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ORIGINAL ARTICLE

Improvement of the quality of buffalo's milk soft cheese by camel's whey protein concentrate

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ABSTRACT

Objective: The objective of this study was to investigate the impact of whey protein concentrate (WPC) derived from camel's milk on cheese yield, some chemical, microbial, and organoleptic properties of low salt soft cheese during refrigerated storage.
Materials and Methods: Cheeses made from buffalo's milk without and with adding 4,000 and 8,000 µg/ml WPC.
Results: Addition of WPC significantly increased the yield, titratable acidity, and decreased pH of the resultant cheese samples. Cheese treated with 8,000 µg/ml WPC had the highest effect on the reduction of the total bacterial count, coliform, molds, and yeast up to 29th day of storage in comparison to the 25th day and 17th day in cheese with 4,000 µg/ml and control samples, respectively. The organoleptic evaluation indicated that adding of WPC improved flavor, body, and texture and appearance of the cheese.
Conclusion: The present study demonstrated that the application of camel's WPC at 8,000 µg/ml in cheese can improve organoleptic and microbiological proprieties of low salt soft cheese and prolong its shelf-life at refrigerated storage up to 29 days in comparison to 25 days and 17 days in cheese treated with 4,000 µg/ml WPC and control cheeses, respectively. So, the present WPC has a potential for preservation as a food ingredient and natural food preservative.

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Camel's milk; whey protein; soft cheese

Introduction

Nowadays the consumer is usually looking for healthier food with natural additives, so he started avoiding the application of synthetic preservatives [1]. Application of natural preservatives of plants, animals, or microbial origin is commonly used for the production of safe and high-quality food that fulfill consumer demands. Lysozyme (egg white, and figs), saponins and flavonoids (herbs and spices), bacteriocins, chitosan (shrimp shells) and lactoferrin, casein, and whey (milk) are considered the best example for natural antimicrobial agents [2].

Milk is one of the most important nutrients which is characterized by not only high nutritive value but also having many of antimicrobial proteins. Camel's milk is an important food as it has a functional and healthy benefit due to the presence of active biological substances. However, the majority of people consume cow's milk regularly than camel's milk due to the fact that cows and buffaloes give much more milk and require less maintenance and labor. Unfortunately, people are not aware about the nutritive value and therapeutic effects of camel's milk. Camel's milk is regarded to be the most important and major source of proteins, especially for people who accommodate in the desert area of the world. These proteins present mainly in whey of camel's milk with high concentration and are rich in antimicrobial ingredients including lysozyme, lactoferrin, lactoperoxidase, and peptidoglycan recognition protein [3]. In addition, immunoglobulin IgA and IgG have powerful antimicrobial efficacy against some bacterial and viral pathogens. Consumption of milk of camel's origin has a positive effect on reducing diseases that are caused by nutritional deficiencies in the adult [4].

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Whey protein (WP) concentrate (WPC) is preferred to use in cheese manufacturing as it increases its yield and improves its organoleptic characters. Furthermore; it contains some antimicrobial proteins that improve the quality of the final product. Camel's milk has a higher WP concentration than milk from other animal species, especially the content of albumin and lactoferrin [5].

Cheese is mainly characterized by highly nutritive value. It is considered the best source for protein and some minerals, especially calcium and phosphorus which are mainly required for human health and nutrition. At the same time, it is mainly susceptible to physical, chemical, and biochemical spoilage due to its high nutritive value. Therefore, the preservation of such product is very essential. In addition, soft cheese considered a vehicle for food spoilage organisms which cause bad effects on flavors, color, and body and texture [6,7]. To overcome the fast spoilage of cheese and prolong the shelf-life, natural antimicrobial preservatives are now applied in the dairy industry [8].

Considering these aspects, the current work was aimed to evaluate the effect of WPC separated from the milk of camel's origin on soft cheese quality through assessing the organoleptic, acidity indices, and microbiological status of soft cheese during refrigerating storage.

Materials and Methods

Preparation of WPC

Camel's milk was obtained from Halayeb and Shalteen at the New Valley Governorate. Milk had been centrifuged at 5,000 × g/30 min at 10°C for removing fat content. WP was obtained by adding 10% (v/v) acetic acid to skimmed milk till the pH was closed at 4.6 and then incubated at 37°C for 10 min. It was neutralized using 1M sodium acetate and then centrifuged at 10,000 × g/5 min at 4°C. The casein pellet was rested at the bottom of the centrifugation tube, while the whey (supernatant) floats at the top. The resultant whey, which contained the undenatured WPs, was saturated with 80% ammonium sulfate to precipitate the WPs [9]. WPs are then lyophilized (freeze dried) using a laboratory-scale freeze dryer at −75°C for almost 6–24 h (WPC).

Cheese manufacturing

Low salt soft cheese (3%) was made according to the method of El-Sheikh et al. [10]. The manufactured cheese was divided into three groups; the first was regarded as control (C), the second was inoculated by 4,000 μg/ml camel's WPC (T1), and the third was inoculated by 8,000 μg/ml camel's WPC (T2). The three groups were kept at 42°C till proper curd was obtained, then the curd was kept draining for 18 h in some previously sterilized stainless-steel frames lined with cheese cloth.

The obtained cheese with their respective whey was packaged in pre-sterilized aluminum cups and tightly covered with aluminum foil paper then kept at the refrigerator. Cheeses were sampled fresh (zero time) and at 7th, 14th day then every 4 days till the spoilage signs were visible. The experiment was repeated in triplicates and an average result for each group was tabulated.

Cheese analyses

Yield: The cheese yield was calculated as per the formula described by Hanafy et al. [11].

Chemical examination

Cheese samples were examined for pH value and titratable acidity (TA). The titrimetric method was used for the determination of TA according to AOAC [12]. Briefly, in 250 ml conical flask, 100 ml of distilled water and 1 ml of 1% alcoholic solution of phenolphthalein were mixed with 5 gm of cheese sample. The sample was then titrated with 0.1N sodium hydroxide until the faint pink appeared and persisted for 30 sec. The amount of alkali used was recorded and TA% was calculated. In addition, pH meter (Jenway 3051 pH meter) was used for the determination of the pH values of cheese samples. It was calibrated by standard buffer solutions pH 4.00 and pH 7.00 at 25°C before each measure.

Microbiological examination

Cheese samples (10 gm) were homogenized with sodium citrate (2%) and then added to 90 ml of 0.1% buffered peptone water (Biolife Italiana, Italy). Ten-fold serial dilutions were prepared and plated on plate count agar and violet red bile lactose (Oxoid, UK) and aerobically incubated at 37°C for 24–48 h for total mesophilic bacterial count and coliform, respectively, as described by British standards Institution [13]. Dextrose tryptone agar medium (HiMedia, India) was used for the enumeration of aerobic spore-formers. The samples were incubated at 37°C for 24–48 h; whereas yeasts and molds were determined on Sabaroud dextrose agar (Lab M), after the addition of 0.1 g/l chloramphenicol, incubated aerobically at 25°C for 5–7 days [14]. The specific colonies were counted, and data were demonstrated as Log_{10} cfu/gm.

Organoleptic examination

Nine panelists have applied the sensory evaluation of examined cheese samples for an overall score of 100 points through 45 points for flavor, 35 points for body and texture, and 20 points for appearance International Dairy Federation [15].

Statistical analysis

One-way analysis of variance was used for statistically comparing among the groups. The results were considered significantly different with $p < 0.05$ [16]. The experiment was repeated in triplicates and average results for each group was tabulated

Results and Discussion

The current experiment was applied to evaluate the effect of WPC derived from camel's milk on soft cheese quality at different concentrations. The current results showed that WPC could improve keeping quality of white soft cheese at concentrations more than 4,000 µg/ml.

Effect of camel's WPC on cheese yield

It is clear from Figure 1 that the addition of camel's WPC to milk intended for cheese making significantly increased the yield of cheese. The higher of the added WPC was, the significantly higher the cheese yield. The increasing rates % of cheese yield were 19%, 25%, and 29% in control and groups of 4,000 and 8,000 µg/ml camel's WPC, respectively. These results were agreed with those reported by El-Sheikh et al. [10], Hanafy et al. [11], and Othman [17]. Similar trend was also given by Sakr and Mehanna [18] and Ismail [19] via using WPC in the manufacture of Cheddar and Ras cheese.

The improvement of cheese yield may be related to the addition of WPC and/or denatured WPs that resulted from severe heat-treatment milk. Such treatments lead to the integration of WPs into cheese and/or higher retention of serum in cheese matrix, resulted in a pronounced increase in cheese yield [20]. Henriques et al. [21] stated that the concentration of milk proteins not only WPs but also caseins have a great effect on improvement in the cheese yield.

Generally, cheese yield is increased by a high content of milk fat and protein, integration of WPs, lactose, ash, and moisture [22].

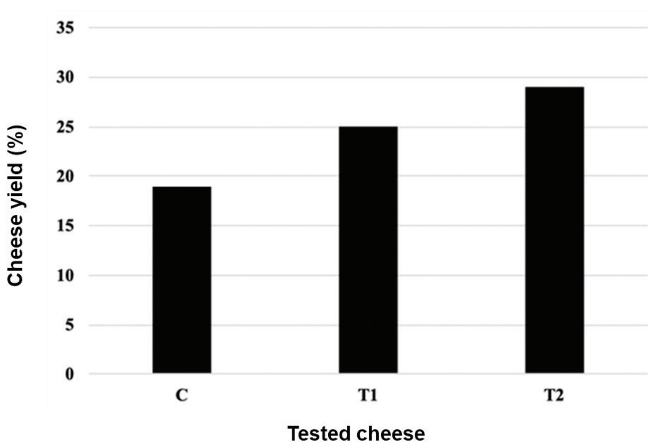

Figure 1. Impact of camel's WPC on cheese yield. C = Control, T1 = Cheese with 4,000 µg/ml WPC, T2 = Cheese with 8,000 µg/ml WPC.

Acidity profile of examined soft cheese

Measuring milk acidity is mainly reflecting milk quality physically, chemically, and microbiologically for the manufacture of different dairy products [23]. Acidity characteristics of fresh white soft cheese samples during the storage period were shown in Table 1. Acidity increased continuously until the end of the refrigerated storage period in tested groups ($p \leq 0.05$). However; the control cheese samples had a higher pH value and lower TA% than the cheese samples containing WPC ($p \leq 0.05$). The pH values for control samples (C) were 6.45 ± 0.02, 6.28 ± 0.02, 6.05 ± 0.01 at zero-day, 7th, and 14th day of storage, respectively. However, they decreased gradually along the storage period and reached to 5.86 ± 0.02 at the 17th day of storage (Table 1). While pH value in treated samples with WPC (T1) 4,000 µg/ml was 6.30 ± 0.03 at zero-day and reached to 5.54 ± 0.02 at the end of refrigerated storage (21st days). The addition of WPC (T2) 8,000 µg/ml had significantly lower pH values than control which reached to 5.47 ± 0.02 at 29th days of refrigerated storage. These results were agreed with Othman [17]. Tashakori et al. [24] observed that there was a higher acidity % in Feta cheese containing WPC in comparison to control. Yazici and Akgun [25] found that strained yogurt with WPC had lower pH values than control. Similar trend was also observed by Ismail [19] in Ras Cheese treated with WPC. These results may be due to the higher concentration of protein and other buffering constituents in supplemented samples than control and/or the increase in available nutrients from WPs may promote the activity and growth of yogurt bacteria [26]. However, generally, such a pH decrease in all groups may be due to the higher growth of lactic acid bacteria that ferments lactose to lactic acid, thus leading to acid production [27].

Addition of WPC in (T2) had a slight increase ($p < 0.05$) in TA%. It increased from 0.28 ± 0.02, at zero time to 0.46 ± 0.02 at the end of refrigerated storage (Table 1). Protein content has a great reflection on acidity % of milk, where a high TA% suggested a high concentration of proteins and/or buffering constituents in treated samples than control and/or the increase in available nutrients from WPs [11,28].

Microbiological changes of cheese during storage period

Soft white cheese is widely consumed by the Egyptian population, but it is one of limited shelf-life dairy products because of its high water activity and low salt level [29], and consequently higher microbial spoilage may occur during cheese making, storage, and handling [30].

Table 2 showed the total bacterial count (TBC) in cheese in the presence of WPC. The TBC in control samples increased gradually from zero-day of production and reached to 3.28 ± 0.2 Log_{10}cfu/gm and reached its

Table 1. Influence of camel's WPC s of camel milk on pH and titratable acidity of soft cheese.

Storage time	pH			TA%		
	C	T1	T2	C	T1	T2
Zero time	6.45* ± 0.02^{A}	6.30 ± 0.03^{B}	6.25 ± 0.02^{C}	0.23 ± 0.02^{C}	0.27 ± 0.03^{B}	0.28 ± 0.02^{A}
7	6.28 ± 0.02^{A}	6.21 ± 0.02^{AB}	6.18 ± 0.04^{C}	0.25 ± 0.03^{A}	0.30 ± 0.02^{B}	0.35 ± 0.04^{C}
14	6.05 ± 0.01^{B}	6.08 ± 0.02^{A}	6.09 ± 0.02^{A}	0.32 ± 0.01^{C}	0.32 ± 0.02^{AB}	0.36 ± 0.02^{A}
17	5.86 ± 0.02^{C}	5.95 ± 0.01^{B}	6.00 ± 0.01^{A}	0.34 ± 0.02^{A}	0.35 ± 0.01^{AB}	0.38 ± 0.03^{C}
21	S	5.78 ± 0.01^{B}	5.85 ± 0.01^{A}	S	0.37 ± 0.01^{A}	0.40 ± 0.01^{C}
25	S	5.54 ± 0.02^{C}	5.63 ± 0.03^{B}	S	0.43 ± 0.02^{A}	0.42 ± 0.01^{AB}
29	S	S	5.47 ± 0.02^{A}	S	S	0.46 ± 0.02^{A}
33	S	S	S	S	S	S

C = Control, **T1** = Cheese with 4,000 µg/ml WPC, **T2** = Cheese with 8,000 µg/ml WPC, **S** = spoiled sample. *The value given was the mean ± standard error (SE).
^{ABC} Values in the same row having different superscripts differ significantly ($p < 0.05$).

Table 2. TBCs (Log_{10}cfu/gm) in the examined cheese samples during their refrigerated storage.

Storage (days)	Total Bacterial Counts (Log_{10} cfu/gm) (Mean ± SE*)		
	C	T1	T2
Zero time	3.28 ± 0.24^{C}	3.18 ± 0.07^{B}	3.18 ± 0.05^{AB}
7	3.65 ± 0.17^{C}	3.43 ± 0.02^{B}	3.35 ± 0.01^{A}
14	4.53 ± 0.20^{C}	2.89 ± 0.01^{AB}	2.74 ± 0.07^{A}
17	4.78 ± 0.20^{C}	2.45 ± 0.04^{A}	2.34 ± 0.18^{C}
21	S	2.28 ± 0.25^{A}	2.16 ± 0.15^{AB}
25	S	2.15 ± 0.25^{AB}	2.08 ± 0.11^{A}
29	S	S	2.00 ± 0.03^{A}
33	S	S	S

C = Control, **T1** = Cheese with 4,000 µg/ml WPC, **T2** = Cheese with 8,000 µg/ml WPC, **S** = spoiled sample, ***SE** = Standard error.
^{ABC} Values in the same row having different superscripts differ significantly ($p < 0.05$).

Table 3. Influence of camel's WPC on coliform counts in the examined cheese samples during their refrigerated storage.

Storage (days)	Total coliform counts (Log_{10} cfu/gm) (Mean ± SE*)		
	C	T1	T2
Zero time	<1	<1	<1
7	1.65 ± 0.05^{C}	<1	<1
14	2.53 ± 0.03^{C}	1.45 ± 0.01^{AB}	<1
17	3.28 ± 0.03^{C}	1.89 ± 0.04^{AB}	1.14 ± 0.1^{A}
21	S	2.18 ± 0.05^{B}	1.85 ± 0.05^{AB}
25	S	2.25 ± 0.05^{AB}	2.00 ± 0.03^{A}
29	S	S	2.48 ± 0.02^{A}
33	S	S	S

C = Control, **T1** = Cheese with 4,000 µg/ml WPC, **T2** = Cheese with 8,000 µg/ml WPC, **S** = spoiled sample, ***SE** = Standard error.
^{ABC} Values in the same row having different superscripts differ significantly ($p < 0.05$).

maximum level at the end of refrigerated storage to be 4.78 ± 0.20 Log_{10}cfu/gm. However, the TBC in T1 and T2 were lower than control samples from 7th day with a mean count of 3.43 ± 0.02 and 3.35 ± 0.01 Log_{10}cfu/gm, respectively, till the end of storage. In addition, they reached to 2.15 ± 0.25 Log_{10} cfu/gm for T1 at 25th day and 2.00 ± 0.03 Log_{10}cfu/gm at 29 day of refrigerated storage. This indicated that TBC of the treated groups was significantly affected by camel's milk WPC and decreased with the increase of their concentrations.

Regarding coliforms, Table 3 showed the effect of using camel's WPC in the manufacture of low salt soft cheese on coliforms count. It could not be detected in any cheese samples at zero days. The mean coliforms count was 2.53 ± 0.03 and 1.45 ± 0.01 Log_{10}cfu/gm in control and T1 only at the 14th day of storage, respectively. However, coliform could be detected in all groups until the end of storage with lower counts in treated groups in comparison to control samples.

The extended shelf-life of low salt soft cheese with 8,000 µg/ml camel's WPC up to the 29th day of refrigerated storage with restricted and relatively low coliforms may result from the suppressive effect of antimicrobial components that present in milk WP milk [31]. In the same context, camel's whey contains higher protein % than that of cow's whey. These results reflected the concentration of protein in the final undenatured WP. It ranged from 34.4% in cow's milk to 55% in camel's milk [32].

Yeast and mold play an important role in the spoilage of dairy products primarily in fermented milk and cheese [33]. At the first week of manufacture, yeast and mold growth was not detected in control and treated samples in all as shown in Tables 4 & 5. However, their counts increased gradually in control samples from 7th day of storage until reached its maximum level 1.98 ± 0.02 and 2.58 ± 0.01 Log_{10}cfu/gm for mold and yeast, respectively, at the end of refrigerated storage. These

Table 4. Effect of camel's WPC on mold count in examined cheese samples.

Storage (days)	Total mold counts (Log_{10} cfu/gm) (Mean ± SE*)		
	C	T1	T2
Zero time	<1	<1	<1
7	<1	<1	<1
14	1 ± 0.01^{A}	<1	<1
17	1.98 ± 0.02^{B}	1.24 ± 0.02^{AB}	<1
21	S	1.60 ± 0.05^{B}	<1
25	S	1.95 ± 0.05^{AB}	1.30 ± 0.05^{A}
29	S	S	1.78 ± 0.02^{A}
33	S	S	S

C = Control, **T1** = Cheese with 4,000 μg/ml WPC, **T2** = Cheese with 8,000 μg/ml WPC, **C** = Control, **S** = spoiled sample, ***SE** = Standard error.
^{ABC} Values in the same row having different superscripts differ significantly ($p < 0.05$).

Table 5. Effect of camel's WPC on yeast count in examined cheese samples.

Storage (days)	Yeast count (Log_{10} cfu/gm) (Mean ± SE*)		
	C	T1	T2
Zero time	<1	<1	<1
7	<1	<1	<1
14	2.45 ± 0.01^{C}	1.15 ± 0.01^{B}	<1
17	2.58 ± 0.01^{C}	1.49 ± 0.04^{AB}	1 ± 0.0^{B}
21	S	1.78 ± 0.05^{B}	1.45 ± 0.0^{B}
25	S	2.15 ± 0.05^{C}	1.95 ± 0.02^{A}
29	S	S	2.39 ± 0.01^{A}
33	S	S	S

C = Control, **T1** = Cheese with 4,000 μg/ml WPC, **T2** = Cheese with 8,000 μg/ml WPC, **C** = Control, **S** = spoiled sample, ***SE** = Standard error.
^{ABC} Values in the same row having different superscripts differ significantly ($p < 0.05$).

results exceed the permissible limit (<10 Log_{10}cfu/gm for mold and <40 Log_{10}cfu/gm for yeast) suggested by the Egyptian Organization for Standardization and Quality [34]. However, in T1 and T2 (treated groups), the mold and yeast count decreased gradually and reached to 1.95 ± 0.05 and 1.78 ± 0.02 Log_{10}cfu/gm for mold and 2.15 ± 0.05 and 2.39 ± 0.01 Log_{10}cfu/gm for yeast at 25th and 29th day of refrigerated storage, respectively. The current results may be related to many antimicrobial substances in WPC. There are high significant differences ($p < 0.01$) between the effect of WPC on the count of mold as well as on yeast count. These results were agreed with results demonstrated by Naidu [35] and Samaranayake et al. [36] who reported that lactoferrin which one of antimicrobial substance in WPC of camel milk had antifungal effect

Generally, camel's WP contains a large number of antimicrobial agents, such as lyzosyme, α-lactalbumin, serum albumin, lactophorin, immunoglobulin, and lactoferrin more than those in cow's and goat's milk in reverse to ß-lactoglobulin. In the same context, goat's WP contains double amount of lysozyme than that of cow's WP [37]. Camel's WPs showed the superiority among other used WPs and this may be attributed to their constituent from lactoferrin. Lactoferrin of camel milk's has more potent antibacterial efficacy against wide varieties of bacteria than lactoferrin from milk of other animal species. Beaulieu et al. [38] which in turn it could preserve cheese for long period.

On the other hand, aerobic spore formers were not detected in all examined cheese samples at all stages of shelf life. This may be due to using good quality milk and good hygienic measures during the processing of cheese and storage. This result agreed with Awad [39] and Gamiel [40].

Effect of camel's WPC on organoleptic characters of soft cheese

The organoleptic evaluation is an important method for the determination of the quality of dairy products and consumer acceptance [41]. In this study, the addition of camel's WPC improved the flavor of the cheese samples. The flavor score of cheese samples prepared with 8,000 μg/ml scores was higher than those of 4,000 μg/ml and control cheese samples storage period (Fig. 2A). These results were similar to those reported by Hanafy et al. [11] who demonstrated that the flavor score after the addition of WPC was better than the control samples in soft cheese. El sheikh et al. [10] concluded that WPC is a good fat replacer in skimmed milk cheese which positively improves mouth feel of cheese. Sakr and Mehanna [18] found that adding WPC significantly improved the flavor of low-fat Ras cheese.

Body and texture are an important sensory attribute of dairy products like cheese. In this study, groups with camel's WPC have a better score than those without WPC (Fig. 2B). This improvement increased with an increase in the concentration of WPC. This agreed with Hanafy et al. [11], who reported that cheese containing 6% WPC showed low hardness and gumminess than cheese treated with 2% WPC and control samples. Lobatto-Calleros et al. [42] found that WPC caused significant modification on the body and texture of partial and full skimmed soft cheese. In other study, replacing fat with WPC increased the hardness, cohesiveness, gumminess, and chewiness values of soft cheese [43]. In the same context, Tashakori et al. [24] found that cheese prepared by the addition of 20% WPC improved the body, texture, and consistency of spread cheese. However, Henriques et al. [21] stated that body and texture were similar in control and treated cheese with WP. These differences

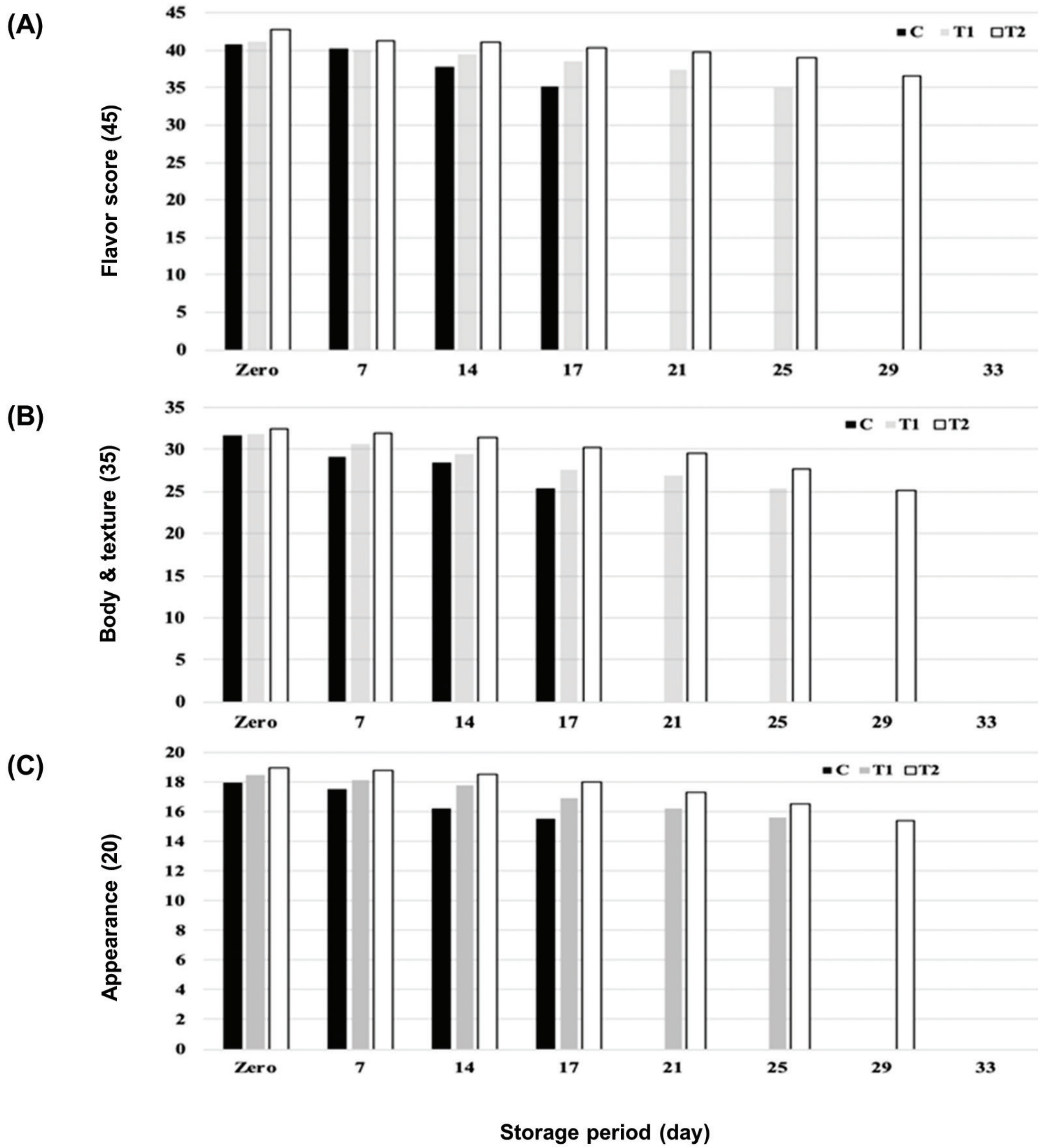

Figure 2. Organoleptic properties of different cheese groups during their refrigerating storage. (A) Flavor score, (B) body and texture score and (C) appearance score. C: Control, T1 = Cheese with 4,000, T2 = Cheese with 8,000 μg/ml WPC.

may be due to the type and characters of the adding WPC and its concentration as well as the procedures of cheese making and chemical composition of resultant cheese.

Evaluation of the appearance of control and camel's WPC-treated cheese was shown in Figure 2C. It is clear that the integration of WPC with cheese milk markedly improved the appearance of cheese samples comparing to control. However, the highest ($p < 0.05$) score was recorded in the cheese sample treated with 8,000 μg/ml WPC till the 29th day of refrigerating storage.

This result indicated that cheese prepared with 8,000 μg/ml camel's WPC was superior in organoleptic properties to that prepared with 4,000 μg/ml camel's WPC and control cheese samples when fresh as well as, this superiority continued till 29th day of refrigerated storage. Such variation was significant at $p < 0.05$ (Figure, 2). WP concentration has a direct proportion with the organoleptic characters of cheese [21]. In addition, WP acts as a fat replacer so that the quality of low-fat content cheese is improved [44].

Camel's milk is hardly coagulated so the production of cheese from it was not an easy process; therefore, researchers used a mixture of camel's and bovine milk. The bovine milk is commonly used for cheese production as it has good coagulation [45]. However; in the current study, the WPC derived from camel's milk displayed significantly antimicrobial activities. It reduced the populations of coliform, TBC, yeast, and molds during refrigerated storage of cheese. Moreover, it preserved soft cheese for 29 days in comparison to 17 days in the control cheese group. In addition, it also improved the organoleptic properties of cheese. These activities increased by increasing the WPC concentration.

Conclusion

WPC extracted from camel's milk could be applied as a natural antimicrobial agent that elongated the life of soft cheese, especially at the concentration of 8,000 μg/ml. Moreover; WPC would be used as a replacer for synthetic substances to meet consumer's needs.

Conflict of interests

There are no potential conflicts of interest among the authors with respect to the research, authorship, and/or publication of this article.

Authors' contribution

Hend Ahmed Elbarbary and Marwa Awad Saad designed the concept for this research and scientific paper. Marwa Awad Saad was provided camel's milk and chemical substances. All authors participated in all examinations of groups. They also participated in manuscript's draft and revision. All authors have read and approved the final manuscript.

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