#### QUALITY OF YOGHURT MADE FROM COW'S MILK FORTIFIED WITH WHEY PROTEIN ISOLATE BY

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#### **SUMMARY**

Five yoghurt treatments were made to study the effect of fortifying cow's milk with whey protein isolate on the quality of voghurt, control voghurt was made by adding 3% non-fat dry milk to cow's milk while the other four treatments were made by fortifying cow's milk with 0.5, 1.0, 1.5 and 2.0% whey protein isolate respectively and 3.0% nonfat dry milk to each treatment. All yoghurt treatment was stored in refrigerator for 12 days and was sampled when fresh and at 3, 6, 9 and 12 days for chemical, rheological, microbiological analysis and sensory evaluation. The obtained results indicated that adding whey protein isolate to cow's milk caused a significant increase of total solids, total protein, ash contents and titratable acidity, while decreased pH values and whey syneresis of yoghurt treatments and these effects were proportional to the rate of adding whey protein isolate. Also, the values of Hardness, adhesiveness, cohesiveness, springiness and gumminess of the resulting yoghurt had increased and this increase was proportional to the rate of fortification. Adding whey protein isolate up to 1.5% increased the scores of organoleptic properties and treatment that made with adding 1.5% whey protein isolate was the most acceptable yoghurt treatments. Total solids, total protein, ash and fat contents of all yoghurt treatments did not change significantly, (P > 0.05) during storage period, while titratable acidity increased. Whey separation decreased during storage period up to the sixth day of storage period then increased up to the end of storage period, while the scores of organoleptic properties were almost stable up to the ninth day of storage period.

Key words: Cow's milk, non-fat dry milk, whey protein isolate, yoghurt, syneresis, organoleptic properties.

#### **INTRODUCTION**

Yoghurt is the most popular fermented milk produced all over the world. Supplementing voghurt with probiotic bacteria and prebiotics increased the health and nutritional benefits of yoghurt. Recently the production and consumption of yoghurt has been increased tremensdously in Egypt. The nutritional importance of yoghurt is based not only on the nutritive value of the milk from which it is made and the chemical changes of milk components occurring during fermentation but also some beneficial effects such as prophylactic and healing (Birollo et al., 2000; Ayar et al., 2006; Chandan, 2006 and Shah, 2007). There is large quantity of whey are produced during cheese making, whey was considered the most important pollutant of the dairy industry. Most of whey produced in Egypt was discharged directly into the sewage system, but according to the Egyptian environmental low that was issued recently, dairy effluents should be

treated before its drainage into the sewage system. Therefore, recovery of whey proteins which represent 20 % of milk proteins can be very important. Whey protein products have been used in the manufacture of many dairy and nondairy products because of their valuable health and technological benefits. Whey proteins can be used as an emulsifying, thickening, gelation, foaming, and water binding agent resulting in manufactured products with similar and desired characteristics compared to those produced with classical ingredients.

In view of a for mentioned the objective of this study were to investigate the possibility of making a good quality yoghurt that made from cow's milk using whey protein isolate and monitor the changes of chemical, microbiological and organoleptic properties during cold storage.

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## MATERIALS AND METHODS

#### Materials: Bacterial strains

Active Streptococcus thermophilus (EMCC 1043) and Lactobacillus delbrueckii subsp. bulgaricus (EMCC 1102) were obtained from Cairo Mircen, Ain Shams University, Egypt. Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus were active-ted individually by three successive transfers in sterile 10% reconstituted non-fat dry milk.

### Manufacture of yoghurt

Fresh cow's milk was obtained from the herd of agricultural secondary school, Shibin El-kom, Egypt, Fresh cow's milk was standardized to 3% fat. The preliminary experiment showed that the best yoghurt quality was made by supplementing cow's milk with 3.0% nonfat dry milk. Standardized (3.0 % fat) cow's milk was fortified with 3.0 % non-fat dry milk. This milk was divided into 5 treatments. These treatments were fortified with 0.0, 0.5, 1.0, 1.5, and 2.0 % whey protein isolate (C, T1, T2, T3 and T4, respectively). Non-fat dry milk (Dairy America, California, USA) and whey protein isolate (Arla Food Ingredients, Skander, Denmark) were added to milk and stirred thoroughly, then filtered through cheesecloth. All milk batches were heated to 85° C for 20 min, then cooled to 42° C and inoculated with 1.5% Streptococcus thermophilus and 1.5% Lactobacillus delbrueckii subsp.bulgaricus. The inoculated batches were packed in plastic cups and incubated at 42° C until complete coagulation. All yoghurt treatments were stored in a refrigerator  $(6^{\circ}C\pm 1)$  for 12 days and were sampled when fresh and at 3, 6, 9 and 12 days for chemical, microbiological, rheological analysis and sensory evaluation. The whole experiment was triplicated.

#### Microbiological analysis:

The total bacterial counts were determined using standard plate count agar (Marth, 1978). Streptococci were enumerated on yeast lactose agar medium (Skinner and Quensel, 1978). Lactobacilli were determined using MRS agar medium (De man *et al.*, 1960). Moulds and yeasts were enumerated on Potato Dextrose agar (acidified) medium (Difco, 1953).

#### **Chemical analysis:**

pH value, titratable acidity and fat content were determined according to A.O.A.C(2012), while total solids, ash and total protein were determined according to A. O. A. C (2012).

#### **Rheological properties:**

Synerasis was determined according to the method of Danneberg and Kessler (1988) with slight modification. One hundred grams of yoghurt in plastic cup were cut into four sections and transferred into funnel fitted with 120 mesh metal screen. The amount of whey drained into a graduated cylinder was measured after 120 min. at room temperature ( $20 \pm 1^{\circ}$ C) for all yoghurt treatments stored for 1, 3, 6, 9 and 12 days.

Textural parameters are determined using Texture Analyzer TMS-Pro (Food Technology Corporation, sterling, Virginia, USA). equipped with (250lbf) load cell and connected to a computer programmed with Pro<sup>TM</sup> texture analysis software (program, DEV TPA withhold). The texture of yoghurt samples was evaluated in triplicate of each batch of a set yoghurt sample prepared in a 100-ml cup at a temperature of 4°C. A flat rod probe was subjected to two subsequent cycles (bites) of compression-decompression. The probe used in "Texture Profile Analysis" (TPA) was 49.95 mm. diameter, double compression test to penetrate 50% depth, at speed of 1 mm/s and of penetration using cycle or hold programs. Data were collected on computer and the texture profile parameters were calculated from LFRA texture analyzer and computer interface. Calculation described by Bourne (2003) was used to obtain the texture profile parameters. The parameters stimulating included hardness (measure of force required to achieve a given deformation), adhesiveness (the work necessary to overcome the attractive forces between the surface of a food and surface of other materials with which it comes in contact, e.g., the teeth, palate and tongue, cohesiveness (a measure of strength of internal bonds making up the body of the product). springiness (a measure of the rate at which a deformed material returns to its original

dimensions after the deforming force is removed), chewiness (the energy required to masticate a solid food material to a state ready for swallowing) and guminess (the energy required to disintegrate a semisolid food to a state ready for swallowing) (Fox *et al.*, 2017).

#### Sensory evaluation:

Yoghurt was judged by ten panelists from the staff members of Dairy Science and Technology Department, and Food Science and Technology Department, Faculty of

Titratable acidity of all yoghurt treatments increased by fortifying cow's milk with whey protein isolate ( $p \le 0.05$ ). There was positive correlation between the rate of fortification with whey protein isolate and the titratable acidity of yoghurt (Tables 1, 5). Yoghurt treatment (T4) that was made by adding the highest amount of whey protein isolate (2.0%) had the highest titratable acidity (Tables 1, 5). These results might be due to the stimulating effect of whey protein on the growth of lactic acid bacteria and consequently increasing the development of acidity (Gaudreau et al., 2013; Zhao and Shah, 2014; Muniandy et al., 2016 and Akgul, 2018). Titratable acidity of all yoghurt treatments increased gradually  $(p \le 0.05)$  as storage period progressed (Tables 1, 5). These results are in agreement with those reported by Ali et al. (2014); Chatterjee et al. (2016); Elkot (2017); Al-aswad et al.(2018); Blassy and Abdeldaiem (2018); Abdalla and Ahmed (2019); El-Garhi et al. (2019) and Saleh et al. (2019).

pH values of yoghurt treatments as affected by adding whey protein isolate and storage period followed an opposite trends to those of titratable acidity (Tables 1, 5)

Total solids and total protein contents increased significantly (P $\leq$ 0.5) by increasing the amount added of whey protein isolate (Tables 1, 5). These results are in agreement with those reported Ali *et al.* (2014); Wang *et al.* (2015) and Bierzuńska and Sokolińska (2018). Total solids and total protein contents didn't change significantly (P > 0.5) during

Agriculture, Menoufia University. Results were recorded on a score sheet described by (Kebary and Hussein, 1999).

#### Statistical analysis:

Data were analyzed using completely randomized block design and  $2\times3$  factorial design. Newman-Keuls test was used to make the multiple comparisons (Steel and Torrie, 1980) using Costat program. Significant differences were determined at  $p \le 0.05$ .

#### **RESULTS AND DISCUSSION**

storage period. These results are in accordance with those of Al-aswad *et al.* (2018); Blassy and Abdeldaiem (2018) and Abdalla & Ahmed (2019).

There were no significant differences among yoghurt treatments in fat content which means adding whey protein isolate did not affect significantly (P > 0.05) the fat contents of the resulting yoghurt treatments (Tables 1, 5) (Shamsia, 2010 and Ali *et al.*, 2014). These results are in agreement with those reported by Blassy and Abdeldaiem (2018) Abdalla and Ahmed (2019) and El-Garhi *et al.* (2019).

There were slight differences in ash content among yoghurt treatments which mean that adding of whey protein isolate affected significantly ( $p \le 0.05$ ) the ash content of all yoghurt treatments (Tables 1, 5). Ash content of all yoghurt treatments did not change significantly during storage period (P>0.5) These results in agreement with those reported by Kebary *et al.* (2012); Ali *et al.* (2014) and Abdalla & Ahmed (2019).

Whey syneresis of all yoghurt treatments decreased significantly ( $p \le 0.05$ ) by adding whey protein isolate (Tables 3, 5). There was negative correlation between whey syneresis and the rate of adding whey protein isolate (Tables 3, 5) (Lee and Lucey, 2010; Henriques *et al.*, 2013 and Akgul, 2018). These results might be due to increasing the total solids content (Khalil and Blassy, 2017; Abdalla and Ahmed, 2019 and Saleh *et al.*, 2019), increasing the water holding capacity

(Henriques *et al.*, 2013; Delikanli and Ozcan, 2014; Jeewanthi *et al.*, 2015; Ghanimah, 2018; Akgul, 2018 and Nastaj *et al.*, 2019) and increasing the gel strength of yoghurt as a result of decreasing the casein to whey protein ratio, which enables to the shift form compact structure to larger aggregates by interaction with casein micelles created a more rigid gel structure in yoghurt (Delikanli and Ozcan, 2014). Whey syneresis of all yoghurt treatments decreased as storage period proceeded

and reached their minimum values at the sixth day of storage period, then increased up to the end of storage period (Tables 3, 5). This increase of whey syneresis might be due to the contraction of curd as a result of developed acidity during storage, that help to expel the whey from the curd. These results in agreement with those reported by Blassy and Abdeldaiem (2018); Abdalla and Ahmed (2019) and Saleh *et al.* (2019).

		Titrata	ble acid	ity (%)		pH values						
Yoghurt treatments		Storag	e period	(days)		Storage period (days)						
	1	3	6	9	12	1	3	6	9	12		
<b>C</b> *	4.85	4.73	4.56	4.43	4.28	4.85	4.73	4.56	4.43	4.28		
T1	4.76	4.62	4.50	4.43	4.30	4.76	4.62	4.50	4.43	4.30		
T2	4.70	4.85	4.53	4.42	4.38	4.70	4.85	4.53	4.42	4.38		
Т3	4.66	4.58	4.46	4.38	4.34	4.66	4.58	4.46	4.38	4.34		
T4	4.61	4.50	4.46	4.33	4.26	4.61	4.50	4.46	4.33	4.26		
	Total solids content (%)					Total protein content (%)						
		Storag	e period	(days)		Storage period (days)						
C*	13.32	13.31	13.36	13.37	13.35	3.56	3.58	3.58	3.57	3.56		
T1	13.78	13.77	13.78	13.79	13.78	4.02	4.03	4.05	4.02	4.01		
T2	14.22	14.23	14.24	14.22	14.23	4.45	4.43	4.45	4.44	4.42		
Т3	14.65	14.68	14.64	14.66	14.66	4.91	4.90	4.92	4.91	4.90		
T4	15.11	15.12	15.13	15.11	15.13	5.35	4.35	5.33	5.34	5.33		
	Fat content (%)					Ash content (%)						
		Storag	e period	(days)		Storage period (days)						
<b>C</b> *	3.1	3.0	3.0	3.1	3.1	0.82	0.83	0.84	0.84	0.83		
<b>T1</b>	3.0	3.0	3.0	3.0	3.1	0.84	0.84	0.86	0.85	0.84		
T2	3.0	3.1	3.1	3.0	3.0	0.88	0.87	0.89	0.88	0.87		
Т3	3.0	3.0	3.0	3.0	3.0	0.91	0.92	0.94	0.94	0.95		
T4	3.1	3.0	3.0	3.0	3.0	0.98	0.97	0.98	0.99	0.99		

Table (1): Chemical composition of yoghurt fortified with WIP during storage.

 $\bigcirc$ Each value in the table was the mean of three replicates.

\*C: yoghurt made from cow's milk fortified with 3% non-fat dry milk.

T1, T2, T3, T4 and T5 yoghurt treatments made from cow's milk fortified with 3% nonfat dry milk and

0.5, 1.0, 1.5, and 2.0 % whey protein isolate, respectively.

The effect of adding whey protein isolate on textural parameters is shown in Table (2). Hardness of yoghurt treatments increased significantly ( $P \le 0.05$ ) by fortifying the milk with whey protein isolate. This increase was proportional to the rate of fortification with whey protein isolate (Chatterjee et al., 2016 and Nastaj et al., 2019). These results could be attributed to the formation of protein-casein complexes that improve their firmness by protein network formation (Mahomud et al., 2017) and / or the high concentration of thiol groups and consequently creating the disulphide bonds during yoghurt production that increase the final gel strength (Matumoto-Pintro et al., 2011 and Tsevdou et al., 2013).

Adhesiveness has a positive effect on the thickness and is an important factor governing the stability of yoghurt. Fortification of milk with whey protein isolate caused a significant increase of the adhesiveness of the resulting yoghurt treatments (Table 2) there was a positive correlation between the value of adhesiveness and the rate of fortification with

whey protein isolate. Similar results were reported by Delikanli and Ozcan (2014) and Nastaj et al. (2019) who reported that voghurt fortified with whey protein isolate had higher hardness values showed more compact structure with more adhesiveness and this resulted in the good mouthfeel and improved the stability of yoghurt during storage. On the other hand fortification of milk with whey protein isolate increased significantly ( $P \le 0.05$ ) the values of cohesiveness, springiness and gumminess of the resulting yoghurt and these increase were proportional to the rate of fortification. These results might be due to the formation of fine network that contains very small pores. These results are in agreement with those reported by Sandoval-Castilla et al. (2004) and Delikanli and Ozcan (2014). Also it has been reported that fortification of milk with whey proteins that was used in the manufacture of yoghurt improved the physiccal, textural and rheological properties of the resultant yoghurt (Singh, 2007; Guggisberg et al., 2007 and Landge, 2009).

Yoghurt treatments <sup>◊</sup>	Fracture (N)	Hardness (N)	Adhesi- veness (mj)	Cohesiveness (Ratio)	Springiness (mm)	Gumminess (N)	Chewiness (mj)
C*	4.5 <sup>e</sup>	4.5°	1.227 <sup>e</sup>	0.40 <sup>b</sup>	10.60 <sup>c</sup>	1.8°	33.25 <sup>e</sup>
<b>T1</b>	7.1 <sup>d</sup>	7.1 <sup>b</sup>	2.064 <sup>d</sup>	0.44a <sup>b</sup>	11.70 <sup>c</sup>	2.8°	51.79 <sup>d</sup>
T2	7.9°	8.0 <sup>b</sup>	4.540 <sup>c</sup>	0.49a <sup>b</sup>	13.50 <sup>b</sup>	3.2 <sup>b</sup>	58.69°
Т3	8.3 <sup>b</sup>	8.3 <sup>b</sup>	4.828 <sup>b</sup>	0 52a <sup>b</sup>	15.60 <sup>a</sup>	3.8ª	69.99 <sup>b</sup>
<b>T4</b>	8.8ª	10.1ª	5.146 <sup>a</sup>	0.56ª	16.59ª	4.7ª	86.83ª

Table (2): Textural parameters of yoghurt fortified with whey protein isolate.

 $\checkmark$  See table (1).

a, b different letters in the same column means the treatment are significantly different. Significant at 0.05 level (0.05).

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	ŀ	Effect of storage period(days)										
Properties of	Mean	Multiple comparisons•					Mean	Multiple comparisons•				
yognurt <sup>.</sup>	squares	$\mathbf{C}^{\Diamond}$	<b>T1</b>	T2	<b>T3</b>	T4	squares	1	3	6	9	12
Titratabe acidity (%)	0.0627*	D	С	В	А	А	0.1865*	E	D	C	В	А
PH value	0.055*	А	AB	Α	В	С	0.432*	А	В	С	D	E
Total solids (%)	9.274*	E	D	С	В	А	0.229	Α	Α	Α	Α	А
Total protein (%)	4.319*	Е	D	С	В	А	0.056	Α	Α	Α	Α	А
Fat (%)	0.0779	А	А	Α	А	А	0.0180	Α	Α	Α	А	А
Ash (%)	0.3105*	А	А	ABC	BC	С	0.1299	Α	Α	Α	Α	А
Seneraseis (%)	488.52*	А	В	С	D	E	74.22*	А	В	С	В	А
Organoleptic properties												
Flavor	20.879*	С	В	В	А	В	4.079*	Α	Α	Α	AB	В
Body&texture	6.179*	С	BC	AB	А	BC	1.679*	Α	Α	Α	AB	AB
Appearance	0.420*	В	В	В	А	AB	0.419*	Α	Α	Α	В	В
Acidity	2.579*	В	В	AB	А	В	4.379*	Α	Α	Α	Α	В
Total	85.49*	D	С	В	А	В	29.699*	А	Α	Α	AB	В

Table: (5) Statically analysis of yoghurt fortified with whey protein isolate.

See table (1)

• 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.

\*Significant at 0.05 level (0.05).

Counts total bacteria, Lactobacilli and Streptococci of yoghurt treatments increased significantly by adding whey protein isolate (Tables 3, 5). There was positive correlation between the total bacterial counts and the rate of adding whey protein isolate. Treatment T4 that was made by adding the highest amount of whey protein isolate exhibited the highest counts of total bacterial counts. This increase of total bacterial, Lactobacilli and Streptococci counts could be attributed to the stimulating effect of whey protein isolate on the growth of bacteria and consequently increasing the total bacterial counts (Kailasapathy and Supriadi, 1996; Gaudreau et al., 2013; Muniandy et al., 2016 and Akgul, 2018). On the other hand the obtained results indicated that total bacterial, Lactobacilli and Streptococci counts of all voghurt treatments increased during the first three days of storage period and reached their maximum counts at the third day of storage period, then decreased gradually up to the end of storage period. This decrease might be due

to the development of acidity during storage period and /or the cold storage. Similar trends were obtained by Kebary *et al.* (2010).ElKot (2017) and Saad & Elkhtab (2019).

All yoghurt treatment samples were free from moulds and yeasts during first nine days of storage period, then they apeard towards the end of storage period (Table 3). These results are in agreement with those reported by Mehriz *et al.* (1993) who detected moulds and yeast only at the end of storage period. Similar trends were optained by Ali *et al.* (2014); Priyadarshani and Muthumuniarachchi (2018) and Saad & Elkhtab (2019).

Scores of organoleptic properties (flavor, body & texture, acidity and appearance) of yoghurt treatments fortified by whey protein isolate are presented in Table (4). The obtained results revealed that the score of flavor, body and texture, appearance and total scores of organoleptic properties followed similar trends. Fortification of yoghurt treatments up to 1.5 % WPI increased the scores of organoleptic properties while increasing the rate of fortification above that decreased the scores of organoleptic properties. Treatment T3 that was made by fortification cow's milk with 1.5 % whey protein isolate was the most acceptable yoghurt treatment although other voghurt treatments were accepted by the panelists (Tables 4, 5). These results are confirmed with the results of texture parameters, where adding whey protein isolate improved the texture parameters of yoghurt treatments. It has been reported that adding whey proteins to yoghurt improved the texture of the resulting yoghurt and improved the mouth feel of this yoghurt (Megenis et al., 2006; Sodini et al., 2005; Guggisberg et al., 2007; Aziznia et al., 2008 and Landge, 2009). On the other hand scores of all yoghurt treatments did not change significantly during the first nine days of cold storage, while they decreased slightly after that up to the end of storage period (Tables 4, 5). These results are in agreement with reported by ElKot (2017); Khalil and Blassy (2017); Al-Aswad *et al.* (2018) and Blassy & Abdeldaiem (2018).

It could be concluded that fortification of cow's milk with whey protein isolate increased titratable acidity, total solids content, protein content, total bacterial counts, Lactobacilli counts, Streptococci counts and improved the texture parameters, while decreased pH and whey syneresis, but did not affect the fat and ash content. Adding whey protein isolate up to 1.5 % increased the scores of organoleptic properties. Therefore it is possible to make good quality yoghurt from cow's milk by adding up to 1.5 % whey protein isolate.

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# تأثير تدعيم اللبن البرى ببروتينات الشرش المعزولة على صفات اليوجورت

يحتوى الشرش الناتج من صناعة الجبن على حوالى 20% من البروتينات، ولقد بذلت جهود كبيرة لفصل هذا البروتين للإستفادة منه إقتصاديا وكذلك تقليل مخاطر التلوث الناتج من تصريف الشرش المحتوى على بركتينات الشرش، واليوم توجد منتجات عديدة من الشرش وبروتينات الشرش تستخدك فى كثير من الصناعات منها تدعيم منتجات الألبان وذلك للإستفادة من الفوائد الغذائية والصحية والتكنولوجية العديدة لبروتينات الشرش ولذلك يهدف هذا البحث إلى تدعيم اليوجورت المصنع من اللبن البقرى ببروتينات الشرش للإستفادة منها فى تحسين خواص اليوجورت المصنع من اللبن البقرى ببروتينات الشرش اليوجورت العينة الكنترول صنعت من اللبن البقرى ولقد تم تصنيع 5 معاملات من المربع الأخرى فقد صنعت من اللبن البقرى المضاف له 3% من اللبن الفرز المجفف أما المعاملات المعزولة. ولقد تم تخزين كل المعاملات فى الثلاجة لمدة 12 يوم حيث حلق عنه 30. 9، 12 يوم كيميائيا وميكروبيولوجيا وريولوجيا وكذلك التققيم الحسى، ولقد أوضحت النتائج المتحصل عليها بعد تحليلها إحصائيا مايلى:-

- 1- أدى إضافة بروتينات الشرش المعزولة إلى زيادة نسب كل من الحموضة والجوامد الصلبة الكلية والبروتين الكلى والرماد، فى حين لم تؤثر على نسبة الدهن.
  - 2- أدى إضافة بروتينات الشرش المعزولة إلى خفض قيم الــ pH وكذلك انفصال الشرش.
- 3- إزدادت درجات التحكيم الحسى لمعاملات اليوجورت بإضافة بروتينات الشرش المعزولة وكانت أكثر المعاملات قبولا هي المعاملة المصنعة بإضافة 1.5% من بروتينات الشرش المعزولة.
- 4- لم تتغير نسب كل من الجوامد الصلبة الكمية والبروتين الكلى والدهن والرماد لكل معاملات اليوجورت أثناء فترة التخزين، في حين إزدادت نسبة الحموضة وانخفضت قيم الـ pH.
- 5- إنخفضت نسب إنفصال الشرش أثناء الأيام الست الأولى من التخزين ثم إزدادت تدريجيا بعد ذلك حتى نهاية فترة التخزين.
- 6- لم تتغير درجات التحكيم لكل معاملات اليوجورت معنويا أثناء التخزين لمدة 9 أيام ثم إنخفضت قليلا بعد ذلك.