powder milk by WPC 82.5% protein to 30, 60 and 90% more protein in the ice cream mix. They found that overall texture acceptance for all treatments was more desirable compared with control. Sensory evaluation revealed that substitution to 30% more protein was the best ratio, and they concluded that it is possible to produce acceptable ice cream with higher levels of protein.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Appearance (5)</th>
<th>Texture (5)</th>
<th>Flavor &amp; mouth feel (10)</th>
<th>Total (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.67</td>
<td>4.43</td>
<td>8.33</td>
<td>17.43</td>
</tr>
<tr>
<td>Tr. 1</td>
<td>4.83</td>
<td>4.57</td>
<td>8.33</td>
<td>17.73</td>
</tr>
<tr>
<td>Tr. 2</td>
<td>4.73</td>
<td>4.33</td>
<td>8.27</td>
<td>17.33</td>
</tr>
<tr>
<td>Tr. 3</td>
<td>4.87</td>
<td>4.67</td>
<td>8.00</td>
<td>17.54</td>
</tr>
<tr>
<td>Tr. 4</td>
<td>4.67</td>
<td>4.13</td>
<td>6.67</td>
<td>15.47</td>
</tr>
<tr>
<td>Tr. 5</td>
<td>4.43</td>
<td>4.03</td>
<td>6.00</td>
<td>14.46</td>
</tr>
<tr>
<td>Tr. 6</td>
<td>3.97</td>
<td>3.67</td>
<td>5.33</td>
<td>12.97</td>
</tr>
</tbody>
</table>

LSD P ≤ 0.05 = 0.0175, 0.0175, 0.0264 and 0.7646 for appearance, texture, flavor and total score respectively.

Water holding capacity (WHC) of Yogurt has been shown in table 4. WHC significantly (P≤0.005) increased from control sample to
treatments. Treatments 2, 4 and 6 had higher WHC than treatments 1, 2 and 3. As the added amount of Ca citrate or WPC increased, the WHC increased. Donna Gorski (1997) mentioned that whey protein has outstanding gelation and water-binding properties and emulsifying properties. Water-binding, gelling increasing with increasing of protein content. The water-binding and emulsification properties of WPC are useful in adding lubricity and good mouth feel. In addition, Ca of Ca citrate play a principle role in forming a colloidal network that can trapping the water in the matrix of Yogurt body. Gurmeet (2007) found that fortification of pasteurized yogurt mix with 50 mg Ca/100 ml of calcium lactate, significantly (P≤0.005) increased the water holding capacity (WHC) by 7.76 % on 1st day of storage.

Table 4: Water holding capacity (WHC) of Yogurt

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>Tr. 1</th>
<th>Tr. 2</th>
<th>Tr. 3</th>
<th>Tr. 4</th>
<th>Tr. 5</th>
<th>Tr. 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHC%</td>
<td>86.8</td>
<td>91.9</td>
<td>94.3</td>
<td>92.1</td>
<td>94.8</td>
<td>92.6</td>
<td>95.1</td>
</tr>
</tbody>
</table>

LSD P≤ 0.05 = 0.1751

REFERENCES


تم تحضير البئر من اللبن الجاموسي المعدل إلى 4% دهن وتم تزويمه بإضافة ستات الكالسيوم لرفع نسبة الكالسيوم في البئر بـ 50، 100% ومركز بروتينات الشرش (80%) بروتين، أظهرت النتائج زيادة في سبب كل من المواد للسلبية والبروتين الكلي وبروتينات النبات بـ 0.2، 0.3، 0.5%، أظهرت النتائج زيادة من كل من ستات الكالسيوم ومركز بروتينات الشرش بينما لم يتغير نسبة الهذين في جميع المعاللات. تراوحت نسبة الحمضية بين 0.85، 0.89% وهو والذي المنطقي الموجود في البئر المعدل بينما كان الأخفاض في رقـ الـ pH نتيجة تكون الحمضية واضحا في العينة الكتربول أما في المعاللات فقد كان انخفاض الـ pH أقل وقد أعزى ذلك للفعل المنظم لبروتينات الشرش المعتادة. أظهرت نتائج التحكم الحسي على المنتج أن خواص المظهر والتركيب والتكية على الحساس في المحاذا كانت أفضل في تلك المعاللات التي أضيف فيها كميات أقل من كل ستات الكالسيوم ومركز بروتينات الشرش وكذلك العينة الكتربول مقارنة بباقي المعاللات. وقد بنيت النتائج زيادة في نسبة البئر على الاحتفاظ بالماء في المعاللات التي تم فيها إضافة كل من ستات الكالسيوم ومركز بروتينات الشرش. وقد إزدادت الحفاظ بالماء، وقد زادت نسبة الاحتفاظ وقد أعزى ذلك لـ الاحتفاظ بالماء، وذلك قدرة الكالسيوم على بناء شبكة البروتينات التي تكون البئر وتثبيت الماء داخلها.