International Journal of
Food Sciences
(IJF)

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IPRJB
INTERNATIONAL PEER REVIEWED JOURNAL AND BOOK PUBLISHING

International Journal of Food Sciences
2789-7680 (online)
Vol. 5, Issue 1, No. 2, pp 16 - 32, 2022
www.iprjb.org

IMPACT OF HYDROLYZED CAMEL´S WHEY PROTEIN CONCENTRATE ON SOFT CHEESE QUALITY

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Abstract

Purpose: The current study's aim is to to investigate the effect of hydrolyzed whey protein concentrate (WPC) derived from camel's milk on quality and organoleptic properties of soft cheese during refrigerated storage.

Methodology: Two concentrations (10 and 20 mg/g) of camel´s WPC and its hydrolysates [pepsin (P) and pepsin-trypsin (P-T) hydrolysates] were incorborated in to soft cheese and their effects on total bacterial, phsychrophilic, aerobic spore formers, coliforms and yeast & mold counts were calculated till end of refrigerated storage peroid. Also, flavour, texture and appearance of treated cheese groups were evaluated compared to control one.

Findings: The results revealed that the higher concentration of WPC and its hydrolysates, the more significant decrease in the microbial load and increase the shelf life up to 34th days with P-T hydrolysate (20 mg/g) compared with the control with a shelf-life of 18th days only at refrigerated temperature (4^{o}C).This hydrolysate showed also the highest degree of hydrolysis (DH%) of 34.06% ± 1.53 and protein concentration of 30.72%± 3.16. The maximum score for body, and texture and appearance was recorded for the cheese sample containing P-T hydrolysate (20 mg/g), while the maximum flavour score was recorded to pepsin (P) hydrolysate (10 mg/g), compared with unhydrolyzed WPC concentrations and control soft cheese groups.

Unique contribution to theory, practice and policy: This study was conducted to elaborate antimicrobials from camel´s WPC after enzymatic hydrolysis which could serve as a potential natural presrvatives in soft cheese without altering the sensory characteristics.

Keywords: *Camel´s WPC, enzymatic hydrolysis, antimicrobial activity, soft cheese, refrigerated storage*

INTRODUCTION

Camel's milk has a higher whey protein concentration than milk from other animal species, especially proteins with higher biological and health benefits such as peptidoglycan recognition protein, immunoglobulins IgA and IgG, whey acidic proteins (WAP), lactoferrin, lysozyme and serum albumin (Kappeler *et al*., 2005). The higher concentration of protein hydrolysates and bioactive peptides released from enzymatic hydrolysis of camel whey can exert a powerful antimicrobial activity against some bacterial, fungal and viral pathogens (Sołowiej *et al*., 2010). Many studies focused on the effective antimicrobial activity of pasteurized camel milk against many foodborne pathogens (Ayyash, 2016).

The bioactive peptides of milk protein hydrolysates have many different mechanisms for inhibition of microorganisms. These mechanisms include production of inhibitory compounds, competition for binding sites and nutrient competition. From these inhibition activities, the production of organic acids that able to destroy the cell membrane of pathogens (Haller *et al.*, 2001).

Cheese can be contaminated by different varieties of spoilage microorganisms such as psychophilic bacteria or fungi which not only limit the shelf life of the product but also, render the nutritious and taste of cheese inedible with objective visual and organoleptic changes with public health hazards for consumers (Borges *et al*., 2008). To overcome these bad effects and prolong the shelf-life of cheese, there is a great concern about natural antimicrobial agents applied in the dairy industry (Da Silva *et al*., 2012).

Considering these aspects, the current work was aimed to investigate the impact of using of camel WPC hydrolysates on the quality and organoleptic properties of soft cheese during refrigerated storage.

MATERIALS AND METHODS

Preparation of camel´s WPC

Camel's milk (3.54% total protein, 3.3% fat) was obtained from the herd of Sidi-Barani areas, Matrouh Governorate, North West Coast, Egypt. Milk was centrifuged at 12,000 rpm at 4°C for 30 min (refrigerated benchtop centrifuge model Sigma 3-16KL, Central Lab, Fac.Vet.Med, Benha University, Egypt) to remove fat content. Skimmed milk was then subjected to isoelectric precipitation at pH 4.5 by the addition of 10% (v/v) acetic acid (1M) and then incubated at 37°C for 1 hr to enhance casein precipitation. The obtained supernatant was adjusted to pH 7.0 with 1ml NaOH(1M) and re-centrifuged at 13,000 rpm for 30 min at 4˚C. The micellar casein pellet was precipitated at the bottom layer, while the whey was migrated to the supernatant layer. The resultant liquid whey solution was dialyzed against distilled water using a porous membrane with a molecular weight cut-off (MWCO) of 8 kDa (Sigma-Aldrich Chemical Company, Nasr City, Egypt) for 72 hr at 4°C. The retentate of the dialysis containing un-denatured whey protein concentrate (WPC) was then lyophilized (freeze-dried) using a laboratory-scale freeze dryer at -60°C, 10 Pa pressure for 24hr (Esquire Biotech, Chennna, International Scientific Research Center, Dokki, Giza, Egypt) to obtain camel WPC powder (Wang *et al*., 2020).

Preparation of the camel´s WPC hydrolysates

The hydrolysis of the camel´s WPC by both pepsin enzyme (from porcine gastric mucosa with activity of 3000 U/g), and trypsin enzyme (from pancrease with activity of 2000 U/g) were obtained from Sigma-Aldrich Chemical Company, Nasr City, Egypt). According to

the method of Wang *et al* (2020), camel´s WPC suspension of 3.0% (wt/vol) was prepared by dispersing WPC powder in distilled water and was divided into 2 groups for enzymatic hydrolysis (G1: using pepsin enzyme alone and G2: using both pepsin and trypsin enzymes together).The pH was adjusted to the optimum for each enzyme (pH 2 for pepsin using HCl (1 M); pH 7.72 for trypsin using Sodium phosphate buffer(0.1M).The hydrolysis of whey protein was then carried out at the optimal temperature for each enzyme (37°C for pepsin; 42°C for trypsin) in water bath under constant agitation of 500 rpm (PHOENIX Magnetic Stirrer RSM14HP model, Central Lab, Fac.Vet. Med, Benha University, Egypt).

The hydrolysis process was carried out at the same enzyme to substrate ratio (2%(w/w) for the same 3 hr of hydrolysis for each enzyme and then enzymes were inactivated by heating at 85°C for 5 min. The hydrolysates were centrifuged at 10,000 rpm at 4°C for 30 minutes and immediately freeze dried. The control group of unhydrolysed WPC was prepared under the same hydrolytic conditions without addition of enzymes. All obtained groups were designed as unhydrolyzed camel´s WPC, P-hydrolysates (pepsin generated WPC hydrolysates), and P-T hydrolysates (pepsin and trtpsin generated WPC hydrolysates).

Characterization of camel´s WPC hydrolysates

Determination of protein concentration of camel´s WPC and its hydrolysates

Protein concentration of camel´s WPC and WPC hydrolysates was determined with Kjeldahl method (AOAC, 2005).

Degree of hydrolysis (DH%)

The degree of hydrolysis of camel whey hydrolysates was determined as the method recorded by Silvestre *et al*. (2013). About 20 ml of protein hydrolysate was added to 20 ml of trichlorocacetic acid (10% TCA). Then obtained mixture was centrifuged at 7800 rpm for 15min. The calculation of the degree of hydrolysis (DH%) was conducted as follows:

DH (%) = Solubilized protein content in TCA / Total protein content in original sample x 100.

Determination of soft cheese quality fortified with camel´s WPC and its hydrolysates:

Fourteen liters of full cream buffalo's milk (4.30% total protein, 7.50% fat) was obtained from the herd of Faculty of Veterinary Medicine, Benha University.The bulk milk was heat treated at 63°C for 30 min. Low salt soft cheese (3%) was prepared as described by Denis *et al*. (1997) and divided into seven groups (2 liter of each) as follows;

(G_0): Group without any additives (control).

(G_1): Group treated with 10 mg/g of unhydrolyzed camel´s WPC.

(G_2): Group treated with 20 mg/g of unhydrolyzed camel´s WPC.

(G_3): Group treated with 10 mg/g of P-WPC hydrolysates.

(G_4): Group treated with 20 mg/g of P -WPC hydrolysates.

(G_5): Group treated with 10 mg/g of P-T hydrolysates.

(G_6): Group treated with 20 mg/g P-T hydrolysates.

After curd formation & drainage of the whey, the cheese groups were stored at the refrigerator temperature (4°C) and were examined for microbiological and organoleptic properties when fresh and after 7, 14 day then every 4 days till the signs of spoilage were appeared. All the trials and examination were repeated for 3 times.

Microbiological examination

Ten g of soft cheese were added to 90 ml of Sodium citrate solution (2%) in sterile flasks and homogenized in a Stomacher for 2 to 4 minutes (APHA, 1992). 10-fold serial dilutions were prepared and 1 ml of each dilution was taken and plated on nutrient agar and incubated at 37°C/24-48 hrs and at 7°C for 7–10 days for the enumeration of total bacterial count (TBC) and psychrophilic bacterial count, respectively as described by ISO (2008). Coliforms count was conducted according to the method described by FDA (2002). Aerobic spore former count was conducted according to the method described by APHA (1992); whereas yeasts and molds were determined according to ISO (2008).

Organoleptic evaluation

The organoleptic properties of soft cheese samples were carried out according to IDF (1995). Seven Staff members of Food Hygiene and Control Department, Fac.Vet. Med, Benha University were evaluated the cheese for appearance (20 points), body and texture (35 points), and flavor (45points) with an overall acceptability (100 points).

Statistical analysis

Differences among means were tested for significance ($P<0.05$) as described by Hill and Lewicki (2007). Statistical analysis of the data was carried out employing analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Protein concentration and DH% in camel WPC hydrolysates

The degree of hydrolysis (DH) of whey protein hydrolysates considerds as a vital parameter to calculate the percentage of broken peptide bonds which affects on the biological activity of generated hydrolysates (Aluko, 2018).Thus, the DH% of camel´s WPC hydrolysates were expressed by measuring the amount of peptides in the trichloroacetic acid (TCA) supernatant and expressed as a ratio of percent of total protein weight in the hydrolyzate. Camel WPC hydrolysates possessed some remarkable characteristics, the mean vaues of DH% for P- hydrolysates and P-T-hydrolysates were 14.93 ± 1.50 and 34.06% ± 1.53 while the mean values of protein content were 28.93% ±3.04 and 30.72% ± 3.16, respectively. However, mean values of 25.23%± 0.64 of protein content and 8.47%± 2.06 of DH% were evaluated in unhydrolyzed camel´s WPC (Figure.1).

International Journal of Food Sciences
2789-7680 (online)
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www.iprjb.org

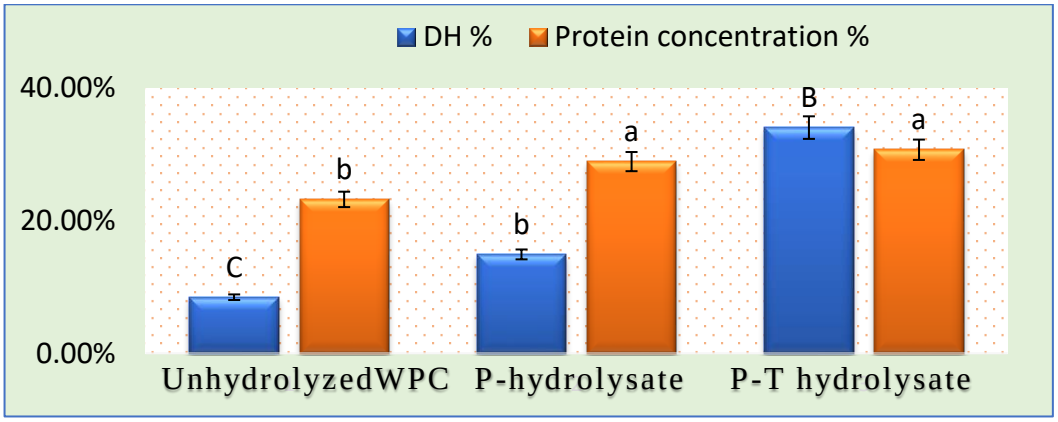

Figure 1: Protein concentration % and degree of hydrolysis (DH%) of unhydrolyzed camel´s WPC and its hydrolysates (P-hydrolysates {pepsin generated camel´s WPC hydrolysates}; P-T hydrolysates [pepsin and trypsin generated camel´s WPC hydrolysates}. Values are expressed as means ± SD of 3 independent determinations. Different letters indicate a significant difference among different hydrolysates ($p < 0.05$).

The low DH% of unhydrolyzed camel´s WPC, could be related to a mild denaturation and hydrolysis occurred during the heat inactivation (Zúñiga *et al.*, 2010). However, the higher protein content in P-T hydrolysates may be due to the synergism between both pepsin-trypsin enzymes for breaking more peptide bonds in camel whey proteins releasing higher concentrations of smaller more soluble peptides (Chalamaiah *et al.*, 2010).This came in agreement with Rossini *et al.* (2009) who demonstrated the increased protein content of hydrolysates with progressed hydrolysis time depending on enzyme specificity and the protein substrate and the same results were reported by Ovissipour *et al.* (2012).

The effect of camel WPC hydrolysates on soft cheese quality

Soft cheese may be contaminated with several spoilage microoranisms affecting on cheese quality and shelf life (Cokal *et al.*, 2012). On other hand, several camel whey proteins and their hydrolysates have multiple binding sites with higher affinity for microbial membranes and lipopolysaccharides of many spoilage microorganisms causing its destruction (Diarra *et al.*, 2003).

Table (1) illustrated that the mean values of the TBC were the highest values in G_0 (control cheese group) and were increased from 3.18± 0.12 $\log_{10}$ cfu/g at zero day (day of production) untill reached to 5.64±0.05 $\log_{10}$ cfu/g at 18th day of refrigerated storage. On the other hand, G_6 (cheese treated with 20 mg/g of P-T hydrolysates) showed the lowest TBC along the storage period with the mean values of 3.18±0.003 $\log_{10}$ cfu/g at 26th day and increased to 4.22±0.15 $\log_{10}$ cfu/g at 34th day.

International Journal of Food Sciences
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www.iprjb.org

Table 1: The total bacterial counts in different cheese groups during their refrigerated storage (4°C).

Storage time (Day)	G_0	G_1	G_2	G_3	G_4	G_5	G_6
Zero	3.18±0.12^{bdA}	3.17±0.02^{acA}	3.19±0.01^{cA}	3.16±0.03^{a} dA	3.06±0.25 dA	3.02±0.37^{c} eA	3.00±0.35^{d} A
7	4.04±0.02^{acA}	4.13±0.45 bcABC	4.25±0.18 bAB	3.82±0.06^{c} BC	3.7±0.23^{c} dABC	2.95±0.36^{c} eC	2.83±0.42^{dC}
14	5.42±0.05^{dcAC}	4.13±0.13 bB	4.12±0.07 acAB	4.36±0.09^{b} C	4.19±0.04^{cC}	3.09±0.10^{c} dA	3.13±0.06^{bc} dA
18	5.64±0.05^{abAD}	4.36±0.06 bcB	4.26±0.14 aB	4.41±0.13^{b} A	4.26±0.07^{cA}	3.49±0.26^{b} cdCD	3.16±0.16 dD
22	S	4.52±0.33 bA	4.34±0.35 abAB	4.55±0.02^{b} BC	4.73±0.30^{bAB}	4.18±0.59^{a} bcdABC	3.17±0.06^{bc} dC
26	S	4.59±0.08 aAD	4.78±0.41 aAB	4.71±0.34^{a} bcBC	4.57±0.04^{aC}	4.25±0.05^{b} CD	3.18±0.003^{c} CD
30	S	S	S	S	4.90±0.03^{aA}	4.35±0.02^{a} dA	3.10±0.10^{bB}
34	S	S	S	S	S	S	4.22±0.10^{aA}
38	S	S	S	S	S	S	S

G_0: control group (Soft cheese without any additives); G_1: Soft cheese treated with 10 mg/g unhydrolyzed camel´s WPC; G_2: Soft cheese treated with 20 mg/g of unhydrolyzed camel´s WPC; G_3: Soft cheese treated with 10 mg/g of P-hydrolysates (pepsin generated camel´s WPC hydrolysates) ;G_4: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin generated camel´s WPC hydrolysates) ; G_5:Soft cheese treated with 10 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates) ; G_6: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates).

^{abcdef} the differences between the values in the same raw are statistically significant ($p < 0.05$) from each other.

^{ABCDE} the differences between the values in the same column are statistically significant ($p < 0.05$) from each other.

S: Spoiled samples. *The values indicated are the mean ± S.E.

Table (2) showed the mean values of psychrophilic organisms in examined cheese samples. At the day of production, the psychrophilic counts were detected at lower levels that not exceed 1 Log_{10}cfu/g either in control or treated groups. With respect to G_0 (control cheese sample), there was a gradual increase in mean values of psychrophilic count from 1.85±0.19 Log_{10}cfu/g at 7th day till reached to 2.39± 0.15 Log_{10}cfu/g at 18th day of refrigerated storage. However, psychrophilic bacteria could not be detected in both G_5 (cheese treated with 10 mg/g of P-T hydrolysates) and G_6 (cheese treated with 20 mg/g of P-T hydrolysates) beginning from zero day (day of production) till 22th day of storage. After that, the mean values increased untill reached to 1.7 ±0.20 and 0.89±0.46 Log_{10}cfu/g for G_5 and G_6 at 30th and 34th day of storage, respectively.

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Table 2: The psychrophilic bacterial counts in different cheese groups during their refrigerated storage (4°C)

Storage time (Day)	G_0	G_1	G_2	G_3	G_4	G_5	G_6
Zero	0.78±0.40^{bA}	0.92±0.46 bA	0.96±0.48 abA	0.89±0.45^{a} A	0.79±0.40^{aA}	ND	ND
7	1.85±0.19^{abA}	1.06±0.58 abA	0.83±0.44 bA	0.89±0.46^{a} A	0.78±0.40^{aA}	ND	ND
14	1.96±0.10^{aA}	1.20±0.76 abA	1.10±0.67 abA	1.04±0.61^{a} A	1.00±0.58^{aA}	ND	ND
18	2.39±0.15^{aA}	1.81±0.93 abAB	1.64±0.16 abB	1.00±0.58^{a} AB	1.10±0.67^{aAB}	ND	ND
22	S	1.99±0.52 aA	1.76±0.92 abA	1.13±0.69^{a} A	1.12±0.06^{aA}	ND	ND
26	S	2.39±0.23 aA	2.20±0.2^{a} AB	1.66±0.15^{a} ABC	1.40±0.20^{aBC}	0.99±0.51 abABC	0.82±0.42^{aC}
30	S	S	S	S	1.65±0.83^{aA}	1.7±0.89^{ab} A	0.89±0.46^{aA}
34	S	S	S	S	S	S	0.89±0.46^{aA}
38	S	S	S	S	S	S	S

G_0: control group (Soft cheese without any additives); G_1: Soft cheese treated with 10 mg/g unhydrolyzed camel´s WPC; G_2: Soft cheese treated with 20 mg/g of unhydrolyzed camel´s WPC; G_3: Soft cheese treated with 10 mg/g of P-hydrolysates (pepsin generated camel´s WPC hydrolysates) ;G_4: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin generated camel´s WPC hydrolysates) ; G_5:Soft cheese treated with 10 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates) ; G_6: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates).

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^{ABCDE} the differences between the values in the same column are statistically significant ($p < 0.05$) from each other.

S: Spoiled samples. *The values indicated are the mean ± S.E.

ND: Not detected.

Table (3) showed the effect of addition of camel´s WPC and its hydrolysates on coliform counts in soft cheese. The counts of coliform were detected at lower levels not exceed 1 Log_{10}cfu/g in all cheese samples at zero day (day of production). There was a gradual increase in mean values of coliforms in G_0 (control group) from 2.61±0.06 log_{10}cfu/g at 7^{th} day till it reached to 4.16±0.01 log_{10}cfu/g at 18^{th} day of refrigertaed storage. However, P-T hydrolysates had strongest inhibitory effect against coliforms which decreased gradually from 18^{th} day with mean value of 2.83±0.30 log_{10}cfu/g till reached to 1.38±0.69 log_{10}cfu/g at 30^{th} day of storage for G_5 (cheese treated with 10 mg/g of P-T hydrolysates).

Regarding to G_6 (cheese treated with 20 mg/g of P-T hydrolysates), coliform could not be detected beginning from 26th day till 34th day of refrigertaed storage.

Table 3: The total coliform counts in different soft cheese groups during their storage (4°C).

Storage time (Day)	G_0	G_1	G_2	G_3	G_4	G_5	G_6
Zero	0.81 ± 0.25^{cA}	0.78 ± 0.40^{cA}	0.67 ± 0.33^{cA}	0.74 ± 0.38^{dA}	0.75 ± 0.33^{cA}	0.77 ± 0.33^{aA}	0.77 ± 0.31^{bcA}
7	2.61 ± 0.06^{aA}	1.46 ± 0.20^{abB}	0.74 ± 0.37^{cC}	2.49 ± 0.13^{cA}	1.96 ± 0.32^{aAB}	0.40 ± 0.41^{afC}	0.75 ± 0.38^{bC}
14	3.28 ± 0.13^{bA}	1.88 ± 0.37^{abBC}	1.16 ± 0.09^{bC}	2.51 ± 0.12^{cB}	2.05 ± 0.25^{aBD}	2.27 ± 0.09^{deB}	1.26 ± 0.23^{aCD}
18	4.16 ± 0.01^{acA}	2.75 ± 0.32^{aBC}	2.73 ± 0.15^{aB}	3.79 ± 0.34^{abABC}	3.38 ± 0.16^{bC}	2.83 ± 0.30^{dBC}	0.24 ± 0.67^{abD}
22	S	2.04 ± 0.04^{aAC}	2.46 ± 0.24^{aC}	3.57 ± 0.03^{aAB}	2.88 ± 0.41^{abAC}	1.38 ± 0.04^{bcB}	0.67 ± 0.33^{bD}
26	S	1.10 ± 0.10^{B}	0.99 ± 0.44^{bcB}	3.17 ± 0.12^{bA}	2.83 ± 0.13^{abAC}	1.23 ± 0.23^{bB}	ND
30	S	S	S	S	2.21 ± 0.15^{aA}	1.38 ± 0.69^{abdB}	ND
34	S	S	S	S	S	S	ND
38	S	S	S	S	S	S	S

G_0: control group (Soft cheese without any additives); G_1: Soft cheese treated with 10 mg/g unhydrolyzed camel´s WPC; G_2: Soft cheese treated with 20 mg/g of unhydrolyzed camel´s WPC; G_3: Soft cheese treated with 10 mg/g of P-hydrolysates (pepsin generated camel´s WPC hydrolysates) ;G_4: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin generated camel´s WPC hydrolysates) ; G_5:Soft cheese treated with 10 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates) ; G_6: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates).

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ABCDE the differences between the values in the same column are statistically significant ($p < 0.05$) from each other.

S: Spoiled samples. *The values indicated are the mean ± S.E.

ND: Not detected.

Table (4) declared that the addition of camel WPC and its hydrolysates to soft cheese significantly decreased aerobic spore former counts compared to control group. The counts of aerobic spore former could not be detected in any cheese groups at zero day (day of production). However, the count observed in G_0 (control group) beginning from 7th day with mean value of 0.78 ± 0.40 $\log_{10}$ cfu/g, then it increased till reached to 2.32 ± 0.08 $\log_{10}$ cfu/g at 18th day. However, in G_6 (cheese treated with 20 mg/g of P-T hydrolysates), the aerobic spore formers completely disappeared throughout the storage period.

Table 4: The aerobic spore former counts in different soft cheese groups during their storage (4°C).

Storage time (Day)	G_0	G_1	G_2	G_3	G_4	G_5	G_6
Zero	ND	ND	ND	ND	ND	ND	ND
7	0.78 ± 0.40^{bA}	0.94 ± 0.48^{aA}	0.93 ± 0.47^{aA}	ND	ND	ND	ND
14	2.09 ± 0.07^{aA}	1.00 ± 0.00^{aB}	0.33 ± 0.34^{aB}	0.47 ± 0.40^{aB}	0.86 ± 0.43^{aB}	0.47 ± 0.45^{aB}	ND
18	2.32 ± 0.08^{aA}	1.19 ± 0.09^{aB}	1.00 ± 0.54^{aAB}	0.84 ± 0.43^{aB}	0.77 ± 0.40^{aB}	0.41 ± 0.40^{aB}	ND
22	S	0.74 ± 0.37^{aA}	0.68 ± 0.64^{aA}	0.33 ± 0.31^{aA}	0.37 ± 0.33^{aA}	0.95 ± 0.48^{aA}	ND
26	S	ND	ND	ND	ND	ND	ND
30	S	S	S	S	ND	ND	ND
34	S	S	S	S	S	S	4.22 ± 0.10^{aA}
38	S	S	S	S	S	S	S

G_0: control group (Soft cheese without any additives); G_1: Soft cheese treated with 10 mg/g unhydrolyzed camel´s WPC; G_2: Soft cheese treated with 20 mg/g of unhydrolyzed camel´s WPC; G_3: Soft cheese treated with 10 mg/g of P-hydrolysates (pepsin generated camel´s WPC hydrolysates) ;G_4: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin generated camel´s WPC hydrolysates) ; G_5:Soft cheese treated with 10 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates) ; G_6: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates).

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S: Spoiled samples. *The values indicated are the mean ± S.E.

ND: Not detected.

The yeast and mould counts were observed in Table (5) revealed that in all examined cheese groups, the counts not exceed 1 log10cfu/g at zero day (day of production), then it increased gradually in the progress of storage beginning from 7th day till the end of storage. These results were matched with the EOS (2005) which demonstrated that the initial yeast counts should not exceed 400 cfu/g, however the mould counts should not exceed 10 cfu/g in fresh cheese at day of manufacture. In G_0 (control group), yeast and mold counts increased gradually from 7th day of storage with the mean value of 2.89±0.34 log10 cfu/g till reached to its maximum mean value of 4.56±0.02 log10 cfu/g at 18th day of refrigerated storage. These results agreed with findings of Ledenbach and Marshall (2010). However, the highest antifungal effect was observed in G_6 (cheese treated with 20 mg/g of P-T hydrolysates) with the mean value of 1.38±0.53 log10 cfu/g at 34th day of storage.

Table 5: The total yeast and mold counts in different soft cheese groups during their storage (4°C).

Storage time (Day)	G_0	G_1	G_2	G_3	G_4	G_5	G_6
Zero	0.37±0.04^{abdA}	0.34±0.03^{abcdA}	0.34±0.03^{aA}	0.34±0.03^{fA}	0.54±0.23^{dA}	0.44±0.30^{eA}	0.71±0.36^{abA}
7	2.89±0.34^{cA}	2.78±0.12^{abdA}	2.60±0.44^{cA}	1.07±0.19^{eAC}	1.33±0.20^{dBC}	2.30±0.17^{abcdeAC}	0.72±0.32^{abAB}
14	3.38±0.02^{bA}	2.57±0.15^{cB}	2.37±0.32^{bB}	1.27±0.09^{bdfAB}	1.82±0.09^{cAC}	1.54±0.03$^{abcd AC}$	0.43±0.58^{abCD}
18	4.56±0.02^{aA}	3.81±0.04^{bB}	3.45±0.29^{bA}	2.37±0.05^{bfA}	2.35±0.22^{bC}	1.59±0.29^{dcbCD}	0.28±0.69^{abD}
22	S	4.43±0.10^{abA}	4.36±0.15^{aA}	4.43±0.72^{abcAB}	3.58±0.16^{bB}	2.67±0.67^{abcdABC}	1.43±0.72^{abC}
26	S	4.31±0.15$^{a A}$	4.11±0.11^{abB}	4.55±0.03^{afC}	4.10±0.05^{aD}	2.73±0.27^{cE}	1.49±0.76^{abE}
30	S	S	S	S	4.11±0.06^{aA}	3.17±0.02^{bB}	1.67±0.33^{bAB}
34	S	S	S	S	S	S	1.38±0.53^{abB}
38	S	S	S	S	S	S	S

G_0: control group (Soft cheese without any additives); G_1: Soft cheese treated with 10 mg/g unhydrolyzed camel´s WPC; G_2: Soft cheese treated with 20 mg/g of unhydrolyzed camel´s WPC; G_3: Soft cheese treated with 10 mg/g of P-hydrolysates (pepsin generated camel´s WPC hydrolysates) ;G_4: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin generated camel´s WPC hydrolysates) ; G_5:Soft cheese treated with 10 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates) ; G_6: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates).

^{abcdef} the differences between the values in the same raw are statistically significant ($p < 0.05$) from each other.

^{ABCDE} the differences between the values in the same column are statistically significant ($p < 0.05$) from each other.

S: Spoiled samples. *The values indicated are the mean ± S.E.

Organoleptic evaluation of soft cheese samples fortified with CWPH

The organoleptic properties of cheese are the main cheese characteristics which influence the acceptability of product (Hicsasmaz *et al.*, 2000). Camel whey proteins and their hydrolysates have improved organoleptic properties associated with their better solubility, emulsifying capacity, fat binding and water holding properties (Al-Shamsi *et al.*, 2018). The mean flavor values for all examined cheese groups were shown in Figure (2A). The results of the flavor analysis indicated that the cheese groups treated with camel´s WPC and its hydrolysates were better than control group. G_3 (cheese treated with 10 mg/g of P-hydrolysates) was achieved the highest flavor score throughout storage. The mean value of flavor score in G_3 (cheese treated

with 10 mg/g of P- hydrolysates) was 44.33±0.67 at zero day (day of production) and decreased gradually till reached to 42.00± 0.58 at 26th day of storage, while the lowest flavor score was obseved for G_0 (control group) with mean value of 36.67±0.88 at 18th day of storage.

Regarding to the texture, data presented in Figure (2B) revealed that the addition of camel´s WPC hydrolysates and increasing their concentrations significantly affects the body and texture of yoghurt (Figure 2B), ($p < 0.05$). G_0 (control group) gained the lowest score of body and textures either fresh or throughout storage with mean values beginning from 31.33±0.66 at zero day (day of production) till reached to 27.67±0.33 at 18th day of storage. However, G_6 (cheese treated with 20 mg/g of P-T hydrolysates) exhibited the highest body and texture score at zero day (day of production) with mean value of 33.00±0.00 and maintained their high score till reached to the mean value of 32.32±0.33 at 34th day of storage.

With respect to cheese appearance, Figure (2C) illustrated that at zero day (day of production), the identical and homogeneous appearance of the cheese groups was observed with no visual differences between control and these treated cheese groups ($P>0.05$). Beginning from the 7th day, a more pronounced attractive white color of soft cheese was developed by P and P-T camel´s WPC hydrolysates followed by unhydrolyzed WPC treated groups in comparison to control group ($P<0.05$). G_0 (control group) with mean value of 19.67 ±0.33 at zero day (day of production), decreased to 11.31 ±0.52 at 18th day of refrigerated storage. However, G_6 (cheese treated with 20 mg/g of P-T hydrolysates) had the highest appearance scores when fresh and maintained the highest appearance scores with the same mean value of 20.00±0.00 throughout refrigerated storage, then it slightly decreased till reached to mean value of 19.63±0.12 at 34th.

As shown in Figure (2D), G_3 (cheese treated with 10 mg/g of P-hydrolysates) and G_6 (cheese treated with 20 mg/g of P-T hydrolysates maintained the highest organoleptic overall scores with mean values of 96.97±0.21 and 95.66 ±0.84, respectively ($P>0.05$) from zero day (day of production) to 14th day of refrigerated storage. The total scores in G_3 were gradually decreased after 14th day till reached to mean value of 89.64±0.91 at 30th day. However, G_6 reached to mean value of 90.51 ±0.51 at 34th day. On the other hand, G_0 (control group) gained the least organoleptic scores throughout storage with mean value ranged from 92.21±0.84 at time of cheese manufacture till reached to 74.99±0.43 at 18th day of storage. There are significant differences in the mean overall organoleptic scores between G_6 and G_0 ($p< 0.05$).

International Journal of Food Sciences
2789-7680 (online)
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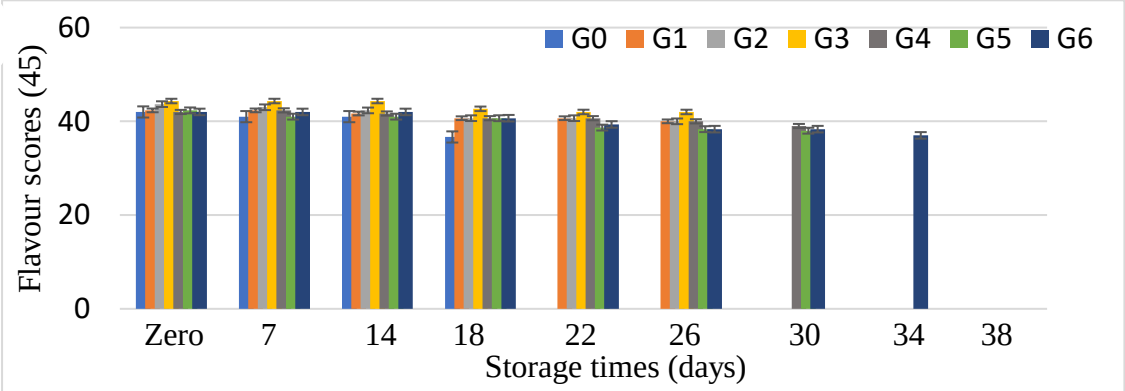
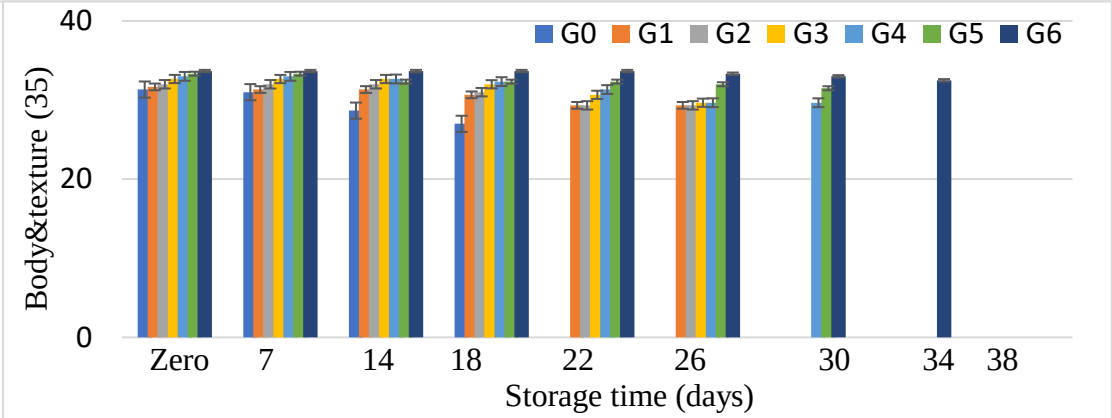
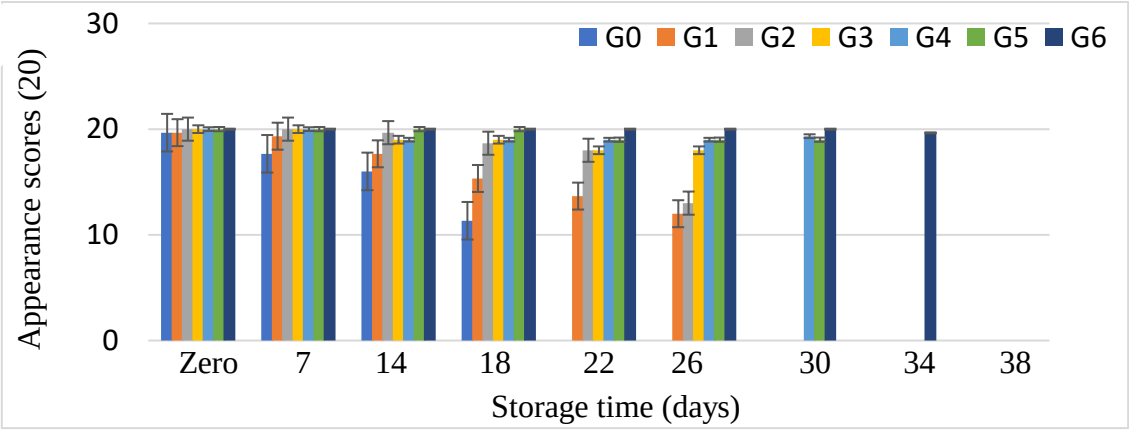
(A)



(B)



(C)



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(D)

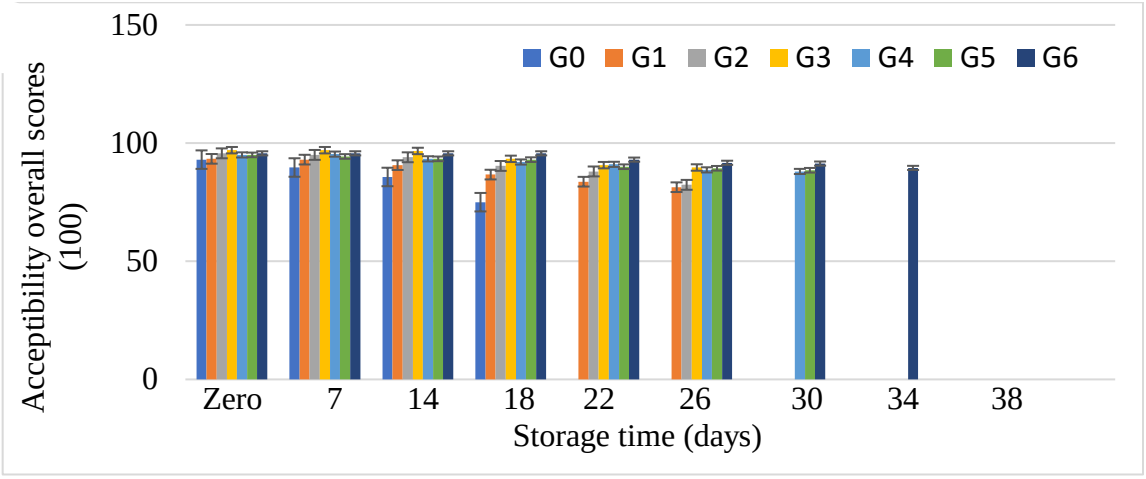

Figure 2: Organoleptic properties of different cheese groups during their refrigerating storage. (A) Flavor score, (B) body and texture score, (C) appearance score and (D) overall acceptibility score. G_0: control group (Soft cheese without any additives); G_1: Soft cheese treated with 10 mg/g unhydrolyzed camel´s WPC; G_2: Soft cheese treated with 20 mg/g of unhydrolyzed camel´s WPC; G_3: Soft cheese treated with 10 mg/g of P-hydrolysates (pepsin generated camel´s WPC hydrolysates) ;G_4: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin generated camel´s WPC hydrolysates) ; G_5:Soft cheese treated with 10 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates) ; G_6: Soft cheese treated with 20 mg/g of P-T hydrolysates (pepsin and trypsin generated camel´s WPC hydrolysates).

DISCUSSION

The enzymatic hydrolysis of camel whey proteins generated several bioactive peptides **(**Alhaider *et al.*, 2013). These hydrolysates have an improved antimicrobial activity against many food borne microorganisms (Salami *et al.*2010). Camel´s WPC had been prevoiusly applied to improve the quality of soft cheese (Elbarbary and Saad, 2019). However, there were no in vivo studies carried out on camel whey protein hydrolysates in food system. This current study revealed the antagonistic effect of camel´s WPC hydrolysates against TBC in soft cheese which might be attributed to their peptide fractions especially those released from lactoferrin, lysozyme, lactoperoxidase and immunoglobulins after enzymatic hydrolysis (Benkerroum *et al.*, 2004). Similar findings were reported by and Elbarbary and Saad (2019). According to EOS (2005), TBC should not exceed 10^5 cfu/g in soft cheese. The results for all treated cheese samples were within these permissible limits until end of refrigerated storage, while the higher levels of TBC (>10^5 cfu/g) were showed in control group. A significant reduction of psychrophilic counts in all treated cheese groups may attributed to effectivness of peptides in camel whey proteins hydrolysates mainly potent camel Lactoferricin peptides as demonstrated by Pirkhezranian *et al.* (2020). The greater anti-coliform activity may be attributed to the bactericidal peptide fragments released from camel lactoferrin hydrolysates as lactoferricin and lactoferrampin, which had greatest activity against coliforms (Jrad *et al.*, 2020).In addition, the complete dissapearance of coliforms in G_6 (cheese treated with 20 mg/g of P-T hydrolysates) at the end of storage may be attributted to unfolding of some compact globular whey proteins after synergestic pepsin-trypsin hydrolysis mainly α-

lactalbumin and immunoglobulins generating bactericidal peptides (Abd El-Rahim, 2020). This came in accordance with findings revealed by El-Bayoumi (2019). The potent inhibitory effect of WPC hydrrolysates against aerobic spore formers in cheese may be related to the storng effectivness of lactoferrampin peptide fractions (268-288) obtained from camel lactoferrin against spore formers (van der Kraan *et al*., 2004). According to EFSA (2005), the maximum permissible limit for aerobic spore formers should not exceed 10^3 spores/g in cheese. This was agreed with Elbarbary *et al*. (2018). Compared to unhydrolyzed WPC treated and control groups, **t**he greater antibacterial activity of camel´s WPC hydrolysates may related to their richness with hydrophobic bioactive peptides which inhibit bacterial growth either by penetrating the bacterial membrane and the release of lipopolysaccharides or causing DNA denaturation of bacterial cell (Manzoni, 2019). Also, the potent antifungal activity of camel´s WPC hydrolysates in soft cheese may be attributed to their potent peptides which inhibit fungal cell wall division either through chitin and heparine binding abilities of these peptides or through direct contact with fungal cell membrane causing its damage (Ider *et al*., 2020). The damage of fungal hyphae and conidia by action of camel´s lactoferrin and lactoperoxidase, in addition of the higher levels of Lactobacillus spp. and Bifidobacteria that isolated from camel milk may play role in fungicidal potency of camel´s WPC (Crisp *et al*., 2006; Iqra et al., 2020). These results were in same line of work of Maaroufi *et al*. (2015). But these results were in contrast to those reported by Ismail *et al.* (2015) who revealed the failure of liquid bovine whey up to 20% against fungal contamination in feta cheese.

With respect to organoleptic properties of treated soft cheese, the better flavour of G_3 (cheese treated with 10 mg/g of P-hydrolysates) may be related to the exposed hydrophobic amino acid residues and peptides with lower molecular weight taste nucleotides present in camel´s WPC hydrolysates which may responsible for improved emulsion and creamy mouth-feel of soft cheese (Elkot, 2019). In addition, the highest antioxidant activity of camel whey protein hydrolysates which may prevent lipid peroxidation and undesirable off flavors as demonstrated by Salami *et al.* (2010). These results agreed with Mortazavi *et al* (2010). Also, the better texture and appearance in G_6 (cheese treated with 20 mg/g of P-T hydrolysates) may be attributed to the greater emulsifying and water binding properties of WPC hydrolysates which improve lubricity and creaminess texture of soft cheese (Ibrahim *et al*., 2019). Similar results were reported by Yadav *et al*. (2015) and Borges *et al.* (2020).These agreed with findings of Desouky and EL-Gendy (2019) whom confirmed on the better organoleptic properties of processed cheese fortified with 10% camel milk powder.

CONCLUSION AND RECOMMENDATION

The current results revealed that camel´s WPC and its hydrolysates could be incorborated as sensory enhancers and natural antimicrobial agents for extending the shelf life of soft cheese, especially P-T hydrolysates at 20 mg/g up to 34th days. Moreover; WPC and its hydrolysates would be used as a replacer for synthetic substances to meet consumer's needs. Further studies should be carried out for purification and identification of the peptides sequences in antimicrobial camel´sWPC hydrolysates to be applied as effective preservatives in dairy industry.

ACKNOWLEDGEMENTS

The authors are grateful to The International Scientific Research Center, Dokki, Giza, Egypt, also more thanks to my supervisors in the Department of Food Hygiene and Control in Faculty of Veterinary Medicine at Benha University for providing all laboratory facilities and support throughout the work.

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