

Utilization of Egg-shell Powder as a Calcium Fortifier in Stirred Dibis Probiotic Yoghurt

By

Khaled H. Salman,⁽¹⁾ Ali I. A. Mansour,⁽¹⁾ Adel A. Tammam⁽²⁾ and Fathy E. El-Gazzar⁽²⁾

⁽¹⁾ Dairy Science Department, Faculty of Agriculture, Al-Azhar University (Assiut).

⁽²⁾ Dairy Science Department, Faculty of Agriculture, Assiut University.

ABSTRACT

Date syrup (Dibis) and egg-shell powder were prepared from Siwi date variety and white egg chicken, respectively. Stirred dibis probiotic yoghurt (SDPY) was prepared by adding different concentrations of egg-shell powder (0.5, 0.6, 0.9 and 1.1%) to milk and mixed with 1.5% skim milk powder, 0.4% gelatin and 10% dibis then inoculated with probiotic bacteria ABT-5 (*Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus*) and held at $6\pm 2^{\circ}\text{C}$ for 9 days. Our results showed that the date syrup was high in total solids & acidity; and rich in Ca, K, P, Mg and Fe. While, egg-shell powder was alkaline and rich in Ca, P, Mg and Fe. The presence of egg-shell powder in probiotic yoghurt may increase the TS, density, Ca, Mg, Fe and net utilization calcium (NU Ca). It is clear from our results that as the storage period progressed, the SN/TN of all treatments gradually increased for all treatments. There are significant differences among all the treatments and during storage periods for all chemical analyses ($p > 0.05$). Count of *L. acidophilus* and *Bif. Bifidum* was increased in SDPY. Samples of the SDPY without egg-shell powder found to be superior after 9 days of storage at $6\pm 2^{\circ}\text{C}$ among the other samples. Generally, egg-shell powder could be used up to 0.9% to make SDPY with 10% dibis and held at $6\pm 2^{\circ}\text{C}$ for 6 days.

Key words: Egg-shell – Probiotic bacteria – Dibis – Stirred yoghurt.

INTRODUCTION

Yoghurt is fermented milk consumed all around the world. This “biotechnological” food is considered by nutritionists as having high nutritional value. It lacks lactose and has a significant concentration of Ca^{++} and positive bioactive effects in products treated with prebiotic ingredients and probiotic bacteria. The “natural” plain yoghurt is produced by adding lactic acid bacteria, which increase the lactic fermentation. Among all milk fermented products, yoghurt is well-known compared with others, and has more acceptability for the consumer around the world (Coisson *et al.*, 2005).

A physiological functional food can be defined as a food derived from naturally occurring substances that should be consumed as a part

of the daily diet, and thus provides health benefits in reducing the risk of developing disease (Schmidl, 1993). A nutraceutical is any substance in food or a part of a food that provides medical or health benefits in reducing the risk of developing disease (De Felice, 1995). Yoghurt is a complete food, can serve as an ideal source to carry these nutraceuticals. However, functional foods are generally considered as those foods which are intended to be consumed as a part of the normal diet and that contains biologically active components, which offer the potential of enhanced health or reduced risk of disease. Examples of functional foods include foods that contain: specific minerals, vitamins, fatty acids or dietary fiber foods with added biologically active substances such as phytochemicals or other antioxidants and probiotic bacteria that have live beneficial (FAO, 2007).

Calcium is one of the essential minerals for the human body; therefore its daily intake values and relation to bone health are attaining growing awareness among research community. Research has demonstrated that adequate calcium intake is not only essential for bone growth and development, but also important for the regulation of cell function, nerve conduction, muscle contraction and blood coagulation (Thys-Jacobs *et al.*, 1999). 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). Calcium intake can be increased by consuming foods naturally rich in calcium such as dairy foods, certain vegetables, calcium fortified foods or their combinations. Dairy foods are the major source of calcium in U.S. diet (Gerrior *et al.*, 2004). They also improve overall nutritional quality of diet by providing substantial amounts of certain essential nutrients. However, recent food consumption trends a steady decrease in milk consumption combined with a steady increase in low calcium drinks such as soft drinks (Miller *et al.*, 2001). On the other hand, Solomon *et al.* (1994) and Schaafsma *et al.* (2000) reported that chicken egg-shell powder have been used by humans for a long period as a food additive and it might be an attractive source for human nutrition.

Therefore, the present study was undertaken to utilize probiotic bacteria, dibis and egg-shell powder to make functional dairy foods and to evaluate the influence of these additives on chemical, microbiological, rheological and sensory properties of SDPY during storage at $6\pm 2^{\circ}\text{C}$ for 9 days.

MATERIALS AND METHODS

Whole fresh buffalo's milk was obtained from the herd of the animal production department, faculty of Agriculture, Al-Azhar University (Assiut branch).

Probiotic cultures ABT-5 were provided by the Chr. Hansen Co., contains the following species: *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus*.

Siwi Dates Variety, calcium fortification source (from chicken white egg-shell), skim milk powder and gelatin were obtained from the local market, Egypt.

The pulp of Siwi date was separated from kernel, weighted, washed twice and extracted by water rate 1-2 (pulp : water) at 70°C for two hours with stirring at intervals. Mixture was placed in molds and then pressed. The juice was filtered in cheese clothes (double layer), concentrated using water bath at 70°C until total soluble solids reaches 72% to avoid spoilage of the date syrup on storage. Date syrup was sterilized by tindilization and storage at room temperature until use it.

Chicken white egg-shell was washed, sterilized in autoclave at 121°C for 20 min. After drying at 60°C over night, it was well grinded. The egg-shell powder was sifted by 0.125 mm.

Buffalo's milk (6% fat) was mixed with 1.5% skim milk powder and 0.4% gelatin, and then divided into six equal portions. Every part was heated to 90±1°C for 15 min, rapidly cooled to 42-44°C, addition 10% of date syrup (w/w) for all parts was carried out, except the negative control, and then addition the egg-shell powder was added as following:

C⁻ - negative control.

C⁺ - positive control.

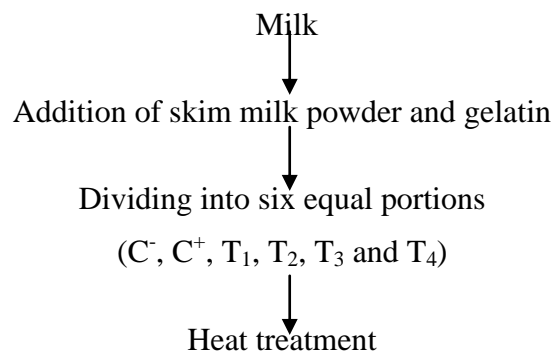
T₁- Adding 0.5% (w/w) egg-shell powder.

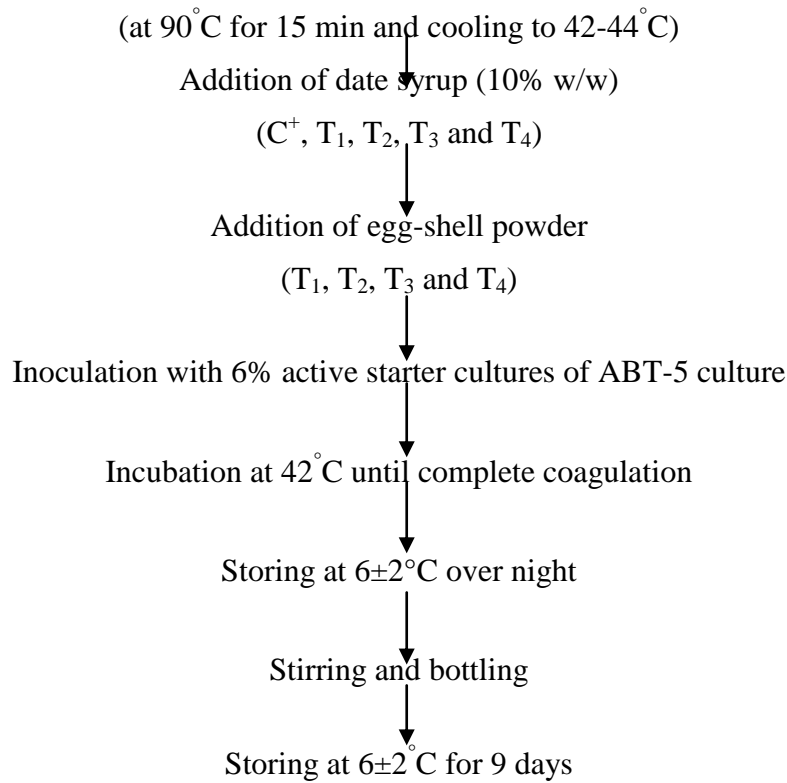
T₂- Adding 0.7% (w/w) egg-shell powder.

T₃- Adding 0.9% (w/w) egg-shell powder.

T₄- Adding 1.1% (w/w) egg-shell powder.

Each part was inoculated with 6% active starter cultures of ABT-5 culture according to Klupsch (1985). The inoculated batches were incubated at 42°C until complete coagulation. After coagulation, samples were stored at 6±2°C over night, stirred and bottled according to El-Sonbaty *et al.* (2008). All batches were kept at the same temperature for 9 days, and analyzed at fresh and after 3, 6 and at the end of storage.





Total solids and titratable acidity were carried out according to AOAC (2000) and IDF (1987) respectively. Fat content was determined according to IDF (1986). Carbohydrates content was determined according to Dubois et al. (1956). Total Nitrogen and soluble nitrogen were estimated as described by IDF (1993). pH Measurement was determined using a pH meter (model 68 ESD 19713), USA. Calcium and Magnesium contents were estimated according to the method of Ntailianas and Whitney (1964). Phosphorus content was determined according to Tiessen and Moir (1993). Potassium content was estimated by Flame photometer according to the method of Page (1982). Determination of Iron was done by Atomic Absorption Spectrophotometer according to (Sotera and Stux, 1979). Net utilization calcium (NU Ca) was calculated according to Ahmed (2009) as the following equation:

$$\text{NU Ca\%} = \frac{\text{Ca in fermented milk} - \text{Ca in milk}}{\text{Ca in fermented milk}} \times 100$$

For detecting the total bacterial count, the general plate count technique outlined in the standard methods for examination of dairy products was adopted according to Marshal (1992). Serial dilutions of each sample in citrate buffer were plated on tryptone soya agar (TSA), pH 7.0±0.2 and incubated first at 30°C for 3 days, then at 37°C for another 2 days. lactobacilli count was estimated on the selective medium for lactobacilli (MRS) as suggested by De Man *et al.* (1960). The plates were incubated at 37°C for 48 hours. Streptococci count was determined by M17 agar

medium as suggested by IDF (1997). Bifidobacteria count was enumerated according to Dave and Shah (1996) using modified MRS agar medium (m-MRS), supplemented with 0.05% L-Cysteine HCL and 0.3% lithium chloride. The plates were incubated at 37°C for 48 hours under anaerobic condition. Coliform bacteria and moulds & yeasts were enumerated according to IDF (1985a) and IDF (1985b), respectively.

Curd syneresis was determined as described by Farooq and Haque, (1992). Density was calculated using the regular equation as follows:

$$\text{Density (gm/cm}^3\text{)} = \frac{\text{Weight}}{\text{Volume}}$$

The organoleptic evaluation of plain stirred probiotic yoghurt (PSPY), SDPY and SDPY with egg-shell powder were assessed by a panel test of 10 persons of staff members of the Dairy Department, Faculty of Agriculture, Al-Azhar University (Assiut branch) as fresh, 3, 6 and 9 days of storage at 6±2°C according to the scheme described by Kebary and Hussein (1999).

The measurements of all previous tests were done in triplicate.

Results were evaluated statistically using the software program; the SAS system for windows, release 8.02 TS level 02M0, SAS Institute Inc., Cary, NC, USA (SAS, 1999).

RESULTS AND DISCUSSION

Data (Table 1) illustrate the chemical composition of Siwi date syrup. The data revealed that the total soluble solid (TSS) of date syrup was high, this is principally due to the high level of sugars and relatively low moisture content; while, it had low fat and total nitrogen.

Table 1: Chemical and microbiological analyses of Siwi date syrup:

Components		Ratio
Moisture (%)		27.96
Total soluble solids (%)		72.00
Carbohydrates (%)		69.39
Fat (%)		1.77
Total nitrogen (%)		0.124
Acidity (%)		0.29
pH		4.20
Density gm/cm ³		1.630
Minerals (on wet basis) (mg/100 gm)	Calcium (Ca)	163
	Potassium (K)	291
	Phosphors (P)	176
	Magnesium (Mg)	136
	Iron (Fe)	4.5
Total bacterial count (cfu/ml)	Before Tindilization	343.33
	After Tindilization	100.00
Moulds and Yeasts Count (cfu/ml)	Before Tindilization	143.33
	After Tindilization	ND*

* ND:- Not detected

On the other hand, the date's syrup is rich in calcium, potassium, phosphors, magnesium and iron. The present data are in agreement with those of Mustafa *et al.* (1983) and El-Shobery *et al.* (2012) and higher than that found by Al-Saidy *et al.* (1982). In addition, the results of Khalil *et al.* (2002) indicated that the dibis is a good source of amino acids as well as many important elements such as; K, Na, Ca, Mg, Fe and Zn. In addition the results showed that the total bacterial count decreased after tindilization, while moulds & yeasts were not detected.

Data (Table 2) show the chemical composition of egg-shell powder. The data revealed that the total nitrogen (TN) of egg-shell powder was high; this is due to the low moisture content. The obtained data showed that the prepared egg-shell powder had high pH value; therefore the expected storage ability will be short. On the other hand, the egg-shell powder is rich in calcium, phosphors, magnesium and iron. Similar results obtained by Schaafsma *et al.* (2000).

Table 2: Chemical composition of egg-shell powder:

Components		Ratio
Total nitrogen (%)		0.45
pH		9.51
Density gm/cm ³		1.350
Minerals (on dry basis) (mg/100 gm)	Calcium (Ca)	38500
	Phosphors (P)	146
	Magnesium (Mg)	360
	Iron (Fe)	230

Data (Table 3) illustrate the chemical composition of stirred probiotic yoghurt with the addition of date syrup and with different concentrations of egg-shell powder during storage periods at 6±2 °C for 9 days.

The data revealed that the TS content of PSPY was affected by the addition of dibis during storage periods. These results are similar to the results obtained by Hashmi *et al.* (2011) and El-Shobery *et al.* (2012). The TS content of SDPY and SDPY with egg-shell powder was higher than that in PSPY (Table 3).

On the other hand, the TS content of SDPY with egg-shell powder found to increase with an increasing egg-shell concentration. In addition, there was an increase of TS content with an increasing the storage period up to 9 days for all treatments (Table 3).

The data (Table 3) revealed that the fat content of PSPY and SDPY was found to decrease with an increasing the storage period at 6±2 °C for 9 days for all treatments. On the other hand, the fat content of SDPY was less using dibis. This might be due to low fat content in dibis. Similar results were obtained by El-Shobery *et al.* (2012).

The data in the same Table revealed that the acidity and pH values of PSPY and SDPY were affected by the addition of dibis & egg-shell powder during the storage conditions. The acidity of SDPY was higher than that in PSPY, and increased during storage period at $6\pm 2^{\circ}\text{C}$ for 9 days. This may be due to the more carbohydrate content of dibis being converted into acid in fermentation process. The acidity of SDPY found to be decreased with an increasing of egg-shell powder concentrations. This may be due to the pH of egg-shell powder (9.51). While, the pH values of PSPY were higher than that in SDPY. O'Neil *et al.* (1979); Zekai & Erdogan (2003) and Hashmi *et al.* (2011) reported that acidity and the pH values of yoghurt showed similar changes. When pH decreased alcoholic aroma and acidic taste increased on yoghurt samples resulted in decrease flavour scores.

Table 3: Effect of different egg-shell powder concentrations on chemical composition of stirred probiotic yoghurt held at $6\pm 2^{\circ}\text{C}$ for 9 days.

Components	Storage (days)	Control		Percentage of egg-shell powder				L.S.D
		C ⁻	C ⁺	0.5	0.7	0.9	1.1	
TS %	Fresh	17.67	19.46	19.64	19.78	19.78	19.94	0.051 ¹
	3	18.75	22.18	20.43	21.25	21.88	22.21	
	6	19.86	23.48	22.48	22.60	22.79	23.68	0.062 ²
	9	21.05	24.82	24.24	24.29	24.31	24.59	
Fat %	Fresh	6.0	5.0	5.2	4.9	4.9	5.0	0.141 ¹
	3	6.0	5.0	4.9	4.8	4.8	4.8	
	6	5.8	4.8	4.8	4.7	4.7	4.7	0.173 ²
	9	5.7	4.7	4.7	4.7	4.7	4.5	
Acidity %	Fresh	1.04	1.16	1.18	1.16	1.09	1.08	0.008 ¹
	3	1.23	1.28	1.24	1.24	1.19	1.17	0.010 ²
	6	1.25	1.30	1.31	1.30	1.24	1.18	
	9	1.28	1.36	1.35	1.32	1.26	1.20	
pH	Fresh	4.52	4.38	4.76	4.82	4.82	4.84	0.010 ¹
	3	4.30	4.28	4.73	4.78	4.78	4.80	0.011 ²
	6	4.28	4.25	4.69	4.74	4.77	4.79	
	9	4.27	4.18	4.62	4.67	4.70	4.72	
SN %	Fresh	0.055	0.053	0.051	0.054	0.052	0.055	0.001 ¹
	3	0.061	0.056	0.056	0.055	0.063	0.066	0.001 ²
	6	0.068	0.063	0.061	0.067	0.074	0.067	
	9	0.076	0.075	0.068	0.080	0.080	0.074	
TN %	Fresh	1.04	0.98	0.97	1.00	1.00	0.99	0.013 ¹
	3	1.01	0.95	0.93	0.94	0.94	0.95	0.016 ²
	6	0.98	0.92	0.90	0.93	0.94	0.94	
	9	0.93	0.90	0.87	0.90	0.88	0.90	
SN/TN%	Fresh	5.31	5.42	5.25	5.41	5.18	5.53	0.238 ¹
	3	6.03	5.93	6.01	5.81	6.62	6.90	0.291 ²
	6	7.00	6.86	6.83	7.18	7.87	7.26	
	9	8.21	8.40	7.82	8.87	9.17	8.27	

C⁻: control.

¹ L.S.D. for times

C⁺: control + 10 % dibis (w/w).

² L.S.D. for treatments

On the other hand, the pH values were increased with an increasing of the egg-shell powder concentrations, while it was decreased during the storage period (9 days).

Results in Table 3 revealed that SN and TN of stirred probiotic yoghurt were affected by the addition of dibis & egg-shell powder during storage periods at $6\pm 2^{\circ}\text{C}$ for 9 days. The SN and TN of PSPY were higher than that in SDPY. On the other hand, SN found to increase with an increasing the storage period at $6\pm 2^{\circ}\text{C}$ for 9 days for all treatments. While, the TN values decreased with an increasing the storage period for 9 days for all treatments. In addition, it is clear from these data that as the storage period progressed, the soluble nitrogen coefficient (SN/TN) was gradually increased for all treatments.

Generally, there are significant differences among all the treatments and during storage periods for all chemical analyses except density (Table 8).

Data presented in Table 4 illustrate the major minerals of PSPY and SDPY with varying concentrations of egg-shell powder during storage periods at $6\pm 2^{\circ}\text{C}$ for 9 days. The obtained data showed that the calcium, potassium, phosphors, magnesium, iron and Net utilization calcium (NU Ca) of PSPY and SDPY with egg-shell powder increased with an increasing the storage period for 9 days for all treatments. Unpublished data observed that the calcium content of milk was 161 mg/100 gm.

Table 4: Effect of different egg-shell powder concentrations on minerals (mg/100 gm) of stirred probiotic yoghurt held at $6\pm 2^{\circ}\text{C}$ for 9 days (on wet basis).

Components	Storage (days)	Control		Percentage of egg-shell powder			
		C ⁻	C ⁺	0.5	0.7	0.9	1.1
Ca	Fresh 9	181.00	173.11	327.18	419.46	490.94	551.09
		187.76	179.89	334.00	427.28	498.11	558.27
K	Fresh 9	174.65	202.40	202.31	202.16	203.05	203.00
		186.05	213.00	214.04	213.99	213.95	214.00
P	Fresh 9	169.54	172.78	172.88	173.00	173.10	173.11
		173.16	177.24	177.29	177.41	177.85	178.00
Mg	Fresh 9	13.02	22.00	22.02	21.99	22.24	22.52
		14.02	23.21	23.04	23.00	23.21	23.08
Fe	Fresh 9	0.48	0.80	3.02	3.02	3.03	3.04
		0.51	0.83	3.04	3.05	3.06	3.06
NU Ca	Fresh 9	11.05	7.00	50.79	61.62	67.21	70.79
		14.25	10.50	51.80	62.32	67.68	71.16

On the other hand, the values of potassium, phosphors, magnesium and iron were higher in SDPY with egg-shell powder than that in PSPY. This may be due to increase of these minerals in dibis and egg-shell powder concentrations. Also, the values of calcium and NU Ca were lower in SDPY than that in PSPY and SDPY with egg-shell powder. Similar results were obtained by El-Shobery *et al.* (2012). In addition,

the values of calcium, phosphors, iron and NU Ca increased with increasing of egg-shell powder for all treatments.

Table 5 shows that the total bacterial, *L. acidophilus*, *S. thermophilus* and *Bif. bifidum* counts of stirred probiotic yoghurt were affected by addition of dibis and during storage periods. There were increased in SDPY than in PSPY. These results are similar to the results obtained by Hashmi *et al.* (2011). In addition, the total bacterial and *L. acidophilus* counts were decreased during storage period for 9 days, while *Bif. bifidum* counts were increased at fresh and when stored at 3 days, then decreased up to 9 days of storage in all treatments. On the other hand, the data show that there was an increase of *Bif. bifidum* count up to 3 days of storage, then decreased up to 9 days for all treatments. Regarding egg-shell powder, the data revealed that there was an increase in counts of *L. acidophilus*, and *Bif. bifidum* of SDPY with egg-shell than that of PSPY.

Table 5: Effect of different egg-shell powder concentrations on microbiological properties (Log CFU/ml) of stirred probiotic yoghurt held at 6±2°C for 9 days.

Microbial type	Storage (days)	Control		Percentage of Egg-shell powder			
		C ⁻	C ⁺	0.5	0.7	0.9	1.1
Total bacterial count	Fresh	9.30	9.46	9.29	9.34	9.42	9.83
	3	9.23	9.32	9.22	9.19	9.22	9.38
	6	9.09	9.22	9.22	9.16	9.17	9.14
	9	8.38	9.12	9.19	9.14	9.14	9.12
<i>L. acidophilus</i> count	Fresh	8.84	8.93	9.15	9.14	9.93	9.15
	3	8.02	8.38	9.04	9.09	9.18	9.09
	6	7.79	7.89	8.93	9.02	9.15	8.97
	9	7.75	7.85	8.91	8.97	8.92	8.96
<i>S. thermophilus</i> count	Fresh	9.00	9.00	8.98	9.08	9.32	9.28
	3	9.05	9.39	9.15	9.08	9.16	9.13
	6	8.96	9.16	9.13	9.07	9.10	9.07
	9	8.89	9.03	9.11	9.06	9.10	9.06
<i>Bif. bifidum</i> count	Fresh	6.81	6.88	6.95	6.97	7.01	6.95
	3	6.82	6.97	6.99	7.03	7.06	7.02
	6	6.68	6.87	6.93	6.94	6.98	6.93
	9	6.56	6.83	6.87	6.93	6.97	6.91
Moulds and Yeasts count	Fresh	ND*	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND
	6	3.04	2.98	3.02	3.03	3.08	3.10
	9	3.11	3.08	3.09	3.10	3.11	3.14

As shown in Table 5, the data indicated that moulds & yeasts were not detected in fresh or after 3 days of storage for all treatments. Whereas, these were detected and increased gradually after 6 days of storage and with progressing the storage up to 9 days. In addition, there counts were higher in PSPY than that in SDPY. On the other hand, there counts increased with an increasing the concentration of egg-shell

powder in all treatments. Coliform bacteria were not detected in all experimental samples.

The obtained data showed that there was a decrease on syneresis of samples with an increase of egg-shell powder concentrations. Also, syneresis decreased during storage periods up to 9 days of storage for all treatments. Similar trend was found by Farooq and Haque (1992).

Regarding density, the data showed that the density of PSPY and SDPY with egg-shell powder increased with an increasing the egg-shell powder concentration. This may be due to the increase of total solids and density in egg-shell powder. Also, the density increased with an increasing the storage period up to 9 days for all treatments. On the other hand, this value increased in SDPY than in PSPY. This may be due to increase of component in dibis. Similar results were obtained by El-Shobery *et al.* (2012). In addition there are significant differences among all the treatments and during storage periods for all rheological properties.

Table 6: Rheological properties of stirred probiotic yoghurt held at 6±2°C for 9 days.

Properties	Storage (days)	Control		Percentage of egg-shell powder				L.S.D.
		C ⁻	C ⁺	0.5	0.7	0.9	1.1	
Syneresis (ml/100 gm)	Fresh	38.00	36.00	36.67	38.00	32.67	30.67	2.712 ¹
	3	39.00	34.33	33.33	34.33	30.00	33.67	
	6	33.00	32.67	33.00	33.67	28.67	26.33	3.137 ²
	9	34.67	34.00	31.00	27.33	21.67	21.67	
Density (gm/cm ³)	Fresh	1.11	1.11	1.11	1.11	1.12	1.13	0.079 ¹
	3	1.13	1.15	1.12	1.12	1.15	1.15	
	6	1.14	1.15	1.14	1.14	1.17	1.16	0.096 ²
	9	1.14	1.16	1.16	1.16	1.18	1.18	

Data in table 7 revealed that the organoleptic properties such as; flavor, body & texture and appearance & colour of PSPY and SDPY were affected by addition of dibis and egg-shell powder respectively, during storage periods.

Flavour of stirred probiotic yoghurt was influenced by adding of dibis and egg-shell powder. The obtained data showed that the SDPY had higher scores than that in PSPY in fresh and after storage up to 9 days. In addition, the sample with 1.1% egg-shell powder had lower scores than that in other concentrations of egg-shell powder. This may be due to further increase in concentration resulted in an increasing the alcoholic aroma and acidic taste of SDPY with egg-shell powder. On the other hand, the SDPY found to be superior at storage period at 6±2°C at the end of storage period (9days) among all the other samples.

The data revealed that the score of PSPY was good in body and texture as compared to all treatments with dibis or egg-shell powder. The presence of dibis in SDPY reduced the score for body and texture. This may be due to separation of whey and to the production of acids, which, reduced the coagulation and to the formation of soft and loose textured curd. On the other hand, the SDPY with 0.7% of gg-shell powder had the higher values than that in other samples with concentration of egg-shell powder, while the sample with 1.1% of egg-shell powder had the lower values.

Table 7: Sensory quality of stirred probiotic yoghurt held at 6±2°C for 9 days.

Properties	Storage (days)	Control		Percentage of egg-shell powder			
		C ⁻	C ⁺	0.5	0.7	0.9	1.1
Flavor (45)	Fresh	33.88	41.50	36.63	38.00	37.50	34.25
	3	36.40	37.40	35.90	37.70	39.00	33.90
	6	36.40	41.60	38.00	39.20	39.60	33.80
	9	36.90	42.40	33.80	33.40	31.60	29.00
Body & Texture (30)	Fresh	27.75	25.63	25.38	25.00	24.88	25.25
	3	24.40	23.80	23.20	23.30	24.10	24.30
	6	26.40	26.00	25.00	26.20	24.40	24.20
	9	25.60	25.40	24.40	25.20	24.00	22.00
Appearance (15)	Fresh	13.38	13.38	12.88	12.88	13.00	13.00
	3	12.50	12.00	13.40	12.30	12.70	11.80
	6	13.40	12.40	12.00	12.00	12.60	11.80
	9	14.20	12.60	11.80	11.80	11.00	9.80
Acidity (10)	Fresh	6.50	8.13	7.88	7.38	7.88	7.38
	3	7.40	7.50	7.00	7.10	7.00	7.00
	6	6.60	7.00	8.00	7.20	7.00	7.00
	9	8.20	8.40	8.20	7.20	7.20	7.20
Overall Score (100)	Fresh	81.51	88.64	82.77	83.26	83.26	79.88
	3	80.70	80.70	79.50	80.40	82.80	77.00
	6	82.80	87.00	83.00	84.60	83.60	76.80
	9	84.90	88.80	78.20	77.60	73.80	68.00

The data revealed that the appearance score of PSPY was good as compared to SDPY of all treatments. The presence of dibis in SDPY is reduced the score for appearance. This may be due to separation of whey and the production of acids, which give the reduced coagulation and to the formation of soft and loose textured curd. On the other hand, the SDPY with 0.5% of egg-shell powder had higher values than that in other samples. While the sample with 1.1% of egg-shell powder stored at 9 days had the lower values.

The obtained data indicated that the SDPY had higher scores than that in PSPY in fresh and after storage up to 9 days. On the other hand, the sample with 1.1% egg-shell powder had lower scores than that in other concentrations of egg-shell powder. This may be due to further increase in concentration resulted in increasing the alcoholic aroma and acidic taste of SDPY and SDPY with egg-shell powder.

As shown in Table 7, the SDPY at fresh and after storage for 9 days had superior scores, followed by sample stored for 6 days. While the control sample stored for 3 days had the lowest value of overall scores. In addition, the SDPY had higher values than that in PSPY. On the other hand, the SDPY with egg-shell powder had higher values when stored up to 6 days, and then reduced after 9 days of storage. Generally, the data concluded that we can use egg-shell powder to make stirred probiotic yoghurt with 10% dibis up to 0.9% and held at $6\pm 2^{\circ}\text{C}$ for 6 days.

Table 8: Statistical analyses of stirred probiotic yoghurt held at $6\pm 2^{\circ}\text{C}$ for 9 days.

Probiotic yoghurt properties	Effect of treatments					
	Multiple comparison					
	C ⁻	C ⁺	T ₁	T ₂	T ₃	T ₄
Dry matter %	F	B	D	E	C	A
Fat %	A	C	B	CD	CD	D
Titratatble acidity %	B	C	A	A	D	E
pH	E	D	C	B	B	A
Soluble nitrogen %	BC	D	E	C	A	AB
Total nitrogen %	A	BC	C	B	B	B
SN/TN %	D	BC	D	BC	A	C
Syneresis (ml/100gm)	A	A	A	A	B	B
Density gm/cm ³	A	A	A	A	A	A
Probiotic yoghurt properties	Effect of times					
	Multiple comparison					
	Fresh	3 days	6 days	9days		
Dry matter %	D	C	B	A		
Fat %	A	A	A	B		
Titratatble acidity %	C	B	A	A		
Ph	A	B	C	D		
Soluble nitrogen %	D	C	B	A		
Total nitrogen %	A	B	C	D		
SN/TN %	D	C	B	A		
Syneresis (ml/100gm)	A	A	B	C		
Density gm/cm ³	AB	B	AB	A		

For each effect the different letters in the same row means the multiple comparisons are different from each other, letter A is the highest mean followed by B, C, etc.

REFERENCES

- Ahmed, Y. A. 2009. A study on yoghurt and yoghurt derivatives. Ph.D. Thesis, Fac. Agric., Al-Azhar Univ.
- Al-Saidy, M. A., K. A. Al-Dujaili, and A. M. Majced. 1982. Evaluation of date syrup (dibs) as a substitute for table sugar in bread making. *J. Biological Sci.* 13(1): 93-107.
- A.O.A.C. 2000. Association of Official Analytical Chemists. Official Methods of Analysis of Association of Official Agriculture Chemists. 17th Wisconsin: George Banta Co. Inc.
- Coisson, J.D., F. Travaglia, G. Piana, M. Capasso, and M. Arlorio. 2005. Euterpe oleracea juice as a functional pigment for yogurt. *Food Research International* 38: 893-897.
- Dave, R. I., and N. P. Shah. 1996. Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus* and *bifidobacteria*. *J. Dairy Sci.* 79: 1529-1536.
- De Felice, S. L. 1995. The nutritional revolution: its impact on food industry R&D. *Trends Food Sci. Technol.* 6: 59-61.
- De Man, J.C., M. Rogosa, and M. Sharpe. 1960. A medium for the cultivation of lactobacilli. *Journal Applied Bacteriology* 23:130-135.
- Dubois, M. K. A., Gilles, J. K., Hamilton, P.A., Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350-356.
- El-Shobery, M. A., A. I. A. Mansour, and K.G.Zaki. 2012. Studies on using of date syrup (Dibis) in yoghurt making. *Minia Journal of Agriculture Res. & De.* 32(1):177-194.
- El-Sonbaty, A. H., K. M. K. Kebary, R. M. Badawi, and Hweda, A. El-Sayed. 2008. Effect of stabilizers on the quality of stirred yoghurt. *Egyptian J. Dairy Sci.* 36: 185-195.
- FAO, 2007. Report on functional foods. http://www.fao.org/ag/agn/agns/files/Functional_Foods_Report_Nov2007.pdf.
- Farooq, H., and Z. U. Haque. 1992. Effect of sugar esters on the textural properties of non fat low caloric yoghurt. *J. Dairy Sci.* 75: 2676-2680.
- Gerrior, S., L. Bente, and H. Hiza. 2004. Nutrient content of the US food supply 1909-2000, Home Economics Research Report No. 56. p:42. Washington DC: US Department of Agriculture, Center for Nutrition Policy and Promotion.

Website:<http://www.cnpp.usda.gov/Publications/FoodSupply/FoodSupply1909-2000.pdf> (assessed on 15th January, 2007).

- Hashmi, S. I., S. S. Kadam, and R. V. Kale. 2011. Studies on effect of different varieties of date palm paste incorporation on quality characteristics of yoghurt. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 10(6): 2371-2381.
- International Dairy Federation (FIL-IDF 73A: 1985a) Milk and milk products. Enumeration of coliforms- colony counts technique and most probable number technique at 30°C.
- International Dairy Federation (FIL-IDF: 94A 1985b) Milk and milk products. Detection and enumeration of yeasts and moulds.
- International Dairy Federation (FIL-IDF 5B: 1986) Cheese & processed cheese. Determination of fat content, Schmid-Bondzynski-ratzlaff method. Standard. Brussels.
- International Dairy Federation (FIL-IDF 21B: 1987). Milk, cream, fermented milks, and evaporated milk – total solids. 41 square Vergote, 1040 Brussels, Belgium.
- International Dairy Federation (FIL-IDF 20B: 1993) Milk. Protein determination, determination of nitrogen content. Kjeldahl method and calculation of crude protein content. Standard. Brussels.
- International Dairy Federation (FIL-IDF 117B: 1997) Yoghurt. Enumeration of characteristic microorganisms. Colony count technique at 37°C.
- Keব্য, K. M. K., and S. A. Hussein, 1999. Manufacture of low fat Zabady using different fat substitutes. *Acta Alimentaria* 28: 1-14.
- Khalil, K.E., M. S. Abdel-Bari, N. E. Hafiz, and E. Y. Ahmed. 2002. Production, evaluation and utilization of date syrup concentrate (Dibis). *Egyptian J. Food Sci.* 30(2): 179-203.
- Klupsch, H. J. 1985. Production process for a whey drink. German Federal Republic Patent Application, DE 3326, 347.
- Marshall, R. T. 1992. *Standard Methods for the Examination of Dairy Products*. 16th ed. Am. Publ. Health Assoc., Washington, DC.
- Miller, G. D., J. K. Jarvis, and L. D. Mc Bean. 2001. The importance of meeting calcium needs with foods. *J. Am. Coll. Nutr.* 20(2 Suppl.): 168-185.
- Mustafa, A. I., A. M. Hamad, and M. S. Al-Kahtani. 1983. Extraction of date syrup (Dibis) and its utilization in bakery products and juice. *Proc. of the 1st Symp. on Date Palm, K.F. Univ., March 23-25, 1982, Al-Hassa, Saudi Arabia* pp. 534-543.

- Ntailianas, H. A., and M. C. I. Whiteny. 1964. Calcein as an indicator for the determination of total calcium and magnesium and calcium alone in the same aliquot of milk. *J. Dairy Sci.* 47:19-27.
- O'Neil, J. M., D. H. Klen, and L.B. Har. 1979. Consistency and compositional characteristics of commercial yoghurt. *J. Dairy Sci.* 62:1032-1036.
- Page, A. L. 1982. *Methods of Soil analysis. Part 2: Chemical and microbiological properties*, (2nd Ed). Am. Soc. At Agron. Inc. Soil Sci. Soc. Of Am., Madison, Wisconsin, USA.
- SAS. 1999. *Statistical analysis system, User's guide for personal computers, Version 8.2 Edition* SAS Institute, Cary, N.C.
- Schaafsma, A., I. Pakan, G.J.H., Hofsted, F.A.J., Muskiet, E., Van Der Veer, and P.J.F. De Vries. 2000. Mineral, amino acid, and hormonal composition of chicken eggshell powder and the evaluation of its use in human nutrition. *Poultry Sci.* 79: 1833-1838.
- Schmidl, M. K. 1993. Food products for medical purposes. *Trends Food Sci. Technol.* 4: 163-168.
- Solomon, S. E., M. M. Bain, S. Cranstoun, and V. Nascimento. 1994. Hens egg shell structure and function. p. 1-24. *In* R. G. Board R. Fuller (ed.). *Microbiology of the Avian Egg*. Chapman and Hall, London, England.
- Sotera, J.J. and R. L. Stux. 1979. *Atomic absorption methods manual. Vol.1. Std. Cond. For flame. Operation. Int. Lab. Inc. Andover, MA., USA.*
- Thys-Jacobs, S., D. Donovan, A. Papadopoulos, P. Sarrel, and J. P. Bilezikian. 1999. Vitamin D and calcium dysregulation in the polycystic ovarian syndrome. *Steroids* 64: 430-435.
- Tiessen, H., and J. O. Moir. 1993. Characterization of available P by sequential extraction. *In* M.R. Carter (ed.) *Soil Sampling and Methods of Analysis*. Lewis Publishers, Boca Raton, Fl. pp: 75-86.
- Wilson, J. F. 2004. New treatments for growing scourge of brittle bones. *Ann. Intern. Med.* 140: 153-156.
- Zekai, T. and K. Erdoga. 2003. Physical, chemical, microbiological and sensory characteristics of some fruit flavored yoghurt. *YYU Vet Fak Derg* 14(2): 10-14.

الاستفادة من مسحوق قشر البيض لتدعيم مشروب زيادي الدبس الحيوي بالكالسيوم

خالد حمدي سلمان^(١) ، على إبراهيم علي منصور^(١) ، عادل علي تمام^(٢) ، فتحي السيد الجزار^(٢)
^(١) قسم الألبان - كلية الزراعة - جامعة الأزهر - فرع أسيوط
^(٢) قسم الألبان - كلية الزراعة - جامعة أسيوط

تم استخلاص الدبس من البلح السيوي ، مسحوق قشر البيض الأبيض لاستخدامهما في صناعة مشروب زيادي الدبس الحيوي ، وذلك بإضافة الدبس الذي تم تركيزه إلى ٧٢% بنسبة إضافة ١٠% إلى اللبن المعامل حراريا ، ثم إجراء عملية التخمير بإضافة بادئ الزيادي الحيوي مع وجود عينة مقارنة بدون إضافة دبس وأخرى مضاف إليها الدبس ، كما تم استخدام مسحوق قشر البيض بنسب ٠.٥ ، ٠.٧ ، ٠.٩ ، ١.١% ، وقد تم تخزين العينات على درجة حرارة $6 \pm 2^\circ \text{C}$ ولمدة ٩ أيام ، وقد أوضحت النتائج المتحصل عليها ما يلي :-

- ١- الدبس المتحصل عليه يحتوي على نسبة عالية من الجوامد الكلية والحموضة ، كما يعتبر غني في بعض الأملاح المعدنية مثل الكالسيوم والبوتاسيوم والفسفور والماغسيوم والحديد .
- ٢- مسحوق قشر البيض المتحصل عليه كان غنيا في الكالسيوم ، الفوسفور ، الماغنسيوم والحديد .
- ٣- أدت إضافة مسحوق قشر البيض إلى زيادة كل من الجوامد الكلية ، الكثافة وكذلك أملاح الكالسيوم والماغسيوم والحديد .
- ٤- زيادة فترة التخزين أدت إلى زيادة نسبة النتروجين الذائب إلى النتروجين الكلي في جميع المعاملات.
- ٥- تبين من التحليل الاحصائي أن هناك فروق معنوية بين المعاملات بالنسبة لجميع التحليلات الكيماوية وكذلك أثناء فترة التخزين.
- ٦- وجدت زيادة في أعداد كل من *L. acidophilus* و *Bif. bifidum* في مشروب زيادي الدبس الحيوي المدعم بالكالسيوم عنها في عينات مشروب الزيادي الحيوي غير المدعم .
- ٧- لوحظ عدم وجود أي من الخمائر والفطريات في جميع المعاملات حتى ثلاثة أيام من التخزين ، بينما حدثت زيادة تدريجية بزيادة مدة التخزين بعد ستة أيام وحتى نهاية فترة التخزين .
- ٨- من الناحية الحسية وجد أن مشروب زيادي الدبس الحيوي قد حصل على أعلى درجات تحكيم عند التخزين على درجة حرارة $6 \pm 2^\circ \text{C}$ لمدة تسعة أيام في جميع المعاملات .
- ٩- بناء على تلك النتائج ينصح بإضافة مسحوق قشر البيض حتى ٩% لعمل مشروب زيادي الدبس الحيوي مع إضافة ١٠% دبس ، والتخزين على درجة حرارة $6 \pm 2^\circ \text{C}$ حتى ستة أيام .