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QUALITY ATTRIBUTES OF PROTEIN FORTIFIED YOGHURT

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ABSTRACT

The aim of this study was to evaluate the quality attributes of yoghurt made from cow's milk (3.6%fat and 3.37% protein) fortified with different levels of milk protein (0.5, 1.0 or 1.5%) as skim milk powder (SMP), sodium caseinate (NaCn) and dried whey protein concentrate (WPC); or ultrafiltrated (UF) to similar protein levels.

The results indicated that titratable acidity (TA), acetaldehyde (AC) and diacetyl (DA) contents as well as the firmness (Fr), as inversely indicated from the penetration value, consistency coefficient (CC) and yield stress (YS) of yoghurt raised as the protein fortification level increased, by which the reduction in pH values were delayed. SMP-fortified yoghurt had the highest contents of TA, AC and DA and the lowest pH value, followed by WPC, UF and NaCn, which caused the greatest Fr and YS followed by UF. Proportional increases in TA and all rheological parameters were determined, while pH value decreased continuously until the end of SP. The increment in the AC and DA contents retreated after the 7^{th} and 14^{th} day respectively. Gradual increment in *Streptococcus.. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* counts associated with protein elevation level was determined. SMP in yoghurt promoted the highest viability conditions for both strains followed by WPC, UF and NaCn. The duration of storage period (SP) for 21 days led to gradual reduction in counts of both strains. In conclusion, the yoghurt with increased 1.0% protein by UF, attained the highest total sensory scores, and was kept with stable sensory quality until the 14^{th} day and stilled acceptable until the end of storage period.
Keywords: Yoghurt, quality, protein, fortification, physiochemical, microbiology, rheology, sensory

INTRODUCTION

Yoghurt is defined by Codex Alimentarius (2003) as a coagulated milk product that results from the fermentation of lactic acid in milk by *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*. The nutrient composition of yoghurt is based on the nutrient composition of the milk where it is derived, by the species and strains of bacteria used in the fermentation, the source and type of milk solids that may be added before fermentation, and the temperature and duration of the fermentation process.

It has been argued that protein from yogurt is more easily digested than protein from milk, as bacterial pre-digestion of milk proteins in yogurt may occur. This argument is supported by evidence of a higher content of free amino acids, especially proline and glycine, in yogurt than in milk. The activity of proteolytic enzymes and peptidases is preserved throughout the shelf life of the yogurt. Thus, the concentration of free amino groups increases up to twofold during the first 24 h and then doubles again during the next 21 day of storage at 7 °C (Loones,1989). Some bacterial cultures have been shown to have more proteolytic activity than others do. For example, *L. bulgaricus* was shown to have a much higher proteolytic activity during milk fermentation and storage than does *S. thermophilus*, as indicated by elevated concentrations of peptides and free amino acids after milk fermentation (Beshkova *et al.*, 1998). During fermentation, both heat treatment and acid production result in fine coagulation of casein, which may also contribute to the greater protein digestibility of yoghurt than that of milk. Proteins in yoghurt are of excellent biological quality, similar to those in milk, because the nutritional value of milk proteins is well preserved during the fermentation process (Hewitt and Bancroft, 1985).

Both the caseins and the whey proteins in yoghurt are rich sources of all the essential amino acids, and the intestinal availability of nitrogen has been reported high (Gaudichon et al. ,1994). It is also, reported that proteins from both milk and yoghurt were rapidly hydrolyzed after ingestion, but the gastroduodenal transfer of dietary nitrogen was slower when yogurt was fed, as oppose to when milk was fed (Gaudichon *et al.*, 1995).

Milk proteins are known to exert a wide range of nutritional, functional and biological activities that make them potential ingredients of health-promoting foods. There are many benefits resulting from the addition of milk proteins in yoghurt formulations. These benefits include: improved flavor, texture improvement , nutritional enrichment , reduced syneresis , extended shelf-life , prebiotic effect and nutraceutical benefits. The appearance and texture of yogurt is dependent upon numerous factors: total solids, protein content, type of protein, fat content and the type and concentration of any thickeners or stabilizer that are added (Kuehn et al.,2006). The casein micelles in yogurt form different matrices depending upon the concentration of the other proteins. When milk is fortified with whey protein concentrate (WPC) and heat treated, fine protein floccules are observed. When casein, skim milk powder, or milk protein concentrates are added, no floccules are observed. When milk is heated, ß-lactoglobulin is denatured and reacts with a-casein to form an insoluble complex. When milk is fortified with WPC, the concentration of ß-lactoglobulin greatly exceeds the concentration of a-casein. As a result, other protein complexes, such as ß-lactoglobulin and a-lactalbumin complexes will form. In yogurt fortified with WPC, it is the ß-lactoglobulin and a-lactalbumin complex, rather than the casein complex, that probably stabilizes the yogurt, resulting in different consistency. Fortification of yogurt with WPC results in yogurt with a better texture and consistency. Yogurt fortified with casein or skim milk protein often have a firmer gel, but yogurts fortified with WPC tend to be smoother and have a better appearance.

Food matrix components, such as proteins (Gianelli et al. 2005) are known to interact with flavor compounds. Proteins are added to foods primarily because of their functional properties, such as emulsifying and stabilizing capacities, and because of their nutritional value. However, interactions between proteins and flavors are known to influence the perceived flavor of a food product (Land, 1996). Protein ingredients not only reduce the perceived impact of desirable flavors, but also may transmit undesirable off-flavors to foods (Semenova et al. 2002). In addition, proteins may change the texture of a food that is gelling, and thus decrease the flavor perception due to inhibition of mass transfer (Wilson and Brown 1997).

Cow's milk is the main raw milk supply for dairy industries in many countries. Due to that, cow's milk is relatively characterized with thin body because of its solids deficiency, the obtained yoghurt is usually suffering from a pronounced weak consistency and wheying off defects. Therefore, solids enrichment became necessary when yoghurt was designed to be made from cow's milk for overcoming such disadvantages. The fortification of yoghurt with SCaCN improved the firmness and adhesiveness. Higher values of viscosity were also obtained in probiotic yogurts with SCaCN during storage. However, WPC enhanced water-holding capacity more than the caseinate. Addition of SCaCN resulted in a coarse, smooth, and more compact protein network; however, WPC gave finer and bunched structures in the scanning electron microscopy micrographs. The use of SCaCN decreased texture scores in probiotic yogurt; probably due to the lower water-holding capacity and higher syneresis values in the caseinate-added yogurt sample. Therefore, the textural characteristics of probiotic yogurts improved depending on the ingredient variety (Akalin *et al.,* 2012).

The aim of this study was to evaluate the quality attributes of yoghurt made from cow's milk (3.6%fat and 3.37% protein) fortified with different levels of milk protein (0.5, 1.0 or 1.5%) as skim milk powder (SMP), sodium caseinate (NaCn) and dried whey protein concentrate (WPC); or ultrafiltrated (UF) to similar protein levels.

MATERIALS AND METHODS

Materials

The materials used in this study include:

Fresh cow's milk (3.60% fat and 3.37% protein) was obtained from the herd of High Institute of Agric. Co-operation, Shoubra El-Kheima. Skim milk powder (SMP, 36% protein) produced in Denmark was purchased from the local market. Dried whey protein concentrate (WPC, 82% protein) produced by SFK DATABLAD, Hvidovre and Viborg, Denemark, was obtained from local market.Sodium caseinate (NaCn, Lactovit Co., Germany, 84% protein) was kindly obtained from Arab Dairy Co., Egypt. Freeze - dried yoghurt starter culture (3.63) containing *Streptococcus thermphilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was obtained from Cagilificio Clerici, Cadorag, Italy

Microbial transglutaminase enzyme (100 units / g protein) derived from *Streptovertierllium* sp. was obtained from Gewuerzmueller GmbH, Salzburg, Bergheim, Germany

Experimental procedures

Activation of yoghurt starter culture

Yoghurt starter culture (YSC) was activated at 42°C using antibiotic free SMP reconstituted to 12% total solids (TS) and autoclaved at 120°C for 10 min. After incubation at 42°C for 4-5 h, the obtained culture was freshly used.

Preparation of protein fortified yoghurt

Thirteen treatments including the control were designed, where cow's milk was fortified with 0.5, 1.0 or 1.5% protein by adding SMP, Na caseniate or WPC; or via pasteurized milk ultrafiltration at 50°C (as recommended by

Maubois *et al.*(1971) using CARBOSEP UF-unit (type 2S 37, France) with zirconium oxide membrane area 1.68 m^2 at Agric. Secondary school, Giza.
The yoghurt bases were prepared as described by Tamime and Robinson (1999). They were heat treated at 85°C for 5 min., cooled down to 42°C, inoculated with 2% of fresh active YSC, filled into 100 ml polystyrene (PS) containers, covered, and incubated until complete coagulation (through about 3 h.). Later on, yoghurt packs were transferred to the refrigerator (5±1°C), where they were kept for the periodical analyses. Three replicates were carried out for each treatment.

Analytical methods

Physiochemical analysis

pH values were measured using a pH meter (HANNA Instruments, USA). Titratable acidity and soluble nitrogen were determined according to AOAC (2007). Acetaldehyde content was determined as described by Lees and Jago (1969). Diacetyl was determined as given in Lees and Jago (1970).

Microbiological analysis:

The count of *Str. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were carried out using M17 and MRS agar media, respectively, as described by Gueimonde *et al.,* (2003). Both cultures were incubated anaerobically for 2 days at 37°C.

Rheological profile

The firmness of yoghurt was measured using penetrometer model SUR, BERLIN, PNR as described by Bourne (1982). The depth to which a loaded perfortated disc penetrates into the yoghurt curd in a given time was measured (using cone weight 35g). The depth of penetration (0.1 mm, penetrometer unit, PE) was a function of the firmness of yoghurt curd. The measurements were always carried out at about 18°C and the depth of penetration was measured after 5 sec.

Rheological properties of yoghurt during cold storage period were measured at 10°C using a rotary viscometer (RHEOTEST, type RV and Pruefgeraetewerk Medingn, Dresden) as described by Toledo (1980). Consistency coefficient was calculated from the descending flow curve to express the data corresponding the yoghurt using the following equation:

$$\log\delta = \log\kappa + n\log\gamma$$

Where :

δ = Shearing stress

γ = Sshearing gradient or shear rate,

n = Flow behaviour index and

κ = Consistency coefficient or consistency index

Yield value or yield stress was calculated by fitting the shear stress-shear rate data to the Casson equation (Bourne, 1982):

$$\sqrt{\delta} = \sqrt{\delta o} + \eta a \sqrt{\gamma}$$

Where

δ	=	Shearing stress,
δo	=	Yield stress,
ηa	=	Apparent viscosity and γ = shear rate
γ	=	Shear rate

Organoleptic evaluation

Sensory evaluation of yoghurt samples was carried out by regular score panels including the staff members of Food Science Department, Faculty of Agriculture, Ain Shams University according to Tamime and Robinson (1999), using yoghurt evaluation scheme III approved by the American Dairy Science Association

Statistical analysis:

The data obtained were exposed to proper statistical analysis according to statistical analyses system user's guide (SPSS, 1998).

RESULTS AND DISCUSSIONS

Physiochemical Analysis

Titratable acidity and pH values

Data presented in Table (1) are the titratable acidity (TA) and pH values of yoghurt during storage period as affected by the level and source of protein fortification.

Table (1): Titratable acidity (TA) and pH value of yoghurt during storage period as affected by the level and source of milk protein fortification

storage period (days)	Level and source of protein fortification												
	control	0.5%				1.0%				1.5%			
		SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC
	TA (as lactic acid%)												
0	0.71	0.94	0.78	0.77	0.81	0.99	0.83	0.81	0.85	1.01	0.90	0.85	0.99
7	0.83	1.12	0.86	0.86	0.88	1.15	0.91	0.90	0.97	1.39	0.99	0.95	1.05
14	0.95	1.46	1.05	0.99	1.12	1.48	1.15	1.11	1.19	1.51	1.23	1.22	1.24
21	1.15	1.50	1.20	1.18	1.22	1.58	1.28	1.21	1.33	1.60	1.34	1.30	1.41
		pH value											
0	4.42	4.24	4.90	4.86	4.30	4.54	5.04	5.0	4.97	4.67	5.12	5.10	5.02
7	4.04	4.21	4.70	4.33	4.29	4.24	4.73	4.34	4.32	4.38	4.87	4.35	4.33
14	3.97	4.00	4.67	4.11	4.08	4.13	4.70	4.13	4.10	4.24	4.81	4.14	4.11
21	3.91	3.99	4.51	4.09	4.07	4.05	4.64	4.11	4.08	4.00	4.74	4.12	4.09

The obtained results reveal that, although the proportional increasing in the protein fortification level led to significant increase in TA of yoghurt, the increased level of protein delayed the reduction in the pH value. This phenomenon could be ascribed to the strengthened buffering capacity gained as the protein content raised. Similar findings were reported by Green *et al.* (1981) and Haggag and Fayed (1988).

Concerning the protein source, the results indicated that SMP caused the highest TA and hence the lowest pH value among other fortification sources. That could be due to the relatively high lactose content of SMP. Many authors stated that, the fortification with milk powder can lead to excessive acidity (Tamime and Deeth, 1980; Lankes *et al.,* 1998 and Tamime and Robinson, 1999). The TA of yoghurt fortified by WPC fall in the second order followed by UF and NaCn, respectively.

The pH values of UF yoghurt were the highest followed by NaCn-, WPC-, and SMP-containing yoghurt. This could be due to that the colloidal salts in UF retentate are in the native form and remained active with respect to the buffering capacity. Therefore, at a given protein content, the UF yoghurt exhibited further buffering capacity *more than* other studied protein sources. Similar observations were reported by Schmutz and Puhan (1979); Mehaia and Cheryan (1983) and Haggag and Fayed (1988). Along storage period, all yoghurt samples exhibited gradual increase in the TA and hence decrease in pH values without any exception ($P<0.001$).

Acetaldehyde and diacetyl

Table (2): represent the acetaldehyde (AC) and diacetyl (DA) contents of yoghurt along storage period as affected by the level and source of protein used in yoghurt fortification.

Table (2): Acetaldehyde and diacetyl contents of yoghurt during storage as affected by the level and source of milk protein fortification

storage period (days)	Level and source of protein fortification												
	control	0.5%				1.0%				1.5%			
		SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC
	Acetaldehyde (µml/100 g)												
0	286.20	363.93	336.72	322.53	364.32	395.4	330.5	310.43	393.55	470.11	315.36	284.3	451.32
7	370.5	443.67	402.72	393.56	415.8	475.6	385.51	377.49	448.09	498.9	377.5	361.16	494.32
14	259.11	341.11	325.4	291.21	313.21	370.71	320.55	287.43	345.71	415.42	272.11	272.11	412.69
21	222.45	322.53	305.43	272.64	298.7	351.83	301.65	266.52	310.52	399.41	262.41	252.42	389.10
	Diacetyl (µml/100 g)												
0	13.88	15.49	17.00	11.91	14.80	16.68	16.56	11.46	15.38	19.80	17.52	11.91	18.53
7	14.30	16.20	18.48	12.09	15.86	18.09	19.09	11.68	17.26	20.75	19.58	12.16	19.01
14	15.01	18.50	22.16	16.18	17.66	20.99	22.36	15.14	19.61	23.07	22.36	14.15	20.81
21	14.90	16.82	19.93	13.95	16.86	19.61	20.29	13.19	18.27	21.73	20.59	13.10	19.55

The obtained results show that yoghurt fortification with protein led to significant increase in both AC and DC contents, however, the differences in AC contents between 0.5 and 1.0% fortified protein yoghurts were not significant.

Rasic and Kurmann (1978) confirmed that increased solids content achieved by milk çoncentration or by adding SMP favorably influence the production of AC.Also, Likewise Estevez *et al.* (1988) found that, level of AC was positively correlated to the level of milk solids of yoghurt.

These increments were actually dependent on the kind of protein source, which played a significant role and possessed effective factors for AC and DC production ($P<0.001$). Thereby, the statistical analysis confirmed that

SMP was the most protein source, which promoted AC and DC formation in yoghurt .

The high levels of lactose and ash associated with SMP promote aroma production in higher levels than those produced in UF-, WPC- or NaCn fortified yoghurt. Rasic and Kurmann (1978) reported that rate of AC production is highly dependent on acidity level. While, the NaCn fortified yoghurt exhibited the lowest figures in these respects. WPC-yoghurt took place the second order in both two properties, while UF-yoghurt had AC content lower than, and DA content similar to, these of WPC fortified yoghurt.

The development of AC continued up to 7th day of storage period, then gradual decrease was occurred as the storage period prolonged. Because of AC is a transitory component, its level tends to be reduced again. Rasic and Kurmann (1978) reported that a decrease of the AC content occurs during the storage of yoghurt. On the contrary, DA of yoghurt was increasingly produced until the 14th day of the storage period, then reduced again at the end of the storage period (21 days at 5±1°C), but still higher than the initial figures. This means that DA was more stable than AC. Similar findings were reported by El- Kenany (1996); and Mistry (2002).

Soluble nitrogen

Table (3): show the soluble nitrogen / total nitrogen of yoghurt during storage period as affected by the level and source of fortifying protein.

Table (3): Soluble nitrogen content of yoghurt during storage as affected by the level and source of milk protein fortification.

storage period (day)	control	Level and source of protein fortification								
		0.5%			1.0%			1.5%		
		SMP	NaCn	WPC	SMP	NaCn	WPC	SMP	NaCn	WPC
0	4.85	4.92	4.85	5.05	4.86	4.73	5.31	4.82	4.68	5.36
7	5.36	5.32	5.11	5.58	5.24	5.08	5.69	5.17	5.01	5.75
14	7.19	7.05	6.85	7.25	7.01	6.78	7.33	6.92	6.65	7.35
21	9.47	9.58	7.25	9.66	9.66	7.33	10.47	9.85	7.35	10.52

Data confirmed that gradual increase in soluble nitrogen was associated with the increase of storage period. Moreover, WPC as protein source gave the yoghurt with the highest soluble nitrogen value followed by SMP and NaCn respectively. The total amino acid content of yogurt does not differ substantially from milk but the free amino acid content is higher due to proteolytic activity of microorganisms (Rasic & Kurmann, 1978).Generally the soluble nitrogenous constituents increased with increasing in the storage period. El-Shibini et al. (1979) found the same trend,

Microbiological Analysis

LAB count is shown in Table (4) and presented in Figures (2 and 3) as log of colony forming unit (cfu) for *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in yoghurt during the storage period.

Table (4): lactic acid bacterial count (log cfu/g) of yoghurt during storage as affected by the level and source of milk protein fortification

storage period (days)	control	Level and source of protein fortification											
		0.5%				1.0%				1.5%			
		SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC
	Streptococcus thermophilus												
0	8.29	8.36	8.30	8.27	8.33	8.40	8.28	8.26	8.35	8.43	8.25	8.24	8.40
7	8.26	8.33	8.26	8.20	8.30	8.36	8.24	8.22	8.31	8.40	8.23	8.20	8.35
14	7.52	8.14	7.97	7.88	8.06	8.18	7.96	7.79	8.11	8.22	7.96	7.61	8.16
21	7.20	7.97	7.83	7.44	7.85	8.04	7.72	7.24	7.93	8.10	7.66	7.25	8.08
	Lactobacillus delbrueckii subsp.*bulgaricus*												
0	7.28	7.34	7.28	7.26	7.32	7.36	7.27	7.24	7.33	7.42	7.24	7.23	7.38
7	7.24	7.30	7.24	7.22	7.27	7.33	7.15	7.01	7.31	7.38	7.07	6.91	7.34
14	6.99	7.08	6.96	6.81	7.03	7.09	6.85	6.73	7.06	7.18	6.74	6.64	7.15
21	6.43	6.76	6.61	6.30	6.74	6.86	6.59	5.78	6.84	6.95	6.40	5.46	6.93

The data indicated that protein level, at which the yoghurt was fortified until 1% did not lead to any significant difference in *Str. thermophilus* count. However, significant increase was recorded when the protein fortification level raised there more. While, significant gradual increase ($P<0.001$) in *Lb. delbrueckii* subsp. *bulgaricus* count was positively correlated to the protein level of yoghurt. That might be due to some nutritional growth factors, which is introduced indirectly with the protein sources used for fortification, which perhaps met the nutritional requirement of such

Rasic and Kurmann (1978) reviewed that protein containing sulphur amino acids influenced favorably the growth of *Lb. delbrueckii* subsp. *bulgaricus* by lowering the oxidation / reduction potential (E_h) through increasing sulphydryl groups made available by heating.

Concerning protein sources, the fortified yoghurt with SMP gave the highest count of both strains of starter ($P<0.001$), followed by WPC and UF respectively. The NaCn yoghurts yielded the lowest counts in this respect. Similarly, Dave and Shah (1998) found that fortification of yoghurt with 2% WPC supported the growth of *Streptococcus thermophilus* and multiplication of this organism was faster, which could have been the reason for the shorter incubation time needed to reach pH of 4.5 for samples.

Along storage period, at the beginning, it could be noticed that, *Str.* count is always higher than that of *Lb. delbrueckii* subsp. *bulgaricus*, whether when yoghurt was fresh or at a certain storage period. Thereafter, gradual reductions in counts of both strains were recorded until the end of the storage period (21 days). The obtained results are in coincidence with those reported by Rasic and Kurmann (1978), who indicated that total count of viable yoghurt culture varied in range of 200×10^6 to 1000×10^6 cfu / ml of fresh yoghurt. During the storage of yoghurt at 5°C, the number of lactic acid bacteria decreases reaching 1×10^6 cfu/ ml.

Rheological profile

Data illustrated in Table (5) are the penetration values, those inversely indicating the firmness of set yoghurt as well as the consistency coefficient and yield stress of its stirred form during storage period as affected by the level and source of fortifying milk protein.

Table (5): Penetration values of set yoghurt as well as consistency coefficient and yield stress of its stirred type during storage period as affected by the level and source of milk protein fortification

storage period (days)	Level and source of protein fortification												
	control	0.5%				1.0%				1.5%			
		SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC
	Penetration (mm)												
0	28.0	27.0	24.5	25.0	25.5	26.0	24.0	24.3	24.6	25.6	23.6	24.0	24.4
7	27.0	26.5	23.8	24.3	24.8	25.7	23.5	24.0	24.3	24.8	22.8	23.5	23.8
14	26.6	26.0	23.5	24.0	24.2	25.2	23.0	23.6	23.9	24.3	22.0	23.0	23.4
21	25.8	25.2	23.0	23.5	23.8	24.8	22.5	23.2	23.2	24.0	21.5	22.5	22.8
	Consistency coefficient (dyne.sec./cm^2)												
0	13.76	14.87	18.8	17.9	15.66	17.35	19.9	18.98	18.95	18.8	21.5	20.77	19.46
7	16.50	16.6	19.3	18.4	17.45	18.25	21.4	20.01	19.21	19.31	22.47	22.0	19.74
14	18.9	18.5	20.91	20.4	19.72	19.1	22.0	21.6	20.23	20.0	23.0	22.45	21.27
21	20.9	20.15	21.5	21.2	21.06	20.30	22.5	22.0	21.59	21.49	23.5	22.78	22.70
	Yield stress (dyne/cm^2)												
0	26.83	58.67	96.04	77.26	60.178	76.02	132.71	97.33	88.65	84.16	185.77	118.77	95.05
7	55.05	64.96	103.42	85.37	71.22	84.44	179.02	124.47	98.24	109.29	224.1	202.49	110.63
14	90.61	96.59	155.5	141.13	107.98	104.86	203.34	186.86	121.11	121.35	244.6	221.71	135.55
21	122.04	112.02	182.79	168.2	126.11	125.5	224.34	200.78	139.87	132.01	280.22	239.01	142.65

Firmness of set yoghurt

As indicated from the data shown in Table (5), there are gradual strengthening in the set yoghurt firmness as the protein level raised and inversely, penetration values decreased ($P<0.001$). Prentice (1992) discussed the yoghurt fortification with dried milk powder, WPC, or concentration by UF, and confirmed that, whichever method was used, it was the increase of protein that was the principal factor influencing the texture. When milk powder is used for enrichment, a dense matrix of chains and aggregates of small casein micelles developed. With UF the casein strings were strengthened by the inclusion of more particles, which resulted in a greater stiffness of the network. Increasing the DM content from 12 to 15% resulted in an increase in the firmness of set yoghurt by a factor of nearly 2 . This forward relationship was found to be significantly dependent on the protein source ($P<0.001$). Where NaCn yoghurt exhibited the firmest body followed by that made by UF milk concentration. While SMP imparted the yoghurt the highest penetration value, i.e. the weakest firmness trait. Moreover, the prolonging of the cold storage period was associated with increasing in the yoghurt firmness ($P<0.001$) as reflected from the penetration values given in Table (5).

Consistency coefficient of stirred yoghurt

As appeared from the Table (5), similar to the firmness criterion of set yoghurt, the consistency coefficient, expressed from the descending flow curve of yoghurt to stand for stirred yoghurt, raised as the protein content of yoghurt increased ($P<0.001$). The increase rate was significantly depending on the protein source, which gave the highest value, when fortified with NaCn or concentrated by UF technique ($P>0.05$). SMP caused the lowest figures in this respect as similarly observed before in the firmness. Furthermore, the consistency coefficient increased gradually as the cold storage period of yoghurt progressed. These observations agree with those reported by Abrahamsen and Holmen (1981); Rhom and Schmid (1993); Vlahopoulu and Bell (1993); Fayed *et al.* (1996) and Tamime and Robinson (1999).

Yield stress of stirred yoghurt

As shown in Table (5) the yield stress of stirred yoghurt behaved trends similar to those of consistency coefficient, where it increased by increasing the protein level and was higher in NaCn fortified yoghurt, followed by that in UF yoghurt. While SMP yoghurts did not vary from those made using WPC ($P>0.05$). Furthermore, the yield stress of yoghurt gradually increased by duration of cold storage period. Similar findings were reported by Vlahopoulu and Bell. (1993) and Fayed *et al.* (1996) .

Organoleptic quality

Table (6) shows the judging scores of yoghurt during storage as affected by the level and source of protein.

Table (6): Appearance, consistency, flavor and total sensory scores of yoghurt during storage as affected by the level and source of milk protein fortification

storage period (day)	Level and source of protein fortification												
	control	0.5%				1.0%				1.5%			
		SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC	SMP	UF	NaCn	WPC
	Appearance score (out of 5 points)												
0	4.5	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.5	5.0	5.0	5.0	4.0
7	4.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.5	5.0	5.0	5.0	4.0
14	4.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.5	5.0	5.0	5.0	4.0
21	4.0	4.5	5.0	5.0	5.0	5.0	5.0	5.0	4.5	5.0	5.0	5.0	4.0
	Body and Texture score (out of 5 points)												
0	3.5	5.0	5.0	4.5	4.0	5.0	5.0	5.0	4.5	5.0	5.0	5.0	5.0
7	4.0	5.0	5.0	4.5	4.0	5.0	5.0	5.0	4.5	5.0	5.0	4.5	5.0
14	4.0	5.0	5.0	5.0	4.0	5.0	5.0	5.0	4.5	5.0	5.0	4.5	4.5
21	4.0	5.0	5.0	5.0	4.0	5.0	5.0	5.0	4.5	4.5	5.0	4.5	4.5
	Flavour score (out of 10 points)												
0	9	10	9	8	10	10	10	8	10	8	10	8	9
7	9	10	9	8	10	9	10	8	10	8	10	8	9
14	9	10	9	8	10	9	10	8	10	8	10	8	9
21	9	9	9	8	10	9	10	8	10	7	10	7	9
	Total scores (out of 20 points)												
0	17.0	20	19.0	17.5	19.0	20	20	18	19	18	20	18	18
7	17.0	20	19.0	17.5	19.0	19.0	20	18	19	18	20	17.5	18
14	17.0	20	19.0	18.0	19.0	19.0	20	18	19	18	20	17.5	17.5
21	17.0	19.0	19.0	18.0	19.0	19.0	20	18	19	16.5	20	16.5	17.5

Regarding the yoghurt appearance, the results revealed that yoghurt fortification with protein at any level starting from from 0.5% led to prevent the wheying off observed in the control sample. Full marks were given to the appearance of all protein fortified yoghurt regardless its level, whether being 0.5% or more (P>0.05), except of that fortified with WPC, where slight yellowness was recorded for samples containing 1% WPC, and the color developed to be pronounced yellowness as the WPC level raised.

The appearance's score decreased significantly along the first two weeks of storage (P<0.001) because some syneresis were remarked, especially in the control sample. While the differences between the last two weeks in appearance's score were not significant (P>0.05). Concerning the consistency score, data indicated that, the sensory consistency improvement had began to be noticed at the starting protein fortification level, (0.5%), while the increasing of the level to 1% did not lead to any further enhancement in the body and texture of yoghurt. With progressing the protein fortification level to 1.5%, the consistency score decreased but remained higher than of the control (0.0% protein fortification). Different reasons were recorded depending on the protein source (P<0.001). The addition of 1.5% NaCn-protein resulted in too firm yoghurt body. While WPC caused relatively weak body when was added to yoghurt at the level of 0.5%, although the score of yoghurt body raise as the WPC level increased. Statistical analysis demonstrated that SMP- containing yoghurt was in the consistency as good as that fortified by UF concentration (P>0.05) followed by WPC and NaCn, respectively. Nevertheless, the changes in yoghurt consistency occurred due to prolong storage period, were sensory insignificant (P>0.05).

The palatability of yoghurt was the best when it was fortified with 1.0% protein regardless its sources, followed by 0.5 and 0.0% (control). While, those supplemented with 1.5% protein from any protein source studied have gained the lowest flavor score.

Moreover, among the protein sources, UF concentration or WPC fortification imparted yoghurt the highest flavour score (P>0.05) followed by SMP and NaCn, respectively.

Furthermore, the yoghurt samples kept their palatability unchanged until the 2nd week of storage period. Then slight reduction in flavour score at the end of the storage period (21 days). The over acid taste was the main observed defect in cold stored samples fortified with SMP. Similar observations were reported by Tamime and Deeth (1980); Tamime and Robinson (1999) and Lankes *et al.,* (1998).

Generally, the total sensory score given in Table (6) confirm that, a superior yoghurt quality could be yielded when it is made from the milk fortified with 0.5 or 1.0% protein, especially by means of UF technique to ensure a stable organoleptic quality along two weeks at refrigeration storage. Fortification with SMP came in the second order in this respect.

Finally, the foregoing findings led obviously to the conclusion that, yoghurt could be successfully made from milk fortified with at least 0.5% protein, especially by means of UF concentration.

CONCLUSION

The results indicated that titratable acidity (TA), acetaldehyde (AC) and diacetyl (DA) contents as well as the firmness (Fr), as inversely indicated from the penetration value, consistency coefficient (CC) and yield stress (YS) of yoghurt raised as the protein fortification level increased, by which the reduction in pH values were delayed. SMP-fortified yoghurt had the highest contents of TA, AC and DA and the lowest pH value, followed by WPC, UF and NaCn, which caused the greatest Fr and YS followed by UF. Proportional increases in TA and all rheological parameters were determined, while pH value decreased continuously until the end of SP. The increment in the AC and DA contents retreated after the 7^{th} and 14^{th} day respectively. Gradual increment in *Streptococcus. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* counts associated with protein elevation level was determined. SMP in yoghurt promoted the highest viability conditions for both strains followed by WPC, UF and NaCn. The duration of storage period (SP) for 21 days led to gradual reduction in counts of both strains. In conclusion, the yoghurt with increased 1.0% protein by UF, attained the highest total sensory scores, and was kept with stable sensory quality until the 14^{th} day and stilled acceptable until the end of SP.

REFERENCES

Abrahamson, R.K. and T.B. Holmen (1981). Goat's milk yoghurt made from non-homogenized and homogenized milk, concentrated by different methods. J. Dairy Res., 48: 457-463.

Akalın , A.S.; Unal , G.; Dinkci; N.; and Hayaloglu, A.A. (2012). Microstructural, textural, and sensory characteristics of probiotic yogurts fortified with sodium calcium caseinate or whey protein concentrate. J. of dairy Science, Volume 95, Issue 7, Pages 3617–3628.

AOAC (2007). Association of Official Analytical Chemists. Official Method of Analysis. (18^{th} Ed.), pp. 302-850. Benjamin Franklin Station Washington, D.C., USA.

Beshkova D. M.; ED,Simova ; G.I. Frengova ; Z.I. Simov and E.F. Adilov. (1998) Production of amino acids by yogurt bacteria. Biotechnol. Prog.14:963–965.

Bourne, M.C., (1982). In "Food Texture and Viscosity Concept and Measurement". Academic Press Inc., New York, USA.

Codex Stan 243-2003 (2003). Codex standard for fermented milks.

Dave, R. I and N.P. Shah (1998). Ingredient supplementation effect on viability of probiotic bacteria in yoghurt. J. Dairy Sci, 81: 2804-2816.

El-Kenany, Y.M. (1996).Properties of cultured buffalos cream manufacture with different starter cultures. Ann. of Agric. Sc., Moshtohor, 34: 285-299.

El-Shibini ,S. ; H.F. El-Dein and A. A. Hofi (1979) Effect of storage on the protein of zabadi. Egyptian J. Dairy Sci. 7:9-16.

Estevez, C.R.; A. Goicoechea and S. Jimenez-Perez (1988). Aroma development in yoghurt made by different methods. Deutsche Molkerei- Zeitung, 109: 733-735.

Fayed, A.E. (1986). Protein fortification of some dairy products. Ph.D. Thesis, Fac. of Agric. Ain Shams Univ., Egypt.

Fayed, A.E.; I.M. Roushdy and A.I. Metwally (1996). Physical and Organoleptic properties of yoghurt made from standardized buffalo's milk fortified with total milk protein. 6^{th} Conf. Agric. Dev. Res., Ain Shams Univ., Cairo, Dec. 17-19. Ann. Agric. Sci., Sp. Issue, 109-124.

Gaudichon ,C. ; N. Roos ; S. Mahé; H. Sick ; C. Bouley and D. Tomé (1994) Gastric emptying regulates the kinetics of nitrogen absorption from ^{15}Nlabeled milk and ^{15}N-labeled yogurt in miniature pigs. J Nutr;124:1970–1977.

Gaudichon,C, S. Mahé; N. Roos ; H. Sick ; C. Bouley and D. Tomé (1995) Exogenous and endogenous nitrogen flow rates and level of protein hydrolysis in the human jejunum after [^{15}N] milk and [^{15}N] yogurt ingestion. Br J Nutr;74:251–60.

Gianelli, M.P.; M. Flores, and F. Toldra (2005) Interaction of soluble peptides and proteins from skeletal muscle with volatile compounds in model systems as affected by curing agent J. Agric. Food Chem 53:1670-1677.

Green, M.L. ; F.A.Glover ; E.M.W. Scurlock ; R.J. Marshall and D.S. Hatfield (1981). Effect of use of milk concentrated by ultrafiltration on the manufacture and ripening of Cheddar cheese. J. Dairy Res., 48: 333-341.

Gueimonde, M.; L. Alonso; T. Delgado; J.C. Bada-Gancedo; C. Reyes-Gavilan (2003). Quality of plain yoghurt made from refrigerated and CO_2- treated milk. Food Res. Int., 36: 43-48.

Haggag, H.F. and A.E. Fayed (1988). Production of Zabady from ultrafiltered buffalo's milk. Food Chem., 39: 29-36.

Hewitt , D. and H.J. Bancroft. (1985) Nutritional value of yogurt. J Dairy Res. 52:197–207.

Kuehn ,J.; T. Considine and H. Singh (2006) Interactions of milk proteins and volatile flavor compounds: Implications in the development of protein foods J. Food Sci. 71:R72-R82

Land, D.G. (1996) perspectives on the effects of interactions on flavor perception :an overview. In McGorrin, R.J.and Leland, J.V. Eds Flavor food interactions, American Chemical Soc., Washington D.C. pp. 2-11.

Lankes, H.; H.B. Ozer and R.K. Robinson (1998). The effect of elevated milk solids and incubation temperature on the physical properties of natural yoghurt. Milchwissenschaft, 53: 510-513.

Lees, G. J. and G. R. Jago (1969). Methods for the estimation of acetaldehyde in cultured dairy products. Aust. J. Dairy Technol., 24: 181-185.

Lees, G. J. and G. R. Jago (1970). The estimation of diacetyl in the presence of other carbonyl compounds. J Dairy Res., 37: 129-132.

Loones A. (1989)Transformation of milk components during yogurt fermentation. In: Chandan RC, ed. Yogurt: nutritional and health properties. McLean, VA: National Yogurt Association,:95–114.

Maubois, J. L ; G. Moquost; J. L . Thapon ; M.C. Humilier ; A.Chopin; H.Gouded ranche; C. Dupas; E. Blance-patin; M. Piot and J. Fauqant (1971). Preparation of chees from a concentrate obtained by ultrafiltration of milk. Lait, 51: 495-533.

Mehaia, M. A and M. Cheryan (1983). Coagulation studies of ultrafiltration – concentrated skim milk. Milchwissenschaft, 38: 708-710.

Mistry, V.V. (2002). Manufacture and application of high milk protein powder. Lait, 82: 515-522.

Prentice, J. H. (1992).In "Dairy Rheology A concise Guide". VCH Pub., Inc., New York, USA.

Rasic, J.L. and J.A. Kurmann (1978). In "yoghurt, Scientific Grounds, Technology, Manufacture and Preparations". Tech. Dairy Pub. House, Copenhagen, Denmark.

Rhom, H. and W. Schmid (1993). Influence of dry matter fortification on flow properties of yoghurt. 1. Evaluation of flow curves. Milchwissenschaft, 48: 556-559.

SAS, (1996). Statistical Analysis System. SAS user's guide. Statistics. SAS Inst. Inc. Ed., Cary, NC, USA.

Schmutz, M and Z. Puhan (1979). Rennet coagulation of ultrafiltrated milk. Deutsche Molkerei-Zeitung, 100: 254-258.

Semenova, M.G.; A.S. Antipova ; T.A. Misharina and R.V.Golovnya (2002) Binding of aroma compounds with legumin. Food Hydrocolloids 16:557-584

SPSS (1998). Statistical Package for Social Science SAPSS Inc., 444 North Michingan Avenue, Chicago, Illinois 60611, USA.

Tamime, A.Y. and H.C. Deeth (1980). Yoghurt: Technology and biochemistry. J. Food Prot., 43: 939- 977.

Tamime, A.Y. and R.K. Robinson (1999). Yoghurt, "Science and Technology". 2nd Ed. CRC press wood lead pub. Limited, Cambridge, England.

Toledo, T.T.(1980). In "Fundamental of Food Process Engineering". AVI pub. Co. West port, Connecticut, USA.

Vlahopoulu, I. and A.E. Bell (1993). Effect of various starter culture on the viscoelastic properties of bovine and caprine yoghurt gel. J. Soc. dairy Technol. 46: 61-63.

Wilson, C.E. and W.E. Brown (1997) Influence of food matrix structure and oralbreakdown during mastication on temporal perception of flavor .J. Sens Stud. 21:69-86.

خواص الجودة لليوجهورت المدعم بالبروتين

عثمان عبد العظيم عيطة ، يارا عبد اللطيف حسين ، عاطف السيد فايد و محمد عبد الرازق النواوى
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استهدف البحث تدعيم اللبن البقري ببروتين اللبن بنسب مختلفة ومصادر مختلفة ودراسة أثر ذلك على الخواص البيوكيميائية و البكتريولوجية و الريولوجية بالإضافة الى الحسية. حيث تم تدعيم اللبن البقري بنسب بروتين ٠,٥%، ١,٠%، ١,٥% بإضافة مساحيق اللبن الفرز ، أو كازينات أو الصوديوم ، أو مركز بروتينات الشرش، أو بتركيز اللبن باستخدام تقنية الترشيح الفائق لرفع نسبة البروتين بنفس المعدلات السابقة.

ولقد أوضحت النتائج أنه بزيادة نسبة البروتين المضاف زاد محتوى اليوجهورت الناتج من الحموضة و الاسيتالدهيد والداي استيل مع انخفاض قيم pH ، و تحسنت الخواص الريولوجية من حيث صلابة اليوجهورت المتماسك و معامل القوام، وجهد القص الإبتدائي لليوجهورت المقلب بزيادة تركيز البروتين. وقد كانت العينات المدعمة بنسبة ٠,٥% بروتين هى الأفضل من حيث المظهر بينما حصلت العينات المدعمة بنسبة ١,٠% بروتين على الأفضلية من حيث القوام ومجموع درجات التقييم الحسي.

و قد احتوت العينات المدعمة باستخدام كازينات الصوديوم على القيم الأقل من حيث الحموضة و الاسيتالدهيد و الداي استيل بالاضافة للخواص الحسية من حيث درجات القوام والنكهة ودرجات التحكيم الكلية. بينما حصلت على أعلى القيم من حيث الصلابة ومعامل القوام وقيم جهد القص الابتدائي. في حين تساوت القيم للعينات المدعمة بتركيز اللبن بالترشيح الفائق مع العينات المدعمة باستخدام اللبن الفرز المجفف من حيث الحصول على أعلى درجات القوام والنكهة وكان هناك أفضلية للعينات المدعمة بتركيز اللبن بالترشيح الفائق من حيث الخواص الحسية الكلية.

أما بالنسبة لتأثير التخزين على الخواص السابقة فقد صاحب زيادة مدة التخزين حتى ٢١ يوم زيادة في نسبة الاسيتالدهيد (حتى اليوم السابع) و نسبة الداي استيل (حتى اليوم الرابع عشر) والقيم الخاصة بالصلابة ومعامل القوام وجهد القص الابتدائي، وكذلك الدرجات الخاصة بالمظهر. ولم تتغير الدرجات الخاصة بالقوام. بينما انخفضت درجات النكهة بنهاية مدة التخزين.

و من النتائج المتحصل عليها يتضح أن الأفضلية كانت للعينات مدعمة البروتين بنسبة ٠,٥% على الأقل بواسطة تركيز اللبن بالترشيح الفائق.