

## **EFFECT OF MILK SUPPLEMENTATION WITH VARIOUS TYPES OF MILK PROTEINS ON PHYSICOCHEMICAL AND MICROBIOLOGICAL PROPERTIES OF BIO-FERMENTED CAMEL'S MILK.**

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### **ABSTRACT**

In the present work, the influence of milk supplementation on physicochemical, microbiological and sensory properties of probiotic camel's yoghurt during refrigerated storage period of 21 d was studied. Three powders: sodium caseinate (SCN), whey protein concentrate (WPC) and skim milk powder (SMP) at three different ratios (1, 2 and 4%) were tested as supplementation. The results indicated that, the highest ( $P<0.05$ ) titratable acidity, acetaldehyde and diacetyl values was found in bio-yoghurt supplemented with 4% WPC during storage period. Also, the addition of 4% WPC improved the viability of *S. thermophilus*, *Lb. delbrueckii subsp. bulgaricus*, *Bifidobacterium animalis subsp. lactis BB-12* and *Lb. acidophilus LA-5* more than SCN or SMP bio-yoghurts. On the other hand, the bio-yoghurts fortified with 4% SCN had the highest ( $P<0.05$ ) viscosity, gel firmness and the lowest whey syneresis values in comparison with other treatments during storage. Organoleptic tests indicate that, the bio-yoghurts fortified with 4% SCN had significantly ( $P<0.05$ ) higher scores in appearance and body and texture while the bio-yoghurts fortified with 4% WPC had higher acidic taste and flavor scores. Both the bio-yoghurts supplemented with 4% SCN or WPC showed better physical and similar overall acceptability scores compared to other treatments. The results suggested that the addition of 4% SCN or WPC could be used to produce acceptable bio-yoghurt made from camel's milk.

**Keywords:** Camel milk, Bio-yoghurt, Whey protein concentrate, Sodium caseinate.

### **INTRODUCTION**

The camel (*Camelus dromedarius*) is of considerable socio-economic value in many arid and semi-arid areas of the world and its milk comprises a significant part of human dietary habits in these areas. Camel milk is unique from other ruminant milk in terms of composition as well as functionality as it contains high concentration of immunoglobulin's and insulin. It is high in vitamins (A, B-2, C and E) and minerals (sodium, potassium, iron, copper, zinc and magnesium) and low in cholesterol (Kamal et al., 2007 and Al-Hashem, 2009). Fresh and fermented camel milks have been used in different regions in the world including Africa and the Middle East as a treatment for a series of diseases. The positive health effects of milk proteins can be presented as anticarcinogenic (Magjeed, 2005) and antidiabetic (Agrawal et al., 2007), and has been recommended to be consumed by children who are allergic to bovine milk (El-Agamy et al., 2009).

Camel's milk is different from other ruminant milk and it does not form coagulum in acidic environment (Wangoh, 1993). Thus, fermented

camel milk products are difficult to produce because of the problem in milk coagulation. Mohamed and Larsson-Raznikiewicz (1990) found that camel milk coagulum failed to reach a gel-like structure even after 18 h incubation with lactic acid culture. This may be because camel milk contains a greater content of antimicrobial components such as lysozyme, lactoferrin and immunoglobulins than do bovine or buffalo milk (Benkerroum, 2008). Furthermore, camel's milk has slightly lower casein content than cow's milk, with a very low ratio of beta-CN to kappa-CN than in cow milk (Kappeler et al., 1998). All these factors influence the rheological properties of the heat treatment and enzymatic coagulation in camel's milk that is almost semi liquid.

Therefore, research is necessary for identification of food additives which can be used to optimize viscosity and texture in camel milk yoghurt. Therefore, one of the most important steps in the production of camel yogurts is to increase the total solids content of the yoghurt mixes by the addition of a source of milk proteins.

However, milk supplements with milk proteins can affect the texture and the physical properties of the yoghurt. Yoghurt produced from camel milk (with no additives) was reported to have a thin and very soft texture due to the low total solids content in camel yoghurts (Hashim et al., 2009). Recently, some researchers attempt to put off syneresis and improvement the texture by increasing total solids constituents of camel milk, by the addition of milk powder (Mortada and Omer, 2013) and stabilizers such as alginate, pectin and gelatin (Hashim et al., 2009). In addition whey protein polymers/isolates are also used as gelling agents in stirred camel yoghurt (Sakandar et al., 2014).

Dry dairy ingredients such as skim milk powder (SMP), whey protein concentrate (WPC), sodium caseinate (SCN) are commonly used to increase the solids content of the yoghurt mix. Nevertheless, fortification with these ingredients affects production costs. The use of stabilizers including gelatin or gums may affect the consumer perception of yoghurt. The use of stabilizers is also prohibited in some European countries (De Vuyst and Degeest, 1999).

It is common practice to use skim milk powder (SMP) to fortify yoghurt, but other dried milk protein ingredients, such as, whey products and caseinates are also used (Isleten and Karagul-Yuceer, 2006 and 2008). The added milk protein assists in providing a firmer body and reduces whey separation (Mistry and Hassan, 1992). Whey protein concentrate modifies the texture profile, water holding capacity, buffering capacity and fermentation time, as compared to yoghurts containing only skim milk powder, at the same level of protein addition (Antunes et al., 2004 and Damin et al., 2008). On the other hand, yoghurts fortified with SCN displayed better physical and sensory properties than control yoghurts (Isleten and Karagul-Yuceer, 2006). Peng et al., (2013) found that yoghurts made with additional casein-based ingredients were firmer and showed less syneresis than yoghurts that were fortified at the same protein level with whey protein-based ingredients.

However, a comparison of the effects of different type of milk proteins on the physical, chemical and microbial properties of camel's milk fermented product has not been studied.

Therefore, the objective of this study was to compare the effects of fortification with different types of milk proteins (SCN, WPC or SMP) at three different ratios (1, 2 and 4%) on the physiochemical, microbiological as well as sensory characteristics of set-type probiotic yoghurt made from camel's milk. The bio-yoghurts were evaluated when fresh and after storage at  $4\pm 1^{\circ}\text{C}$  for 1, 7, 14 and 21 days.

## MATERIALS AND METHODS

### Ingredients and Strains:

Fresh camel's milk was obtained from Matrouh Governorate, North West Coast, Egypt. For camel milk fortification, three dried milk protein ingredients were used: Skim milk powder (low heat); made in the California Dairies, Inc., Fresno, California, USA was purchased from the local market at Cairo, Egypt. Whey protein concentrate (WPC80) was purchased from Davisco Foods International, Inc.(Eden Prairie, Minnesota 55344, USA). Sodium caseinate (Alanate 180, Fonterra Co-operative Group, New Zealand).

The composition of the dried milk protein ingredients used for fortifying yoghurt is shown in Table 1.

**Table 1. Composition of fresh camel's milk and dried milk protein ingredients\*<sup>a</sup> used in this study.**

Components %	Total Solids %	Protein %	Fat %	Lactose %	Ash %
Fresh camel milk	12.21	3.16	3.3	4.94	0.87
Skim milk powder (SMP)	95.5	35.37	0.8	50	8.2
Whey protein concentrate (WPC)	94.5	81.4	5.4	8.0	2.6
Sodium Caseinate (SCN)	95.5	91	0.9	0.2	3.7

\*Specifications obtained from the manufacturers.

Direct vat culture of commercial lyophilized 'FD-DVS NU-TRISH<sup>®</sup> ABY-2 culture (containing *Bifidobacterium animalis subsp. lactis BB-12*, *Lb. acidophilus LA-5*, *Streptococcus salivarius subsp. thermophilus* and *Lb. delbrueckii subsp. bulgaricus*) were obtained from Chr-Hansen Company (Horsholm, Denmark), by Misr Food Additives (MIFAD), Egypt. The cultures were maintained according to the manufacturer's instructions at  $-18^{\circ}\text{C}$ .

### Yoghurt manufacture:

Probiotic fermented camel's milk was manufactured according to the method reported by (Tamime and Robinson 1999). The bio-yoghurt was prepared by using whole camel milk. Camel's milk was divided into 4 parts, the first portion was used as control and the other three parts was supplemented with 1, 2 and 4 % w/w of WPC, SCN or SMP, respectively. All mixes were heated to  $60^{\circ}\text{C}$ , homogenized at 15 MPa, then heat-treated in a thermostatically controlled water bath at  $85^{\circ}\text{C}$  for 30 min and cooled to  $42^{\circ}\text{C}$  in an ice bath. For each experimental, according to Chr-Hansen's

recommended procedure, a fifty-unit pouch of direct vat commercial lyophilized ABY-2 culture was suspended in 1 L sterilized skim milk powder and incubated at 42 for 4 h before use, then 4.0 mL of this inoculum was inoculated into 1 L of camel milk heat-treated. The inoculated milks were poured into 150 g plastic cups with lids and incubated at 42°C for 8 h. After fermentation, bio-yoghurt samples were cooled down and transferred to a refrigerator at 4±1°C, then stored at this temperature over 21 d for the physicochemical, textural, microbiological and sensory analyses. The experiment was replicated 3 times on different days.

#### **Chemical Composition Analyses:**

##### **Chemical analysis:**

Samples of camel's bio-yoghurt were analyzed for total solid, protein contents, titratable acidity and pH value. The total solid, protein and titratable acidity were measured using the methods of AOAC 990.20, 991.20 and 947.05, respectively (AOAC, 2000). The titratable acidity was expressed as % lactic acid. The pH of the bio-yoghurt samples was measured with a pH meter equipped with a glass electrode (model pH 211; Hanna Instruments). Diacetyl (spectrophotometric method) according to (Less and Jago, 1970) and Acetaldehyde (spectrophotometric method) by using method of (Lees and Jago, 1969). All the analyses were performed in triplicate. Analyses were performed in triplicate after storing the product for 1, 7, 14 and 21 days at 4±1°C.

##### **Rheological measurements:**

##### **Apparent Viscosity (mPa.s):**

Apparent viscosities of camel's bio-yoghurt were measured on cup at 10°C with a Brookfield viscometer (model DV II+ Pro Brookfield Engineering Laboratories, Inc., Middleboro, MA) after 1, 7, 14 and 21 days of storage at 4±1°C. The spindle used (no.4 spindle at 10 rpm) in 150 g of bio-yoghurt. The spindle was allowed to rotate in the sample for 60 s at 10°C of shearing. The apparent viscosity reading in millipascal second (mPa.s) was noted from the digital output of the viscometer. The measurements were performed in triplicate for each sample. (Donkor et al.,2007).

##### **Gel Firmness:**

The gel firmness of bio-yoghurts was measured by using a texture analyzer (TA-XT2 model, Stable Micro Systems, Godalming, Surrey, U.K.) using a single compression cycle test with a 5-kg load cell. The probe used was a 3.5cm diameter aluminum cylinder. Pretest and test speed were fixed at 1 mm/s and penetration depth was 3.0 cm (Sandoval-Castilla et al., 2004). The measurements were performed as soon as the samples were removed from the refrigerator. The firmness of the bio-yoghurt samples was expressed in gram.

##### **Syneresis index:**

The analysis was carried out within 24 h after the fermentation was completed. The syneresis index (determined in triplicate) of whey was carried out by gravity according to (Tsevdou et al., 2013). This involved placing a 100 ml bio-yoghurt samples in a Buchner funnel lined with a Whatman filter paper number #1(Whatman International Ltd., Maidstone,

England) for 3 h at 4°C. After drainage, the volume of whey collected in a beaker was measured and used as an index of syneresis.

**Microbiological analyses:**

For each run, bio-yoghurt were analyzed after 1,7,14 and 21 days of storage at 4±1°C. Fermented milk samples (0.1 mL) were added to 9.9 mL sterile tryptone diluent (0.1% w/v). Appropriate dilutions were made and subsequently pour-plated in duplicate onto selective media.

Counts of *S. thermophilus* were enumerated on M17 agar containing 5g/L lactose (Oxoid Ltd., Basingstoke, UK) (Torriani et al., 1996). The pH of the medium was 6.9 ± 0.1. The inoculated plates were incubated aerobically at 37° C for 48 h.

*Lactobacillus delbrueckii subsp. bulgaricus* were enumerated on acidified (pH 5.2) MRS agar (deMan, Rogasa, Sharpe) (Difco Laboratories) supplemented with 0.5 g/L cysteine HCl (Dave and Shah, 1996). Plates were incubated under anaerobic conditions using AnaeroGen in plastic anaerobic jars (Gas-pack, Anaerogen ; Oxoid,UK.). ) at 37°C for 72 h (Torriani et al., 1996).

Enumeration of *Lb. acidophilus* was on MRS agar (deMan, Rogasa, Sharpe) (Difco Laboratories) supplemented with 0.5g/L cysteine HCl, (Merck, Germany), and anaerobical conditions incubation at 37°C for 72 h (Lankaputhra et al., 1996).

Enumeration of *Bifidobacterium animalis subsp. lactis* was determined according to (Lankaputhra et al., 1996) using MRS-NNLP (nalidixic acid, neomycine sulfate, lithium chloride, paromycine sulfate) and vancomycine (Sigma Chemical Co., St Louis, MO) agar. Plates were incubated for 48 h at 37 °C in anaerobic conditions. Plates containing 30-300 colonies were enumerated and recorded as colony forming units (CFU) per gram of sample. All bacterial counts were conducted in triplicate.

**Sensory evaluation:**

A number of 10 trained panelists from the staff members at Desert Research Center (DRC) who consume yoghurts regularly in their diets and have previous experience in taste evaluation were selected to rate sensory properties of camel's bio-yoghurts. Bio-yoghurt samples were organoleptically examined according to the scheme described by (Farak et al., 2007). The samples were organoleptically scored using score card for flavour (45points), body and texture (35 points), appearance and color (10 points) and acidity (10 points). Panelists evaluated all bio-yoghurt samples after storage for 1, 7, 14 and 21 days at 4±1 °C.

**Statistical analysis:**

Statistical analysis for experimental data was analyzed as factorial arrangement, analysis of variance (ANOVA) was applied, and Duncan's multiple range test was used to determine the differences using SPSS® 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Standard error of the means was derived from the error mean square term of the ANOVA, which was used

the least significant difference (LSD) test. Differences were considered significant at ( $P < 0.05$ ). All experiments were conducted in triplicate.

## **RESULTS AND DISCUSSION**

### **The chemical composition of experimental bio-yoghurts:**

The chemical composition of the control (not supplemented with milk proteins) and bio-yoghurt fortified with different levels of whey protein concentrate (WPC), sodium caseinate (SCN) and skim milk powder (SMP), during storage period at ( $4 \pm 1^\circ\text{C}$ ) is shown in Table 2.

It was clear that, the means of total solids and proteins contents of all bio-yoghurt samples gradually increased with increasing storage period. These results are in agreements with (Salama, 2002, Badran et al., 2004 and Hassan and Imran, 2010) which may be due to evaporation of water and loss of moisture .

Furthermore, the fortification type and level of the ingredients had significant differences ( $P < 0.05$ ) among bio-yoghurt samples in total solids and proteins .The level of total solids and protein content in treated bio-yoghurts with 4% WPC or SCN was significantly ( $P < 0.05$ ) higher than that other bio-yoghurt samples. Similar changes in the composition of the products were also observed due to the type and level of ingredients (Gülfem and Akalin, 2013).

On the other hand, the pH values of all bio-yoghurt samples decreased slightly during storage and did not drop under 4.4 at the end of storage, possibly due to the low acidifying activity of the yoghurt and probiotic cultures, which is generally considered detrimental to the survival of probiotic bacteria (Dave and Shah, 1997). Also, may be because camel milk contains a greater content of antimicrobial components such as lysozyme, lactoferrin and immunoglobulin's (Benkerroum, 2008).

The highest pH values was found in control and treated samples with 1% SCN during the first day of storage period while, the lowest pH value was found in samples treated with 4% WPC during the end of the storage period. Similar results were obtained by (Dave and Shah 1998ab) they reported that, decrease in pH was higher in yoghurt containing WPC than that of the control yoghurt .

On the other hand, a gradual increase in titratable acidity was noted for all bio-yoghurts during storage .The level of titratable acidity in treated bio-yoghurts with 4% WPC was higher than those other bio-yoghurt samples. Thus increasing of the WPC addition resulted in reduced acidity. This might be because of a greater lactic acid bacteria growth when WPC is added to milk (Martin-Diana et al., 2003). Also, it may be due to higher buffering action of whey proteins. Similar results were obtained by (Shah et al., 1995 and Bozanic and Tratnik, 2001) for probiotic yoghurt and fermented bifido milk, respectively. Salaün et al., (2005) have reported that buffering capacity was a major factor affecting the variations in pH of dairy products. Furthermore, it has been also suggested that addition of WPC to yoghurt increases the buffering capacity at around pH 4.

Furthermore, one of the most important aroma compounds in yoghurt is acetaldehyde. For optimal flavour in yoghurt, the acetaldehyde

concentration should be between 23 and 41 mg/kg of yoghurt (Tamime and Deeth, 1980). Significant changes in relation to storage time were found in diacetyl and acetaldehyde of all bio-yoghurt samples. The results showed that the maximum acetaldehyde and diacetyl contents were found in bio-yoghurt treated with 4% WPC during 7 days storage. These results are in agreement with that finding of (Salama, 2002 and Badran et al., 2004) where found that, acetaldehyde and diacetyl contents gradually increased during 3 days of storage and then decreased in the end of storage period. However, during storage the amount of acetaldehyde decreased because of the hydrolysis by microbial enzymes in order to form other substances such as ethanol (Güler-Akin, 2005).

#### **Rheological properties:**

##### **Apparent viscosity:**

Generally, the viscosity values of the experimental bio-yoghurts increased up in the first 14 days followed by a decrease at the end of the storage Table 3. Increasing viscosity was also observed in concentrated (Abu-Jdayil and Mohameed, 2002) and non-fat plain yoghurt (Isleten and Karagul-Yuceer, 2006) throughout storage period. These authors explained that the increasing viscosity during storage could be due to the protein rearrangement and protein-protein contact. Similarly, (Akalin et al., 2008) observed fluctuations in the viscosity values of their samples during storage.

During storage, the highest viscosity values were found in the bio-yoghurt samples fortified with SCN or WPC whereas the control and bio-yoghurts supplemented with SMP was least viscous. Isleten and Karagul-Yuceer (2006) reported that the use of SCN to fortify milk was the most effective means of increasing yogurt viscosity. Fortification with SCN resulted in yoghurt products with higher viscosity and stronger networks and less syneresis than yoghurt enriched with SMP, but reduced smoothness and rougher texture compared to yoghurt made by addition of SMP (Remeuf et al., 2003).

However, the fortification level of the ingredients had a significant effect ( $P < 0.05$ ) on the viscosity of the bio-yoghurt samples. Viscosity values decreased in the bio-yoghurt samples in the following order control < SMP < WPC < SCN. In addition, the highest viscosity values were found in the samples fortified with 4% SCN. This may be attributed to the higher protein and total solid contents of the samples (Martin-Diana et al., 2003). Yoghurt is suggested to have weak bonding, but SCN supplementation tends to change the gel structure and increase in viscosity (Sodini et al., 2004 and Damin et al., 2009).

In our study, bio-yoghurts supplemented with WPC had also shown a higher viscosity than the SMP or control bio-yoghurt. According to (Remeuf et al., 2003 and Akalin et al., 2008) higher whey protein content and its denaturation during heat treatment highly influence viscosity due to an increase in the protein-binding capacity that results in a higher gel viscosity during coagulation.

**Table 2. Chemical composition (mean<sup>a</sup> ±SD) of experimental control and supplemented bio-yoghurts during storage period at (4 ±1°C).**

Ingredient types	Rate %	Storage days	Total solids (%)	Protein (%)	pH	Titrateable acidity%	Diacetyl (mg/kg)	Acetaldehyde (mg/kg)
Control	0	1	12.53±0.18	3.38±0.07	4.90±0.10	0.76±0.02	0.41±0.07	24.10±1.83
		7	12.60±0.11	3.43±0.10	4.83±0.06	0.83±0.03	0.76±0.08	32.81±1.07
		14	12.89±0.51	3.49±0.04	4.70±0.10	0.86±0.02	0.58±0.06	22.13±1.46
Whey Protein Concentrate (WPC)	1	1	13.35±0.12	4.20±0.09	4.73±0.06	0.81±0.01	0.46±0.02	26.13±1.96
		7	13.53±0.11	4.35±0.05	4.60±0.10	0.92±0.02	0.84±0.02	35.81±0.27
		14	13.70±0.27	4.41±0.08	4.57±0.05	0.95±0.01	0.62±0.06	23.80±1.87
	2	1	14.11±0.35	4.77±0.06	4.73±0.14	0.86±0.02	0.48±0.01	25.00±2.65
		7	14.36±0.30	4.83±0.06	4.67±0.15	0.85±0.02	0.88±0.02	37.00±2.00
		14	14.48±0.18	5.07±0.15	4.63±0.12	0.96±0.01	0.63±0.03	21.67±1.15
	4	1	14.56±0.29	5.30±0.17	4.57±0.12	1.07±0.03	0.44±0.08	20.83±1.61
		7	16.61±0.33	6.93±0.06	4.63±0.12	0.87±0.02	0.52±0.06	27.67±2.52
		14	17.15±0.84	7.20±0.17	4.57±0.06	0.93±0.03	0.94±0.07	40.33±2.08
Sodium Caseinate (SCN)	1	1	17.68±0.86	7.27±0.15	4.53±0.06	1.06±0.05	0.70±0.10	25.07±4.44
		7	18.46±1.14	7.33±0.15	4.47±0.12	1.15±0.05	0.49±0.06	20.07±1.68
		14	13.53±0.13	4.33±0.15	4.90±0.17	0.77±0.02	0.39±0.03	23.33±2.08
	2	1	13.74±0.16	4.57±0.06	4.82±0.03	0.84±0.01	0.78±0.04	33.00±1.00
		7	14.06±0.51	4.70±0.10	4.73±0.06	0.87±0.03	0.60±0.02	23.50±0.87
		14	14.43±0.86	5.03±0.12	4.63±0.06	1.15±0.05	0.37±0.13	18.73±0.40
	4	1	14.31±0.45	4.77±0.06	4.93±0.15	0.80±0.02	0.45±0.03	20.67±1.53
		7	14.54±0.32	4.87±0.06	4.80±0.17	0.83±0.03	0.81±0.01	32.33±1.53
		14	14.98±0.44	5.03±0.12	4.77±0.06	0.88±0.02	0.58±0.06	24.33±2.31
Skim Milk Powder (SMP)	1	1	15.40±0.11	5.23±0.06	4.63±0.06	1.18±0.03	0.37±0.02	19.67±1.53
		7	16.78±0.24	6.83±0.06	4.83±0.15	0.79±0.03	0.50±0.04	22.43±2.23
		14	17.01±0.67	7.10±0.10	4.67±0.15	0.81±0.02	0.91±0.05	33.33±1.15
	2	1	17.35±0.24	7.27±0.06	4.57±0.06	0.88±0.02	0.63±0.07	20.40±0.96
		7	18.12±1.30	7.47±0.06	4.57±0.06	1.20±0.01	0.44±0.07	18.57±0.86
		14	13.53±0.13	3.70±0.10	4.77±0.06	0.79±0.02	0.42±0.02	23.33±2.08
	4	1	13.94±0.16	3.87±0.12	4.73±0.12	0.85±0.02	0.79±0.03	34.33±1.53
		7	14.34±0.33	4.13±0.06	4.67±0.15	0.87±0.03	0.60±0.02	25.00±0.87
		14	14.59±0.26	4.23±0.15	4.60±0.10	1.20±0.00	0.40±0.09	20.50±1.80
Total Main effects (means)	1	1	14.19±0.47	4.30±0.10	4.87±0.12	0.81±0.01	0.47±0.02	19.67±1.15
		7	14.37±0.27	4.43±0.12	4.77±0.15	0.84±0.02	0.82±0.01	35.17±1.04
		14	14.68±0.49	4.60±0.00	4.73±0.06	0.88±0.03	0.59±0.01	24.33±2.31
	2	1	15.61±0.25	4.77±0.15	4.60±0.10	1.20±0.01	0.42±0.06	20.67±0.58
		7	16.78±0.24	5.07±0.15	4.67±0.15	0.80±0.05	0.49±0.03	22.43±2.23
		14	17.14±0.36	5.20±0.17	4.57±0.06	0.84±0.04	0.92±0.05	36.50±0.50
	4	1	17.47±0.51	5.63±0.15	4.53±0.06	1.09±0.18	0.67±0.04	20.40±0.96
		7	17.75±0.18	6.07±0.15	4.53±0.06	1.23±0.06	0.47±0.06	20.13±1.50
		14	12.80 <sup>b</sup> ±0.37	3.46 <sup>c</sup> ±0.08	4.76 <sup>a</sup> ±0.14	0.89 <sup>b</sup> ±0.15	0.53 <sup>c</sup> ±0.19	24.39 <sup>b</sup> ±5.62
Control		15.17 <sup>a</sup> ±1.79	5.53 <sup>a</sup> ±1.23	4.62 <sup>c</sup> ±0.09	0.96 <sup>a</sup> ±0.11	0.62 <sup>a</sup> ±0.18	26.99 <sup>a</sup> ±6.99	
WPC		15.36 <sup>a</sup> ±1.58	5.60 <sup>a</sup> ±1.16	4.65 <sup>a</sup> ±0.13	0.92 <sup>b</sup> ±0.09	0.57 <sup>b</sup> ±0.18	24.19 <sup>b</sup> ±5.54	
SCN		15.37 <sup>a</sup> ±1.49	4.67 <sup>b</sup> ±0.70	4.55 <sup>b</sup> ±0.10	0.94 <sup>a</sup> ±0.12	0.59 <sup>ab</sup> ±0.17	25.08 <sup>b</sup> ±6.31	
Main effects (means)								
Control	0		12.80 <sup>b</sup> ±0.37	3.46 <sup>c</sup> ±0.08	4.76 <sup>a</sup> ±0.14	0.89 <sup>b</sup> ±0.15	0.53 <sup>c</sup> ±0.19	24.39 <sup>b</sup> ±5.62
WPC	1		13.66±0.32	4.42±0.20	4.62 <sup>cd</sup> ±0.09	0.95 <sup>bc</sup> ±0.11	0.59 <sup>cd</sup> ±0.18	26.55 <sup>b</sup> ±6.11
	2		14.38 <sup>cd</sup> ±0.30	4.99 <sup>c</sup> ±0.24	4.65 <sup>bcd</sup> ±0.13	0.94 <sup>bcd</sup> ±0.09	0.61 <sup>bcd</sup> ±0.18	26.13 <sup>bc</sup> ±6.95
	4		17.48 <sup>a</sup> ±1.01	7.18 <sup>b</sup> ±0.20	4.55 <sup>d</sup> ±0.10	1.00 <sup>a</sup> ±0.12	0.66 <sup>a</sup> ±0.20	28.28 <sup>a</sup> ±8.18
SCN	1		13.94 <sup>cd</sup> ±0.56	4.66 <sup>d</sup> ±0.28	4.77 <sup>a</sup> ±0.13	0.91 <sup>de</sup> ±0.15	0.53 <sup>d</sup> ±0.18	24.64 <sup>cd</sup> ±5.53
	2		14.81 <sup>b</sup> ±0.53	4.98 <sup>b</sup> ±0.20	4.78 <sup>b</sup> ±0.15	0.92 <sup>cd</sup> ±0.16	0.55 <sup>cd</sup> ±0.18	24.25 <sup>de</sup> ±5.41
	4		17.32 <sup>a</sup> ±0.83	7.17 <sup>a</sup> ±0.25	4.66 <sup>bcd</sup> ±0.15	0.92 <sup>cd</sup> ±0.17	0.62 <sup>abc</sup> ±0.19	23.68 <sup>de</sup> ±6.11
SMP	1		14.10 <sup>de</sup> ±0.47	3.98 <sup>d</sup> ±0.24	4.69 <sup>abc</sup> ±0.12	0.93 <sup>cd</sup> ±0.17	0.55 <sup>cd</sup> ±0.17	25.42 <sup>cd</sup> ±5.69
	2		14.71 <sup>bc</sup> ±0.66	4.53 <sup>c</sup> ±0.21	4.74 <sup>ab</sup> ±0.14	0.93 <sup>bcd</sup> ±0.16	0.57 <sup>cd</sup> ±0.16	24.96 <sup>cd</sup> ±6.53
	4		14.81 <sup>b</sup> ±0.48	5.49 <sup>b</sup> ±0.43	4.58 <sup>de</sup> ±0.10	0.97 <sup>b</sup> ±0.20	0.64 <sup>ab</sup> ±0.19	24.87 <sup>cd</sup> ±7.18

<sup>a</sup> Means are average from three independent trials.

Means in the same column with different superscripts letters significantly differ ( $P<0.05$ ).

**Gel firmness:**

The firmness of yoghurt is dependent on total solids content and protein content of the product (Abu-Jdayil, 2003), and on the type of protein (Cho et al., 1999). According to the results, the firmness of the all bio-yoghurt samples gradually increased during 14 days of storage and then decreased in the end of storage period Table 3. This situation may be attributed to increased water holding capacity of milk proteins with time storage (Damin et al., 2008). However, the fortification level of the ingredients had a significant effect ( $P<0.05$ ) on the bio-yoghurt firmness. The firmness of bio-yoghurt fortified with 4% SCN was higher than that of bio-yoghurt supplemented with WPC or SMP, whereas the control bio-yoghurt was least firm ( $P<0.05$ ) probably due to the lowest protein content, which contributes to the firmness of the product. On the other hand, the promoting agent of SCN on firmness of yoghurt can be attributed to its ability to form larger aggregates. Damin et al., (2009) reported an increase in firmness values linearly with the sodium caseinate level which is in agreement with our results.

Interestingly, the control and fortified bio-yoghurts with 1 % (w/v) SCN, WPC or SMP which had <13.5% total solid had a very weak gel. This characteristic of soft gel could be due to the low solid content. Therefore, the increase in total solid through additions of SCN, WPC or SMP facilitated the gel formation. The less-open gel structure formed with high protein content would produce an aggregate network with high gel firmness (Gozález-Martínez et al., 2003).

Furthermore, this result supports that, the use of 4% SCN in the fortification of bio-yoghurt caused the hardest structure. Our results are also in agreement with (Puvanenthiran et al., 2002) they reported that the firmness of yoghurt made with sodium caseinate was higher than that of yoghurt made with a SMP-fortified milk base. Moreover, the benefit of WPC on the firmness of bio-yoghurt was observed when it was compared to the use of SMP. The increase in firmness of the yoghurt gel by addition of WPC can be explained by higher protein content and increased water-binding capacities by denatured whey proteins in the finished product (Bhullar et al., 2002).

**Whey Syneresis:**

For all tested samples, the values of whey syneresis decreased gradually during the 3 weeks of refrigerated storage and thus, these values were also dependent on the storage period Table 3. With regard to the effect of the ingredient type and the addition rate on the whey syneresis of bio-yoghurts were found statistically significant ( $P<0.05$ ). This can be attributed to the increase in the protein and total solids levels (Amatayakul et al., 2006a). Bio-yoghurts fortified with 4% SCN had the lowest syneresis values, whereas control bio-yoghurts had the highest whey separation ( $P<0.05$ ). Akalin et al., (2012) suggested that an increase in the compactness of yoghurt microstructure, as the casein-to-whey protein ratio was reduced, led to immobilization of a high level of free water. Furthermore, higher total solid

causes an increase in density, and reduces pore size in the protein matrix of the yoghurt gel. This leads to a reduction of syneresis and improvement of the water holding capacity of the gel (Sodini et al., 2004 and Amatayakul et al., 2006b).

**Microbiological viability of the experimental bio-yoghurts:**

The viability of *S. thermophilus* and *Lb. delbrueckii subsp. bulgaricus* starter cultures, of control and fortified bio-yoghurts during storage period at ( $4 \pm 1^\circ\text{C}$ ) was shown in Table 4. The results indicated that, the counts of *S. thermophilus* reached its maximum increment during the 7 days and then declined slightly in all bio-yoghurts until the end of storage period. Oliveira et al., (2002) reported similar results for counts of *S. thermophilus* in fermented lactic beverages containing probiotic bacteria.

The fortification level of the ingredients had a significant difference ( $P < 0.05$ ) in the counts of *S. thermophilus* in all bio-yoghurts batches. In general, the highest viable counts of *S. thermophilus* were enumerated in bio-yoghurts supplemented with 4% WPC. Improved viability could be due to the amino nitrogen present in WPC and this is in agreement with the faster reduction of pH observed in WPC bio-yoghurts during fermentation. Dave and Shah, (1998a) detected the positive effect of addition of WPC on viable counts of *S. thermophilus* in yoghurt containing bifidobacteria. Also, Marafon et al., (2011) reported that, after 28 days of storage at  $4^\circ\text{C}$ , counts of *S. thermophilus* were higher in yoghurt supplemented with WPC than counts in yoghurt with added skim milk. Increase in the viability of *S. thermophilus* was also determined in probiotic-fermented milks supplemented with up to 4% of WPC (Martin-Diana et al., 2003 and Lucas et. al., 2004).

Generally, the highest viable counts of *Lb. delbrueckii subsp. bulgaricus* were enumerated in bio-yoghurts fortified with 4% WPC, whereas control and bio-yoghurts supplemented with SCN had the lowest values with significant difference ( $P < 0.05$ ) between all bio-yoghurts. This data is in accordance with (Akalin et al., 2007) which reported an increase of 1 log cfu/ml in the growth of *Lb. delbrueckii subsp. bulgaricus* in yoghurt with 1.5% whey protein concentrate added compared to no whey protein concentrate addition. Besides providing peptides and amino acids, the addition of whey protein acted as a buffer agent, preventing sudden changes in the acidity of the media and avoiding lethal pH levels for *Lb. delbrueckii subsp. bulgaricus* (Dave and Shah, 1998ab). According to Nadal et al.,(2010) the addition of whey proteins can improve the buffering capacity of a media, thus reducing the effect of acid environments for the bacterial strain. On average the survival rate of *S. thermophilus* was better than *Lb. delbrueckii subsp. bulgaricus* this might be because the *S. thermophilus* was better competitor than the latter for utilization of limiting nutrients (Rajagopal and Sandine, 1990).

**Table 3. Rheological properties (mean<sup>a</sup> ±SD): viscosity (mPa), gel firmness (g) and syneresis (100 ml) of the experimental control and supplemented bio-yoghurts during storage period at (4 ±1°C).**

Ingredient types	Rate %	Storage days	Viscosity (mPa)	Gel firmness (g)	Syneresis (100 ml)
Control	0	1	15.21±0.83	25.23±2.42	29.07±3.04
		7	17.09±0.80	26.13±1.95	26.80±1.80
		14	18.21±1.06	31.70±2.19	25.40±1.10
		21	18.18±0.64	29.27±0.29	24.03±0.93
Whey Protein Concentrate (WPC)	1	1	23.20±1.01	32.50±1.67	26.10±0.66
		7	24.90±0.62	35.50±3.24	24.83±1.10
		14	25.77±4.51	37.90±3.20	23.53±2.68
		21	21.93±0.85	35.77±1.61	21.60±0.75
	2	1	27.87±1.36	41.87±1.99	22.97±0.74
		7	28.57±1.31	44.57±0.32	20.30±0.62
		14	32.57±2.17	47.17±2.17	20.27±0.76
		21	30.87±2.25	46.30±3.08	19.27±0.55
	4	1	32.93±2.50	49.13±1.93	19.37±0.23
		7	34.47±1.65	53.10±0.70	17.63±1.25
		14	35.67±2.50	56.93±1.11	17.43±0.21
		21	34.80±1.54	52.90±1.22	17.33±0.85
Sodium Caseinate (SCN)	1	1	25.14±0.87	36.90±0.75	24.07±1.26
		7	26.92±0.65	39.53±0.45	21.97±1.25
		14	28.05±0.95	42.57±1.52	20.33±1.96
		21	28.11±0.53	41.53±0.93	20.17±1.15
	2	1	32.17±2.12	46.03±1.02	19.83±2.22
		7	33.83±2.51	47.87±1.22	18.67±1.07
		14	36.30±2.16	49.10±0.62	17.73±0.59
		21	34.93±1.89	50.80±2.00	16.80±0.36
	4	1	37.50±1.39	56.37±0.76	18.33±0.86
		7	41.17±0.83	58.43±0.76	17.23±0.31
		14	43.90±1.37	59.80±0.17	16.47±0.55
		21	42.93±1.12	55.93±1.53	16.30±0.17
Skim Milk Powder (SMP)	1	1	18.07±1.11	28.83±1.02	27.57±0.65
		7	23.20±2.01	29.73±0.72	26.50±0.52
		14	24.20±0.85	33.67±0.74	25.20±0.79
		21	23.23±1.03	32.63±1.91	23.67±1.25
	2	1	22.20±4.00	32.80±0.52	25.10±0.92
		7	23.20±2.01	35.60±1.23	24.37±0.87
		14	24.87±0.49	36.87±0.50	23.00±1.23
		21	22.37±0.81	35.33±1.44	22.10±1.00
	4	1	26.30±0.85	42.67±1.29	20.43±0.58
		7	27.83±3.51	44.63±0.64	19.63±0.15
		14	30.10±2.14	46.60±1.61	18.97±0.72
		21	27.67±0.80	44.17±2.25	18.10±0.85
Total Main effects (means)					
Control			17.17 <sup>a</sup> ±1.46	28.08 <sup>a</sup> ±3.14	26.33 <sup>a</sup> ±2.54
WPC			29.46 <sup>b</sup> ±4.94	44.47 <sup>b</sup> ±7.84	20.89 <sup>c</sup> ±2.99
SCN			34.25 <sup>c</sup> ±6.33	48.74 <sup>c</sup> ±7.54	18.99 <sup>d</sup> ±2.50
SMP			24.44 <sup>d</sup> ±3.50	36.96 <sup>d</sup> ±5.99	22.89 <sup>b</sup> ±3.06
Main effects (means)					
Control	0		17.17 <sup>a</sup> ±1.46	28.08 <sup>a</sup> ±3.14	26.33 <sup>a</sup> ±2.54
WPC	1		23.95 <sup>b</sup> ±2.55	35.42±2.97	24.02 <sup>b</sup> ±2.17
	2		29.97 <sup>c</sup> ±2.50	44.98 <sup>c</sup> ±2.79	20.70 <sup>c</sup> ±1.55
	4		34.47 <sup>d</sup> ±2.07	53.02 <sup>d</sup> ±3.09	17.94 <sup>d</sup> ±1.09
SCN	1		27.06 <sup>e</sup> ±1.42	40.13 <sup>e</sup> ±2.41	21.63 <sup>e</sup> ±2.05
	2		34.31 <sup>f</sup> ±2.44	48.45 <sup>f</sup> ±2.14	18.26 <sup>f</sup> ±1.60
	4		41.38 <sup>g</sup> ±2.75	57.63 <sup>g</sup> ±1.82	17.08 <sup>g</sup> ±0.96
SMP	1		22.18 <sup>h</sup> ±2.76	31.22 <sup>h</sup> ±2.32	25.73 <sup>h</sup> ±1.69
	2		23.16 <sup>i</sup> ±2.24	35.15 <sup>i</sup> ±1.77	23.64 <sup>i</sup> ±1.49
	4		27.98 <sup>j</sup> ±2.31	44.52 <sup>j</sup> ±1.98	19.28 <sup>j</sup> ±1.05

<sup>a</sup> Means are average from three independent trials.

Means in the same column with different superscripts letters significantly differ ( $P<0.05$ ).

On the other hand, the highest viable counts in the *Lb. acidophilus* and *B. Lactis* Bb-12 population in all bio-yoghurts were observed during 7 days of refrigerated storage then decline throughout storage as affected by type and percentage of supplementation milk proteins Table 4. Generally, the highest viable counts of *Lb. acidophilus* were enumerated in bio-yoghurts fortified with 4% WPC, whereas the control had the lowest values with significant difference ( $P<0.05$ ) between all bio-yoghurts. Oliveira et al., (2001) evaluated the effect of supplementation with WPC and casein hydrolyzed on the microbial stability of probiotic bacteria in fermented milk. Only the WPC supplementation maintained high cell counts of *L. acidophilus*. Also, Adriane et al., (2005) observed that high numbers of *Lb. acidophilus* remained viable during refrigerated storage (5°C for 21days) of yoghurt produced by partial replacement of SMP with WPC. In addition, Lankaputhra and Shah, (1995) have reported that *Lb. acidophilus* showed good survival in acidic conditions. Generally, the highest viable counts of *B. Lactis* were enumerated in bio-yoghurts fortified with 4% WPC, whereas control and bio-yogurts supplemented with SCN had the lowest value, which was found statistically significant according to the general mean value of storage ( $P<0.05$ ).

Dave and Shah, (1998b) reported that the viable counts of bifidobacteria were enhanced by addition of WPC at a ratio of 2% in bio-fermented milk. WPC was also shown to increase the growth and viability of Bifidobacterium species in goat's milk, and the content of WPC added to milk was found to be significant ( $P<0.05$ ) (Martin-Diana et al., 2003). Also, Dave and Shah, (1998a) showed that the viability of bifidobacteria in yoghurt supplemented with WPC was improved by >3 log cycles as compared to the control yoghurt. The WPC serve as a source of peptides and amino acids when heat treated in the yoghurt mix. In addition, whey proteins are rich in sulphur containing amino acids, which are liberated during the heat treatment and lower the redox potential (Dave and Shah, 1998a). Such conditions are favourable to probiotic viability.

On the other hand, (Dave and Shah, 1998b) showed that the higher viability of bifidobacteria, possibly due to proteolytic activity of *Lb. delbrueckii* subsp. *bulgaricus* resulting in availability of free amino acids which have been reported to be essential growth factors for bifidobacteria. In addition, increase viability of probiotic bacteria in camel fermented milk because of camel's milk enrichment, with some nutrients for probiotics such as free amino acids and peptides (Omer et al., 2007 and Natasa et al., 2008). Generally, the counts of viable bifidobacteria in this study were within the recommended range (6-8 log<sup>10</sup> cfu/mL) as reported by (Vasiljevic and Shah, 2008 and Marafon et al., 2011).

Table 4. Changes in viable counts (cfu/g<sup>-1</sup>, meana ±SD) of *Streptococcus thermophilus*, *Lb. bulgaricus* , *Lb. acidophilus*, LA-5 and *Bifidobacterium* Bb-12, of experimental control and supplemented bio-yoghurts during storage period at (4 ±1°C).

Ingredient type	Rate %	Storage days	<i>S. thermophilus</i>	<i>Lb. delbrueckii subsp. bulgaricus</i>	<i>Lb. acidophilus</i>	<i>B. animalis subsp. lactis</i>	
Control	0	1	7.10±0.63	6.68±0.35	6.83±0.19	6.79±0.60	
		7	7.31±0.64	6.81±0.23	7.22±0.66	6.86±0.62	
		14	6.90±0.08	6.50±0.68	6.64±0.54	6.53±0.05	
		21	6.78±0.11	6.44±0.73	6.48±0.30	6.29±0.43	
Whey Protein Concentrate (WPC)	1	1	7.74±0.49	7.29±0.77	7.31±0.39	7.18±0.20	
		7	8.19±0.93	7.57±0.52	7.53±0.29	7.30±0.45	
		14	7.43±0.09	7.23±0.57	7.18±0.52	7.19±0.62	
	2	1	7.35±0.56	7.17±0.63	6.98±0.03	6.87±1.06	
		7	7.85±0.20	7.44±0.40	7.38±0.52	7.27±0.51	
		14	8.29±0.30	7.68±0.24	7.80±0.14	7.56±0.65	
	4	1	7.62±0.14	7.19±0.55	7.43±0.57	7.29±0.59	
		7	7.56±0.05	7.05±0.71	7.26±0.54	7.03±0.07	
		14	8.04±0.08	7.68±0.13	7.62±0.09	7.40±0.38	
	Sodium Caseinate (SCN)	1	1	8.41±0.31	7.89±0.82	7.91±0.07	7.77±0.26
			7	7.76±0.63	7.43±0.55	7.74±0.62	7.33±0.44
			14	7.67±0.57	7.27±0.66	7.64±0.46	7.11±0.63
2		1	7.34±0.57	6.85±0.10	7.12±0.58	6.69±0.47	
		7	7.53±0.44	6.99±0.01	7.22±0.43	6.81±0.04	
		14	7.20±0.41	6.71±0.12	7.04±0.45	6.60±0.18	
4		1	7.05±0.09	6.41±0.43	6.67±0.15	6.42±0.53	
		7	7.67±0.51	7.08±0.34	7.26±0.58	6.97±0.64	
		14	7.86±0.25	7.16±0.17	7.41±0.48	7.16±0.55	
Skim Milk Powder (SMP)		1	1	7.53±0.25	6.67±0.17	7.28±0.45	6.69±0.18
			7	7.30±0.50	6.52±0.61	6.99±0.80	6.58±0.01
			14	7.74±0.50	7.23±0.57	7.42±0.56	7.04±0.70
	2	1	7.92±0.10	7.39±0.49	7.66±0.19	7.12±0.39	
		7	7.70±0.55	6.94±0.37	7.34±0.72	6.78±0.22	
		14	7.36±0.54	6.70±0.12	7.24±0.58	6.57±0.36	
	4	1	7.63±0.37	7.10±0.41	7.16±0.45	6.80±0.04	
		7	7.55±0.43	7.17±0.47	7.38±0.56	6.99±0.73	
		14	7.21±0.07	6.99±0.98	7.20±0.54	6.64±0.07	
	Total Main effects (means)	1	1	7.18±0.85	6.52±0.50	6.75±0.21	6.58±0.65
			7	7.51±0.34	7.19±0.90	7.34±0.35	7.22±0.52
			14	7.87±0.07	7.26±0.50	7.54±0.53	7.33±0.57
2		1	7.31±0.14	7.05±0.45	7.35±0.43	7.04±0.30	
		7	7.19±0.58	6.82±0.14	7.18±0.55	6.74±0.19	
		14	7.81±0.41	7.31±0.39	7.62±0.13	7.16±0.66	
4		1	8.16±0.75	7.38±0.58	7.85±0.28	7.54±0.18	
		7	7.73±0.60	7.00±0.55	7.56±0.07	7.30±0.36	
		14	7.46±0.42	6.87±0.10	7.40±0.48	6.79±0.24	
Control			7.02 <sup>a</sup> ±0.44	6.61 <sup>a</sup> ±0.49	6.79 <sup>c</sup> ±0.49	6.62 <sup>c</sup> ±0.47	
WPC			7.83 <sup>b</sup> ±0.50	7.41 <sup>b</sup> ±0.54	7.48 <sup>b</sup> ±0.43	7.27 <sup>b</sup> ±0.51	
SCN			7.52 <sup>b</sup> ±0.44	6.89 <sup>b</sup> ±0.41	7.22 <sup>b</sup> ±0.50	6.79 <sup>b</sup> ±0.42	
SMP			7.55 <sup>b</sup> ±0.50	7.05 <sup>b</sup> ±0.51	7.36 <sup>bc</sup> ±0.44	7.01 <sup>bc</sup> ±0.47	
Main effects (means)							
Control	0		7.02 <sup>a</sup> ±0.44	6.61 <sup>a</sup> ±0.49	6.79 <sup>b</sup> ±0.49	6.62 <sup>b</sup> ±0.47	
WPC	1		7.66 <sup>abcd</sup> ±0.61	7.32 <sup>abc</sup> ±0.56	7.25 <sup>bcd</sup> ±0.37	7.13 <sup>abcd</sup> ±0.59	
	2		7.83 <sup>ab</sup> ±0.34	7.34 <sup>ab</sup> ±0.50	7.47 <sup>abc</sup> ±0.46	7.29 <sup>ab</sup> ±0.48	
	4		7.97 <sup>a</sup> ±0.49	7.57 <sup>a</sup> ±0.56	7.73 <sup>a</sup> ±0.35	7.40 <sup>a</sup> ±0.45	
SCN	1		7.28 <sup>bc</sup> ±0.40	6.74 <sup>bc</sup> ±0.30	7.01 <sup>bc</sup> ±0.43	6.63 <sup>b</sup> ±0.34	
	2		7.59 <sup>abcd</sup> ±0.40	6.86 <sup>bc</sup> ±0.42	7.24 <sup>bcd</sup> ±0.53	6.85 <sup>cd</sup> ±0.44	
	4		7.66 <sup>abcd</sup> ±0.44	7.07 <sup>bcd</sup> ±0.45	7.42 <sup>abc</sup> ±0.50	6.88 <sup>cd</sup> ±0.45	
SMP	1		7.39 <sup>cd</sup> ±0.48	6.95 <sup>bc</sup> ±0.60	7.12 <sup>cd</sup> ±0.46	6.75 <sup>bc</sup> ±0.45	
	2		7.47 <sup>bcd</sup> ±0.40	7.08 <sup>bcd</sup> ±0.51	7.35 <sup>abcd</sup> ±0.42	7.06 <sup>abcd</sup> ±0.43	
	4		7.79 <sup>abc</sup> ±0.55	7.14 <sup>bcd</sup> ±0.44	7.61 <sup>ab</sup> ±0.30	7.20 <sup>abc</sup> ±0.44	

<sup>a</sup> Means are average from three independent trials.

Means in the same column with different superscripts letters significantly differ (P<0.05).

**Sensory evaluation:**

A summary of the ratings for sensory attributes (flavor, body and texture, appearance and acidic taste) of control and supplemented bio-yoghurt of 10 trained panelists during 21 days of storage period at ( $4 \pm 1^\circ\text{C}$ ) is shown in Table 5.

According to the results, significant changes in relation to storage time were found in all sensory attributes (flavor, body and texture, appearance and acidic taste) of all bio-yoghurts samples. On the other hand, the fortification type and level of the ingredients had significant differences ( $P < 0.05$ ) among bio-yoghurt samples in flavour scores. The bio-yoghurts fortified with 4% WPC had higher flavor than other bio yoghurts. Similar results were obtained for the control and WPC-supplemented probiotic yoghurt by (Akalin et al., 2008). The addition of whey powder also did not have a negative effect on the yoghurt flavour (Gülfem and Akalin, 2013).

Acetaldehyde is a main flavour component of most cultured dairy products (Ostlie et al., 2003). The bio-yoghurt fortified with 4% WPC showed more acceptability for flavour scores than bio-yoghurts fortified with SCN, SMP or control that might be due to the higher count of probiotic bacteria and greater ability of *Lb. acidophilus* in production of acetaldehyde and diacetyl in supplemented bio-yoghurt with WPC.

In general, the present work demonstrates that substitution with probiotic ingredients has a greater influence on flavor and aroma. Our results are also in agreement with (Akalin et al., 2012) they observed that, the metabolism of the probiotic culture can result in the production of components that may contribute positively to the aroma and taste of the product.

Furthermore, bio-yoghurts fortified with 4 % WPC had higher significant differences ( $P < 0.05$ ) in acidic taste scores than other bio-yoghurts. Lactic acid produced by *Lb. delbrueckii subsp. bulgaricus* and *Lb. acidophilus* may also be responsible for higher acidity taste of 4 % WPC bio-yoghurt in this study.

Generally, the effect of addition rate and ingredient type on bio-yoghurts body and texture scores was found statistically significant ( $P < 0.05$ ). Bio-yoghurts fortified with SCN, WPC or SMP at a rate of 1% showed the lowest scores for texture, probably due to soft gel that was noted by the panellists. On the other hand, large differences were observed in body and texture scores between samples: the supplemented bio-yoghurt with 4% SCN received higher scores in body and texture, while control bio-yoghurts was the least acceptable, tasters objecting to its liquid texture, and non-typical yoghurt taste. These results confirm that supplementation of the milk base with SCN increases the body and texture of the product, a fact that is well perceived by panellists; (Sodini et al., 2005 and Isleten and Karagul-Yuceer, 2006) reported similar findings. In addition, the increase in the firmness of bio-yogurt fortified with SCN throughout storage may have been improved body and texture scores. Marafon et al., (2011) also reported that the supplementation of the milk base with milk proteins increased the consistency of the probiotic yoghurt in terms of sensory attributes; however, the values decreased during 28-d storage.

Table 5. Sensory evaluation (mean<sup>a</sup> ±SD) of experimental control and supplemented bio-yoghurts during storage period at (4 ±1°C).

Ingredient type	Rate %	Storage days	Flavour (45)	Body and Texture (35)	Appearance (10)	Acidity(10)	Overall Acceptability (100)	
Control	0	1	28.14±2.61	20.71±1.89	6.00±0.58	6.00±0.01	60.86±3.29	
		7	30.57±3.41	21.71±1.50	6.29±0.49	6.14±0.38	64.71±4.75	
		14	32.00±1.41	21.86±0.69	6.29±0.49	6.43±0.53	66.57±1.40	
		21	31.43±1.72	21.29±1.38	6.14±0.69	6.57±0.53	65.43±2.82	
Whey Protein Concentrate (WPC)	1	1	34.00±2.89	22.57±1.72	6.14±0.38	6.57±0.98	69.29±3.30	
		7	35.86±3.39	23.57±1.51	6.43±0.53	7.00±1.00	72.86±4.67	
		14	35.43±1.81	24.00±1.15	6.43±0.79	7.57±0.53	73.43±2.57	
	2	1	32.86±1.07	25.29±1.60	6.29±0.49	7.71±0.49	72.14±2.54	
		7	35.29±2.21	24.29±1.11	6.29±0.76	6.86±0.90	72.71±3.50	
		14	36.29±1.11	25.57±1.90	6.57±0.53	7.57±0.53	76.00±2.24	
	4	1	36.71±2.14	26.29±1.70	7.00±1.00	7.86±0.38	77.86±2.73	
		7	35.43±2.44	27.43±1.81	6.86±1.07	8.14±1.07	77.86±1.07	
		21	37.43±3.10	26.29±1.70	7.14±0.38	8.14±0.38	79.00±3.06	
	Sodium Caseinate (SCN)	1	1	40.86±3.39	27.57±1.51	7.29±0.76	8.43±0.53	84.14±3.18
			7	39.57±2.44	28.57±1.51	7.29±0.76	8.71±0.49	84.14±2.27
			21	36.00±1.53	30.57±1.13	7.14±0.69	8.86±0.38	82.57±2.07
2		1	33.14±2.41	25.14±2.12	7.57±0.53	6.14±0.69	72.00±2.83	
		7	34.71±1.50	25.71±2.14	7.86±1.07	6.43±0.79	74.71±2.29	
		14	34.29±2.63	27.00±2.71	8.14±0.69	6.71±1.11	76.14±3.76	
4		1	32.14±1.07	28.71±3.35	8.00±0.58	6.86±0.69	75.71±2.98	
		7	34.00±0.82	26.57±2.07	8.00±0.58	6.29±0.49	74.86±2.34	
		21	35.29±2.21	27.57±3.31	8.43±0.79	6.57±0.98	77.86±4.53	
Skim Milk Powder (SMP)		1	1	35.00±2.24	28.86±3.29	8.71±0.49	6.86±0.69	79.43±4.24
			7	33.43±1.27	29.86±1.35	8.29±0.76	7.29±0.49	78.86±2.85
			21	36.43±2.64	28.43±1.51	8.43±0.79	6.43±0.79	79.71±3.73
	2	1	38.86±1.35	30.43±2.23	8.57±0.53	6.57±0.79	84.43±2.23	
		7	38.14±1.46	31.43±0.98	9.14±0.38	7.14±0.38	85.86±1.95	
		21	35.14±1.07	32.00±1.00	8.71±0.76	7.43±0.53	79.14±1.50	
	4	1	31.14±0.69	21.86±0.69	6.43±0.53	6.29±0.49	65.71±1.50	
		7	31.86±1.07	21.86±1.35	6.71±0.95	6.57±0.79	67.00±2.45	
		14	33.00±1.41	22.43±1.72	7.29±0.76	6.71±0.49	69.43±2.23	
	Total Main effects (means)	1	1	30.86±1.07	22.57±1.40	7.29±0.49	7.00±1.15	67.71±2.43
			7	32.14±1.21	24.14±2.19	7.00±0.01	6.43±0.53	69.71±2.14
			21	33.86±3.02	24.86±1.07	7.43±0.53	6.71±0.49	72.86±3.76
2		1	35.29±1.89	25.29±0.49	7.14±0.69	6.86±0.69	74.57±1.90	
		7	32.57±2.51	26.14±2.79	7.00±0.82	7.43±0.53	73.14±2.97	
		21	34.57±1.62	25.86±1.68	7.29±0.49	6.57±0.53	74.29±2.21	
4		1	35.00±2.31	26.71±1.89	7.71±0.95	6.86±0.38	76.29±3.82	
		7	37.57±1.13	26.71±2.14	7.71±0.76	7.29±0.76	79.29±3.55	
		21	32.43±1.62	28.00±2.71	7.57±0.79	7.57±0.53	75.57±2.82	
Total Main effects (means)								
Control				30.54 <sup>a</sup> ±2.73	21.39 <sup>a</sup> ±1.42	6.18 <sup>a</sup> ±0.55	6.29 <sup>a</sup> ±0.46	64.39 <sup>a</sup> ±3.79
WPC				36.31 <sup>b</sup> ±3.09	26.00 <sup>b</sup> ±2.63	6.74 <sup>b</sup> ±0.78	7.79 <sup>b</sup> ±0.95	76.83 <sup>b</sup> ±5.49
SCN			35.05 <sup>b</sup> ±2.55	28.48 <sup>b</sup> ±3.02	8.32 <sup>b</sup> ±0.76	6.73 <sup>b</sup> ±0.78	78.57 <sup>b</sup> ±4.99	
SMP			33.36 <sup>b</sup> ±2.50	24.70 <sup>b</sup> ±2.64	7.21 <sup>b</sup> ±0.75	6.86 <sup>b</sup> ±0.71	72.13 <sup>b</sup> ±4.77	
Main effects (means)								
Control	0		30.54 <sup>a</sup> ±2.73	21.39 <sup>a</sup> ±1.42	6.18 <sup>a</sup> ±0.55	6.29±0.46	64.39 <sup>a</sup> ±3.79	
WPC	1		34.54 <sup>ab</sup> ±2.62	23.86 <sup>a</sup> ±1.74	6.32 <sup>a</sup> ±0.55	7.21±0.88	71.93 <sup>a</sup> ±3.58	
	2		35.93 <sup>b</sup> ±2.02	25.89 <sup>ab</sup> ±1.95	6.68 <sup>a</sup> ±0.86	7.61 <sup>a</sup> ±0.88	76.11 <sup>bc</sup> ±3.21	
	4		38.46 <sup>b</sup> ±3.19	28.25 <sup>b</sup> ±2.12	7.21 <sup>ab</sup> ±0.63	8.54 <sup>a</sup> ±0.51	82.46 <sup>b</sup> ±3.32	
SCN	1		33.57 <sup>a</sup> ±2.15	26.64 <sup>a</sup> ±2.84	7.89 <sup>a</sup> ±0.74	6.54 <sup>a</sup> ±0.84	74.64 <sup>a</sup> ±3.28	
	2		34.43 <sup>ab</sup> ±1.81	28.21 <sup>a</sup> ±2.79	8.36 <sup>a</sup> ±0.68	6.75 <sup>ab</sup> ±0.75	77.75 <sup>a</sup> ±3.85	
	4		37.14 <sup>b</sup> ±2.21	30.57 <sup>b</sup> ±1.99	8.71 <sup>a</sup> ±0.66	6.89 <sup>ab</sup> ±0.74	83.32 <sup>b</sup> ±3.30	
SMP	1		31.71 <sup>a</sup> ±1.33	22.18 <sup>a</sup> ±1.31	6.93 <sup>a</sup> ±0.77	6.64 <sup>a</sup> ±0.78	67.46 <sup>a</sup> ±2.47	
	2		33.46 <sup>a</sup> ±2.47	25.11 <sup>a</sup> ±1.91	7.14 <sup>a</sup> ±0.59	6.86 <sup>ab</sup> ±0.65	72.57 <sup>a</sup> ±3.19	
	4		34.89 <sup>ab</sup> ±2.47	26.82 <sup>a</sup> ±2.16	7.57 <sup>a</sup> ±0.74	7.07 <sup>ab</sup> ±0.66	76.36 <sup>ab</sup> ±3.52	

<sup>a</sup> Means are average from three independent trials.

Means in the same column with different superscripts letters significantly differ ( $P<0.05$ ).

However, the texture of the bio-yoghurt with WPC was liked more than the bio-yoghurt supplemented with SMP. Whey proteins have been reported to influence the sensory properties of yogurt depending on the source and concentration. In particular the whey proteins have been reported to increase the thickness and flavor (Isleten and Yuceer, 2006).

On the other hand, bio-yoghurts fortified with 2 and 4 % SCN had higher appearance than other bio-yoghurts. The white color of dairy products is due to the light scattering into the casein micelles and fat globules. When the number of the scattering particles is increased the white color intensity also increases. SCN addition increased the brightness and reduced the yellow color intensity of yoghurt samples (Dimitris et al., 2014). Also, Piyawan et al., (2009) reported that the increased protein coagulation enhanced the light absorption that resulted in the lighter tones.

Generally, the highest overall acceptability scores were obtained in bio-yoghurts supplemented with SCN, while the lower scores was observed in control bio-yoghurt, whereas control bio-yoghurt caused an unpleasant taste and appearance. However, the fortification level of the ingredients had a significant effect ( $P<0.05$ ) on the bio-yoghurt overall acceptability. In summary, the highest ( $P<0.05$ ) overall sensory scores were observed for bio-yoghurt made with 4% WPC or SCN compared to other treatments. Overall, 4% WPC and SCN can be recommended in manufacture of higher protein yoghurts compared to SMP.

## **CONCLUSION**

This study demonstrates that the SCN was the most effective in improving the rheological properties. WPC and SMP are also able to increase the gel strength of yoghurt but not as effectively as the fortification with SCN. On the other hand, WPC supplementation of camel's milk beneficially influences *St. thermophilus*, *Lb. bulgaricus*, *L. acidophilus*, LA-5 and *B. Lactis* Bb-12 growth during fermentation, as well as its enhanced viability more than fortification with SCN and SMP during fermented milk storage. The highest ( $P<0.05$ ) overall sensory scores were observed for bio-yoghurt made with 4% WPC or SCN compared to other treatments. However, further research is recommended to evaluate and compare micro textural of the camel's bio-yoghurts prepared from WPC, SCN or SMP to confirm these results including industrial trials.

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### تأثير إضافة أنواع مختلفة من بروتينات اللبن على الخصائص الفيزيوكيميائية و الميكروبيولوجية للبن النوق الحيوي

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تهدف هذه الدراسة الى تحسين خواص اليوجورت الحيوي المصنع من اللبن النوق وذلك بتدعيمه بمركبات بروتينات اللبن المجففة مثل كازينات الصوديوم ، بروتينات الشرش او اللبن الفرز المجفف وذلك بنسب إضافة (٢,١ او ٤%) وكذلك معرفة تأثير إضافتها على الخواص الفيزيوكيميائية ، نشاط السلالات البكتيرية والخواص الحسية خلال ٢١ يوم من التخزين المبرد على  $4 \pm 1^\circ \text{C}$  مقارنة بالعينات الغير معاملة . وقد أظهرت النتائج المتحصل عليها ما يلي:-

١- تدعيم يوجورت النوق الحيوي بنسبة ٤% من مركز بروتينات الشرش أدى إلى زيادة معنوية ( $P < 0.05$ ) في نسبة الحموضة المعايرة و الأسيتالدهيد والداي أسيتالدهيد وكذلك أدى إلى زيادة معنوية في أعداد بكتيريا *S. thermophilus*, *Lb. delbrueckii subsp. bulgaricus*, و *Bifidobacterium animalis subsp. lactis* BB-12 خلال ٢١ يوم من التخزين.

٢- أوضحت النتائج أن زيادة نسبة التدعيم بمركز كازينات الصوديوم إلى نسبة ٤% أدى إلى زيادة معنوية ( $P < 0.05$ ) في اللزوجة وصلابة في الخثرة وقلة المترشح من الشرش عن باقي المعاملات خلال ٢١ يوم من التخزين.

٣- أشارت نتائج التحكيم الحسي إلى أن تدعيم يوجورت النوق الحيوي بنسبة ٤% من مركز كازينات الصوديوم حصلت على أعلى درجات التحكيم لخواص القوام والتركيب والمظهر بينما اليوجورت الحيوي المدعم بنسبة ٤% من مركز بروتينات الشرش حصلت على أعلى درجات التحكيم لخواص الرائحة والحموضة خلال ٢١ يوم من التخزين. وأوضحت نتائج التحكيم الحسي إلى أن كل من يوجورت النوق الحيوي المدعم بنسبة ٤% من مركز كازينات الصوديوم أو ٤% من مركز بروتينات الشرش قد حازا على أعلى درجات التقبل العام مع عدم وجود فروق معنوية ( $P < 0.05$ ).