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(Original Article)

Effect of Whey Protein Concentrate on Bio-Yogurt Properties

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Abstract

This study was conducted on bio-yogurt made from full cream buffalo's milk with the addition of different percentages of whey protein concentrate WPC at (2, 4 and 6 %). The chemical, microbiological, rheological and sensory properties were evaluated during storage at refrigerator temperature (5±2°C) for 15 days. Analysis was performed on fresh samples as well as those that had been stored over three storage periods (5, 10 and 15 days). The obtained results indicated that the addition of whey protein concentrate (WPC) led to an increase in total solids, as well as total nitrogen and soluble nitrogen, in addition to the acidity levels, while the addition of WPC led to a decrease in the amount of separated whey. Microbiologically, there was an increase in the total count of bacteria, *Bifi. bifidum*, *Lb. Acidophilus*, *Stre. thermophilus*, while these numbers decreased with increasing storage periods in all the treatments. On the other hand, the coliform group was not detected in all treatments. The results from sensory evaluation recorded that the bio-yogurt added to 4% WPC gained the highest score, followed by the bio-yogurt with 2% WPC in most treatments.

Keywords: *Bio-yogurt, Whey protein concentrate, Chemical properties, Rheological properties, Probiotic.*

Introduction

Yogurt is one of the most popular fermented dairy products worldwide and has gained widespread consumer acceptance as a healthy food (Bouhadi *et al*., 2021). It has been increasingly popular among consumers for its high nutritional value and health benefits associated with bioactive peptides and lactic acid bacteria (Gu *et al*., 2020). Furthermore, yogurt has numerous ways to promote healthy digestion such as lowering the risk of type 2 diabetes, protecting against colorectal and stomach cancers, preventing osteoporosis, improving the immune system and reducing high blood pressure and plasma levels of LDL cholesterol (Marketeer, 2018). Therefore, manufacturers and scientists continue to search for value-added ingredients to produce functional yogurts with more beneficial features (Fazilah *et al*., 2018). Because of their nutritional worth and technological functionality, whey proteins are one of the most prized ingredients.

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They contain a lot of bioactive peptides, which have biological effects such as anti-inflammatory, anti-hypertensive, and antioxidant, as well as antibacterial qualities. The development of functional foods uses these peptides as an active ingredient. (Shayanti and Sanjeev, 2020). Additionally, according to Ramos *et al*., (2016), the primary components of whey protein fractions are lactoglobulin (-Lg), -lactalbumin (-La), immunoglobulins (IGs), bovine serum albumin (BSA), lactoferrin (LF), and lactoperoxidase (LP). It has been found that whey protein concentrate (WPC), stimulates the growth of *Bifidobacteria* (El-Batawy *et al*., 2019).

The objective of this study is to produce a high-quality bio-yogurt by using WPC and evaluate the effect of adding different concentrations of it on the chemical, microbiological, rheological and organoleptic properties of the produced bio-yogurt during cold storage for 15 days at refrigerator temperature (5± 2°C).

Materials and Methods

Materials

Fresh buffalo milk was obtained from the herd of the animal production department, faculty of Agriculture, Al-Azhar University (Branch of Assiut). WPC contains (80% protein, 5.0% moisture, 2.0% ash and 4% fat) and was obtained from the local market. Probiotic cultures ABT-5 (*Lactobacillus acidophilus*, *Bifi. bifidum* and *Str. thermophilus*) were provided by the Chr. Hansen's, Denmark.

Bio-yogurt manufacture

The bio-yogurt was manufactured as follows: the milk was heated to 90 °C for 15 min. The milk was divided into four parts then 2, 4, 6% WPC were added; the fourth part was treated as a control. All parts cooled to 42°C for adding 2.5% ABT yogurt culture. All treatments were packaged in 100 ml plastic cups then incubated at 42°C for 3-4 until completely curdled. The treatments were kept under refrigeration at (5 ± 2 °C) for analysis up to 15 days.

Methods of analysis

Chemical analysis

Total solids (TS), fat, total nitrogen (TN), water soluble nitrogen (SN) and titratable acidity (AC) of bio-yogurt treatments were determined according to AOAC (2020).

Microbiological analysis

Total bacterial count (TBC)

Total bacterial count (TBC) was enumerated by using Nutrient Agar medium as mentioned by APHA (2004).

Lactobacillus acidophilus

Lactobacillus acidophilus counts were appraised on the selective medium for Lactobacilli (MRS) as mentioned by IDF Standard (1997). The plates were incubated at 37°C for 48 h.

Streptococcus thermophilus

Counts of *Streptococcus thermophilus* were enumerated by using M17 agar medium. The plates were incubated at 30°C for 24-48 h according to IDF Standard (1997).

Bifidobacterium bifidum

Bifidobacterium bifidum counts were detected according to Dave and Shah (1996), using modified MRS agar medium (m-MRS), supplemented with 0.05% L- Cysteine HCL and 0.3% lithium chloride. The plates were incubated at 40°C for 48 h under anaerobic conditions.

Coliform bacteria

Coliform bacteria were detected using MacConkey broth medium and incubated at 37ºC for 48 h as described by Standard IDF (1985).

Yeasts and Molds

Counts of yeasts and molds enumerated using malt extract agar medium according to Oxoid manual (1982) at 25º C for 5-7 days.

Rheological analysis

Determination of syneresis

One hundred grams of the sample was placed on a filter paper using a measuring jar. After 2 h of drainage at 5±2°C, the collected whey was used as the index of syneresis (ml/100 g) according to Farooq and Haque (1992).

Determination of curd firmness

The penetration method described by Shalabi (1987) for the determination of curd firmness was used with some modifications as follows: the height of the yogurt sample at the package was measured using a test tube (1 cm diameter, 15 cm length). The test tube let to penetrate the yogurt sample curd until the end of the package bottom by using lead balls. The total weight (g) of lead balls required for full penetration is related to the firmness of the curd.

Density

Density was calculated using the regular equation as follows:

$$\text{Density } (\text{g}/\text{cm}^3) = \frac{\text{Weight}}{V\text{olume}}$$

Sensory evaluation

Sensory evaluation of bio-yogurt samples was achieved when fresh and during storage by ten panelists. Bio-yogurt samples rating scorecard for evaluation of flavor (60 points), body and texture (30 points) and appearance (10 points) as described by Nelson and Trout (1981).

Results and Discussion

Chemical composition of bio-yogurt

Chemical composition of bio-yogurt made with different ratios of WPC when fresh and during storage at refrigerator temperature (5 ± 2 °C) are presented in Table 1.

Total solids (TS)

Table 1 shows the content of TS% of fresh and stored samples of bio-yogurt with WPC. Samples when fresh have TS: 11.37, 11.54, 11.66 and 11.84% in control, T1, T2 and T3 bio-yogurt samples; respectively. There was an increase in TS% by increasing the added WPC. These results are in agreement with those reported by Bierzuńska and Sokolińska (2018), who found that the addition of WPC increased TS% compared with the control samples. During 15 days of storage, there was a gradual increase in TS% in all samples to 11.93, 11.97, 12.15 and 12.17% in control, T1, T2 and T3, respectively. The increase in TS% could be due to a slight decrease in moisture content as a result of evaporation. Also, could be due to the effect of the syneresis of whey as a result of the increase in acidity.

Fat

Fat content of bio-yogurt treatments with added WPC is mentioned in Table 1. The data shows that fat contents in fresh bio-yogurt were 6.35, 6.20, 6.17 and 6.07 in control, T1, T2 and T3; respectively. Fat content of the control samples was higher than that of bio-yogurt with WPC treatments. Furthermore, there was a slight decrease in fat content by increasing WPC in all treatments (Shenana *et al*.,2007).

Total nitrogen (TN%)

Total nitrogen of fresh and stored samples is presented in Table 1. In fresh samples, the obtained results of TN% were 0.480, 0.533, 0.563 and 0.587% for the control, T1, T2 and T3; respectively. The TN% of bio-yogurt increased at a proportional rate with the increase of WPC in all treatments. This can be attributed to the high protein content of WPC (Shenana *et al*., 2007). After 15 days of storage, there was a slight decrease in TN%, it reached 0.440, 0.463, 0.493 and 0.497% for the control, T1, T2 and T3; respectively. The low percentages of TN during storage periods may be due to the effect of microorganisms on the total protein; subsequently, there were increases in TN% up to the end of the storage. These results are in agreement with those reported by Noureldin *et al*. (2020).

Titratable acidity (TA)

Table 1 shows the average levels of titratable acidity as (%) for bio-yogurt treatments with different percentages of WPC (2, 4 and 6%) compared by the control samples. In fresh samples, the reported acidity was 0.70, 0.74, 0.82 and 0.84% for control, T1, T2 and T3; respectively. From the data, it can be found

that the lowest reported value was for the control samples and the highest was for that of T3. This may be due to the effect of WPC on increasing the levels of lactic acid production from lactose by ABT during incubation time. These results are in agreement with those of Zedan *et al*. (2001) and Muniandy *et al*. (2016). The increase of titratable acidity was more noticeable for all treatments all over the storage time up to 15 days reaching; 0.86, 0.95, 0.96 and 1.10 for the control, T1, T2 and T3; respectively. These could be due to the activity of the starter culture bacteria.

Table 1. Chemical composition of bio-yogurt with different ratios of WPC when fresh and during storage at refrigerator temperature (5±2°C).

Storage Time (days)	Treatments*			
	C	T1	T2	T3
			TS (%)	
Fresh	11.37	11.54	11.66	11.84
5	11.57	11.83	11.98	12.03
10	11.88	11.90	12.03	12.13
15	11.93	11.97	12.15	12.17
			Fat (%)	
Fresh	6.35	6.20	6.17	6.07
5	6.35	6.20	6.17	6.07
10	6.13	6.03	5.90	5.80
15	5.73	5.65	5.57	5.43
			TN (%)	
Fresh	0.480	0.533	0.563	0.587
5	0.457	0.523	0.529	0.557
10	0.450	0.520	0.525	0.552
15	0.440	0.463	0.493	0.497
			SN (%)	
Fresh	0.055	0.057	0.058	0.060
5	0.057	0.058	0.061	0.063
10	0.061	0.064	0.067	0.078
15	0.070	0.073	0.075	0.088
			TA (%)	
Fresh	0.70	0.74	0.82	0.84
5	0.74	0.86	0.85	0.88
10	0.83	0.88	0.91	0.93
15	0.86	0.95	0.96	1.10

*C: Control bio-yogurt, T2: Bio-yogurt with 4% WPC, T1: Bio-yogurt with 2% WPC, T3: Bio-yogurt with 6% WPC

Microbiological properties

Microbiological properties of bio-yogurt made with different ratios of WPC are presented in Table 2 when fresh and during cold storage at temperature (5±2°C) for 15 days.

Total bacterial counts (TBC)

Table 2 shows TBC of bio-yogurt made with different ratios of WPC when fresh and during storage. Total bacterial counts (log cfu/ml) for fresh control samples have the minimum count (8.64 log cfu/ml), while the maximum was for

that of T3 (9.22 log cfu/ml). This increase in TBC was gradual by the increase of added WPC. These could be due to the enhancement effect of WPC on bacteria growth (Muniandy *et al*., 2016). During storage, TBC increased up to the fifth day of storage period and then decreased up to the end of the storage period. These might be due to the development of acidity and cold storage. These results are in agreement with Kebary *et al*. (2010). At the end of storage, all treatments resulted in gradational regression of TBC. The highest count was for treatment T3 recorded 7.30 log cfu/ml, meanwhile, the lowest was for the control samples (6.13 log cfu/ml). These results are in agreement with El-Wahsh (2013), who observed the increase in the total count at the first 7 days of storage followed by a slight decrease among the different treatments until the end of storage. This may be attributed to the development of acidity by the starter cultures affecting the activity of bacteria.

***Lactobacillus acidophilus* count**

Lactobacillus acidophilus counts of bio-yogurt made with different ratios of WPC are presented in Table 2. Results show that counts of *Lb. acidophilus* were slightly different among treatments in fresh samples. The control samples have the minimum counts (6.89 log cfu/ml); obviously, T3 has the maximum counts (8.20 log cfu/ml). These results are agreed with those obtained by Kebary *et al*. (2015), who found that the addition of WPC increased *Lb. acidophilus count* in bio-yogurt as an effect of the content of WPC nutrients such as minerals, vitamins and whey proteins. *Lb. acidophilus* count has a better development in the presence of proteins and peptides. After 7 days of storage, there was an increase in *Lactobacilli* count in all treatments. At the end of storage, there was a slight decrease in *L. acidophilus* count for all treatments to 5.52, 6.46, 7.14 and 7.16 log cfu/ml for the control, T1, T2 and T3; respectively. The decrease in *Lb. acidophilus* count at the end of the storage could be explained as a result of the increase in total acidity (Mangia *et al*., 2014).

***Streptococcus thermophilus* count**

Streptococcus thermophilus counts are recorded in Table 2 for treatments of bio-yogurt made with different ratios of WPC. The obtained data of the *S. thermophilus* counts were slightly different in fresh samples of all treatments. The minimum counts were 6.14 log cfu/ml for control treatment; meanwhile, the maximum was 7.30 log cfu/ml for T3 treatment. This can be explained by the effect of WPC as a growth-promoting factor for *S. thermophilus* as they contain small peptides and sugar can stimulate *S. thermophilus* growth (Saad and Elkhtab, 2019). After 5 days of storage, although there was an increase in *S. thermophilus* counts in all treatments, the highest counts were 8.89 log cfu/ml for T3. After 15 days of storage, there was a decrease in *S. thermophilus* counts being 5.13, 5.87,6.05 and 6.94 log cfu/ml for the control, T1, T2 and T3, respectively.

***Bifidobacterium bifidum* count**

Bifidobacterium bifidum counts of bio-yogurt with different ratios of WPC are mentioned in Table 2. Results in the same Table, clearly show that in fresh samples *Bifi. bifidum* counts were 6.62, 7.18, 7.33 and 7.55 log cfu/ml for the control, T1, T2 and T3; respectively. By extending the storage for 15 days, these counts were decreased. The lowest counts were for the control treatment being 5.35 log cfu/ml and the highest was for the T3; 6.75 log cfu/ml. This could be due to *Bifi. bifidum* is significantly less tolerant to high acidity values. These results are consistent with those mentioned by Shafiee *et al.* (2010) and Abou-Dobara *et al*. (2017).

Table 2. Microbiological properties as (Log cfu/ml) of bio-yogurt with different ratios of WPC when fresh and during storage at refrigerator temperature (5 ± 2°C)

Storage Time (days)	Treatments*			
	C	T1	T2	T3
		TBC		
Fresh	8.64	9.13	9.20	9.22
5	8.73	9.20	9.35	9.88
10	7.13	8.08	8.20	8.37
15	6.13	7.21	7.27	7.30
		Lactobacillus acidophilus		
Fresh	6.89	8.18	8.18	8.20
5	7.73	8.20	9.12	9.64
10	6.17	6.75	7.15	7.17
15	5.52	6.46	7.14	7.16
		Streptococcus thermophilus		
Fresh	6.14	7.03	7.12	7.30
5	7.42	8.18	8.77	8.89
10	6.05	6.21	7.05	7.12
15	5.13	5.87	6.05	6.94
		Bifi. bifidum		
Fresh	6.62	7.18	7.33	7.55
5	7.46	8.52	8.60	8.67
10	6.36	7.59	7.60	7.81
15	5.35	6.46	6.57	6.75
		Coliform group		
Fresh	ND	ND	ND	ND
5	ND	ND	ND	ND
10	ND	ND	ND	ND
15	ND	ND	ND	ND
		Yeasts and Molds		
Fresh	ND	ND	ND	ND
5	ND	ND	ND	ND
10	ND	ND	ND	ND
15	2.42	2.30	2.22	2.10

TBC: total bacterial count, WPC: whey protein concentrate.

Yeasts and Molds count

Table 2 shows yeasts and molds counts of bio-yogurt made with WPC. It can be seen that all treatments were free from mold and yeast up to 10 days of storage. At the end of the storage, mold and yeast counts were 2.42, 2.30, 2.22

and 2.10 log cfu/ml for the control, T1, T2 and T3; respectively. The progress of storage has an effect of molds and yeasts counts. This could be due to the increase in TS and acidity values. The results are agreed with those reported by (Kebary *et al*., 2020).

Coliform count

Coliform count was not detected in all treatments when fresh and during the storage period, which indicates that the yogurt was manufactured under sanitation and hygienic conditions, so it could not detect any contamination by coliform bacteria in all samples.

Rheological properties

Data presented in Table 3 mentioned the rheological properties of bio-yogurt made with different ratios of WPC when fresh and during storage at refrigerator temperature(5±2°C) for 15 days.

Syneresis

The results showed that there were increases of syneresis in bio-your treated with WPC when fresh than that of other treatments. Addition of WPC decreased syneresis of T1, T2 and T3. Although the control samples have the highest syneresis when compared to WPC treatments, among WPC treatments the lowest syneresis was for T3 treatment and the highest was for T1 treatment, (Bryan *et al*., 2019). On the other hand, syneresis of all treatments decreased by extending the storage up to 15 days.

Density (g/cm^3)

Density (g/cm^3) of bio-yogurt treatments with WPC is shown in Table 3. Bio-yogurt samples exhibited lower density values for the control treatment and the higher was for T3 compared with other treatments. Adding WPC increased density of treatments higher than the control. During storage, density values until the fifth day were the same as when fresh, no differences were found. Nevertheless, when samples were stored for 15 days, density values were higher at all. T3 treatment has the highest density value compared to other treatments. This could be due to the increase in TS.

Firmness

Table 3 presents firmness values of bio-yoghurt with added WPC. There were increases in firmness by increasing in WPC added ratio in fresh treatments. Firmness values at fresh samples were 26.92, 39.27, 43.45 and 46.01 g for the control, T1, T2 and T3, respectively. Firmness values were increased by increasing storage. At the end of the storage, firmness values were 44.64, 54.72, 78.27 and 79.40 g for the control, T1, T2 and T3, respectively. This could be due to the higher values of TS% by storage. Mahomud *et al*. (2017) proved that adding whey protein to yogurt reduces whey separation and increases firmness. Interactions between casein micelles and denatured whey proteins via

intermolecular disulfide bonds help improve network connectivity and water retention.

Table 3. Rheological properties of bio-yogurt with different ratios of WPC when fresh and during storage at refrigerator temperature (5±2°C).

Storage Time (days)	Treatments			
	C	T1	T2	T3
	Curd syneresis (ml/100 g)			
Fresh	40.27	36.72	30.29	26.95
5	37.61	33.07	27.93	25.29
10	36.07	30.83	24.64	24.60
15	34.49	30.39	24.93	24.47
	Density (g/cm^3)			
Fresh	1.13	1.16	1.17	1.18
5	1.13	1.17	1.18	1.18
10	1.14	1.17	1.19	1.20
15	1.16	1.19	1.21	1.22
	Firmness (g)			
Fresh	26.92	39.27	43.45	46.01
5	30.43	45.84	55.25	58.48
10	41.34	52.52	75.27	75.68
15	44.64	54.72	78.27	79.40

Sensory evaluations

Sensory evaluations of bio-yogurt with added different ratios of WPC were recorded in Table 4. Sensory properties were attributed for:

Flavor

Treatments of bio-yogurt with 2, 4 and 6% WPC have the higher flavor scores, while the control samples have the lowest score. Higher flavor scores ranged from 58 to 59 for bio-yogurt with 2, 4 and 6% WPC treatments when fresh. During storage, flavor scores were lower for all treatments including the control samples. At the end of storage, it was clear that the addition of WPC up to 6% was accepted for 14 days of storage. As the WPC bio-yogurt treatments had a pleasant flavor with no aftertaste during storage, the control samples have decreased flavor attributes to record the lowest flavor scores.

Body and texture

Concerning body and texture, treatments manufactured with the addition of WPC when fresh obtained higher scores, compared to the control samples. El-Tahra (2014) found that the addition of WPC led to an increase in TS content to improve the consistency and texture of the product. Bio-yogurt made with WPC was stored for 7 days, no differences were observed for body and texture. Although WPC treatments of 2 and 4% have lowered scores by the end of storage, the WPC treatment of 6% has the highest score compared to the other treatments.

Color and appearance

Fresh samples of bio-yogurt with 2, 4 and 6% WPC, as well as the control samples scored color and appearance (9). At the end of storage, color and

appearance of control samples and T2 and T3 have no differences. Color and appearance score has decreased by increasing WPC to 6%. It was observed that higher concentrations of WPC resulted in more yellowish color of the treatment (Mohmmed *et al*., 2022).

Table 4. Sensory properties of bio-yogurt with different ratios of WPC when fresh and during storage at refrigerator temperature (5±2°C).

Treatments	Storage Time (days)	Flavor (60)	Body and texture (30)	Color and appearance (10)	Total (100)
C	**Fresh**	53	27	9	89
	7	51	26	9	86
	14	51	25	9	85
T1	**Fresh**	58	29	9	96
	7	57	29	9	95
	14	57	28	9	94
T2	**Fresh**	59	29	9	97
	7	58	29	9	96
	14	57	28	9	94
T3	**Fresh**	59	29	9	97
	7	55	29	8	92
	14	55	29	8	92

Total scores

Bio-yogurt treatments with added different percentages of WPC were more accepted than the control samples that when fresh. Treatments with 4 and 6% WPC were the most preferable, followed by the treatment of 2% WPC. The control samples have the lowest accepted attribute among all treatments. Even-more sensory total scores decreased during storage up to 14 days for all bio-yogurt treatments of WPC as well as the control samples. The most accepted bio-yogurt treatments were that of 2 and 4% WPC.

Conclusion

The addition of WPC in bio-yogurt manufacture improved the chemical composition of treatments especially total protein and active peptides content. Moreover, WPC increased the bacteriological quality to have numerous health benefits and improved sensory properties of bio-yogurt treatments compared to the control samples. These are in compliance with international requirements and regulations. Therefore, it is possible to produce high quality bio-yogurt by adding 2-4% WPC.

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تأثير الحليب المدعم ببروتينات الشرش المركزة على خصائص الزبادي الحيوي

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الملخص

أجريت هذه الدراسة على الزبادي المدعم حيوياً المصنع من حليب الجاموس مع إضافة نسب مختلفة من بروتينات الشرش المركزة (2 ، 4 ، 6٪)، وقد تم تقييم الخواص الكيميائية والميكروبيولوجية والريولوجية والحسية مع التخزين علي درجة حرارة الثلاجة(5±2°م) لمدة 15 يوم، وتم تحليل العينات طازجة وعلى مدى ثلاث فترات تخزينية (5 ، 10 ، 15 يومًا)، وأشارت النتائج المتحصل عليها إلى أن إضافة بروتينات الشرش المركزة تعمل علي زيادة المواد الصلبة الكلية والنيتروجين الكلي والنيتروجين الذائبوالحموضة بينما أدت الإضافة إلى انخفاض انفصال الشرش، ومن الناحية الميكروبيولوجية كانت هناك زيادة في العد الكلي للبكتريا، *Bifi .bifidum*، *Lb. acidophilus*، *Stre. thermophilus* في حين أن هذه الأعداد تقل مع زيادة فترات التخزين في جميع المعاملات،ولم يتم الكشف عن وجود مجموعة القولون في جميع المعاملات. كما سجلت النتائج المتحصل عليها في التقييم الحسي أن الزبادي الحيوي المضاف إليه 4% بروتينات الشرش المركزة حصل علي أعلي تقييم يليه الزبادي الحيوي المضاف إليه2% بروتينات الشرش المركزة في معظم المعاملات.