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Research Article

Physicochemical, Sensory Properties of Yoghurt High-Protein Using Whey Protein Isolate

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Abstract

Background and Objective: Recent years have seen a growth in the demand for high-protein yoghurt, which may be driven partly by enhanced the product's flavour and texture and the availability of scientific evidence about the health advantages of dairy protein. Yoghurt's protein level was boosted using a mixture of whey protein isolate (WPI) and buffalo milk (BM). This study aimed to determine whether adding whey protein isolate (WPI) to Buffalo milk (BM) would enhance the quality of the resulting high-protein yoghurt. **Materials and Methods:** The total solids content of a yoghurt mix made from buffalo milk and substituted with WPI was 15%, and varied substitution ratios of 0, 20, 40, and 60% WPI. Yoghurts were fermented at 42 C to a pH of 4.6 and then stored at 4 C overnight. **Results:** Nutritional value, physicochemical parameters, and sensory acceptability of the yoghurt samples were investigated. Yoghurt's flavour were enhanced due to WPI, which boosted the formation of diacetyl and acetaldehyde and increased protein content to 10.34% from the control. Viscosity was increased significantly by 60% WPI substitution. Finally, 40% substituted WPI, potentially a natural and economically ratio for making high-protein fermented dairy food highly acceptable.

Keywords: Whey protein isolate, Yoghurt high-protein, Viscosity, Sensory evaluation

INTRODUCTION

High-protein yoghurts and fermented milk have been consumed for centuries in many nations. Named differently, for instance, Torba (Turkey), Ymer (Denmark), Labneh (Eastern Mediterranean), and Chakka (India) are all types of strained or concentrated fermented milk [1]. Yoghurt, a milk-based mix fermented by lactic acid bacteria, is valuable health food for both young and old. Yoghurt proteins are partially hydrolyzed, increasing their availability. Compared with cheeses, whey proteins (mainly α-lactalbumin and β-lactoglobulin) remain in yoghurt. All of these factors contribute to the increased nutritional value of yoghurt [2]. Denaturation of the whey proteins can result in the exposure of reactive amino acid side groups that are normally buried within the native conformation [3] In yoghurt, the denaturation of whey proteins contributes to the overall quality of the final product. Still, that final quality is contingent not only on the whey proteins denaturing but also on their subsequent interactions with casein in the yoghurt mix [4]. The range of total solids in commercial yoghurt is 9 to 30% [5]. The relationship between food and health has been established, and studies have shown that food can reduce some risk factors that affect health. Prevention of disease in the future will be just as important as treatment of diseases today, and many consumers are highly aware of the health properties of foods. The market for health-promoting food is showing promising growth with an annual increase of 7% [6]. With the growing interest in weight wellness, the desire for "clean label" products, and the rising popularity of high-protein yoghurt, this trend is predicted to continue [7]. Due to whey proteins' potential to boost plasma amino acids [8] and induce muscle protein synthesis, high-protein yoghurts with a high amount of whey proteins may be useful in baby, elderly, or sports nutrition [9]. Protein has a stronger influence on satiety than fat or carbohydrate on energy intake. Hence, high-protein yoghurts may be useful in calorie-restricted diets [10]. Different ratios of whey protein to casein may be used to create a variety of high-protein yoghurts .

This work evaluates the approach to supplementing yoghurt with whey protein Isolate combined to produce a yoghurt. Thus, investigates the sensory acceptability and physicochemical properties of yoghurt.

MATERIAL AND METHODS

Yoghurt milk preparations:

buffalo's milk (BM) (15 % total solids, 5.5% fat, 3.8 % protein) and Whey Protein Isolate (WPI) were individually dispersed at 15 % (w/v) in deionized water and stirred for at least 2 h at ambient temperature, and then stored overnight at 4°C to ensure complete dissolution.

Different volumes of the both solutions were mixed together under moderate magnetic stirring at ambient temperature to obtain yoghurt milk containing different contents of whey protein concentrate as follows:

Table (1) Yoghurt milk mixture of skim milk and Whey Protein Iolate

Samples	buffalo's milk (ml)	WPI Dispersion (ml)
BM	100	0.0
BM- WPI1	80	20
BM- WPI2	60	40
BM - WPI3	40	60

BM: Buffalo Milk; BM- WPI1: 80% Buffalo milk and 20% whey protein isolate; BM- WPI2: 60% Buffalo milk and 40% whey protein isolate; BM - WPI3: 40% Buffalo milk and 60% whey protein isolate

Chemical analysis:

Determination of total solids content.: The samples were analyzed for total solids by dry oven at 105°C for 6 hrs as described in [11].

Determination of nitrogen fractions.: Total nitrogen (TN) and non-protein nitrogen (NPN) were determined by the semi-micro kjeldahl method as described by [12].

Determination of pH:. The pH of samples were measured according to [12] using a pH meter with glass electrode . Titratable acidity (TA.(Titratable acidity was measured as described by [13]. Ten grams of yoghurt were placed in a beaker and titrated with 0.1 N NaOH solution using phenolphthalein as an indicator. End point of titration was the transition from colorless to pink .

TA was calculated as follows :

$$\% TA = \frac{9 \times 0.1 \times \text{ml of NaOH}}{\text{Yoghurt weight}}$$

Determination of fat content : The Fat content was determined according to the modified Gerber method as described by [12].

Determination of lactose content: The lactose content of samples was determined by the phenol-sulphuric method of [14].Carbohydrates content were calculated according to [15].

Determination of acetalehyde and diacetyl : Acetaldehyde and diacetyl of the resultant yoghurt were measured usingspectrophotometer (Sp- UV2000, Taiwan) as described by [18].

Viscosity of yoghurt: The viscosity of yoghurt was measured according to [19]. The apparent viscosity of yoghurt were measured using a Bohlin coaxial cylinder viscometer (Bohlin Instrument Inc., Sweden) attached to a work station loaded with software V88 viscometery programme. The viscometer probe, system C30, was placed in the yoghurt samples cup, and measurements of viscosity were carried out at 20°C ±2°C in the up mode at shear rate ranging from 37 to 1238 1/s.

Sensory evaluation: The yoghurt samples were organoleptically evaluated by some panelists from the staff members of the dairy science department, National Research Center, Egypt. They evaluated each yoghurt sample and used a quality rating score card for evaluation of flavor (60 points) and body and texture (30 points) and appearance (10 points) as described by [20].

8.Statistical analysis: The data were analyzed according to Statistical Analysis System [21]. Duncan multiple range tests were carried out for separation among means. All experimental were replicated three times.

RESULTS AND DISCUSSION

Table (2) shows the composition of formulated milk with substituted different ratios of WPI. All treatments had average total solids of about 15 %. Buffalo milk without WPI had an average total protein of 3.8% increased to 5.98, 8.16, and 10.34%, with WPI, substituted at 20, 40, and 60 % (v/v), respectively. On the contrary, lactose content decreased from 5.10% for milk without WPI substitution to 4.11, 3.07, and 2.42% in the same order. These variations in protein and lactose contents can be attributed to the high protein content of WPI (98% protein) compared to buffalo milk (3.8% protein and 5.10% lactose) used in the preparation of yoghurt milk.

Table 2. Gross chemical composition of yoghurt milk-base with different level of WPI supplementation

Test / Sample	Total Solids %	Fat%	Total Protein %	Lactose %
BM	15.00	5.50	3.80	5.10
BM- WPI1	15.01	3.12	5.98	4.11
BM- WPI2	14.98	3.3	8.16	3.07
BM - WPI3	15.05	2.2	10.34	2.42

BM: Buffalo Milk; BM- WPI1: 80% Buffalo milk and 20% whey protein isolate; BM- WPI2: 60% Buffalo milk and 40% whey protein isolate; BM - WPI3: 40% Buffalo milk and 60% whey protein isolate

Yoghurt acidity and pH: The changes of the major contribution of Sr. thermophilus and L.bulgaricus is the production of lactic acid that results from fermentation, but when acid production starts, the acidity content increase proportionally, whereas the pH decrease. Also, the high lactose or carbohydrate content would encourage acid production by starter organisms.

Table (3) shows the pH and acidity values for yoghurt made of buffalo milk with different ratios of WPI substitution (0, 20, 40, and 60%).

Treatments	Acidity %			pH		
	Fresh	7 days	15days	Fresh	7 days	15days
BM	0.780	0.860	0.910	4.60	4.48	4.40
BM-WPI1	0.755	0.855	0.870	4.64	4.51	4.46
BM-WPI2	0.685	0.815	0.840	4.65	4.56	4.50
BM-WPI3	0.665	0.785	0.820	4.70	4.57	4.52

Table (3) shows the pH and acidity values for yoghurt made of buffalo milk with different ratios of WPI substitution (0, 20, 40, and 60%). A cold storage period of up to 15 days affects the acidity and pH values. That means the acidity increased, but pH values decreased through the cold storage period. [22], Stated that the tendency to lower (pH) post-acidification yoghurt after storage was evident due to the continued metabolic activity of microorganisms in all treatments. The development of pH and the acidity throughout storage shows the expected increase in acidity and the consequent pH decrease for all treatments. The acidity of fresh yoghurt showed a decrease with increasing WPI substitution. Fresh yoghurt acidity without WPI substitution showed 0.780% decreased to 0.755, 0.685, and 0.665 %, with increased WPI substitution of 20, 40, and 60%, respectively. These acidity percentages increased during refrigerated storage. It increased to 0.910, 0.870, 0.840, and 0.820 % after 15 days in the same order. On the contrary, pH values were increased with the increase in WPI substitution. Fresh yoghurt had pH of 4.60, 4.64, 4.65, and 4.70 with substitution WPC of 0, 20, 40, and 60%, respectively.The pH values were directly related to the percentage of whey protein addition. Due to the buffer properties of whey protein, the pH values increased with rising levels of whey protein [23]. Also[24] reported t+hat by increasing whey protein content in yoghurt mixes, total proteins were kept constant, increasing the buffering activity of the mix and pH values. These results were in agreement with our findings.

Viscosity: Viscosity is critical in the texture development of yoghurt and cultured milk. The viscosity of yoghurt creates the impression of "richness" to the consumer. It is a crucial attribute in defining mouth feel, flavour release, and refreshing quality of the product. It forms an important parameter in quality control programs for inoculated dairy products.

Fig. 1 showed the apparent viscosity of fresh yoghurt of 107.6, 117.3, 143.5, and 146.5 mPas with WPI substitution of 0, 20, 40, and 60%, respectively, at a shear rate of 37 1/s. The greater viscosity for yoghurt gels was attributed to the increased casein and whey protein interactions due to increased pasteurization temperatures and greater overall protein concentrations [25]. Protein content also determines viscosity [26]. According to our results, yoghurt with higher protein content(Table 1Chemical composition) has displayed greater viscosity. Similar results were found by [26], who found greater viscosity in yoghurts of the type labneh, with higher protein content.

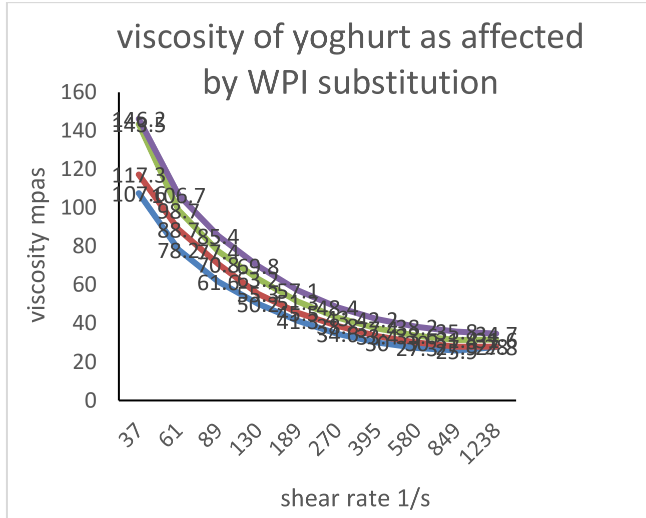

Figure 1. Viscosity of yoghurt as affected by WPI substitution

BM: Buffalo Milk; BM- WPI1: 80% Buffalo milk and 20% whey protein isolate; BM- WPI2: 60% Buffalo milk and 40% whey protein isolate; BM - WPI3: 40% Buffalo milk and 60% whey protein isolate

The apparent viscosity of the yoghurt samples decreased with increasing shear rate, indicating non-Newtonian behaviour. This result follows the previous studies on labneh [27] and fermented [28]. The shear thinning behaviour was expected in yoghurts as the texture of fermented milk products is affected by weak physical bonds and electrostatic and hydrophobic interactions [29]. Therefore, the fall in the apparent viscosity of yoghurts with the shear rate resulted from the destruction of the interactions. Viscosity varies not only with changes in the physical nature of fat but also with the hydration of proteins. Alterations in the size of any dispersed constituents result in viscosity changes. Decreased whey protein content yielded casein micelles with greater sizes in the gel network; thus, the bond types, particle sizes, and protein interactions contributed to yoghurt texture [24]. The levels of total solids content in yoghurt were studied by [30], and they reported that the consistency of yoghurt was improved by increasing the total solids content of the milk base. According to [31,32], the fortification of milk base to 5 g protein/ 100 g improved the rheological properties of the yoghurt. [33] reported that the increase in protein is the principal factor that affects texture and fortifies the milk base with SMP, resulting in the development of the casein network and micelles. In addition, the type and level of protein content affect the texture and rheological characteristics of the product [34,24,35]. Finally, the total solids affected the viability of *Lactobacillus bulgaricus* and *L. acidophilus* in milk and milk whey mixture, as reported by [36]. Increased WPI in yoghurt mix increased bound water and firmness [24,37]. The results showed that whey proteins do not contribute to the gel matrix. Native whey proteins did not support the structure: on the contrary, they acted as "structure breaker" or destructive filler" [35] as an "inert filler" [38] because of their inability to form a cohesive network within the yoghurt matrix. Compared with the control, a less compact casein network was found for the 60% WPI substitution.

Acetaldehyde and diacetyl contents: Diacetyl, produced by microorganisms, also may contribute to the flavour of cultured products [39]. Table 3 shows the acetaldehyde and diacetyl content affected by the WPI level addition and cold storage period. The fresh yoghurt had higher acetaldehyde content of 21.50, 22.50, 22.90, and 24.00 mMol/100g with 0, 20, 40, and 60% WPI substitution, respectively. It then decreased to 10.20, 11.40, 12.20, and 12.40 mMol/100g after 15 days of the storage period in the same order. This may be because WPI contributes to the production of acetaldehyde. [40] reported that the milk ingredients required by the yoghurt starter cultures for producing acetaldehyde are lactose, threonine and methionine. Also, the obtained yoghurt acetaldehyde values are within the normal range recorded by [39]. [41] reported that weakly flavoured yoghurt contained less than 4.0 ppm and well-flavoured yoghurts contained greater than 8.0-ppm acetaldehyde.

Table 4. Acetaldehyde and Diacety contents (mM/100gm) of yoghurt as affected by WPI substitution.

Treatments	Acetaldehyde (mM/100gm)			Diacetyl (mM/100gm)		
	Fresh	7 days	15days	Fresh	7 days	15days
BM	21.50	14.40	10.20	3.80	6.60	6.20
BM-WPI1	22.50	15.60	11.40	3.40	6.40	6.10
BM-WPI2	22.90	16.70	12.20	3.20	5.90	5.20
BM-WPI3	24.00	17.10	12.40	3.00	5.40	5.00

Acetaldehyde increased gradually with increased WPI content in yoghurt milk compared with yoghurt without WPI addition. It had been 21.50, 22.50, 22.90, and 24.00 mMol/100g in fresh yoghurt samples, decreased to 10.20, 11.40, 12.20, and 11.40 mMol/100g after 15 days of storage. On the other hand, the acetaldehyde content of yoghurt samples significantly decreased after the storage period of 14 days. This may be due to dehydrogenase activity at low storage temperature by some lactic Streptococci [42] and Lactobacillus species [43], which reduce acetaldehyde to ethanol. Similar observations were reported by [44]; [41]. Besides, the substitution of different levels of WPI to yoghurt buffalo milk showed an effect on diacetyl production in yoghurt compared to yoghurt made without WPI substitution. However, the results indicated that the acetaldehyde content was higher than the diacetyl content at fresh, 7 and 15 days samples. On the other hand, [45] reported that the low production of diacetyl is thought to be due to a limited ability to synthesize acetyl-CoA, which is involved only in diacetyl biosynthesis. However, diacetyl values are within the range recorded by [41,46]. A positive correlation was found between acidity (Table3) and diacetyl, and a negative correlation was found between acetaldehyde and diacetyl. Opposite to acetaldehyde, the diacetyl content of yoghurt samples increased during the storage period Table 4. This may be because, in mixed culture, diacetyl production is enhanced by the rapid drop in pH, which is associated with the growth of streptococci. Also, to acetyl CoA and TPP-acetaldehyde complex, which may react to form diacetyl [47], these results follow those reported by [48,41,49].Furthermore, diacetyl content increased until the end storage period (14 days) in yoghurts with different levels of WPI. In comparison, controls yoghurt reached maximum diacetyl at 7 days and declined afterwards. However, the differences between all treatments were insignificant.

It could be concluded that the yoghurt containing WPI at a high addition level (50%) affects starter activity and biochemical changes (pH values, acidity, and acetaldehyde and diacetyl contents) during the fermentation process or cold storage period of yoghurt.

Organoleptic properties : Sensory evaluations of yoghurt supplemented with different ratios of WPI substitution were recorded in Tables (5). Total scores, which reflected the general quality of the product, indicated that yoghurt with different ratios of WPI was accepted when fresh. While among the cold storage at 5°C for 15 days, the sensory scores decreased for the WPI yoghurt and the control. It was clear that the addition of WPI up to 40% was still accepted for 15 days, as the yoghurt had a pleasant flavour with no unpleasant aftertaste during these days of storage.

Colour: The yoghurt samples substituted with 20 and 40% with WPI scored maximum colour and appearance 9 and then decreased with increased WPI substitution to 60%. Fresh yoghurt with a higher WPI substitution of 60% scored lower on appearance and colour. It was observed at higher concentrations due to an increase in total solids and acidity; firm curd was not obtained, which spoiled the colour and appearance of the product.

Table 5. Organoleptic properties of yoghurt substituted with WPC during storage.

Ingerd. Conc.	Storage time	Flavor (60)	Body & Texture (30)	Color & Appearance (10)	Total (100)
BM	0 day	53	26	9	88
	7 days	52	28	9	89
	14 days	52	26	9	87
BM-WPI1	0 day	55	28	9	92
	7 days	56	29	9	94
	14 days	55	28	9	92
BM-WPI2	0 day	58	29	9	96
	7 days	59	29	9	97
	14 days	59	29	9	97
BM-WPI3	0 day	56	27	8	91
	7 days	56	28	8	92
	14 days	55	27	9	92

Body and Texture: It is revealed that higher-level concentration of WPI substitution reduced the scoring consistency. Concerning consistency, the highest score of 29 was obtained in the yoghurt sample containing 20% WPI substitution, followed by a sample containing 40% WPI substitution. The obtained sensory consistency scores agreed with that obtained from the viscosity measurements.

Flavour: According to the panellists, yoghurt containing 0, 20, and 40% WPI substitution received the highest flavour scores while the lowest scores were found for 60 % WPI substitution. The highest taste score (56 to 57) was received by yoghurt containing 20 and 40% WPI substitution after 15 days of storage. The most acceptable yoghurt was that made of 20 and 40% WPI substitution.

Overall acceptability: The overall acceptability of fortified yoghurt was found to vary at different concentrations of WPI substitution. The sample fortified with 40% WPI substitution was found superior in all aspects over other samples, followed by the sample fortified with 60% WPC substitution. The results are in good comparison to [50].Table (50). Organoleptic properties of yoghurt substituted with WPC during storage.

CONCLUSIONS

When WPI was substituted for BM in yoghurt mixtures, yoghurt gels with high-protein content and a more palatable structure resulted. These circumstances enhanced the number and strength of contact sites in charge of gel stiffness and resistance to deformation. They produced a complex gel network containing countless aggregating particles. According to this research, adding WPI 40% to milk proteins to replace them enhanced their viscosity, physical properties, and sensory acceptance. As a result, WPI-fortified set yoghurts were softer than control yoghurts. WPIs might be seen as adding value to the process of making yoghurt. The potential of whey proteins in stirred-type yoghurts must be established by more research on the sensory characteristics and storage behaviour of such gels.

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