

## Physicochemical Properties and Viability of Probiotic Bacteria of Functional Synbiotic Camel Yogurt Affected by Oat $\beta$ -Glucan during Storage

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### ABSTRACT

The unique properties of camel milk, qualify this product to be used as a nutraceutical. In this study, functional synbiotic yogurt made from camel milk has been investigated in three levels of fat (0, 2.5 and 5% (w/v)). Probiotic bacteria (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* and *ssp. bulgaricus*.) and  $\beta$ -glucan (prebiotic agent) were added in three levels of concentration (0.5, 1 and 1.5 % (v/v)) and (0, 1 and 2% (w/v)), respectively. The physicochemical properties of the product and viability of probiotic bacteria were measured on the 0, 7th and 14th days. Beta-glucan, fat and storage time had significantly ( $P < 0.05$ ) increasing effects on viscosity, Water-Holding Capacity (WHC) and the viability of probiotic bacteria. These parameters caused decrease in syneresis and pH of yogurt. It was concluded that the addition of oat  $\beta$ -glucan to camel milk to make functional synbiotic yogurt could result in a product of acceptable physicochemical properties.

**Keywords:**  $\beta$ -glucan, Camel milk, Prebiotic, Probiotic, Yogurt.

### INTRODUCTION

In the recent decades, need for using functional foods -Natural or processed food that contains known biologically-active compounds which when in defined quantitative and qualitative amounts provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases of the modern age (Boye, 2015)- is increased. Two main reasons for increasing attention are: changes in life style and incline to use ready foods, and harmful effects occurred by using ready industrial food. Nowadays consumers' demand for healthy dairy products such as: low-fat yogurt, probiotic yogurt and synbiotic yogurt has increased.

Camel is the one of the resister animals to severe conditions such as high temperature and dryness. This animal is suitable for some areas that have unsuitable weather and cannot inbreed another livestock. The amount of camel lactation in dry-warm conditions and the desert is 3.5 (in the desert conditions) to 40 (under intensive management) Liters a day (Hashim *et al.*, 2009). Camel's nourishing and water usage have an effect on constituents and milk flavour (Konuspayeva *et al.*, 2009). Considering nutraceutical properties of camel milk compared with cow milk, it shows five times more potassium and vitamin C, four times more sodium, three times more calcium and magnesium, more value of unsaturated fatty acids, chlorine, folic acid and lactoferrin protein (Faye *et al.*, 2008). Also there are anti-bacterial and anti-virus properties, antibody essence, anti-

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cancer components and bioactive peptides in camel milk that give the ability to fight against some illnesses like cancer, Alzheimer, hepatitis C and HIV (Salami *et al.*, 2011; Meisel 2004; Fiat *et al.*, 1993). Fat and lactose content in camel milk are less than cow milk (Faye *et al.*, 2010). Camel milk has components like insulin and is suitable for diabetic people (Agrawal *et al.*, 2007) and those sensitive to lactose because camel milk has less lactose than cow milk (Khaskheli *et al.*, 2005). Some studies showed that camel milk has medicine components, it is useful for health and could be considered as a suitable choice for human being nutrition, but because of its low shelf life and limited lactate-season, it is necessary to propose an appropriate preservation method to extend its shelf life and to better preserve its qualitative and nutritional properties.

Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO, 2001). Several concentrations of probiotics frequently referred to as therapeutic levels have been suggested, including more than  $10^6$  cfu/mL (Kurmann and Rasic 1991) and over  $10^7$  or  $10^8$  cfu mL<sup>-1</sup> (Karimi *et al.*, 2011). *Lactobacilli* are the most important probiotic microorganisms. Probiotics bacteria have favorable effects on human health such as prevention and control of cancer, reduction of cholesterol, regulation of blood fat, gastric ulcer, facilitation and enhancement of mineral absorption and body immune (Mishra and Mishra 2012). The viability of probiotics in the final product can be improved using prebiotic carbohydrates. When a product has both probiotic and prebiotic agents, it can be called synbiotics (Mishra and Mishra, 2012).

Prebiotics are non-digestive carbohydrates. They can improve intestinal microorganism activity and increase their survival and therefore have a positive effect on consumer health (Vasiljevic *et al.*, 2007). The prebiotic compound studied in this research is  $\beta$ -glucan derived from oats. Beta-glucan is a polysaccharide naturally present in the cell wall (in the endosperm cell walls) of oat,

barley and other grains. The  $\beta$ -glucan contents of oat and barley are 3–7% and 3–11%, respectively (Lyly, 2006). The structure of  $\beta$ -glucan is (1→3) (1→4)- $\beta$ -D-glucan. Beta-glucan has (1→3) glycosidic bonds and suitable solubility in water, due to this it is used for dairy products. Human digestive enzymes cannot be digested  $\beta$  bonds, as a result it is used as a dietary fiber and prebiotic agent (Sahan *et al.*, 2008; Vasiljevic *et al.*, 2007).  $\beta$ -glucan has a moderating effect on postprandial blood glucose and insulin response and it reduces elevated blood cholesterol levels. The  $\beta$ -glucan has good effects on the body including improved intestinal activity (dietary fiber), reducing uric acid and glucose in the blood, stimulating the immune system, reducing blood pressure, cholesterol (HDL) and coronary heart disease (Xua *et al.*, 2013) and the ability to make appropriate texture (Sahan *et al.*, 2008). Beta-glucan can be used to enhance with prebiotic properties, structural additives and fat replacer in low-fat dairy and other products such as pasta, oat flakes, cereals, bakery products and beverages (Lyly, 2006).

The purposes of this study were to investigate the functional properties of synbiotic yogurt made from camel milk. Three levels of fat content [0, 2.5 and 5% (w/v)], three levels of  $\beta$ -glucan content [0, 1 and 2% (w/v)] extracted from oat and three levels of storage time (0, 7 and 14 days) in refrigeration conditions were studied. Starter cultures containing probiotic bacteria were inoculated in three levels [0.5, 1 and 1.5 % (v/v)]. In this study we tried to produce synbiotic yogurt made from camel milk with nutritional properties and to investigate the physicochemical characteristics of this type of yogurt.

## MATERIALS AND METHODS

Camel milk was provided by the Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran (Iran). The results of the chemical analysis

of camel milk are showed in the Table 1. Results were in the range of values determined by Hashim *et al.* (2009). Oats were purchased from the Institute (Taroneh, Qom, Iran) for preparation and processing of medicinal plants. Beta-glucans were extracted from oats (non-enzymatic method) as described by Moura *et al.* (2011).

### Preparation of Yogurt

At first camel milk was standardized by centrifugation (Universal 320, Hettich, Tuttlingen, Germany) to 0, 2.5 and 5% levels of fat then  $\beta$ -glucan in 0, 1 and 2% levels was added to milk, according to the experimental design (Table 2). Camel milk was homogenized with ultra-turrax blender (T25, IKA, Staufen, Germany) with 9,000 rpm speed and pasteurized for 15 minutes at  $75\pm 1^\circ\text{C}$ . Samples were prepared by adding yogurt starter culture containing probiotic microorganisms (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* and *ssp. bulgaricus*.) (ABY1, Cristian Hansen, Hørsholm, Denmark) in 0.5, 1 and 1.5% levels at  $42^\circ\text{C}$ . The mixtures were redistributed into 50 mL sterile plastic cups, incubated at  $42^\circ\text{C}$  until their pH decreased to 4.6, then cooled and stored at  $4\pm 1^\circ\text{C}$  (Mazloomi *et al.*, 2011).

### Apparent Viscosity Measurements

The viscosity of the samples was measured with a spindle (No. 60) rotating at 25 rpm using a viscometer (DV-II+Pro, Brookfield, Middleboro, MA, USA) during storage time at  $4\pm 1^\circ\text{C}$ . The readings were recorded after 30 seconds of the

**Table 1.** Chemical analysis of camel milk.

Factors	Dry material (%)	Ash (%)	Fat (%)	Protein (%)	Lactose (%)	pH	Acidity ( $\text{g L}^{-1}$ )
Amount	$9\pm 0.01$	$0.9\pm 0.001$	$4.1\pm 0.01$	$2.7\pm 0.01$	$3.1\pm 0.01$	$\approx 6.49$	$3.06\pm 0.001$

$N= 3$  and  $P= 0.05$

measurement (Chiavaro *et al.*, 2007).

### Determination of Water-Holding Capacity (WHC)

In order to measure the Water-Holding Capacity (WHC) in samples, 5 g of yogurt was centrifuged (Mikro 220R, Hettich, Tuttlingen, Germany) at 4,500 rpm for 30 minutes at  $10^\circ\text{C}$ . After centrifugation, the supernatant was removed and the pellet was collected and weighed. The WHC was calculated as follows:

$$WHC = \left( 1 - \frac{W_t}{W_i} \right) \times 100 \quad (1)$$

Where,  $W_t$  is the weight (g) of supernatant and  $W_i$  is the initial weight (g) of the sample (Wu *et al.*, 2000; Sahan *et al.*, 2008)

### Chemical Analysis

Total solids, ash, protein and fat contents using AOAC (1990) methods were measured.

### Changes in pH

pH values were determined by using a digital pH meter (GLP22, Crison, Barcelona, Spain).

### Syneresis Measurement

25 grams of yogurt samples were weighed on a 125 mm filter paper (S and S, No. 589, Germany) placed on top of a funnel. Syneresis of whey was carried out by gravity

**Table 2.** Central Composite Design (CCD) design for experiments.

Sample's number	$\beta$ -Glucan (%)	Probiotic inoculum (%)	Fat (%)	Storage time (Day)
1	2	0.5	5	14
2	1	1	0	7
3	1	1	2.5	0
4	1	0	2.5	7
5	2	0.5	0	14
6	1	1	2.5	7
7	0	0.5	5	14
8	0	1.5	5	0
9	2	1.5	5	0
10	0	1.5	5	14
11	1	1	2.5	7
12	1	2	2.5	7
13	1	1	2.5	7
14	2	1.5	5	14
15	1	1	2.5	21
16	0	1.5	0	14
17	0	0.5	5	0
18	3	1	2.5	7
19	0	1	2.5	7
20	1	1	2.5	7
21	1	1	2.5	7
22	1	1	2.5	7
23	0	0.5	0	14
24	0	0.5	0	0
25	0	1.5	0	0
26	2	0.5	5	0
27	1	1	2.5	7
28	2	1.5	0	0
29	2	0.5	0	0
30	2	1.5	0	14

and the quantity (grams) of whey collected in a flask of known weight was used as a syneresis value. The drainage time and temperature was 120 minutes and 25°C, respectively (Sahan *et al.*, 2008).

pour plate technique. The plates in duplicates were incubated anaerobically at 37°C for 72 hours, after this period colonies were counted (Mazloomi *et al.*, 2011; Mishra and Mishra, 2012).

### Microbial Analyses

One g of yogurt with 9 mL of normal saline [a solution of 0.9% (w/v) NaCl (Merck, Darmstadt, Germany)) was mixed and diluted to a concentration of  $10^6$  and  $10^7$ , and then 1 mL of each dilution was repeated in 2 plates containing the MRS-Agar (Merck, Darmstadt, Germany) with 0.15% Bovin-Bile (Sigma-Aldrich, Louis, MO, USA). Bacteria were counted by the

### Statistical Analysis

The method of data analysis was the Response Surface Methodology (RSM) by Design Expert 8 (Version 8.0.7.1, Minneapolis, MN, USA) software and by using ANOVA ( $P < 0.05$ ). The experiment was designed according to Central Composite Design (CCD) (Table 2). All experiments and measurements were

conducted in triplicate and the mean value  $\pm$ SD (standard deviation) is reported.

## RESULTS AND DISCUSSION

### Viscosity

As it can be observed from ANOVA Table (Table 3), the proposed model by software is significant ( $P < 0.05$ ). Prebiotic, fat content and storage time had significant effects ( $P < 0.05$ ) on viscosity of yogurt. However, inoculation level had no significant effect on the viscosity.

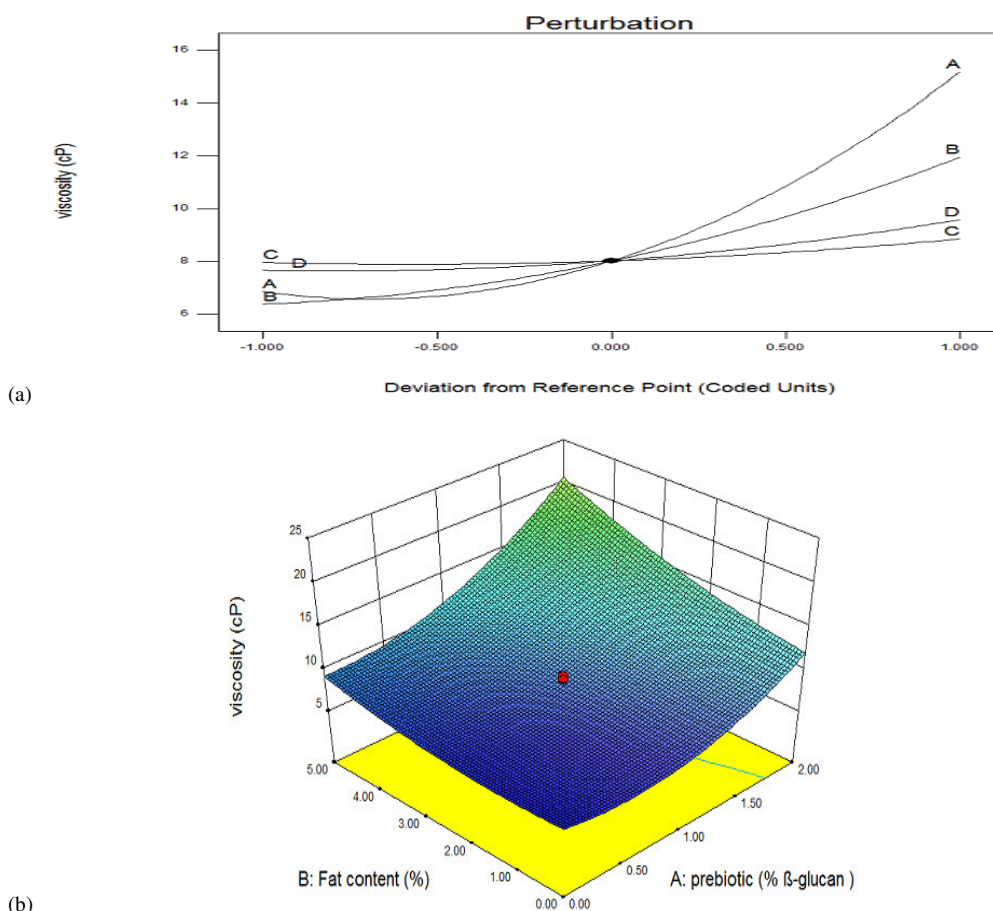
A quadratic model was proposed to predict the flow behavior (viscosity) products.

Viscosity can be obtained using the following Equation (2):

$$\begin{aligned} \text{Viscosity(cP)} = & +8.02+(4.17\times A)+(2.79\times B)+(0.45\times C)+(0.96\times D)+(1.50\times A\times B)- \\ & (0.14\times A\times C)-(0.039\times A\times D)+(0.63\times B\times C)- \\ & (0.50\times B\times D)+(0.19\times C\times D)+(3\times A^2)+(1.15\times B^2)+ \\ & (0.39\times C^2)+(0.60\times D^2) \end{aligned} \quad (2)$$

$A$ ,  $B$ ,  $C$  and  $D$  are the contents of prebiotic ( $\beta$ -glucan), camel milk fat, inoculated probiotic bacteria and storage time, respectively. The proposed model has a high "R-Squared" (0.94) and "Adj R-Squared" (0.88).

As mentioned in other studies, fermented camel milk doesn't have a good texture like yogurt (Jumah *et al.*, 2001), but in this study we attempted to partially solve this problem. Changes in the amount of  $\beta$ -glucan as the prebiotic have the strongest effect on the viscosity (Figure 1a). With the increase of  $\beta$ -



**Figure 1.** Effect of various factors on the viscosity of camel yogurt: (a) Perturbation and (b) 3D graphs.  $A$ ,  $B$ ,  $C$  and  $D$  are prebiotic ( $\beta$ -glucan) percentage, camel milk fat percentage, inoculated probiotic bacteria percentage and storage time, respectively.

**Table 3.** ANOVA Table for viscosity, syneresis, pH, WHC and the viability of probiotic bacteria changes.

Lack of Fit	D2	C2	B2	A2	CD	BD	BC	AD	AC	AB	D-Time (day)	C- Probiotic (%)	B-Fat content (%)	A- prebiotic (% β-glucan)	Model	Source
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	df
0.0936 ns	0.4	0.19	0.95	2.8	0.19	-0.5	0.63	-0.039	-0.14	1.5	0.96	0.45	2.79	4.17	8.82	coef
	0.1377 ns	0.4452 ns	0.0095 *	<0.0001 *	0.7053 ns	0.3305 ns	0.2197 ns	0.9384 ns	0.7841 ns	0.0086 *	0.0313 *	0.3618 ns	<0.0001 *	<0.0001 *	<0.0001 *	p-value
0.1363 ns	—	—	—	—	—	—	—	—	—	—	1	1	1	1	4	df
	—	—	—	—	—	—	—	—	—	—	-3.5	-0.13	-1.71	-8.95	12.21	coef
	—	—	—	—	—	—	—	—	—	—	<0.0001 *	0.8442 ns	0.0094 *	<0.0001 *	<0.0001 *	p-value
0.0914 ns	—	—	—	—	—	—	—	—	—	—	1	1	1	1	4	df
	—	—	—	—	—	—	—	—	—	—	-0.046	-0.093	0.028	-0.072	4.2	coef
	—	—	—	—	—	—	—	—	—	—	<0.0001 *	<0.0001 *	0.0002 *	<0.0001 *	<0.0001 *	p-value
0.8783 ns	—	—	—	—	—	—	—	—	—	—	1	1	1	1	4	df
	—	—	—	—	—	—	—	—	—	—	0.1	-0.09	5.49	2.14	22.35	coef
	—	—	—	—	—	—	—	—	—	—	0.7197 ns	0.7273 ns	<0.0001 *	<0.0001 *	<0.0001 *	p-value
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	df
8.43	0.131	0.0009 *	0.8164 ns	0.1018 ns	0.1069 ns	0.0022 *	0.1550 ns	0.0042 *	0.5200 ns	0.2464 ns	-29	6.07	-10.83	11.33	6.5	coef
0.1414 ns	0.0131 *	0.0009 *	0.8164 ns	0.1018 ns	0.1069 ns	0.0022 *	0.1550 ns	0.0042 *	0.5200 ns	0.2464 ns	<0.0001 *	0.0786 ns	0.0040 *	0.0010 *	<0.0001 *	p-value

\* Significant at (p&lt;0.05), ns non-significant

glucan, viscosity also increased significantly ( $P < 0.05$ ) (Vasiljevic *et al.*, 2007; Souza *et al.*, 2011),  $\beta$ -glucan as a polysaccharide creates a solid network of protein-polysaccharide in camel yogurt that increases the viscosity that can lead to a better texture.

Increasing the camel milk fat increased the viscosity significantly ( $P < 0.05$ ) (Figure 1-a). This result showed the role of texture creating of fat in the dairy product (Vasiljevic *et al.*, 2007; Souza *et al.*, 2011).

Hydration of polysaccharide ( $\beta$ -glucan) in storage time was increased and caused a significant increase ( $P < 0.05$ ) in viscosity (Figure 1-b). This result is similar to other researchers' obtained results on cow milk (Guggisberg *et al.*, 2009; Sahan *et al.*, 2008; Souza *et al.*, 2011).

Different levels of probiotic bacteria did not have significant effects on the viscosity of yogurt. This result was contrary to those obtained by Mishra and Mishra (2012) and Souza *et al.* (2011).

According to Table 3, both  $\beta$ -glucan and fat content and their interaction showed significant effects ( $P < 0.05$ ) on the viscosity of yogurt, so a quadratic model can be applied to predict the viscosity. Figure 1 b showed that the effect of  $\beta$ -glucan concentration on the viscosity was more pronounced at higher concentration of  $\beta$ -glucan. The same effect was observed for the fat content. Highest viscosity was obtained at the highest concentration of fat and  $\beta$ -glucan (Figure 1-b), so due to this interaction, stronger texture and more complex structure in camel yogurt can be obtained. Souza *et al.* (2011) have reached the same conclusion on cow yogurt.

### Syneresis

As it can be observed from the ANOVA Table (Table 3), the proposed model by the software is significant ( $P < 0.05$ ). Probiotic, fat content and storage time had significant effects ( $P < 0.05$ ) on syneresis of yogurt.

However, inoculation level had no significant effect on syneresis.

A linear model was proposed to predict changes in syneresis. Syneresis can be obtained using the following Equation (3):

$$\text{Syneresis(\%)} = +12.27 - (8.95 \times A) - (1.71 \times B) - (0.13 \times C) - (3.50 \times D) \quad (3)$$

A, B, C and D are the contents of prebiotic ( $\beta$ -glucan), camel milk fat, inoculated probiotic bacteria and storage time, respectively.

The model has a high "R-Squared" (0.91) and "Adj R-Squared" (0.90). Two coefficients show that the model is significant and feasible.

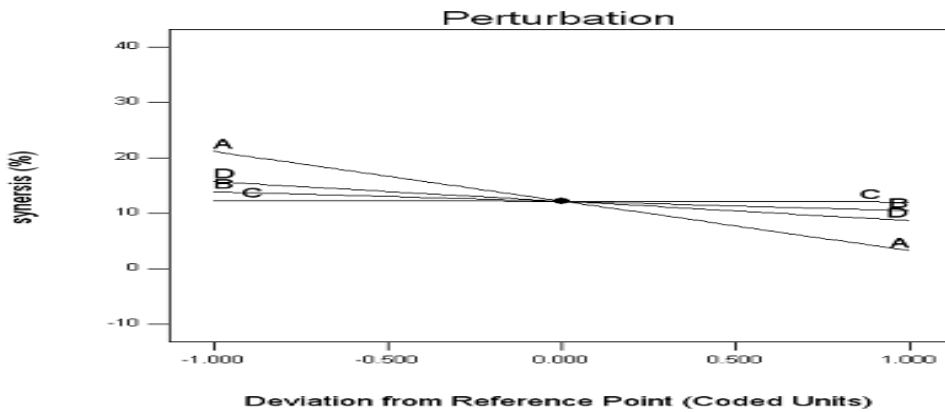
Increasing the prebiotic, fat content and storage time decreased the syneresis. Changes in the amount of  $\beta$ -glucan have the strongest effect on syneresis (Figure 2-a). This result is similar to results of Vasiljevic *et al.* (2007) which were obtained on cow milk. Hydration and network structure creating of  $\beta$ -glucan, for fibrous structure, trapped the water molecules in this network structure and prevented the water from escaping up and reduced the syneresis.

Yogurt syneresis has diminished by lengthening the storage time. According to the results,  $\beta$ -glucan in addition to its prebiotic properties improved the texture and decreased syneresis (Sahan *et al.*, 2008).

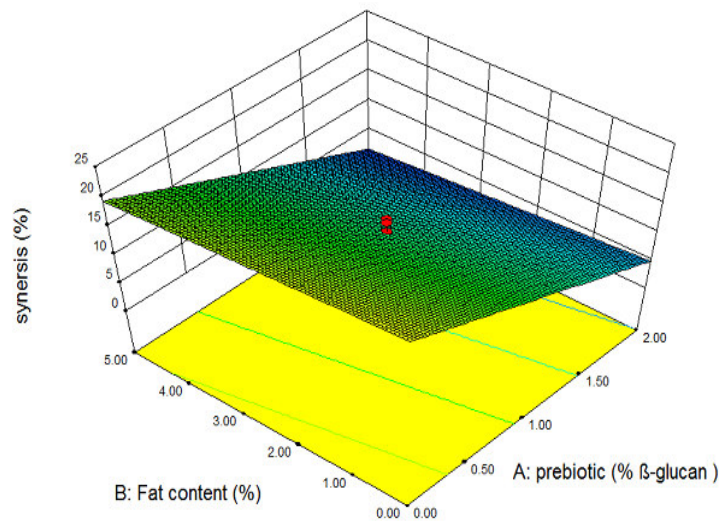
Increasing the prebiotic and fat content decreased syneresis (Amatayakul *et al.*, 2006), but these two factors didn't show any interactions. The linear model is appropriate for syneresis changes (Figure 2-b).

### pH

As it can be observed from the ANOVA Table (Table 3), the proposed linear model by the software is significant with "R-Squared" (0.93) and "Adj R-Squared" (0.92). Probiotic, fat content, inoculation level and storage time had significant effects ( $P < 0.05$ ) on the pH of yogurt. pH and acidity levels were observed in the samples that were appropriate and exactable with consumers.



(a)



(b)

**Figure 2.** Effect of various factors on the rate of camel yogurt syneresis: (a) Perturbation and (b) 3D graphs. A, B, C and D are prebiotic ( $\beta$ -glucan) percentage, camel milk fat percentage, inoculated probiotic bacteria percentage and storage time, respectively.

In Figure 3-a (Perturbation graph), increase in variables such as the inoculation of bacteria, the  $\beta$ -glucan content and time, declined the yogurt pH (Shori and Baba 2011), but fat increased the pH. Probiotic bacteria have the strongest effect on pH. Increasing inoculation content and storage time (Güven *et al.*, 2005), led to an increase in the number of bacteria in the product, and consequently more lactic acid was produced (Güler-akin, 2005). This result was also observed on cow yogurt (Mishra and Mishra, 2012).

Increasing both the prebiotic and fat content increased the pH (Figure 3-b). Beta-glucan (prebiotic) makes a more suitable media for probiotic bacteria growth and they have more activity in this product but fat globules

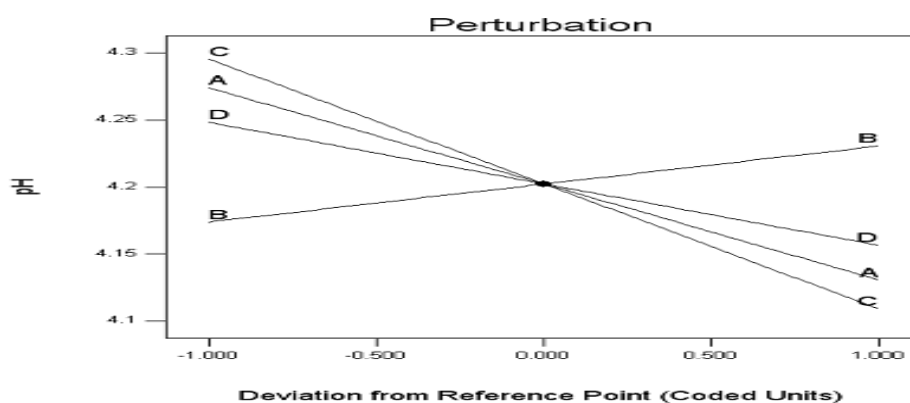
prevented the bacteria from activity. Since there was no interaction between the studied factors, the linear model was chosen.

In contrast to Sahan *et al.* (2008), our results showed that the increase in  $\beta$ -glucan concentration had a significant effect on pH changes. Although in synbiotic products there is a large population of *Lactobacillus* which consume prebiotic materials (here  $\beta$ -glucan) and produce lactic acid, therefore decreasing the pH.

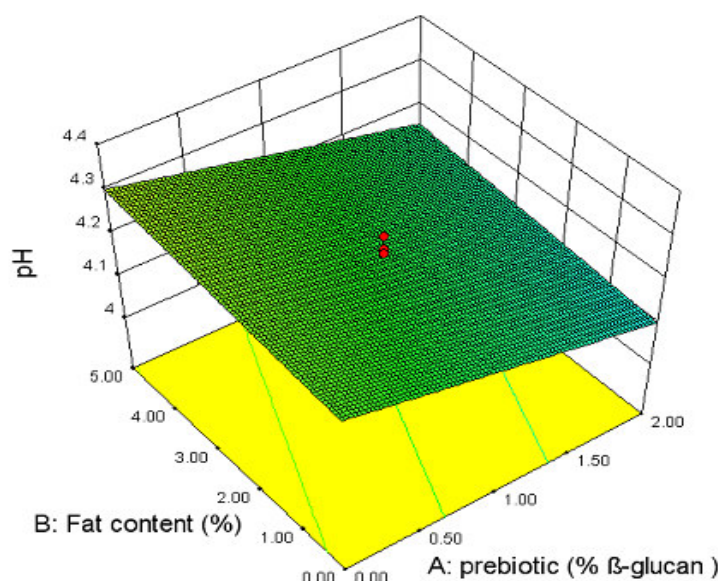
### Water-Holding Capacity (WHC)

As it can be observed from the ANOVA Table (Table 3), the proposed model by the software is significant. Prebiotic and fat





(a)



(b)

**Figure 3.** Effect of various factors on the rate of camel yogurt Ph: (a) Perturbation and (b) 3D graphs. A, B, C and D are prebiotic ( $\beta$ -glucan) percentage, camel milk fat percentage, inoculated probiotic bacteria percentage and storage time, respectively.

content had significant effects ( $P < 0.05$ ) on *WHC* of yogurt. However, inoculation level and storage time had no significant effects on *WHC*.

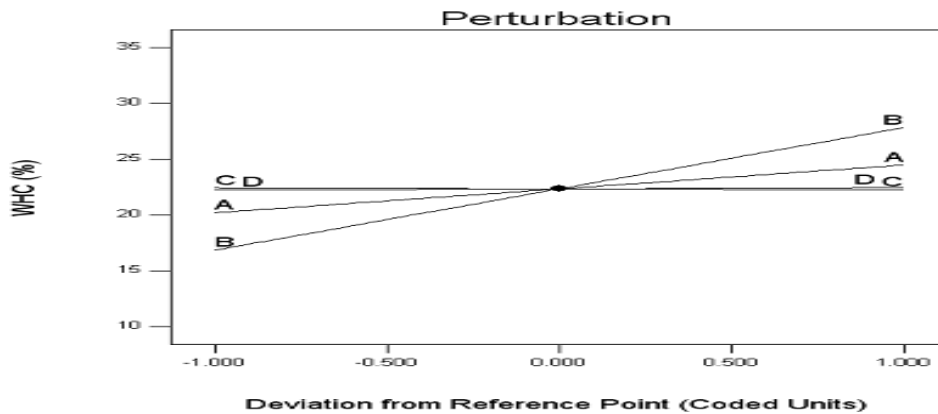
A linear model was proposed to predict the *WHC* of samples and have the appropriate "R-Squared" (0.94) and "Adj R-Squared" (0.93). *WHC* can be obtained using the following Equation (4):

$$\text{WHC}(\%) = +22.56 + (2.14 \times A) + (5.49 \times B) - (0.11 \times C) + (0.10 \times D) \quad (4)$$

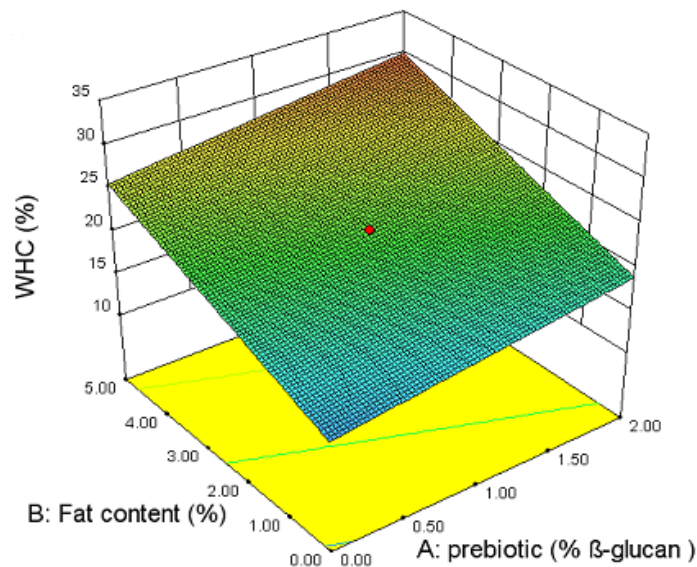
A, B, C and D are the contents of prebiotic ( $\beta$ -glucan), camel milk fat, inoculated probiotic bacteria and storage time, respectively.

The increasing  $\beta$ -glucan and fat content increased the *WHC*. Changes in milk fat levels have the strongest effect on *WHC* changes (Figure 4-a). Beta-glucan as a stabilizer (polysaccharide) and fat can be made the suitable (stronger) texture and it prevented the water from escaping up in this product and improved the camel yogurt *WHC*.

The important result in this study is that the time didn't have a significant effect on the *WHC*. *WHC* decreased during storage time and this yogurt had acceptable quality during storage time. Therefore the industry's important problem was solved and also the



(a)



(b)

**Figure 4.** Effect of various factors on the rate of camel yogurt WHC: (a) Perturbation and (b) 3D graphs. A, B, C and D are prebiotic ( $\beta$ -glucan) percentage, camel milk fat percentage, inoculated probiotic bacteria percentage and storage time, respectively.

same results on cow yogurt were achieved by Sahan *et al.* (2008).

The 3D graph (Figure 4-b) also shows that increasing the concentration of the prebiotic and amount of fat content increased the water-holding capacity, but considering that they didn't have interaction, therefore the suggested linear model was a good model for WHC changes.

### Viability of Probiotic Bacteria

As it can be observed from the ANOVA Table (Table 3), the proposed model by the software is significant. Prebiotic, fat content

and storage time had significant effects ( $P < 0.05$ ) on the viability of probiotic bacteria of yogurt. However, inoculation level had no significant effect on the viability of probiotic bacteria; as a result no more inoculum is needed for increasing the viability of probiotics in yogurt during storage time. Higher viability of probiotic bacteria is achieved by the addition of another factor such as  $\beta$ -glucan.

Salami *et al.* (2011) and Jumah *et al.* (2001) said in their researches that camel milk has antibacterial agents, but we were able to create a suitable medium and adequate nutrient for probiotic bacteria in the functional camel yogurt.

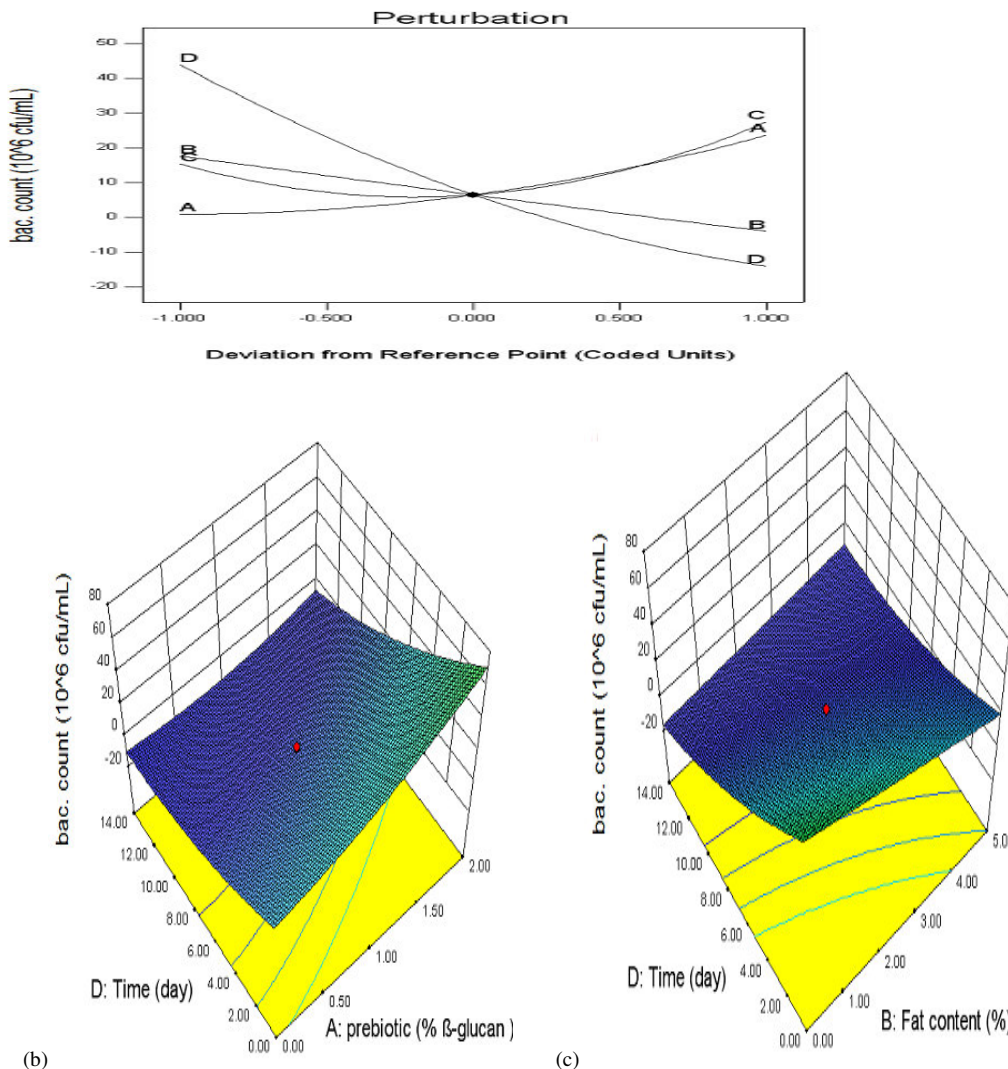
A quadratic model was proposed to predict the viability of probiotic bacteria in camel yogurt ( $R^2= 0.93$  and  $R^2\text{-Adj}= 0.87$ ). The viability of probiotic bacteria can be obtained using the following Equation (5):

$$\begin{aligned} \text{Bac.count (10}^6 \text{ cfu mL}^{-1}) = & +11.50+(11.5\times A)-(9.58\times B)+(6.25\times C)- \\ & (29.33\times D)-(4.12\times A\times B)-(2.25\times A\times C)- \\ & (11.63\times A\times D)-(5.13\times B\times C)+(12.75\times B\times D)- \\ & (5.88\times C\times D)+(4.63\times A^2)- \\ & (0.63\times B^2)+(14.25\times C^2)+(7.50\times D^2) \end{aligned} \quad (5)$$

A, B, C and D are the contents of prebiotic ( $\beta$ -glucan), camel milk fat, inoculated probiotic bacteria and storage time,

respectively.

The effects of various factors on the viability of probiotic bacteria can be observed in Figure 5a. With the increase of  $\beta$ -glucan as the prebiotic agent, available nutrients for bacteria (nitrogen and carbon sources) also increased (Souza *et al.*, 2011) and the viability of probiotic bacteria increased significantly ( $P < 0.05$ ), so more probiotic bacteria can survive in the final product. These results were in contrast with those obtained by Vasiljevic *et al.* (2007) on cow yogurt. Guggisberg *et al.* (2009) demonstrated that  $\beta$ -glucan as a prebiotic agent was more effective for bacterial



**Figure 5.** Effect of various factors on the viability rate of probiotic bacteria in camel yogurt: (a) Perturbation, and (b and c) 3D graphs. A, B, C and D are prebiotic ( $\beta$ -glucan) percentage, camel milk fat percentage, inoculated probiotic bacteria percentage and storage time, respectively.



survival, yogurt texture and formation of protein-polysaccharide network compared to inulin.

Increasing the amount of fat decreased the viability of probiotics. Fat globules can reduce the access of microorganisms to nutrients so the media become inadequate for the probiotic bacteria's growth and activity (Figure 5-a). Guven *et al.* (2005) reported that milk fat improved the yogurt texture, however it had a negative effect on the viability of probiotic bacteria. Therefore, to enhance the viability of probiotic bacteria the fat content of yogurt was decreased and to improve the textural property of yogurt the addition of  $\beta$ -glucan seems to be necessary.

Another variable that had significant decreasing effect ( $P < 0.05$ ) on the viability of probiotic bacteria was storage time (Antunes *et al.* 2005). This can be explained by the consumption and consequently the loss of nutrients for microorganisms therefore, the conditions for their growth and activity became undesirable. Production of organic acids by probiotic bacteria made the condition worst for their growth and activity (Torre *et al.*, 2003).

According to Table 3, interactions between  $\beta$ -glucan-storage time and fat content-storage time showed significant effects ( $P < 0.05$ ) on the viability of probiotic bacteria in yogurt, so a quadratic model can be applied to predict the viability of probiotic bacteria. Figure 5b showed that the effect of  $\beta$ -glucan concentration on the viability of probiotic bacteria was more pronounced at higher concentrations of  $\beta$ -glucan. The inverse effect was observed for the storage time. The highest value for the viability of probiotic bacteria was obtained at the highest concentration of  $\beta$ -glucan and the lowest storage time (Figure 5-b). Figure 5c showed that the effect of fat content on the viability of probiotic bacteria was more obvious at the lower amount of fat. The same effect was observed for the storage time. The highest value for the viability of probiotic bacteria was obtained at the lowest fat content and storage time (Figure 5-c)

## CONCLUSIONS

The important result in this study is that the viscosity increased in high percentage of  $\beta$ -glucan and fat content during time storage, but, syneresis increased when used from low amount of  $\beta$ -glucan and fat content. pH changes were similar to the syneresis changes. Water Holding Capacity (WHC) and bacterial count changes depended on the  $\beta$ -glucan and fat percentage changes in storage time. Results showed that the optimum conditions to obtain the synbiotic yogurt made by camel milk were defined as: adding 2%  $\beta$ -glucan (prebiotic agent) to milk with 1.9% fat content inoculated to 0.5% probiotic bacteria with a storage time of 7 days. The resulted product has the highest viscosity (14.905 cP), water-holding capacity (23.27%) and a viability of probiotic bacteria of  $36 \times 10^6$  cfu mL<sup>-1</sup>. In this product acidity was 8.15 g L<sup>-1</sup> and pH reached to 4.2. Syneresis in this product was acceptable (4.08%).

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## اثر بتا گلوکان جو دوسر بر خواص فیزیکیوشیمیایی و زنده مانی باکتریهای پروبیوتیک ماست فراسودمند سیمبیوتیک از شیر شتر در طول نگهداری

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### چکیده

با توجه به خواص منحصر به فرد شیر شتر، این محصول می تواند به عنوان یک غذا-دارو مورد مصرف قرار گیرد. از این رو در این تحقیق به بررسی تولید ماست فراسودمند سین بیوتیک از شیر شتر با درصد های چربی ۰، ۲/۵ و ۵٪ (w/v) پرداخته شد. باکتری های پروبیوتیک (استرپتوکوکوس ترموفیلوس و لاکتوباسیلوس دلبروکی و بولگاریکوس) و  $\beta$ -گلوکان (عامل پری بیوتیک) و به ترتیب در سه سطح ۰/۵، ۱ (v/v) و ۱/۵٪ و ۰، ۱ و ۲٪ (w/v) به محصول اضافه شد. خواص فیزیکیوشیمیایی محصول و زنده مانی باکتری های پروبیوتیک در روز های اول، هفتم و چهاردهم نگهداری، بررسی گردید.  $\beta$ -گلوکان، چربی و مدت زمان ماندگاری تاثیر افزایشی معنی داری ( $p < 0.05$ ) بر گرانروی، ظرفیت نگهداری آب (WHC) و زنده مانی باکتری های پروبیوتیک داشتند. این متغیرها موجب کاهش آب اندازی و pH ماست تولیدی شدند. در نتیجه، با افزودن  $\beta$ -گلوکان به شیر شتر به منظور تولید ماست فراسودمند سین بیوتیک، محصول تولیدی دارای خصوصیات فیزیکیوشیمیایی مطلوبی می باشد.