The Use of Whey Protein Concentrate and Whey Protein Isolate in The Manufacture of Low Fat Yoghurt

Abbas, F.M.
Dairy Dept., Fac. of Agric., Suez Canal Univ., Ismailia, Egypt.

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Abstract: *Whey Protein Concentrate (WPC) (35% protein in dry matter) and Whey Protein Isolate (WPI) (91.5% protein in dry matter) were used individually at different levels to substitute non fat dry milk in yoghurt manufacture. Addition of WPC reduced the viscosity and consistency, enhanced the buffering capacity and reduced the curd syneresis of the resultant yoghurt. Addition of WPI improved the viscosity and other rheological properties of the resultant yoghurt than yoghurt containing WPC. However, WPI reduced the buffering capacity of yoghurt in alkaline region and reduced the curd syneresis. Yoghurt containing WPC gained less score points for body and textures while that containing WPI treatments obtained nearly the same score points for body and textures as control treatment particularly at yoghurt containing 1.57g WPI/100g.*

***Key words**: whey protein concentrate, whey protein isolate, yoghurt, rheological properties.*

INTRODUCTION

In recent years, milk constituents have become recognized as functional ingredients, suggesting that their consumption has direct and marked effects on health (Gill *et al.*, 2000). Milk contains two major group of proteins; i.e. the caseins and whey proteins. Whey proteins include β-lactoglobulin, α-lactoalbumin, bovine serum albumin, lactoferrin, immunoglobulins. Today whey protein products are popular dietary protein supplements purported to provide antimicrobial activity, immune modulation, improved muscle strength and body composition, and to prevent cardiovascular disease and osteoporosis.

Advances in processing technology, including membrane filtration and Ion Exchange techniques have resulted in development of different finished whey protein products, particularly whey protein concentrates (ranging from 25-85 percent protein) and whey protein isolate (90-95% protein). Relative to other protein sources, whey proteins have high concentration of branched chain amino acids- leucine, iso leucine and valine (Marshall, 2004). These amino acids, particularly leucine, is important factors in tissue growth and repair, leucine has been identified as a key amino acids in protein metabolism during the translation – initiation pathway of protein synthesis (Daenzer *et al.*, 2001). Whey proteins are also rich in the sulfur containing amino acids; cysteine and methionine. With a high concentration of these amino acids, immune function is enhanced through intracellular conversion to glutathione.

The whey proteins were used to enrich yoghurt (Ahmed & Ismail, (1978); Marshall *et al.*, (1982); Abou-Dawood *et al.*, (1984); Guirguis *et al.*,(1984); Abd Rabo *et al.*, (1988); Dannenberg and Kessler (1988); Mehanna and Gone (1988); Abd El-Salam *et al.*,(1990); Hofi *et al.*, (1994); Tamime *et al* (1995)., Fenelon *et al.*, (2000).

Therefore the aim of the present study was to evaluate the possibility of making a functional yoghurt with a number of health benefits from cow's milk fortified with whey protein concentrate or whey protein isolate and to investigate the effect of the rate of replacing non-fat dry milk with both types of whey proteins on the chemical, rheological and organoleptic properties of yoghurt.

MATERIALS AND METHODS

Yoghurt production:

Yoghurt was made according to the formulations given in (Table 1). Commercial whey protein concentrate (WPC 35% protein) was obtained from Misr Milk and Food Company, (Mansoura) and whey protein isolate (WPI 91.5% protein, manufactured and formulated by Bio-Pharma Co. A.R.E). WPC and WPI were used in partial or complete replacement of skim milk powder (Holand) used in the manufactured of control yoghurt (2%fat, 13% S.N.F.).

The milks were then pasteurized 72 °C/5 min and cooled to 40°C .One gram of direct set frozen yoghurt starter (*S. thermophilus* + *L. delbrueckii* subsp. *bulgaricus*)(obtained from CHR-Hansen's, Denmark) was incubated for 1h in 1L of sterilized skim milk at 40°C . Prepared culture was inoculated at the ratio of 50 ml per 1L of yoghurt milk. The milks were then incubated at 43°C until clotting (≈ 3h). Yoghurt were stored at about 5°C and evaluated after 1,5,10 and 15 days. The whole experiment was repeated three times.

Methods of analyses:

The fat, protein and dry matter contents and acidity of milk and other ingredients used in yoghurt manufacture were analyzed by standard methods (Marshall, 1992). During storage, yoghurt samples were checked for pH (Jenway pH meter electrode No. 29010, Jenway limited, England).

Buffer capacity:

Buffer capacity of yoghurt was measured according to Martini *et al.* (1985). Buffer capacity lower than the initial pH (4.1 to 2.0) of yoghurt was obtained by

Table (1): Composition of low fat yoghurt milks containing different levels of whey protein products (g/100g).

Treatments / Ingredients	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Standardized cow's milk (2.1% fat, 9% S.N.F)	95.3	95.3	95.3	95.3	95.3	95.3	95.3
Skim milk powder	4.70	3.13	1.57	0.00	3.13	1.57	0.00
WPC	0.00	1.57	3.13	4.7	0.00	0.00	0.00
WPI	0.00	0.00	0.00	0.00	1.57	3.13	4.7
substitute ratio	0.00	33.3	66.6	100	33.3	66.6	100

T_0: yoghurt milk containing standardized cow's milk+ skim milk powder(control)
T_1: yoghurt milk containing standardized cow's milk and WPC substituted 33.3% of the skim milk powder
T_2: yoghurt milk containing standardized cow's milk and WPC substituted 66.6% of the skim milk powder
T_3: yoghurt milk containing standardized cow's milk and WPC substituted 100% of the skim milk powder
T_4: yoghurt milk containing standardized cow's milk and WPI substituted 33.3% of the skim milk powder
T_5: yoghurt milk containing standardized cow's milk and WPI substituted 66.6% of the skim milk powder
T_6: yoghurt milk containing standardized cow's milk and WPI substituted 100% of the skim milk powder

titrating diluted yoghurt sample (10g sample diluted with 10.0g of distilled water) with 1 N HCl. Half ml increments of 1 N HCl were added to the sample, mixed thoroughly and pH values were recorded. Similarly, buffer capacity values above the initial pH (4.1 to 8.2)of yoghurt were determined by titrating with 1 N NaOH.

Syneresis:

The susceptibility of yoghurt to syneresis was measured by the following two methods:

A- Drainage method reported by Lorenzen *et al.*(2002) where 30g of set-style yoghurt were put on an analysis sieve, placed on the top of a glass beaker by the help of an ice cream scoop. The weight of drained whey after 2 h at 6 °C was related to the initial weight of yoghurt and expressed in percent.

B- Centrifugation method: 50g of set-style yoghurt was centrifuged using IEC PR-700 Centrifuge, USA at 2500 X g for 5 min. The weight of supernatant whey was determined and expressed in percent.

Rheological Properties:

To determine the rheological properties (Viscosity, Consistency and flow index), yoghurt samples were stirred for 5 min to achieve visually homogeneous slurry (Lankes *et al.*, 1998) before measurements. The rheological properties were measured using a Brookfield viscometer (Brookfield Engineering Laboratories, Inc., MA, USA), equipped with a SC4-21spindle running at 25 rpm. Measurements were made in the temperature of 24° C in shear rate ranging from 23.3 to 232.5 S^{-1}.

Organoleptic Properties:

Organoleptic assessment of yoghurt samples included flavour (10 points), Body and texture (10 points), and colour and appearance (5 points). The organoleptic attributes were rated using 25-point scale by panelists from the staff of the Dairy Department, Suez Canal University.

RESULTS AND DISCUSSION

Changes in acidity and pH during storage of yoghurt:

It was noticed that WPI yoghurt (T4, T5, and T6) had relatively high titratable acidity values than WPC yoghurt or control which can be attributed to the high protein content in these treatments which would affect the titratable acidity .In addition, acidity of all yoghurt samples increased gradually along the cold storage to reach maximum values after 15 days. The changes in pH values were negatively related to the changes of titratable acidity.

One day old yoghurt had pH values ranged from 4.4-4.6 in all yoghurt samples. in the 5 th day of storage, the pH did not change and after 15 days the pH dropped slightly to 4.0- 4.1.Hence, the pH of the yoghurt remained almost stable (≈ 4.0) during the 15 days of storage which could be attributed to high buffering capacity of yoghurt (Kailasapathy and supriadi; 1996 and Kailasapathy *et al.* 1996).

Buffering capacity:

All yoghurt samples showed high buffer capacity in acidic conditions (Table 3). The 1N HCl titrant needed to decrease pH from 4.1 to 2.0 was higher than the 1N NaOH titrant needed to increase pH from 4.1 to 8.2. The quantity of 1N HCl titrant or 1N NaOH used in this study was nearly the same that found by Kailasapathy and Supriadi (1996). Addition of WPC or WPI decreased slightly the buffering capacity of yoghurt in the alkaline region.

Generally yoghurt samples showed low buffering capacity in alkaline conditions (Table 2). Although, the addition of WPI to yoghurt milk increased protein concentration, the obtained yoghurt had lower buffer capacity than the control in alkaline condition, that can be attributed to lower buffering power of WPI compared to casein. at high pH.

Table (2): Acidity% and pH values of low fat yoghurt with different levels of whey protein products during cold storage.

Periods \ Treatments	T_0	T_1	T_2	T_3	T_4	T_5	T_6
				Acidity%			
1 day	0.80	0.82	0.82	0.83	0.82	0.85	0.90
5 days	0.85	0.85	0.85	0.84	0.85	0.87	0.90
10 days	0.94	0.86	0.86	0.86	0.90	0.93	0.95
15 days	1.10	1.08	0.92	0.96	1.10	1.14	1.20
				pH			
1 day	4.5	4.5	4.6	4.5	4.4	4.4	4.4
5 days	4.5	4.5	4.6	4.5	4.4	4.4	4.4
10 days	4.2	4.4	4.4	4.4	4.3	4.3	4.3
15 days	4.1	4.1	4.0	4.0	4.0	4.0	4.0

Table (3): Buffering capacity of low fat yoghurt with different levels of whey protein products during the cold storage in acidic and alkaline conditions during cold storage.

Periods \ Treatments	T_0	T_1	T_2	T_3	T_4	T_5	T_6
			In acidic condition(ml 1N HCl)				
1 day	2.00	1.95	2.08	2.00	2.00	2.10	2.00
5 days	2.10	2.00	2.11	2.10	2.05	2.15	2.11
10 days	2.14	2.10	2.18	2.15	2.15	2.13	2.18
15 days	2.20	2.16	2.18	2.13	2.20	2.22	2.22
			In alkaline condition (ml 1N NaOH)				
1 day	1.80	1.77	1.78	1.80	1.70	1.71	1.72
5 days	1.81	1.75	1.81	1.80	1.72	1.74	1.75
10 days	1.87	1.80	1.83	1.84	1.73	1.72	1.74
15 days	1.87	1.84	1.84	1.85	1.73	1.74	1.75

Syneresis:

The syneresis values (Table 4) measured by either the drainage or centrifugation methods showed yoghurt made from pasteurized milk without WPC or WPI (T0) (control treatment) exhibited slightly higher syneresis than that made with WPC. Using of WPC in production yoghurt resulted a product with soft body and low syneresis.

Addition of WPI (T4) resulted in yoghurt of similar syneresis to control treatment. Further decreases in syneresis were obtained by increase the levels of WPI. These results agreed with those reported by Morrise *etal.*, (1995) who found that syneresis is reduced when milk for yoghurt is fortified with 4% WPC compared to 4% skim milk powder . Fenelon *etal.*, (2000) reported that decreasing the ratio of casein to whey protein in the milks decreased the level of syneresis.

Rheological properties:

Rheological parameters (viscosity, consistency index and flow behaviour index) of yoghurt samples during storage at 5°C are presented in Table (5).

Wide differences can be noticed between control treatment (T0) and that from other treatments. The viscosity data (Table 5) illustrate that control yoghurt (T0) showed higher viscosity than yoghurt from other treatments. Addition of WPC in yoghurt manufacture decreased its viscosity due to decreased casein content of milk used, while addition of WPI in yoghurt manufacture improved its viscosity probably because of the high protein content of the yoghurt, Modler and Kalab (1983) reported that yoghurts fortified with skim milk protein often had firm gels, but yoghurts fortified with WPC tended to be smoother. Also Puvanen *et al.*, (2002) reported that as the casein to whey protein ratio was decreased the gels were generally less viscous. The consistency index of yoghurt from different treatments showed similar trend to viscosity while flow behaviour index was inversely related. The determined flow behaviour indexes in all yoghurt were low suggesting pseudoplastic fluids (Abou El-Nour *et al.*, 2004).

Organoleptic properties:

The average results of the organoleptic assessment of different types of yoghurt in terms of flavour, body and texture and appearance are show in Table (6).

Yoghurt made with the use of WPC (T1, T2 and T3) received the lowest scores for appearance compared to other treatments, while control yoghurt was given the highest score. On the other hand, samples made from milk containing WPI received in between scores.

The flavour scores of yoghurt made with WPI (T4, T5 and T6) were nearly the same. These treatments were ranked higher scores than control or WPC treatments. Whey proteins are bland in flavour compared to skim milk, which have less tendency to mask flavour. Also the salt content of WPC had been linked to off flavours when WPC low in protein content was added to yoghurt (Hugunin 1999).

The effect of adding WPC was more apparemt on body and texture of yoghurt, as yoghurt from treatments T1, T2 and T3 received low scores for body and texture. Furthermore when the level of WPC was increased score was decreased. The yoghurt made from WPI received in between scores. Control yoghurt was the only product that received higher score for body and texture.

As a conclusion, addition of WPC /WPI in yoghurt manufacture had no advantage over the use of skim milk powder supplement expect in case of replacing one third skim milk powder with WPI . However addition of WPC /WPI may more advantage in drinking yoghurt formulation due lower viscosity compare to casein.

Table (4): Synersis of low fat yoghurt with different levels of whey protein products during cold storage using drain and centrifuge methods.

Periods / Treatments	T_0	T_1	T_2	T_3	T_4	T_5	T_6
			Drain method (ml/100 g yoghurt)				
1 day	33	34	30	31	33	32	31
5 days	34	34	29	31	33	32	32
10 days	34	35	32	30	34	33	33
15 days	35	36	32	30	34	33	33
			Centrifuge method (ml/100 g yoghurt)				
1 day	36	32	25	20	30	30	28
5 days	34	33	25	23	32	28	26
10 days	36	38	30	25	29	28	28
15 days	40	38	30	28	30	26	28

Table (5): Rheological characteristics of low fat yoghurt with different levels of whey protein products during cold storage.

Periods / Treatments	T_0	T_1	T_2	T_3	T_4	T_5	T_6
			Plastic viscosity (m Pa.S)				
1 day	2061	1017	506	350	1648	1842	1855
5 days	2488	1060	808	356	1855	2175	2134
10 days	2893	1378	808	425	2745	2389	2195
15 days	3282	1431	908	467	2762	2562	2488
			Consistency index (m $Pa.S^n$)				
1 day	1074	663	349	153	1181	1065	859
5 days	1370	693	386	153	1248	1123	1026
10 days	1455	790	404	162	1447	1297	1174
15 days	1778	959	469	202	1494	1418	1412
			Flow index (n)				
1 day	0.45	0.48	0.51	0.58	0.48	0.46	0.46
5 days	0.42	0.46	0.49	0.54	0.48	0.41	0.42
10 days	0.38	0.44	0.45	0.52	0.34	0.38	0.39
15 days	0.31	0.44	0.43	0.52	0.32	0.35	0.36

Table (6): Organoleptic characteristics of low fat yoghurt with different levels of whey protein products during cold storage.

Treatments / Periods	T_0	T_1	T_2	T_3	T_4	T_5	T_6
				Flavour (10 points)			
1 day	9	8	7	7	9	9	9
5 days	8	8	7	6	8	8	8
10 days	8	7	6	6	8	8	8
15 days	6	6	5	5	6	6	6
				Body and texture (10 points)			
1 day	8	4	4	3	6	6	7
5 days	9	5	4	3	7	7	8
10 days	9	5	5	4	8	7	8
15 days	9	7	5	4	8	8	8
				Colour and appearance (5 points)			
1 day	5	4	3	3	5	5	5
5 days	4	4	4	3	5	5	5
10 days	4	4	3	3	5	5	5
15 days	4	3	3	3	4	4	4

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استخدام بروتينات شرش المركزة و المعزولة كل على حده فى صناعة اللبن الزبادى منخفض الدهن

فوزى محمد عباس
قسم الألبان - كلية الزراعة - جامعة قناة السويس – الإسماعيلية – مصر

ينظر حاليا الى مكونات اللبن خاصة البروتين على أنها مكونات وظيفية لما لها من تأثير مباشر على الصحة العامة فاللبن يحتوى على نوعين من البروتين هما الكازين وبروتينات الشرش . وبالمقارنة بأنواع البروتينات الاخرى فإن بروتينات الشرش تحتوى على تركيزات عالية من الاحماض الامينية المتشعبة والتى تعتبر ضرورية لبناء وتجديد الانسجة وأيضا تعتبر بروتينات الشرش غنية بالكبريت لاحتوانها على نسبة عالية من الاحماض الامينية الكبريتية مما يعطيها خواص مناعية لتحول هذه الاحماض الكبريتية داخليا الى جلوتاثيون .

وفى هذه الدراسة تم إحلال بروتينات الشرش المركزة (٣٥٪ بروتين) وبروتينات الشرش المعزولة (٩١,٥ ٪ بروتين) كل على حده محل لبن فرز مجفف فى اللبن المعد لصناعة الزبادى بمستويات إضافة صفر ، ١,٥٧، ٣,١٣ ، ٤,٧ جم / ١٠٠ جم لبن . ووجد أن اضافة بروتينات الشرش المركزة أدت الى زيادة السعة التنظيمية مع ضعف الخواص الريولوجية للزبادى الناتج وبزيادة مستوى الإضافه أدى الى نقص فى اللزوجة والصلابة وحصل الزبادى الناتج على أقل درجات للقوام عن باقى المعاملات .

وبالنسبة لبروتينات الشرش المعزولة فقد أدت اضافتها الى خفض السعة التنظيميه للزبادى الناتج خاصة فى الظروف القاعدية وتحسنت الخواص الريولوجية عن المتحصل عليها سابقا وكادت تقترب من الكنترول عند مستوى إضافه ١,٥٧ جم / ١٠٠ جم لبن.