



## Physicochemical, Rheological and Microbiological Characteristics of Fat-free Yoghurt Enriched with Inulin and Various Milk Protein Sources



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**T**HE current work was conducted to study the feasibility of producing fat-free set yogurt of acceptable quality from reconstituted skim milk using skim milk powder (10% total solids, TS) fortified with inulin (1%) and milk protein concentrate (MPC), whey protein concentrate (WPC), or sodium caseinate (NaCN) at different concentrations of 1, 3, or 4.5%. Control sample was prepared from reconstituting skim milk without adding any ingredients at the same time for comparison. The physicochemical, microbiological, and rheological characteristics of yogurt have been analyzed at 1, 7, and 14 days of cold storage. The findings reported an increase in TS, protein, acidity, and ash content by adding inulin and the increasing added amount of SMP, MPC, WPC, or NaCN. Similar tendency has been reported for the total bacterial count, and sample containing 4.5% WPC presented the highest viable total bacterial count and highest numbers of *S. thermophile* and *L. bulgaricus*. On the contrary, pH and syneresis values have been declined by adding these ingredients. Regarding storage effect, TS, pH, protein, soluble nitrogen, ash, acidity, and syneresis values significantly increased with the progress of storage time. Some texture profile parameters (hardness, adhesiveness, gumminess, and chewiness) increased in yogurt samples fortified with various protein sources, compared to control sample. While cohesiveness and springiness values decreased in yogurt samples.

**Keywords:** Fat-free set yoghurt, Milk protein concentrate, Whey protein concentrate, Sodium caseinate, Inulin, Physicochemical properties, Microbiological and rheological characteristics

### Introduction

Consumers, in recent years, have become more mindful of maintaining good health through diet, thereby inclined toward functional foods that offer additional benefits beyond conventional food. Consumer interest in healthier food products is driven by various factors, including a better understanding of the relationship between diet and health, providing nutritional demands, and lowering disease hazards. Value-added food products, such as low-fat, reduced-fat, and non-fat types, as well as those fortified with functional ingredients, such as dairy-based proteins and dietary fibers that offer particular health and technological profits outside basic nutrition, represent another possible growth area for food products. The excessive consumption of fats is becoming a very urgent problem.

Reducing fat intake in the diet is very opportune (Baker et al., 2022).

Clients are motivated to consume non-fat dairy products to ensure overall respectable health and decrease the hazard of obesity, hypertension, cerebral apoplexy, and coronary heart diseases. Non-fat dairy products promise to expand the dairy industry, especially in specific sectors like yoghurt. The dairy business finds it challenging to formulate these benefits because fat has various beneficial effects on texture, appearance, flavor, and aroma. Dairy products without fat typically have several flaws. Because of these flaws, they are characterized as too complicated, resilient, or coarse and have poor taste and aroma. One of the most significant approaches in producing low-fat and non-fat dairy products is to increase solid

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content to avoid precise textural flaws. Using skim milk powder (SMP) to improve the milk base is common, however, excessive SMP may result in a powdery, astringent flavor in the finished product (Tamime *et al.*, 2001). Furthermore, the formation of excess acidity in the product due to the powder's high lactose content remains a limiting issue for its usage in enrichment. Other milk powders, such as MPC, WPC, and NaCN, offer a variety of functional qualities, including emulsification, foaming, water absorption, viscosity, gelation, and thermal stability. These milk powders have gained popularity as a practical method of increasing total solids in the low-fat yoghurt assortments (Sharma *et al.*, 2012 and Henriques *et al.*, 2013). In this regard, fat replacement is an alternative approach. Soluble fiber has been widely utilized to substitute the fat in dairy products and several soluble fibers, including inulin or oligo-fructose, have found the most successful applications in dairy products such as yoghurt. It offers nutritional qualities and health-promoting properties, including lower-calorie value, dietary fiber, and prebiotic properties. Also, inulin is becoming more popular in dairy and non-dairy products as a bulking ingredient for fat replacement, textural alteration, and organoleptic enhancement (Arora *et al.*, 2015; Abou Ayana & Ibrahim, 2015; Chaito *et al.*, 2016; Safaa Faid, 2017). This study aimed to evaluate the effects of adding some DDCs and inulin on the physicochemical, microbiological, and rheological characteristics of non-fat set yoghurt.

## Materials and Methods

### Materials

Four dried milk protein ingredients were used in this study for milk fortification: SMP (Aktiebolaget Västgöta Mjölkförädlin, Odengatan 6, Sweden), MPC (Westland Milk Products Hokitika, New Zealand), WPC (Davisco Foods International, Le Sueur, Minnesota, USA) and

NaCN (Foodchem International Corporation, China). The inulin Frutafit® HD: Highly dispersible native powdered inulin of medium average chain length (8-13 monomers) properties was imported from Sensus (BrenntagQuímica, SA, Barcelona, Spain). The composition of the dried milk protein ingredients and inulin is shown in Table 1. YC-X11 yoghurt bacterial starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) were provided by Chr. Hansen, Inc., Milwaukee, Wis., USA. The other chemicals, media, and reagents used in this study were of analytical grade and supplied by BDH Analytical Chemicals and Sigma-Aldrich Corporation.

### Methods

#### Preparation of non-fat set yoghurt

The yoghurt base mix was made by reconstituting low-heat skim milk powder to a final concentration of 10% total solids. The reconstituted skim milk was divided into thirteen equal portions 1% inulin and fortified with adding, except one portion which was used as a control sample. The other twelve parts were fortified with SMP, MPC, WPC, and NaCN, at different concentrations of 1.5, 3.0, or 4.5%. All yoghurt mixtures were blended thoroughly with a hand blender until the complete dispersion of all powders. The mixtures were cooled to 5°C and then kept in a refrigerator overnight to complete the hydration of the dried ingredients before use. The yoghurt mixtures were then heat treated in a water bath at 90°C for 10 min, rapidly cooled to 42°C, and inoculated with a 1.5 % activated yoghurt starter culture. The inoculated milk was poured into 250-mL plastic cups with lids, and then the cups were incubated at 42°C until complete coagulation (approximately 3.5 h). After incubation, yoghurts were transferred, and kept in a refrigerator at 5°C for 14 days. All analyses were conducted at 1, 7, and 14 days of cold storage. All analyses have been conducted in triplicate.

TABLE 1. Composition of dried milk protein ingredients and inulin used in this study\*

Constituents (%)	Dried milk protein ingredients				
	SMP	MPC	WPC	NaCN	Inulin
Protein	≥34	≥70	81.4±1.0	≥90	0
Fat	≤1.25	1.4	5.4±1.0	≤2	0
Lactose	≤53	16.2	8.0±1.5	≤1	Carbohydrates ≤ 97%
Ash	≤8	8.2	2.6±0.3	≤6	0.2
Moisture	≤4	<5	4.7±0.3	≤6	3

\*Specifications obtained from the manufacturers. SMP: skim milk powder, MPC: Milk protein concentrate, WPC: whey protein concentrate, NaCN: sodium caseinate

**TABLE 2. Formulation of milk bases and control used for non-fat set yoghurt manufacture, expressed in (g/100g).**

Treatment code	Formulations	Dried milk protein ingredients				
		SMP	MPC	WPC	NaCN	Inulin
<b>Control</b>	SMP	10.0	-	-	-	-
<b>A1</b>	SMP	11.5	-	-	-	1
<b>A2</b>	SMP	13.0	-	-	-	1
<b>A3</b>	SMP	14.5	-	-	-	1
<b>B1</b>	MPC	10.0	1.5	-	-	1
<b>B2</b>	MPC	10.0	3.0	-	-	1
<b>B3</b>	MPC	10.0	4.5	-	-	1
<b>C1</b>	WPC	10.0	-	1.5	-	1
<b>C2</b>	WPC	10.0	-	3.0	-	1
<b>C3</b>	WPC	10.0	-	4.5	-	1
<b>D1</b>	NaCN	10.0	-	-	1.5	1
<b>D2</b>	NaCN	10.0	-	-	3.0	1
<b>D3</b>	NaCN	10.0	-	-	4.5	1

Control\*: Reconstituted skim milk without any additives. SMP: skim milk powder, MPC: Milk protein concentrate, WPC: whey protein concentrate, NaCN: sodium caseinate

#### *Physicochemical analysis*

Chemical analyses including total solids (TS), total nitrogen (TN), ash content, and titratable acidity (TA) were determined according to AOAC (2010). pH value of yoghurt was measured using a digital pH meter (Orion model 410 A, Boston, MA, USA). Syneresis of yoghurt was determined using the drainage whey (mL/100 mL yoghurt) as described by Achanta et al. (2007).

#### *Microbiological analysis*

The total bacterial counts were carried out according to the method described by (Marshall, 2004). The viable bacterial counts of *Streptococcus thermophilus* were counted using the direct plate method and enumerated on M17 agar medium (Oxoid). The plates were incubated at 42°C for 48 hr. On the other hand, *Lactobacillus delbrueckii subsp. bulgaricus* bacteria were counted using MRS agar medium (Oxoid) plus 10% lactose, and then plates were incubated at 37°C for 48 h (IDF, 1997). The results were expressed as a log number of colony-forming units per g (log CFU/g).

#### *Texture profile analysis (TPA)*

Textural parameters of yoghurt samples have been measured using a texture analyzer (TA1000, Lab Pro (FTC's TMS-Pro), USA), and the Specific Expression PC Software calculated the results. Yoghurt samples were measured at 20°C using the TA 17 probe (30 and 25 mm diameter). A two-bite penetration test was operated at a crosshead speed of 1 mm/sec and a penetration distance of 10 mm. The force exerted on the probe was automatically recorded as follows: Hardness (the peak force measured during the first compression cycle),

adhesiveness (the negative force area for the first bite, representing the necessary work to pull the compression plunger away from the sample), cohesiveness (the ratio of the positive force area during the second compression to that during the first compression), springiness (the height that the sample recovers during the time that elapse between the end of the first cycle and the start of the second cycle), and gumminess (the product of hardness and cohesiveness). Chewiness (the product of gumminess and springiness) was also quantified. According to the technique described by Szczesniak et al. (1963) and Bourne (1978).

#### *Statistical analysis*

The data obtained in the present study was analyzed by ANOVA. For all analyses, when a significant difference ( $P < 0.05$ ) was detected in some variable, the data means test was applied to evaluate the difference between the samples. The results were analyzed using the software Statistical Analysis System for Windows (SAS, 2008).

## **Results and Discussion**

#### *Physicochemical properties*

##### *Total solids, protein, and ash contents:*

The data shown in Table 3 reported that the total solids content of non-fat set yoghurt fortified with various milk protein ingredients and inulin increased ( $P < 0.05$ ) by increasing their added amount. The yoghurt sample fortified with 4.5% NaCN had the greatest total solids content. These findings agree with Unal and Akalina (2013) and Aziznia et al. (2008). Total solid contents

increased in all yoghurt samples with the progress of cold storage period. This could mainly be attributed to moisture loss over the storage period. These findings are consistent with that reported by Salariya *et al.* (2010). However, addition of inulin might increase the total solid content by holding more water.

According to data in Table 3, all yoghurts had higher protein levels ( $P < 0.05$ ) than control sample. The protein composition of the various milk protein ingredients used in this study was the same as that of the other three, except for SMP, which had a lower protein level than the others (Table 1). The highest protein level (7.30%) was reported for yoghurt sample fortified with 4.5% NaCN, while the lowest protein level (3.48%) was recorded for control sample. This is mainly attributed to the high protein content of sodium caseinate (NaCN) which used as fortificant, compared to the lower protein content of skim milk powder, milk protein concentrate, and whey protein concentrate. This obviously explain why yoghurt sample fortified with skim milk powder (lowest protein content, 34%) had the lowest protein content. By increasing the additional percentages of various DDCs, the protein content of formulated yoghurts increased significantly ( $p < 0.05$ ). The current findings are in line with that findings of other published articles (Dave & Shah, 1998; Isleten & Karagul-Yuceer, 2008; Akalin *et al.*, 2012; Unal & Bruzantin *et al.*, 2016).

Also, all yoghurt samples showed a slight increase in protein content during storage and these results agree with those previously reported by Salariya *et al.* (2010). It is known that protein is one of the main solid components in yoghurt and the existence of protein is essential for yoghurt body and texture (Sodini *et al.* 2005). The primary purpose of non-fat milk solids added to yoghurt formulas is to increase the amount of milk protein. According to Berber *et al.* (2015), yoghurts with increased protein content had higher viscosity, water-holding capacity (WHC), and hardness values. The same data also demonstrates that adding different dried milk protein ingredients to yoghurt formulations considerably ( $P < 0.05$ ) increased ash levels, compared to control sample. As the quantity of various dried dairy products added to yoghurt recipes increased, the ash level increased as well. Yoghurt enriched with 4.5% MPC had an ash content of 1.12%, which was significantly greater ( $P < 0.05$ )

than that of all other yoghurt samples. When compared to other DDCs, MPC has a higher ash concentration (8.2%), which could be the main factor of this finding. Moreover, ash contents is marginally increased over the cold storage period. The current results are consistent with that reported by Aziznia *et al.* (2008) and Salariya *et al.* (2010).

*The pH values, titratable acidity, and syneresis:*

The pH and titratable acidity values of non-fat set yoghurt fortified with different concentrations of milk protein ingredients and 1% inulin over cold storage are displayed in Table 4. All yoghurt samples had almost similar pH values ranged between 4.44 - 4.57 at the first day of cold storage, of which control yoghurt has the highest pH value (4.57) while yoghurt fortified with 4.5% WPC had the lowest pH value. Antunes *et al.* (2004) recorded similar results. Fortifying yoghurt samples with milk protein ingredients led to significant drop ( $P < 0.05$ ) in pH values and the decline in pH values was proportionally with the added amount of these milk proteoin ingredients. WPC-fortified yoghurts had lower pH values, compared to NaCN-fortified yoghurts. This might be mainly attributed to the buffering capacity of whey protein which is higher than that reported for casein. Similar findings and explanation have been reported by Amatayakul *et al.* (2006). They propose that the buffering capacity of milk is reduced in those with low casein-to-whey protein ratios. This result is in consistent with the current findings.

The current results indicated that pH values of all yoghurt samples gradually dropped during cold storage period. At 14 days of cold storage, pH levels significantly decreased, (Table 4). After 14 days of storage, the pH values of the yoghurt samples enriched with 1.5% WPC were, nevertheless, somewhat higher and more stable than those of the control group. Throughout the storage period, yoghurt samples maintained a consistent pH value because of WPC's buffering capacity. The obtained result were in line with the findings previously reported by Puvanenthiran *et al.* (2002). The findings reported by Isleten & Karagul-Yuceer (2008), reported that the non-fat yoghurt containing WPI had a significantly lower pH value while yoghurt containing NaCN had a higher pH compare at 12 day of cold storage.

**TABLE 3. Total solids, protein, and ash contents of non-fat set yoghurt fortified with different DDCs and inulin during cold storage period.**

Treatment	TS (%)			Protein (%)			Ash (%)		
	Storage period (day)								
	1	7	14	1	7	14	1	7	14
Control	10.23 <sup>j</sup>	10.59 <sup>g</sup>	11.03 <sup>i</sup>	3.48 <sup>m</sup>	3.53 <sup>m</sup>	3.55 <sup>m</sup>	0.81 <sup>j</sup>	0.81 <sup>g</sup>	0.85 <sup>g</sup>
A1	11.54 <sup>h</sup>	11.86 <sup>e</sup>	12.17 <sup>g</sup>	3.90 <sup>l</sup>	3.94 <sup>l</sup>	3.98 <sup>l</sup>	0.90 <sup>g</sup>	0.91 <sup>e</sup>	0.92 <sup>ef</sup>
A2	12.69 <sup>f</sup>	13.11 <sup>c</sup>	13.43 <sup>c</sup>	4.37 <sup>k</sup>	4.40 <sup>k</sup>	4.45 <sup>k</sup>	1.00 <sup>d</sup>	0.99 <sup>d</sup>	1.03 <sup>c</sup>
A3	13.97 <sup>c</sup>	14.32 <sup>b</sup>	14.80 <sup>b</sup>	4.89 <sup>g</sup>	4.90 <sup>g</sup>	4.94 <sup>g</sup>	1.09 <sup>b</sup>	1.10 <sup>b</sup>	1.11 <sup>ab</sup>
B1	11.60 <sup>gh</sup>	11.93 <sup>de</sup>	12.31 <sup>f</sup>	4.51 <sup>j</sup>	4.57 <sup>j</sup>	4.61 <sup>j</sup>	0.91 <sup>fg</sup>	0.91 <sup>e</sup>	0.91 <sup>ef</sup>
B2	12.87 <sup>e</sup>	13.15 <sup>c</sup>	13.47 <sup>de</sup>	5.47 <sup>f</sup>	5.50 <sup>f</sup>	5.54 <sup>f</sup>	1.02 <sup>c</sup>	1.03 <sup>c</sup>	1.04 <sup>c</sup>
B3	14.17 <sup>a</sup>	14.54 <sup>a</sup>	14.92 <sup>a</sup>	6.38 <sup>c</sup>	6.43 <sup>c</sup>	6.49 <sup>c</sup>	1.12 <sup>a</sup>	1.14 <sup>a</sup>	1.13 <sup>a</sup>
C1	11.43 <sup>i</sup>	11.70 <sup>f</sup>	12.04 <sup>h</sup>	4.59 <sup>i</sup>	4.63 <sup>i</sup>	4.67 <sup>i</sup>	0.84 <sup>i</sup>	0.85 <sup>f</sup>	0.88 <sup>f</sup>
C2	12.81 <sup>e</sup>	13.19 <sup>c</sup>	13.52 <sup>d</sup>	5.73 <sup>e</sup>	5.75 <sup>e</sup>	5.79 <sup>e</sup>	0.88 <sup>h</sup>	0.89 <sup>e</sup>	0.90 <sup>ef</sup>
C3	14.07 <sup>b</sup>	14.43 <sup>ab</sup>	14.64 <sup>c</sup>	6.91 <sup>b</sup>	6.97 <sup>b</sup>	6.98 <sup>b</sup>	0.92 <sup>f</sup>	0.92 <sup>e</sup>	0.94 <sup>e</sup>
D1	11.63 <sup>g</sup>	12.04 <sup>d</sup>	12.32 <sup>f</sup>	4.70 <sup>h</sup>	4.75 <sup>h</sup>	4.80 <sup>h</sup>	0.87 <sup>h</sup>	0.90 <sup>e</sup>	0.91 <sup>ef</sup>
D2	13.01 <sup>d</sup>	13.22 <sup>c</sup>	13.45 <sup>de</sup>	5.97 <sup>d</sup>	6.02 <sup>d</sup>	6.08 <sup>d</sup>	0.96 <sup>d</sup>	0.97 <sup>d</sup>	0.99 <sup>d</sup>
D3	14.21 <sup>a</sup>	14.55 <sup>a</sup>	14.83 <sup>b</sup>	7.30 <sup>a</sup>	7.33 <sup>a</sup>	7.36 <sup>a</sup>	1.03 <sup>c</sup>	1.05 <sup>c</sup>	1.09 <sup>b</sup>

**TABLE 4. pH values, titratable acidity (TA), and Syneresis of non-fat set yoghurt fortified with different milk protein ingredients and inulin during cold storage period.**

Treatment	pH values			% (TA)			Syneresis (%)		
	Cold storage period (day)								
	1	7	14	1	7	14	1	7	14
Control	4.57 <sup>a</sup>	4.39 <sup>a</sup>	4.24 <sup>abc</sup>	0.89 <sup>g</sup>	1.08 <sup>g</sup>	1.17 <sup>g</sup>	43.6 <sup>a</sup>	47.3 <sup>a</sup>	52.0 <sup>a</sup>
A1	4.53 <sup>bcd</sup>	4.32 <sup>cde</sup>	4.20 <sup>bcd</sup>	0.93 <sup>efg</sup>	1.06 <sup>g</sup>	1.21 <sup>fg</sup>	40.0 <sup>b</sup>	44.6 <sup>a</sup>	47.3 <sup>b</sup>
A2	4.50 <sup>efg</sup>	4.32 <sup>cde</sup>	4.14 <sup>ef</sup>	1.00 <sup>bcd</sup>	1.20 <sup>abcd</sup>	1.24 <sup>ef</sup>	36.3 <sup>cde</sup>	41.6 <sup>b</sup>	47.0 <sup>b</sup>
A3	4.47 <sup>hg</sup>	4.30 <sup>de</sup>	4.10 <sup>f</sup>	1.04 <sup>ab</sup>	1.23 <sup>a</sup>	1.35 <sup>ab</sup>	34.3 <sup>de</sup>	37.6 <sup>cd</sup>	41.3 <sup>de</sup>
B1	4.56 <sup>ab</sup>	4.38 <sup>ab</sup>	4.25 <sup>ab</sup>	0.90 <sup>fg</sup>	1.11 <sup>efg</sup>	1.26 <sup>def</sup>	38.3 <sup>bc</sup>	41.0 <sup>b</sup>	44.6 <sup>cb</sup>
B2	4.53 <sup>ecd</sup>	4.33 <sup>cd</sup>	4.20 <sup>cd</sup>	0.94 <sup>ef</sup>	1.15 <sup>cdef</sup>	1.30 <sup>bcd</sup>	35.0 <sup>de</sup>	39.3 <sup>bc</sup>	41.6 <sup>de</sup>
B3	4.50 <sup>efd</sup>	4.29 <sup>e</sup>	4.15 <sup>e</sup>	0.99 <sup>cd</sup>	1.17 <sup>cde</sup>	1.35 <sup>ab</sup>	31.0 <sup>f</sup>	32.6 <sup>f</sup>	36.3 <sup>fg</sup>
C1	4.51 <sup>cdef</sup>	4.36 <sup>abc</sup>	4.28 <sup>a</sup>	0.94 <sup>ef</sup>	1.10 <sup>fg</sup>	1.25 <sup>ef</sup>	34.0 <sup>e</sup>	35.6 <sup>de</sup>	40.3 <sup>e</sup>
C2	4.46 <sup>hg</sup>	4.32 <sup>cde</sup>	4.22 <sup>bc</sup>	0.96 <sup>de</sup>	1.15 <sup>def</sup>	1.22 <sup>f</sup>	29.6 <sup>fg</sup>	33.6 <sup>ef</sup>	37.3 <sup>f</sup>
C3	4.44 <sup>h</sup>	4.29 <sup>e</sup>	4.17 <sup>de</sup>	1.03 <sup>abc</sup>	1.19 <sup>abcd</sup>	1.32 <sup>bc</sup>	23.3 <sup>h</sup>	26.0 <sup>g</sup>	30.6 <sup>h</sup>
D1	4.55 <sup>ab</sup>	4.36 <sup>abc</sup>	4.23 <sup>bc</sup>	0.96 <sup>de</sup>	1.17 <sup>bcd</sup>	1.24 <sup>ef</sup>	36.6 <sup>cd</sup>	40.6 <sup>b</sup>	44.0 <sup>cd</sup>
D2	4.51 <sup>def</sup>	4.34 <sup>bcd</sup>	4.20 <sup>cd</sup>	1.00 <sup>bcd</sup>	1.21 <sup>abc</sup>	1.29 <sup>cde</sup>	31.3 <sup>f</sup>	37.0 <sup>cd</sup>	40.3 <sup>e</sup>
D3	4.48 <sup>fg</sup>	4.30 <sup>de</sup>	4.16 <sup>de</sup>	1.07 <sup>a</sup>	1.23 <sup>ab</sup>	1.38 <sup>a</sup>	27.3 <sup>g</sup>	31.6 <sup>f</sup>	34.0 <sup>g</sup>

Table 4 shows a substantial increase ( $P < 0.05$ ) in the percentage of titratable acidity (TA%) in fortified yoghurts, compared to control sample. This increase might be attributed to either the high lactose content, which boosted the activity of lactic acid bacteria, or the potential favorable impact of the added ingredients on bacterial activity. It might also be related to an increase in the total solid contents, mainly protein. The TA% has significantly increased ( $P < 0.05$ ) with the increasing in the added amount of milk protein ingredients. The highest value of titratable acidity was recorded for yoghurt sample supplemented with 4.5% NaCN. According to Yeganehzad et al.

(2007) and (Delikanli & Ozcan 2014), the higher acidity and lower pH in whey protein-fortified yoghurt may be due to the increased milk solids. The titratable acidity was also increased with the progress of cold storage period. The reduction in pH value and the increase in titratable acidity throughout storage period were mainly due to the production of lactic acid produced by the activity of bacteria and yoghurt starter culture. This effect is widely understood and is generated by acidifying bacteria activity. On the other hand, it was noted that pH values and acidity values of low-fat yoghurt produced using inulin as a fat substitute did not change much (Guyen et al.,

2005; Guggisberg *et al.*, 2009; Modzelewska-Kapitula & Klebukowska, 2009). However, the chain length of inulin (small, medium, or long) affects pH values of yoghurt has been previously reported (Aryana *et al.* 2007). Also, yoghurt fortified with 1% inulin showed lower pH values than the control samples over the storage period (Gustaw *et al.*, 2011).

#### *Syneresis*

Syneresis values of non-fat set yoghurt enriched with inulin and milk protein ingredients at cold storage period are shown in Table 4. Control yoghurt, compared to other samples, recorded the greatest syneresis values over the storage period times. The current findings indicated that adding milk protein ingredients reduced significantly ( $P < 0.05$ ) the expelled whey and this was in a dose-dependent manner. As expected, this is mainly attributed to the total solid content which has an inverse relationship with the syneresis values. In this sense, the higher TS content and protein content contributed in reducing the wheying off. Additionally, adding inulin which is well-known as a texturizer ingredient also participated in the reduction of the whey expelled. Inulin also is able to hold more water, particularly during the yoghurt shrinkage period at the end of cold storage period (Aryana *et al.*, 2007).

Yoghurt sample supplemented with 4.5% WPC had the lowest wheying off value. Similar findings were reported by Akalin *et al.* 2012() and (Isleten and Karagul-Yuceer 2006), who found that yoghurt fortified with WPI had the lowest syneresis among milk component fortifiers. Intramolecular disulfide bonds in whey proteins help to maintain their structure. Protein denaturation by heat activates  $\beta$ -lactoglobulin sulfhydryl group, which then interacts with other proteins and itself through sulfhydryl-disulfide bonds. According to Fox *et al.* (2000), proteins have an impact on the rheological characteristics and structure of coagulated milk gels, such as yoghurt and cheese. Reduced syneresis is the result of the gel network becoming more delicate, the cross-link becoming denser, and the pores getting smaller when the casein-to-whey ratio was decreased (Puvanenthiran *et al.*, 2002). According to some researchers, the aggregate network will be more susceptible to syneresis because of the more open gel structure that results from decreased casein concentration (González-Martínez *et al.*, 2002). Also, some previous studies described WPC-enriched matrix as a great network with

many microscopic pores (Remeuf *et al.*, 2003 and Saint-Eve *et al.*, 2006). According to others, there was a more compacted flocculated protein matrix with bigger porosity and whey protein aggregates, as well as more noticeable micelle chains (Krzeminski *et al.*, 2011). As the storage period progressed, whey separation increased in all yoghurt samples, and control recorded the highest syneresis value. These findings are in line with that reported by (Pimentel *et al.* 2012 and Aslam *et al.* 2015). However, Guven *et al.* (2005) found that adding 1% inulin improved consistency and reduced whey separation. Likewise, Aryana *et al.* (2007) indicated that inulin-containing fat-free yoghurt had better body and texture and less syneresis rate, compared to control sample.

#### *Microbiological properties of yoghurts*

The viable bacterial counts of fat-free set yoghurts fortified with different levels of milk protein ingredients and 1% inulin during cold storage period are presented in Table 5. All milk ingredients and inulin have a slight effect on the total bacterial counts of all samples except that sample containing WPC which reported higher TBC compared to that of control sample. The total bacterial number slightly increased by increasing the added amount of milk protein ingredients. Yoghurt sample enriched with 4.5% WPC exhibited the highest total bacterial count. This increase mainly due to the increased total solids which reflects the high nutrients content of that sample including the high lactose content in SMP and MPC treatments. The total bacterial counts were higher in SMP-containing yoghurts, compared to MPC-containing yoghurts, because of the higher lactose content present in skim milk powder. However, WPC yoghurts had the highest viable bacterial counts. The TBC was slightly increased after one week of cold storage then the bacterial counts declined at 14 days. These results are in line with those reported in previous works (Masuda *et al.*, 2005, Unal & Akalin, 2013; Aslam *et al.*, 2015).

Table 5 shows the effect of different concentrations of milk protein ingredients and 1% inulin on viable numbers of *S. thermophilus* (ST) and *L. delbrueckii* subsp. *bulgaricus* (LDB). This effect varies according to type and concentration of the used milk protein ingredient. Yoghurt samples enriched with WPC showed higher viable counts of ST, compared to yoghurt samples enriched with other milk ingredients. The current results indicated that the viability

of these bacteria increased with increasing the added amount of WPC while it decreased with the increased concentrations of NaCN. In this context, the highest viable numbers of ST was recored for yoghurt sample fortified with 4.5% WPC.

Moreover, the current data demonstrated that adding milk protein ingredients increased the viability of LBD. Adding WPC showed the highest viable number of LBD while no significant differences were reported between yoghurts fortified with other milk protein ingredients. (McComas and Gilliland, 2003) studied the growth of yoghurt cultures in milk fortified with whey protein hydrolysates indicating a small effect on viability of LDB and ST, while the probiotic bacteria were grown with different yoghurt cultures. Adding WPC to yoghurt mix seems to sustain a constant cell count of starter culture (ST and LDB) in the inoculum with the dominance of the ST strain over LDB (Lucey & Singh, 1998; Birollo et al., 2000; Oliveira et al., 2001; Damini et al., 2009). After one week of storage, ST and LDB viable counts increased in all yoghurt samples and control then decreased as the storage period progressed. At the end of the storage, the number of ST was more than one log cycle higher than that of LDB.

#### Texture profile analysis of yoghurt

Table 6 shows the textur parameters of yoghurt samples fortified with different concentration of various milk protein ingredients and 1%

inulin. Remarkable changes were observed between yoghurt samples in term of its hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness. Yoghurt showed a marked increase in hardness, adhesiveness, gumminess, and chewiness with reduced cohesiveness and springiness when adding various DDCs and inulin. Differences in texture parameters between enriched yoghurts with multiple types of DDCs, as observed in this study, are likely due to different interactions between protein-protein molecules and variances in the mineral structure of varying milk powders. This mainly due to the varied contents of total solids, protein, and also the protein types, and protein concentrations used in preparing these samples. Similar findings have been obtained by Antunes et al. (2004), who stated that with the rise of whey protein content in milk used for yoghurt manufacture, the hardness of yoghurt increased. The yoghurt samples containing NaCN recorded the highest hardness values from the same Table, while the control yoghurt was the lowest, maybe due to the lowest protein content, which contributes to the product's hardness. Hardness values increased in the yoghurt formulations by increasing the added ratios of the different milk protein ingredients except for the formulations made with WPC, where the degree of hardness increased with WPC added up to 3% while it decreased with 4.5% WPC. However, yoghurt groups enriched with WPC were relatively higher than control.

**TABLE 5. Viable bacterial counts of non-fat set yoghurt fortified with different milk protein ingredients and inulin during cold storage period.**

Treatment	Total bacterial count			<i>Str. thermophilus</i> (ST)			<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> (LSB)		
	Storage period (day)								
	1	7	14	1	7	14	1	7	14
Control	8.33	8.54	8.89	7.96	8.13	7.63	6.57	6.69	6.15
A1	8.56	8.75	7.96	8.01	8.20	7.54	6.61	6.78	6.23
A2	8.61	8.79	8.07	7.93	8.07	7.58	6.73	6.82	6.29
A3	8.75	8.91	8.15	7.79	8.90	7.27	6.82	7.01	6.43
B1	8.41	8.65	7.72	7.94	8.14	7.65	6.47	6.62	5.92
B2	8.47	8.60	7.86	7.81	7.96	7.41	6.59	6.72	6.11
B3	8.62	8.79	8.11	7.62	7.78	7.36	6.71	6.89	6.30
C1	8.65	8.86	7.90	8.09	8.21	7.67	6.79	6.90	6.15
C2	8.83	9.07	8.26	8.26	8.43	7.89	7.15	7.15	6.52
C3	9.12	9.38	8.59	8.47	8.58	7.96	7.43	7.43	6.62
D1	8.47	8.62	8.02	7.89	8.10	7.50	6.83	6.83	6.30
D2	8.64	8.81	7.76	7.73	7.82	7.27	7.12	7.12	6.35
D3	8.71	8.94	8.05	7.51	7.64	7.17	7.19	7.19	6.48

Table 6 also indicated that adhesiveness values increased in all yoghurt formulations compared with the control by increasing the added ratios of the different milk protein ingredients and inulin. Results show that yoghurt sample supplemented with 1.5% NaCN recorded the highest value for adhesiveness. Control yoghurt and SMP-enriched yoghurts had lower adhesiveness scores than the others. This outcome might be explained by the weaker gel that developed as a result of the casein content being reduced, which released bound water (Jayaprakasha *et al.*, 2000). Cohesiveness values decreased in yoghurt samples compared with control, increasing the added percentages of the different milk protein ingredients and inulin. Control sample exhibited the highest value for cohesiveness, while the yoghurt sample containing 4.5% NaCN was the lowest.

The current results reported that springiness value decreased by increasing the percentages of different milk protein ingredients, and inulin added. Control samples recorded the highest springiness value, while the yoghurt sample with 4.5% SMP was the lowest. Gumminess parameter had a similar tendency where control sample recorded the lowest value while sample enriched with 4.5% NaCN had the highest value. The highest chewiness value has been recorded in yoghurt sample containing 4.5% NaCN, while control sample had lowest value. According to textural metrics outlined by several authors, yoghurt supplemented with NaCN had stronger networks and greater stiffness than yoghurt fortified with other milk protein components. Guzmán-González *et al.* (1999) reported that casein-based products produced firmer gels than yoghurts fortified with whey protein. Also, according to Bhullar *et al.* (2002), the yoghurt sample with WPC had a higher water-binding capacity of denatured whey proteins, making it stiffer than the one with SMP. But, during storage, yoghurts fortified with SCaN have become firmer than those fortified with WPC. When WPC or caseinate proteins were added to the milk base, the yoghurts' firmness increased compared to a product fortified with SMP (Sodini *et al.*, 2004). This is mainly due to the increased protein content (increased TS content). Akalin *et al.* (2012) reported that the milk base was fortified with 2% SMP, WPC, NaCN, and a blend of (1:1) WPC and NaCN. The highest values of firmness were reported in yoghurt samples fortified with NaCN, whereas those fortified with SMP had the lowest values. Protein masses produced by

the interaction of casein micelles with denatured whey proteins via intermolecular disulfide bonds developed through the first steps of coagulation, which explains the increased firmness of yoghurt sample containing WPC (Lucey *et al.*, 1999). Damin *et al.* (2009) reported that yoghurt fortified with NaCN had the highest firmness value. The authors reported that a positive correlation of 0.97 was found between protein levels and stiffness in milk supplemented with NaCN, while the corresponding values for supplementation with SMP and WPC were 0.82 and 0.65. Non-fat yoghurt that was firmer and more viscous and less prone to syneresis was often the outcome of the adding milk protein ingredients to reconstituted skim milk (Isleten & Karagul-Yuceer, 2006 and Peng *et al.*, 2009). NaCN-fortified yoghurts have fewer pores and exhibit a relatively coarse and loose structure with largest casein micelles and extensive micelle clusters (Remeuf *et al.*, 2003; Damin *et al.*, 2009). Some authors reported that CaCN-fortified yoghurts showed dense, compact, and finely performed microstructure comparable with yoghurt prepared with WPC (Remeuf *et al.*, 2003). Additionally, Akalin *et al.* (2012) found that probiotic yoghurts with 2% sodium-calcium caseinate had improved textural parameters. Whey protein addition enhanced the textural qualities of non-fat yoghurt by raising its cohesion, elasticity, and hardness values (Delikanli and Ozcan, 2014). Furthermore, culture composition, product's TS and protein level, and also the protein type all have a significant impact on how hard fermented milk turns out (Oliveira *et al.*, 2001). The type of milk supplements had a major impact on the product's firmness, according to the earlier authors, who also noted a considerable interaction between culture's composition and used ingredients. On the other hand, (Güven *et al.*, 2005) reported that low-fat yoghurt containing 2 g inulin/100 g of milk stored at 4 °C for 1 day had the highest consistency compared to samples containing 1 or 3 g inulin. Yoghurt samples containing 1 g inulin/100 mL milk and control yoghurt had the highest firmness values. Also, Guggisberg *et al.* (2009) showed that the firmness and yield stress of low-fat set yoghurt increased with an increase in inulin and fat contents. Nevertheless, it was determined that the maximum inulin amount (4%) was insufficient to replicate whole-milk yoghurt. In contrast, Pasephol *et al.* (2008) indicated that addition of 4% inulin to non-fat set yoghurt resulted in lower yield stress and gel stiffness similar to that of the full-fat yoghurt. They viewed the impact of inulin



addition on yoghurt (lower yield stress value and firmness) as evidence of inulin's interference with protein matrix formation caused by inulin molecules dispersed amid casein micelles. They hypothesized that this impact is analogous to fat globule interference in protein matrix synthesis. Oliveira et al. (2011) showed that inulin behaved as a prebiotic and enhanced firmness of skim fermented milk. Non-fat set yoghurt of this study is similar to the data obtained (Patel and Hati, 2017). Yoghurt samples enriched with NaCN had the greatest casein to non-casein ratios of any enriched yoghurt sample, but have distinct textural features. As a result, variables other than the casein-whey protein ratio appear to impact yoghurt's textural qualities.

The addition of inulin may offer functional properties different from dairy-based protein powders. The effects adding inulins, with varying lengths of chain, on rheological properties of yoghurt have been described by Paseephol et al. (2008). All inulin-containing yoghurts had reduced values for firmness, apparent viscosity, yield stress, complex viscosity,, compared to control sample (nonfat yoghurt without adding inulin). The rheological behavior of the long-chain inulin-supplemented sample was similar to that of a full-fat yoghurt control. The authors hypothesized that the reduced protein content

of inulin-enriched yoghurt and the potential for inulin molecules to be scattered among casein micelles, disrupting the formation of the protein network, might be the causes of the softening impact of inulin on yoghurt structure. In a previous study, Guggisberg et al. (2009) also examined the impact of fat level (0.1 to 3.5%) and long-chain inulin addition (1, 2, and 3%), on the rheology, resistance to penetration, hardness, viscosity, and creaminess of yoghurt and they have proved such results.

#### *Sensory properties:*

Table 7 showed that adding various milk protein ingredients and inulin affected yoghurt's sensory properties. Initially, control sample gained the highest score in terms of taste and minimal body and texture. However, the individual effect of different dried dairy ingredients, except WPC, and inulin positively influenced body and texture values of yoghurt samples. According to these results, the added rate of SMP, MPC, WPC, and NaCN improves the texture grades in fresh yoghurt and during storage periods. NaCN treatments had higher scores compared to other treatments. The data revealed that supplemented yoghurt showed an excellent appearance compared to control. Similar results were obtained by Sady et al. (2007) and Akalin et al. (2012).

**TABLE 6. Values of texture parameters of non-fat set yoghurt fortified with different milk protein ingredients and inulin.**

Treatments	Textural parameters					
	Hardness (g)	Adhesiveness (g.mm)	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (mJ)
Control	114	146.35	0.45	12.47	51.30	639.71
A1	125	166.02	0.43	12.40	53.75	666.50
A2	133	163.22	0.41	12.15	54.53	662.54
A3	152	221.33	0.38	11.16	57.76	644.60
B1	145	169.37	0.41	12.18	59.45	724.10
B2	167	204.51	0.4	11.30	66.80	754.84
B3	171	259.39	0.39	11.23	66.69	748.93
C1	141	257.36	0.43	12.28	60.63	744.54
C2	160	269.82	0.39	11.58	62.40	722.59
C3	145	313.92	0.41	11.92	59.45	708.64
D1	240	345.29	0.44	12.44	105.60	1313.66
D2	349	268.23	0.38	12.25	132.62	1624.60
D3	466	319.66	0.37	11.78	172.42	2031.11

**TABLE 7. Sensory evaluation of non-fat set yoghurt fortified with different milk protein ingredients and inulin during cold storage period.**

Tret.	Flavor (50)			Body & Texture (30)			Appearance & Colour (20)		
	1	7	14	1	7	14	1	7	14
Storage									
Cont.	46.9	38.6	32.3	27.1	24.5	23.3	18.6	17.2	16.2
A1	45.2	38.6	32.6	27.4	25.0	23.4	18.2	17.2	16.4
A2	45.4	39.0	33.8	27.8	25.5	23.6	18.3	17.4	16.8
A3	45.8	40.2	33.3	28.4	26.4	24.4	18.5	17.6	16.8
B1	44.9	39.0	34.8	27.3	26.0	24.0	18.6	17.6	16.3
B2	44.3	37.8	35.0	27.3	26.2	25.1	18.3	17.8	16.9
B3	43.2	36.3	33.2	27.4	26.4	25.3	18.1	17.4	16.6
C1	42.1	37.5	30.0	26.3	25.4	22.6	17.7	17.1	16.6
C2	41.4	35.9	30.8	25.4	24.6	23.3	17.4	16.9	16.6
C3	39.5	32.4	28.4	24.0	24.2	23.2	17.2	16.6	16.6
D1	42.1	34.2	29.6	27.8	25.8	24.3	17.5	17.3	16.6
D2	42.0	33.8	28.6	28.2	25.8	25.4	17.8	17.0	16.6
D3	41.1	33.1	27.4	28.6	27.4	24.9	18.2	17.3	17.0

### Conclusion

Based on the current findings, it could be concluded that adding milk protein ingredients (SMP, MPC, WPC, and NaCN) to non-fat set yoghurt significantly affected its physiochemical characteristics. Also, adding inulin have a good impact on different yoghurt quality parameters. The type and proportions of the used milk protein ingredients showed different impact on the various quality attributes of the obtained yoghurt. It looks good to add the dry dairy components for the manufacturing of non-fat set yoghurt, but it seems to rely greatly on the makeup of the powders used. Finally, mixing the different milk protein ingredients in proper ratios and inulin seems practical and exciting for manufacturing non-fat set yoghurt.

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