



Original article

Bioactive peptides supplemented raw buffalo milk: Biological activity, shelf life and quality properties during cold preservation



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ABSTRACT

This study aimed to prolong the raw buffalo milk handling and cold storage period by controlling the microbes, enhancing sensory properties and their functionality after supplementing bioactive peptides. The additions included hen and duck egg white protein isolates (HPI and DPI), pepper seed protein (PSP), and pepsin-kidney bean protein hydrolysate (PKH). Five milk treatments were prepared and evaluated as non-supplemented milk (M- Control), hen egg white protein isolate-supplemented milk (M-HPI), duck egg white protein isolate-supplemented milk (M-DPI), pepper seeds protein-supplemented milk (M-PSP), and kidney bean hydrolysate-supplemented milk (M-PKH). Pyrogallol, protocatechuic, catechin, benzoic and caffeine were the main phenolic compounds, Apiginin-6-arabinose, naringin, hesperidin, naringenin, kaempferol 3-2-p-comaroyl were the dominant flavonoids in milk samples based on HPLC profile. During 30 days of cold storage, the antioxidant potential of peptides-supplemented milk samples was significantly decreased ($p \leq 0.05$) as decrement of phenolic compounds and flavonoids; the pH was nearly stable, the titratable acidity and total soluble solids (TTS) were ($p \leq 0.05$) raised. PSP and PKH were inhibited ($p \leq 0.05$) the decay of sugars in M-PSP, and M-PKH by reducing 45% of bacterial load as compared to other milk samples. PSP was significantly ($p \leq 0.05$) scavenged 87% of DPPH compared to other peptides. Besides, PSP followed by PKH reduced considerably ($p \leq 0.05$) the growth of tested bacteria, molds, and yeasts. The PSP has significantly increased the whiteness of M-PSP as compared to other milk samples. M-PSP had the highest score in color, taste, and flavor, followed by M-PKH.

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1. Introduction

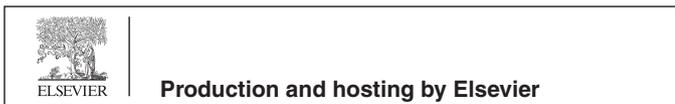
Milk is one of the most vital and nutritional foods for humans, as it contains valuable nutrients (Nicolaou and Goodacre, 2008). Milk is almost sterile when secreted from a vital udder (Gershon and Edward, 2017). Lactoferrin and lactoperoxidase are naturally

found in raw milk, inhibiting the microflora growth for 3–4 h after milking at the ambient temperature, and cooling at 4 °C maintains the milk quality. Refrigeration was considered a problem in developing countries because of the operating costs and the electricity supply deficiency, which can partially be controlled by boiling milk after milking or plunging milk containers in cold water. In the milk collection point, the expert people use the lactoperoxidase system to preserve raw milk from 7 to 8 h at 30 °C and overnight at 20 °C, this system didn't replace pasteurization, and it can be induced by chemical preservative, e.g., H₂O₂ (Alemu and Girma, 2018). Pasteurized milk was maintained for 7–14 days if stored under recommended conditions, but the differences were based on areas and seasons. Consumers check the best before date, but milk can deteriorate before the validation date recorded on the package (Lu et al., 2013). The microbiological standard for milk varies from

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one region to another, i.e., in Egypt, *Staphylococcus aureus* should be less than 100 CFU/mL in pasteurized milk, and *Escherichia coli* should not exist (Egyptian Standard, 2005). Identifying and characterizing chemical or natural additives and their use methods can be essential in developing appropriate techniques for handling milk and maintaining its quality. Chemical and natural additives are antimicrobials, antioxidants, anti-browning, texturizing, flavoring, coloring, and miscellaneous agents and they enhanced milk properties and maintained quality (Branen et al., 2001; Dickson-Spillmann et al., 2011; Randhawa and Bahna, 2009; Wilson and Bahna, 2005). The over-limit usage of chemical preservatives causes adverse damages, i.e., respiratory, dermatological, gastrointestinal, and neural damages (Carocho et al., 2014). However, the supplementation of raw milk with hydrogen peroxide-induced antioxidant and antimicrobial activity of the initial lactoperoxidase enzyme in milk (Arefin et al., 2017), also, potassium sorbate is one of the safer and lower toxicity preservatives used in the food industry as an antimicrobial agent (González-Fandos and Dominguez, 2007; Karabulut et al., 2001; Liu et al., 2014). Antioxidants, e.g., polyphenols, carotenoids, peptides, and vitamins present in plants and by-products, are considering natural additives that can be substituted the synthetic ones (Saad et al., 2021). The phenolic compounds were considered more relevant natural compounds to be used as food preservatives and active ingredients (Baines and Seal, 2012; Caleja et al., 2015a, 2015b; Carocho and Ferreira, 2013; Carocho et al., 2015; Saad et al., 2020). Bioactive peptides are the main defense in most animals. They release the oxidative stress caused by different environmental conditions. They can be used as natural preservatives because of their solubility, stability, and activity in foods (Abdelnour et al., 2020; They et al., 2019). Esterified legume protein isolates were reduced the bacterial load and titratable acidity in raw and pasteurized milk and improved the preservation quality (Sitohy and Osman, 2011). Osman et al. (2013) used 11S soybean protein subunit in milk preservation for 30 days; it was exhibited considerable antibacterial activity. Several peptides with biological activity were applied in food products (Meinert et al., 2016; Meshginfar et al., 2017). Also, Saad et al. (2020) studied the impact of pepsin-white kidney bean protein hydrolysate on minced beef quality, lifetime, and safety. Furthermore, Wan and Xu, (2018) evaluated the physical-chemical and sensory properties of a beverage supplemented with whey protein isolate. Moreover, Rachman et al. (2019) investigated the effects of egg white protein isolate administration on the quality of banana pasta, and they found that quality properties of pasta enhanced than control, and El-Saadony et al. (2020) enhanced the sensory properties and lifetime of cucumber juice with peptides isolated from vegetable and animal sources.

No studies tried to preserve raw milk with HPI, DPI, PSP, and PKH. Therefore, in this work: i) the radical-scavenging and antimicrobial activity of the HPI, DPI, PSP, and PKH were estimated ii) the effect of HPI, DPI, PSP, and PKH on raw milk preservation at cold condition 4 °C for 30 days was investigated, iii) polyphenols and flavonoids profile of HPI, DPI, PSP, and PKH were analyzed by HPLC, iv) the changes of the microbial count, color parameters, and sensory properties of milk were monitored during the storage period for the month.

2. Materials and methods

2.1. Materials

White kidney bean (*Phaseolus vulgaris* L.) seeds, chili pepper (*Capsicum annum* L.) pods, hen and duck eggs were acquired from the local market in Zagazig (Egypt). Raw buffalo milk was directly obtained after milking from a private farm in Abu-Hammad (Shar-

kia, Egypt), then transferred in a sterilized container to the laboratory. Pepsin enzyme, DPPH, Muller Hinton agar (MHA), Muller Hinton broth (MHB), Sabouraud dextrose agar (SDA), MacConkey agar and Plate count agar (PCA) were obtained from Oxoid Ltd (Basingstroke, Hampshire, UK). The milk pathogenic bacterial strains (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Escherichia coli*, and *Campylobacter jejuni*) and pathogenic yeasts and molds (*Candida apsis*, *Candida blankii*, *Candida glabrata*, *Candida glabrosa*, *Candida rugosa*, *Candida stellata*, *Penicillium solitum*, *Penicillium crustosum*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium solani*, and *Fusarium oxysporum*) were used in this work.

2.2. Methods

2.2.1. Protein isolates' preparation

2.2.1.1. PKH preparation. The kidney beans were ground to flour, the flour (10 g) was homogenized in hexane (300 mL) and stirred for 2 h, the solvent was discarded and the precipitate was dried. Total protein was isolated from defatted flour (5 g) according to Johnson and Brekke (1983) with some modifications then lyophilized. The total protein was mixed with pepsin (1:200 w/w), then dissolved in phosphate buffer (pH 2) and incubated at 37 °C for 3 h. The enzyme was inactivated in boiling water. The hydrolysate was centrifuged at 4000 xg for 30 min to obtain the lyophilized supernatant, then kept.

2.2.1.2. DPI and HPI preparation. The duck and hen egg-white were dissolved in water (1:3 v/v), and homogenized for 30 min and then centrifuged at 14,000 undercooling for 20 min. The supernatant was precipitated by polyethylene glycol (10%) then was centrifuged under same conditions. The precipitate was dissolved in Tris-Ca buffer pH 7.8 and kept overnight at 4 °C. The mixture was centrifuged under the same conditions, and the precipitates were washed with Tris-Ca, then mixed with Tris-EDTA, pH 7.8 and kept for 30 min at 4 °C. The supernatant was obtained by centrifugation at 1400g undercooling for 20 min. Then pH was adjusted to 5. The supernatant was dialyzed against Tris buffer pH 8, then was fractionated on the Q Sepharose column by the gradual addition of NaCl concentrations (0.1–0.6 M). HPI and DPI were eluted with NaCl concentrations (0.35–0.45 M) and then lyophilized according to Yoo et al. (2012).

2.2.1.3. PSP preparation. The seeds of pepper were separated, dried, and powdered. The pepper seed flour was defatted with hexane (1:3 v/v), then dried. The PSP was isolated from seeds flour according to Terras et al. (1992) with mild modifications, the seeds flour (50 g) was stirred for 1 h with tenfold of phosphate buffer pH 5.4 (10 mM Na₂HPO₄, 15 mM NaH₂PO₄, 100 mM KCl, 1.5% EDTA) and kept overnight at 4 °C. The supernatant was precipitated by ammonium sulfate (90%) at 80 °C for 15 min. The obtained suspension was centrifuged at (10,000g for 10 min) and the supernatant was dialyzed, lyophilized and kept.

2.2.2. Milk preservation experiment

Raw milk (200 mL) was transferred to sterile screw bottles supplemented with 0.2% (w/v) of obtained peptides. The supplementations were included distilled sterilized water (M-control), hen egg white protein isolate (M-HPI), duck egg white protein isolate (M-DPI), pepper seed protein (M-PSP), and pepsin kidney bean protein hydrolysate (M-PKH). All milk samples were kept at 4 °C for 30 days. Chemical and microbiological analyses were conducted at an interval of (0, 15, and 30) days.

2.2.3. Chemical analysis

2.2.3.1. Chemical parameters assessment. Titratable acidity was calculated as % lactic acid according to standard method 942.15. pH was measured by pH meter, and total soluble solids (TSS) was estimated by Abbe Refractometer (Model 8987, Puji Kuki Ltd., Tokyo, Japan) according to AOAC (2005). Total sugars were estimated as per Chaplin and Kennedy (1994), hydrolysate milk samples or glucose standard (200 µL) were added to 200 µL phenol (5%) and 1 mL sulfuric acid (conc.) and incubated for 30 min, the color absorbance was measured at 490 nm. It was applied in (standard curve: $y = 0.0055x - 0.0187$) to obtain total sugars concentration (µg/mL).

2.2.3.2. Total phenolic compounds (TPC). Polyphenols were assessed in peptides-supplemented milk samples and calculated as µg GAE/mL, following the Folin-Ciocalteu method (Škerget et al., 2005), the absorbance was measured at 750 nm. It was applied in standard Gallic acid linear (equation: $y = 0.005x + 0.1379$).

2.2.3.3. Total flavonoids. One mL of each milk sample was added to 3 mL of ethanoic AlCl₃ then incubated in dark for 1 h as per Ordonez et al. (2006). The absorbance was measured at 430 nm and then applied in the standard quercetin linear (equation: $y = 0.0033x - 0.0045$) to obtain total flavonoid concentration as µg QE/mL.

2.2.3.4. HPLC identification of polyphenols and flavonoids. The phenolic and flavonoids compounds of milk samples were identified by the HPLC Shimadzu series (Shimadzu-prominence-20A, Japan.), the separation column (Gemini, C18, 4.6 × 150 mm × 5µm) with 2 mL/min flow rate. The phenolic and flavonoids content in milk samples was conducted as per Goupy et al. (1999), Mattila et al. (2000), and Hassanin et al. (2020).

2.2.3.5. DPPH radical scavenging activity. The anti-radical activity of peptides suspension or milk samples was assessed using Hatano et al. (1988) with few modifications. One mL of DPPH in ethanol was added to 100 µL of each sample and kept in the dark for 30 min at room conditions (Gülçin et al., 2004). The absorbance was measured at 517 nm against the control and applied in the following equation to obtain DPPH antiradical activity (%).

$$\text{Radical scavenging activity (\%)} = \frac{(\text{A}_{\text{control}} - \text{A}_{\text{sample}})}{\text{A}_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the control and A_{sample} is sample absorbance.

2.2.4. Microbial analysis

2.2.4.1. Antibacterial activity. Antibacterial activity was evaluated by disc diffusion assay as per Akl et al. (2020) and El-Saadony et al. (2021). 100 µL of each pathogenic bacterium was spread at the surface of Muller Hinton agar plates (MHA), and filter paper discs (6 mm) were saturated with each HPI, DPI, PSP, and PKH suspension concentration (200, 400, and 800 µg/mL) then were placed on the Muller Hinton agar surface. The MHA plates were incubated for 24 h at 37 °C. The obtained zones of inhibition (mm) were manually measured using a ruler. The least concentration was inhibited the bacterial growth was minimum inhibitory concentration (MIC) and determined as follow, 500 µL of pathogenic bacteria was added to tubes of 9 mL MHB supplemented with 500 µL of each HPI, DPI, PSP, and PKH suspension concentration (200, 400, and 800 µg/mL), the tubes were incubated for 24 h at 37 °C and the turbidity was measured every 6 h at 600 nm. The least concentration was killed 100% of bacteria was minimum bactericidal concentration (MBC) and was determined as follow 100 µL of each peptide MIC was spread on the surface of MHA plates and were incubated at 37 °C for 24 h and observed the bacterial growth (Janakat et al., 2015).

2.2.4.2. Bacterial count. Microbial count of milk samples supplemented with HPI, DPI, PSP, and PKH 0.2% (w/v) was performed at an interval of (0, 15, and 30 days) of cold preservation at 4 °C (APHA, 1992). Ten mL of each sample was homogenized with 90 mL of saline peptone buffer (0.1% Peptone Water + 0.85% Salt) in a scraw bottle and stirred for 5 min at 25 °C to prepare 10⁻¹ dilution. One ml of the previous suspension was added to 9 mL buffer peptone tube to obtain 10⁻² dilution, further serial dilution to 10⁻⁷. One mL of each dilution was placed in sterile one-use petri-dishes. The plate count ager (PCA) medium was added to the plate and mixed well, the PCA plates were incubated at 30 °C for 48 h to count (mesophilic bacteria) (Ashour et al., 2020; Reda et al., 2020; Sheiha et al., 2020), and at 7 °C for ten days to enumerate (psychrophilic bacteria) (Lee, 2009). 1 mL of each dilution was placed in sterile one-use Petri-dishes, then the Violet Red Bile Agar was added to the plate and mixed well. The plates were incubated at 37 °C for 24 h to count coliforms. All bacterial counts were converted to logarithms (CFU/mL) (Reda et al., 2021).

2.2.4.3. Antifungal activity. The antifungal activities of HPI, DPI, PSP, and PKH were tested against twelve *Candida* and fungi species and evaluated by the agar disc diffusion assay (Elgorban et al., 2016). The tested fungi were cultivated on sabouraud dextrose agar plates (SDA) (Lab M Ltd., Heywood, Lancashire, UK) and the plates were incubated at 37 °C at two days (*Candida* spp.) and 30 °C at 5 days (fungi species). The tested *Candida* were grown on SD broth medium for two days at 37 °C until getting *Candida* inoculum concentration approximately 10⁵ CFU ml⁻¹, fungal species were grown on SDA medium for 5 days at 30 °C, and 6 mm mycelia discs were obtained. 100 µL of *Candida* inoculum was spread over SDA plates, and fungal mycelia discs were put in the center of SDA plates. Discs (6 mm diam.) were saturated with each concentration of HPI, DPI, PSP, and PKH (200, 400, and 800 µg/mL) then was put on the sides of seeded SDA plates, the plates were then incubated at 37 °C for 2 days (*Candida*) and 30 °C for 5 days (Fungi). The antifungal activity was estimated by determining the diameters of inhibition zone around the discs in mm. The minimum inhibitory concentration (MIC) of HPI, DPI, PSP, and PKH was evaluated using the the micro broth dilution method (Alizadeh et al., 2014; El-Saadony et al., 2019). 500 µL of each HPI, DPI, PSP, and PKH concentration (200, 400, and 800 µg/mL) was added to tubes containing 9 mL of sabouraud dextrose broth and inoculated with 500 µL of *Candida* (~10⁵ cfu ml⁻¹) or fungal inoculum (~10³ cfu ml⁻¹). Tubes were incubated at 37 °C for 2 day (*Candida*) or 30 °C for 5 days (fungi). The resultant turbidity was measured at 600 nm using a spectrophotometer (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan). The lowest concentration of HPI, DPI, PSP, and PKH, which inhibited the fungal growth, was recorded as the MIC. In addition, the lowest concentration of HPI, DPI, PSP, and PKH which kills 100% of fungi was considered to be the minimum fungicidal concentration (MFC) (Alizadeh et al., 2014). The MFC was estimated by sub-culturing the MIC levels of HPI, DPI, PSP, and PKH onto sterile SDA plates. The plates were incubated at 37 °C for 2 day (for tests with *Candida*) or 30 °C for 3–5 days (for tests with fungi).

2.2.5. Color measurement

Hunter Lab colorimeter (Color Flex EZ, 45°/0°, USA) was used to estimate the milk samples color, the color parameters values were assessed: L* was expressed as [white (100) /black (0)], a* was expressed as [red (+)/green (-)], b* was expressed as [yellow (+)/bleu (-)], C* (Chroma), WI (whiteness index), h° (Hue angle), and differences (distance between two colors) and these values were calculated using standard equations according to Hunter (1975).

2.2.6. Sensory properties

Sixty members of the Food Science department, Faculty of Agriculture, Zagazig University, Egypt staff, and students have evaluated milk samples' sensory traits as per [Aderinola and Abaire \(2019\)](#). Five attributes were estimated (color, odor, flavor, taste, and overall acceptability) using a 9-point Hedonic scale (9 = like extremely, and, 1 = dislike extremely). Water was applied for each panelist for mouth rinsing after testing each product to avoid the carry-over effect.

2.2.7. Statistical analysis

All experiments were in triplicate. The Two-way ANOVA at $p \leq 0.05$ level and least significant difference (LSD) tests were used to statistically analyze the means of triplicate data using SPSS (V.20).

3. Results

3.1. Antioxidant activity of bioactive peptides

[Fig. 1](#) represented the antiradical activity of HPI, DPI, PSP, and PKH peptides. PSP and PKH were significantly higher antioxidant activity than HPI and DPI peptides. The PSP was exhibited 87% followed by PKH (84%) compared to TBHQ at 91%.

3.2. Antimicrobial activity of bioactive peptides

3.2.1. Antibacterial activity of protein isolates against tested bacteria

The antibacterial activity of DPI, HPI, PSP, and PKH on the pathogenic bacteria (*B. cereus*, *L. monocytogenes*, *S. aureus*, *Y. enterocolitica*, *E. coli*, and *C. jejuni*), expressed as inhibition zones diameter (mm) in [Table 1](#). PSP concentrations (200, 400, and 800) have the highest inhibition zones diameters (IZD) were in the range of 15–28 mm against tested bacteria, followed by PKH and HPI, e.g., (12–27 mm) and (10–25 mm), respectively. Based on the IDZs values in [Table 1](#), *S. aureus* was the most sensitive bacteria intolerance to added peptides; on the other hand, *C. jejuni* was the most resistant bacteria.

The MIC and MBC values of DPI, HPI, PSP, and PKH were observed in [Table 1](#), the lowest values of MIC and MBC were detected in PSP, the MIC values in the range of 85–150 $\mu\text{g}/\text{mL}$, and MBC values were ranged 160–280 $\mu\text{g}/\text{mL}$ as compared to PKH, HPI and DPI.

3.2.2. Antifungal activity

[Table 2](#) showed the IDZs (mm) of DPI, HPI, PSP, and PKH peptides against the tested *Candida* and fungi. The IDZ values of PSP were in the range of 13–25 mm against *Candida* species and they were ranged 16–27 mm in fungal species compared to PKH, DPI, and HPI. The most resistant *Candida* and fungi were *C. glabrata* and *A. niger*, but *C. stellate* and *P. solitum* was the most sensitive species, respectively. The MIC and MFC values were the lowest in PSP against tested *Candida* and fungi species.

3.3. Chemical changes in milk samples during cold preservation

3.3.1. Antioxidant activity, polyphenols, and flavonoids changes during cold preservation

The antioxidant activity of milk was significantly $p \leq 0.05$ incremented due to phenolics and flavonoids in added peptides. Still, the content of antioxidants significantly $p \leq 0.05$ decreased during storage, as shown in [Fig. 2](#). [Table 3](#) showed the changes in antioxidant activity, phenolic compounds, and flavonoids in peptides supplemented-milk (M-HPI, M-DPI, M-PSP, and M-PKH) as compared to non-supplemented milk (M-control) during cold preservation at 4 °C for 30 days. The antiradical potential of M-PSP significantly $p \leq 0.05$ decreased from 85% to 81% as decrement of total phenolic compounds content from 2797 to 932 GAE $\mu\text{g}/\text{mL}$, and the total flavonoids content significantly decremented from 419.4 at zero-day to 186.4 QE $\mu\text{g}/\text{mL}$ after 30 days.

[Table 4](#) presents the HPLC profile of the phenolic compounds and flavonoids in peptides supplemented-milk compared to non-supplemented milk. The results indicated significant differences $p \leq 0.05$ between milk samples, where M-PSP significantly higher in the phenolic compounds content than other milk samples; pyrogallol, protocatechuic, catechol, catechin, and caffeine value were (1.1, 0.13, 0.06, 0.06, and 0.05 mg/mL, respectively), and pyrogallol, protocatechuic, and catechin were in high contents in M- HPI, M- DPI, and M- PKH, other phenolic compounds were in lower contents. The flavonoids with the highest contents occurred in M-PSP. Apignin-6-arabinose, naringin, luteolin-7-glucose, hesperidin, and kaempferol 3-2-p-comaroyl values were (1.2, 0.3, 0.08, 0.7, and 0.07 mg/mL), and other flavonoids in lower contents.

3.3.2. Changes in chemical parameters (pH, TAA, and TSS) during milk preservation

Data in [Fig. 3A](#) showed a significant $p \leq 0.05$ decrease in pH values of milk samples during storage. PSP and PKH significantly controlled the pH decrement with a relative reduction of about 5–10%

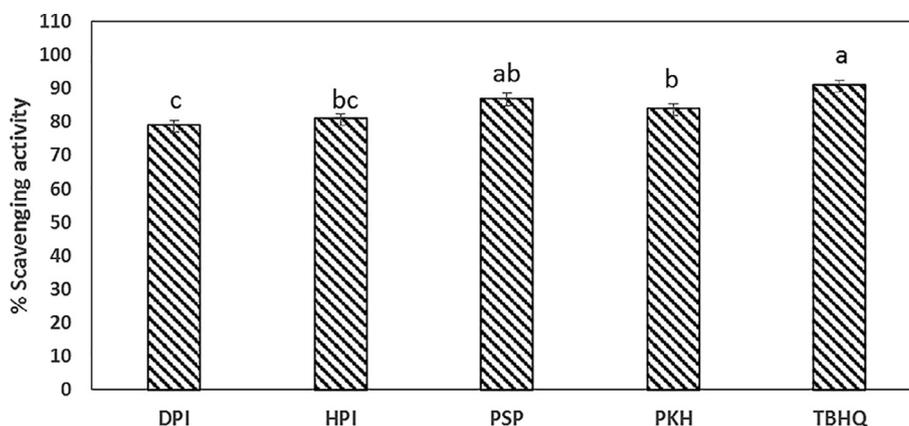


Fig. 1. DPPH radical scavenging activity of isolated proteins (DPI, duck egg protein isolate; HPI, hen egg protein isolate; PSP, pepper seed protein isolate; PKH, pepsin kidney bean protein hydrolysate) compared to Tert-Butylhydroquinone (TBHQ) synthetic antioxidant. Data presented means \pm SE, different lowercase letters indicate significant differences ($p \leq 0.05$).

Table 1
Antibacterial activity of proteins isolates levels (200, 400, and 800 µg/mL) against dairy pathogenic bacteria expressed with inhibition zones (mm/24 h) at 37 °C, MIC, and MBC

Pathogenic bacteria	Protein isolates											
	DPI			HPI			PSP			KBH		
	200	400	800	200	400	800	200	400	800	200	400	800
<i>B. cereus</i>	14ab	17ab	21b	16b	19b	22b	17b	23b	26b	17b	21ab	24b
<i>L. monocytogenes</i>	13b	16b	20b	15b	18b	24ab	16b	21c	25b	16b	20b	23b
<i>S. aureus</i>	15a	19a	25a	18a	21a	25a	19a	25a	29a	19a	22a	28a
<i>Y. enterocolitica</i>	11 cd	14 cd	17d	13 cd	16 cd	20c	14d	19d	22d	14 cd	18c	21c
<i>E. coli</i>	12c	15c	18c	14c	17c	21bc	15c	20c	24c	15c	19b	22c
<i>C. jejuni</i>	10 cd	13 cd	16d	10d	15d	19 cd	14d	17de	21d	12d	17 cd	20d
Bacteria	MIC			HPI			PSP			KBH		
	DPI			MBC			DPI			KBH		
<i>B. cereus</i>	120e	110e	100e	105e	230e	200e	180e	190e				
<i>L. monocytogenes</i>	130d	120d	110d	115d	250d	230d	200d	210d				
<i>S. aureus</i>	100f	95f	85f	90f	190f	185f	160f	175f				
<i>Y. enterocolitica</i>	170b	160b	145b	150b	320b	300b	270b	280b				
<i>E. coli</i>	150c	140c	130c	135c	290c	260c	240c	250c				
<i>C. jejuni</i>	180a	170a	150a	160a	350a	320a	280a	300a				

DPI, duck egg protein isolate; HPI, hen egg protein isolate; PSP, pepper seed protein isolate; PKH, pepsin kidney bean protein hydrolysate; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; Different lowercase letters in same column indicate significant differences ($p \leq 0.05$).

Table 2
Antifungal activity of proteins isolates levels (200, 400, and 800 µg/mL) against dairy pathogenic yeasts and fungi expressed with inhibition zones (mm/4days), MIC, and MFC

Yeasts	Protein isolates												MIC				MFC			
	DPI			HPI			PSP			KBH										
	200	400	800	200	400	800	200	400	800	200	400	800	DPI	HPI	PSP	KBH	DPI	HPI	PSP	KBH
<i>C. apis</i>	14c	16c	19b	15b	17b	20b	16c	19d	22c	15c	17d	19c	120c	110c	90c	100c	210c	200c	170c	190c
<i>C. blankii</i>	13d	15c	18c	14c	16b	19b	15d	17e	21c	16b	18c	20b	150b	130b	100b	120b	280b	250b	190b	220b
<i>C. glabrata</i>	12d	13d	16d	13c	15c	18c	13e	16e	20d	15c	16d	18c	180a	160a	130a	140a	300a	285a	250a	260a
<i>C. glabrosa</i>	15b	17b	19b	16a	18a	21a	18b	20c	23b	16b	18c	20b	100d	90d	80d	85d	200d	170d	160d	168d
<i>C. rugosa</i>	16a	18b	21a	17a	19a	21a	19a	21b	24a	17a	19b	22a	80e	75e	60e	70e	150e	140e	110e	125e
<i>C. stellata</i>	16a	19a	20a	17a	20a	22a	19a	22a	25a	17a	20a	23a	80e	75e	60e	70e	145e	135e	105e	130e
Fungi	Inhibition zones (mm)												Concentration µg/mL							
<i>A. niger</i>	14d	16d	19d	15d	17c	20d	16d	19d	23c	15d	17d	20c	170a	150a	130a	140a	320a	300a	230a	260a
<i>A. flavus</i>	15d	17c	20c	16c	19b	22c	17c	22c	25b	17c	18d	22b	150b	130b	110b	120b	290b	250b	200b	220b
<i>F. solani</i>	17b	18b	22b	16c	18c	24b	19b	25a	28a	19b	20b	24a	100e	95e	80e	90e	190e	170e	150e	165e
<i>F. oxysporum</i>	16c	17c	21c	16c	17c	23c	18c	23b	27a	17c	19c	23b	140c	125c	115c	120c	260c	240c	210c	225c
<i>P. solitum</i>	18a	20a	25a	19a	22a	26a	20a	23b	27a	20a	22a	25a	120d	110d	95d	100d	220d	210d	180d	190d
<i>P. crustosum</i>	17b	18b	23b	17b	20b	24b	19b	20d	23c	18b	20b	22b	90e	85e	75e	80e	170e	165e	140e	150e

DPI, duck egg protein isolate; HPI, hen egg protein isolate; PSP, pepper seed protein isolate; PKH, pepsin kidney bean protein hydrolysate; MIC, minimum inhibitory concentration; MFC, minimum fungal concentration. Different lowercase letters in same column indicate significant differences ($p \leq 0.05$).

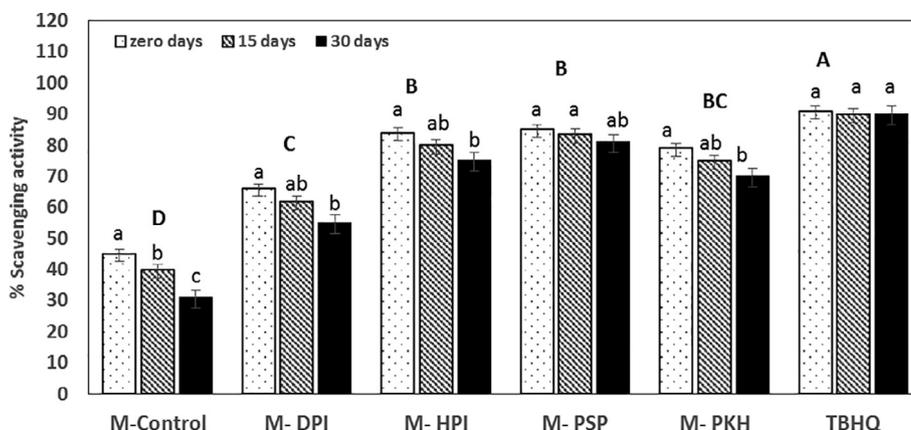


Fig. 2. Changes in the antioxidant activity in peptides-supplemented-milk during storage period of (0, 15, and 30 days) at 4 °C as compared to synthetic antioxidant Tert-Butylhydroquinone (TBHQ), M- Control = non-supplemented milk, M-DPI = duck protein isolate-supplemented milk, M-HPI = hen protein isolate-supplemented milk, M-PSP = pepper seeds protein-supplemented milk, M-PKH = kidney bean hydrolysate-supplemented milk. Different lowercase letters indicate significant differences during storage, uppercase letters indicate differences between scavenging activity of milk samples ($p \leq 0.05$).

in M-PSP and-PKH compared to M-control. At the end of cold storage at 4 °C, the pH of M-control was $p \leq 0.05$ decreased from 5.9 to 5, M-HPI from 5.2 to 4.6, M-DPI from 5.3 to 4.5, M-PSP from 4.9 to 4.4, and M-PKH from 4.7 to 4.4. The titratable acidity significantly

increased in M-control by 0.14 mg/ml lactic acid. Slower increase in M- HPI, M- DPI, M- PSP, and M- PKH, i.e., 0.11, 0.12, 0.06, and 0.08, respectively Fig. 3B, in the same route, the total soluble solids were increased in Fig. 3C.

Table 3

Changes in phenolic and flavonoids content in peptides supplemented-milk during storage period (0, 15, and 30 days) at 4 °C.

Sample	Storage period (day)			Phenolic compounds		
	0	15	30	0	15	30
M-Control	112.15 ± 0.9 ^{dA}	107.00 ± 0.31 ^{dB}	49.85 ± 0.2 ^{dC}	1442.67 ± 0.33 ^{cA}	1320.10 ± 0.8 ^{cB}	447.56 ± 0.2 ^{cC}
M-HPI	259.12 ± 0.8 ^{cA}	247.30 ± 0.15 ^{cB}	115.16 ± 0.3 ^{cC}	1760.39 ± 0.45 ^{bA}	1076.10 ± 0.5 ^{eB}	586.80 ± 0.5 ^{cC}
M-DPI	300.64 ± 0.2 ^{bA}	287.00 ± 0.52 ^{bB}	133.62 ± 0.6 ^{bC}	1494.46 ± 0.3 ^{cA}	1201.10 ± 0.45 ^{dB}	498.15 ± 0.6 ^{dC}
M-PSP	419.42 ± 0.3 ^{aA}	399.12 ± 0.15 ^{aB}	186.41 ± 0.5 ^{aC}	2797.39 ± 0.5 ^{aA}	2274.10 ± 1.2 ^{aB}	932.46 ± 0.9 ^{aC}
M-PKH	258.82 ± 0.5 ^{cA}	247.00 ± 0.23 ^{cB}	115.03 ± 0.7 ^{cC}	1917.03 ± 0.4 ^{bA}	1549.10 ± 0.9 ^{bB}	639.01 ± 0.7 ^{bC}

Data are presented mean ± SD; Mean in the same column with different lowercase letters are significantly different, different uppercase letters in the same row indicate significant differences between storage period $p \leq 0.05$; M- Control = non-supplemented milk, M-DPI = duck protein isolate-supplemented milk, M-HPI = hen protein isolate-supplemented milk, M-PSP = pepper seeds protein-supplemented milk, M-PKH = kidney bean hydrolysate-supplemented milk.

Table 4

Phenolic and flavonoids profile in peptides-supplemented milk samples (µg/mL)

Phenolic compound	M-control	M-DPI	M-HPI	M-PSP	M-PKH
Pyrogallol	125 ^c	99.1 ^d	100 ^d	1118.21 ^a	326.68 ^b
Gallic	30.2 ^b	9.65 ^c	8.99 ^c	52.91 ^a	29.01 ^b
Protocatechuic	27.5 ^d	35.48 ^c	40.2 ^c	138.55 ^a	26.6d
Catechol	33 ^b	27.26 ^c	30.55 ^b	62.87 ^a	32.37 ^b
4-Aminobenzoic	4.5 ^b	2.48 ^d	4.28 ^b	6.28 ^a	3.39 ^c
Catechin	42.22 ^b	44.55 ^b	46.6 ^b	63.55 ^a	43.09 ^b
Chlorogenic	17.55 ^b	6.76 ^e	12.5 ^d	32.1 ^a	16.45 ^c
p-OH-Benzoic	9 ^d	22.82 ^a	20.33 ^b	16.62 ^c	8.42 ^d
Benzoic	49.09 ^a	26.04 ^b	27.44 ^b	ND	ND
Caffeic	8.77 ^a	1.36 ^e	2.4 ^d	4.12 ^b	3.49 ^c
Vanillic	17.32 ^a	16.78 ^b	12.5 ^d	13.12 ^c	12.98 ^d
p-Coumaric	5.12 ^c	5.64 ^b	5.77 ^b	6.15 ^a	ND
Caffeine	ND	ND	ND	50 ^a	46.7 ^a
Ferulic	6.52 ^c	10.32 ^b	9.99 ^b	13 ^a	12.81 ^a
Iso-ferulic	14.45 ^a	3.78 ^d	8.5 ^c	10.3 ^b	9.69 ^b
Alpha-coumaric	1.86 ^e	6.62 ^a	5.12 ^b	2.5 ^c	2.21 ^d
Coumarin	18.1 ^c	8.78 ^d	7.69 ^d	22.7 ^a	21.76 ^b
3,4,5-methoxy-cinnamic	13.63 ^b	6.95 ^d	11.25 ^c	37 ^a	36 ^a
Flavonoids	M-control	M-DPI	M-HPI	M-PSP	M-PKH
Apignin-6-arabinose	539.12 ^c	140.07 ^d	150 ^e	1235.53 ^a	1103.5 ^b
Narengin	46.31 ^d	41.32 ^f	44.2 ^d	361.59 ^a	350 ^b
Rosmarinic	5.3 ^a	4.33 ^b	5.1 ^a	ND	ND
Apigenin-6-rhamnose	ND	2.32 ^d	3.2 ^c	10.6 ^a	8.5 ^b
Luteolin-7-glucose	36.28 ^c	11.54 ^d	12.15 ^d	82.26 ^a	79.55 ^b
Hesperidine	109.09 ^d	111.46 ^d	120.5 ^c	746.2 ^a	722 ^b
Rutin	9.62 ^c	4.23 ^d	5.12 ^d	18.91 ^a	15.9 ^b
Apigenin-7-Glucose	ND	1.4 ^d	2.5 ^c	5.11 ^a	4.2 ^b
Apig-7-O-neohespiroside	11.05 ^b	2.62 ^d	2.9 ^d	21.69 ^a	21.5 ^a
Quercetrin	9.62 ^c	3.05 ^d	4.05 ^d	19.12 ^a	18.5 ^b
Narengenin	53.25 ^a	11.45 ^d	13 ^c	22.61 ^b	21.55 ^b
Quercetin	24.18 ^a	5.23 ^d	5.9 ^d	11.51 ^b	10.9 ^c
Hesperitin	4.13 ^c	6.22 ^a	6.7 ^a	5.28 ^b	4.15 ^c
kampferol 3-2-p-comaroyl	38.39 ^b	5.77 ^d	5.9 ^d	70.23 ^a	69.12 ^a
Acacatin-7 neo-rutinoside	21.36 ^c	10.17 ^d	12 ^d	27.97 ^a	25.13 ^b
Kampferol	11.46 ^a	3.97 ^c	5.5 ^c	10.61 ^b	11.5 ^a
Apigenin	ND	5.27 ^b	5.15 ^b	9.33 ^a	8.56 ^a

ND, not detected; means in the same raw with different lowercase letters are significantly different $p \leq 0.05$.

Table 5 represents the total sugars (µg/mL) changes during milk storage at 4 °C. In M-control, the sugars content significantly ($p \leq 0.05$) decremented from 308.4 to 84.09 µg/mL. In comparison, PSP and PKH ($p \leq 0.05$) inhibited the decay of sugars 279.3 to 105.9 and 382.5 to 202.9 µg/mL in M-PSP, and M-PKH, respectively. These simple sugars increase the total soluble solids (Brix), as shown in Fig. 3C.

3.4. Microbial changes in milk samples during cold preservation

3.4.1. Total viable and coliform bacteria count in milk supplemented with isolated proteins

Data in Table 6 showed that the M-control was acceptable for up to 4–7 days preserved at 4 °C, and the addition of DPI and HPI in M-DPI and M-HPI significantly increased the acceptability to

7–12 days. Moreover, PSP and PKH significantly increased the acceptability up to 18–20 days in M-PSP and M-PKH. The total bacteria and coliform count were significantly affected by DPI, HPI, PSP, and PKH supplementations during the storage period of 0, 15, 30 days at 4 °C. The highest total bacterial count (log CFU/mL) found in M-control during the storage period. The total bacterial count in M-DPI and M-HPI significantly decreased $p \leq 0.05$ from 5.2 to 4.7 and 4.6. The total bacterial count in M-PSP was significantly reduced by 45% compared to control. There were no significant differences between the antibacterial activity of the PSP and PKH.

3.5. Color measurement and sensory evaluation

Table 7 presents color parameters of peptides-supplemented milk samples as compared to control during storage at 4 °C. PSP

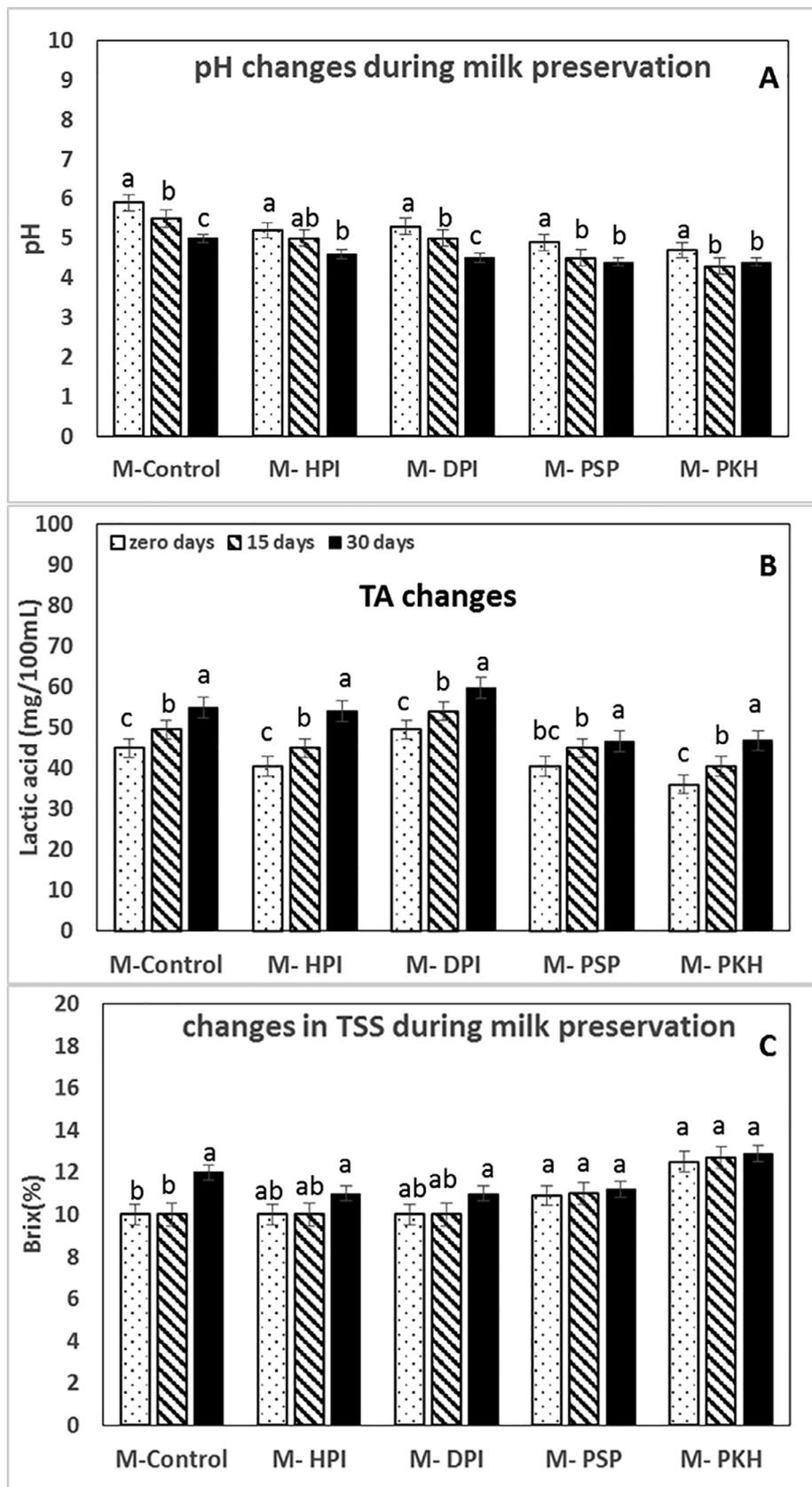


Fig. 3. (A) Changes in pH, (B) in titratable acidity (TA), and (C) in total soluble solids (TSS) of peptides supplemented-milk during storage period of (0, 15, and 30 days) at 4 °C. Different lowercase letters indicate significant differences during storage ($p \leq 0.05$).

Table 5

Changes in total sugars content (µg/mL) in peptides-supplemented milk during storage period of 0, 15, and 30 days at 4 °C

Sample	Storage period (day)		
	0	15	30
M–control	308.43 ± 0.5 ^{ba}	129.19 ± 0.11 ^{bb}	84.09 ± 0.3 ^{cc}
M–DPI	278.25 ± 0.2 ^{ca}	105.04 ± 0.92 ^{cb}	59.94 ± 0.5 ^{dc}
M–HPI	250.51 ± 0.9 ^{da}	88.81 ± 0.15 ^{db}	47.68 ± 0.2 ^{dc}
M–PSP	279.38 ± 0.8 ^{ca}	175.60 ± 0.12 ^{bb}	105.96 ± 0.9 ^{bc}
M–PKH	382.58 ± 0.1 ^{aA}	216.36 ± 0.31 ^{aB}	202.96 ± 0.7 ^{aC}

Data are presented mean ± SD; mean in the same column with different lowercases are significantly different between sugars content in milk samples, different uppercase letters indicate significant difference between storage time affecting sugars content $p \leq 0.05$.

and PKH supplementations were significantly increased $p \leq 0.05$, the whiteness of milk, i.e., 88.7 in the control sample to 88.91, and 88.88, in M-PSP and M-PKH, respectively. M-PSP had the significantly highest score in color, taste, and flavor, followed by M-PKH (Table 8).

4. Discussion

Milk and milk products are sustainable for microbial contamination and chemical deterioration; therefore, the researchers strive to find suitable preservatives to extend the shelf life of milk handling and maintain its quality. Natural additives especially bioactive peptides, are a new trend to achieve this equation. In this study, raw buffalo milk was supplemented with four bioactive pep-

tides: HPI, DPI, PSP, and PKH, then milk samples: M-control, M-HPI, M-DPI, M-PSP, and M-PKH were preserved at 4 °C for 30 days and the chemical, microbial, and sensory changes were noticed during this period. The added bioactive peptides have considerable scavenging activity. Plant-derived peptides (PSP, and PKH) activity depends on their low molecular weight and amino acids that contain OH and SH residues. Alternatively, poultry-derived peptides (HPI, and DPI) have high molecular weight. Sarmadi and Ismail (2010) described the antioxidant action of peptides depended on the hydrophobic aromatic amino acids that donate electrons to radicals and make them stable and the own stability kept, the hydrophobicity improve peptides lipophilic action through hydrophobic residues, also, acidic and basic amino acids act as proton donors and metal ion chelators through their amine and carboxylic residues, also, the tested bioactive peptides exhibited antimicrobial activity and that may return to the positive charge or hydrophobicity action, peptides with positive charges were electrostatically bound to negatively charged compounds on the bacterial cell wall, and the cell wall was demolished (Gobbetti et al., 2004; Jenssen et al., 2006; Lei et al., 2019), and the hydrophobic nature of peptide plays a vital role in disquieting the cell wall and membrane of bacteria, besides, the interaction between phenolic compounds and proteins might be alter the cell wall permeability by inhibiting certain enzymes (Upadhyay et al., 2014). The hydrophobicity of polyphenols and flavonoids may increase their interaction with the cell membrane, causing cell content coagulation and inhibiting the DNA synthesis. Besides, the active groups in the polyphenols and flavonoids may account for their antimicrobial activity (Bouarab-Chibane et al., 2019). for

Table 6

The bacterial count expressed as (Log CFU/mL) in peptides-supplemented milk samples during storage period (0, 15, and 30 days) at 4 °C.

Day	0			15			30		
	Sample	TVC	PBC	CC	TVC	PBC	CC	TVC	PBC
M–control	5.2 ^a ± 0.15	4.5 ^a ± 0.12	2.7 ^a ± 0.13	7.8 ^a ± 0.15	5.0 ^a ± 0.21	3.53 ^a ± 0.18	8.9 ^a ± 0.49	6.0 ^a ± 0.31	4 ^a ± 0.27
M–DPI	4.7 ^b ± 0.25	4.0 ^b ± 0.14	2.3 ^b ± 0.21	5.6 ^b ± 0.29	4.2 ^b ± 0.25	2.84 ^b ± 0.28	6.9 ^b ± 0.51	5.0 ^b ± 0.34	2.9 ^b ± 0.14
M–HPI	4.6 ^b ± 0.23	3.9 ^b ± 0.15	1.9 ^c ± 0.14	4.9 ^c ± 0.31	4.1 ^b ± 0.23	2.44 ^c ± 0.19	6.2 ^b ± 0.81	4.7 ^{bc} ± 0.35	2.7 ^b ± 0.24
M–PSP	3.0 ^c ± 0.19	3.1 ^c ± 0.16	1.8 ^c ± 0.12	3.2 ^d ± 0.27	3.5 ^c ± 0.31	2.16 ^d ± 0.28	4 ^c ± 0.72	3.9 ^c ± 0.36	2.1 ^c ± 0.14
M–PKH	2.9 ^c ± 0.18	3.0 ^c ± 0.18	1.5 ^d ± 0.15	3.1 ^d ± 0.21	3.3 ^c ± 0.25	1.62 ^d ± 0.18	3.8 ^c ± 0.61	3.5 ^{cd} ± 0.34	1.9 ^c ± 0.14

Data are presented mean ± SD; mean in the same column with different lowercase letters are significantly different $P \leq 0.05$; TVC, total viable count; CC, coliform count, and PBC, psychrophilic bacterial count.

Table 7

Color parameters of peptides-supplemented milk samples during cold preservation at 4 °C

Sample	L*	a*	b*	C*	h°	WI	differences	b/a
M–control	88.77 ± 0.7 ^c	-1.99 ± 0.3 ^b	7.5 ± 0.7 ^b	7.75 ± 0.1b	-75.1 ± 0.5a	27.6 ± 0.3a	0.25 ± 0.02ab	-3.76 ± 0.14c
M–DPI	88.71 ± 0.8 ^d	-2.12 ± 0.7 ^a	7.96 ± 0.9 ^a	8.23 ± 0.2a	-75.06 ± 0.6a	27.1 ± 0.4ab	0.27 ± 0.01a	-3.75 ± 0.15c
M–HPI	88.76 ± 1.2 ^c	-1.95 ± 0.5 ^b	7.13 ± 0.3 ^d	7.39 ± 0.2c	-74.64 ± 0.8b	23.5 ± 0.6d	0.26 ± 0.02a	-3.65 ± 0.13d
M–PSP	88.91 ± 0.5 ^a	-1.91 ± 0.19 ^c	7.5 ± 0.5 ^b	7.73 ± 0.1b	-75.68 ± 0.5a	26.3 ± 0.5b	0.23 ± 0.03b	-3.92 ± 0.12a
M–PKH	88.88 ± 0.25 ^b	-1.92 ± 0.29 ^c	7.4 ± 0.22 ^c	7.64 ± 0.4bc	-75.4 ± 0.6a	25.5 ± 0.4c	0.24 ± 0.03b	-3.85 ± 0.17b

Data are presented mean ± SD; Mean in the same column with different lowercase letters are significantly different $p \leq 0.05$; L* was expressed lightness/darkness, a* for redness/ greenness, b* for yellowness/blueness, C* (Chroma), WI (whiteness index), h° (Hue angle), differences (distance between two colors)

Table 8

Sensory traits of peptides-supplemented milk samples during cold preservation at 4 °C

Sample	Color	Taste	Flavor	Over acceptability
M–Control	8.5 ± 0.2 ^c	8.8 ± 0.1 ^b	8.5 ± 1.1 ^c	8.6 ± 0.2 ^b
M- DPI	8.7 ± 0.2 ^b	7.5 ± 0.5 ^c	8.3 ± 0.9 ^d	8.1 ± 0.4 ^c
M- HPI	8.4 ± 0.3 ^d	7.8 ± 0.5 ^c	8.2 ± 0.7 ^d	8.1 ± 0.5 ^c
M- PSP	8.8 ± 0.2 ^a	8.9 ± 0.1 ^a	8.8 ± 0.2 ^a	8.9 ± 0.1 ^a
M- PKH	8.4 ± 0.4 ^d	8.7 ± 0.2 ^b	8.8 ± 0.1 ^b	8.6 ± 0.3 ^b

Data are presented mean ± SD; Means in the same column with different lowercase letters are significantly different $P \leq 0.05$.

these reasons, [Samaranayaka and Li-Chan \(2011\)](#) reported that antioxidants in nutrition improve health and foodstuff quality.

The addition of protein isolates to milk was enhanced the antioxidant activity of milk, prevented lipids oxidation and extended the shelf life ([Winata and Lorenz, 1996](#)). The antiradical mechanisms of antioxidant peptides was investigated by [Esfandi et al. \(2019\)](#), who cleared the mechanisms include electron donation or transfer proton, these mechanisms may act together, or one dominated depended on peptides structure, wherein the aromatic amino acids make radicals stable by donating electron besides, acidic and essential amino acids act as metal ion chelators, also, the antioxidant activity of milk may be explained by [Jakobek \(2015\)](#); [Rawel and Rohn \(2010\)](#) who mentioned that phenolic compounds might interact with added protein isolates to increase the antioxidant and antibacterial activity, the interaction may occur between OH of phenolic compounds and SH and OH side chains in peptides or protein, protein–phenolic interaction may affect the physicochemical properties of protein and phenolic compounds, the complex may increase the peptide activity through blocking some amino acid side chains, besides, increasing the bioavailability and activity of polyphenols. In a previous study, [Cottica et al. \(2015\)](#) and [El-Deeb \(2017\)](#) reported that the antioxidant potential of milk increased with propolis extract. The presence of phenolic compounds in dairy products protects other antioxidant ingredients as their resistant to thermal treatments.

Protein isolates increased the phenolic and flavonoid compounds in milk samples and that can be attributed to the phenolics-protein complex formation ([Jakobek, 2015](#); [Ozidal et al., 2013](#)) that enhanced the antioxidant activity ([Gammoh et al., 2017](#); [Guimarães Drummonde Silva et al., 2017](#)), and this is a call for usage of natural alternatives instead of synthetic preservatives in food ([Bouarab Chibane et al., 2019](#); [Abd El-Hack et al., 2020](#)), and cosmetic applications ([Kočevár Glavač and Lunder, 2018](#)).

Many chemical and microbial changes occurred during raw milk preservation, and natural additives limited these alternations. Protein isolates inhibited lactose decay by fermentative bacteria to glucose and lactic acid, raising the titratable acidity and total soluble solids ([Sivakumar, 2017](#)). Also, the protein isolates reduced the bacterial growth and inhibited the oxidation and some enzymatic reactions occurring in milk. In the previous study, an increase in the titratable acidity and a decrease in pH were observed in milk samples enriched with 0.5%, 0.75% and 1% level of tulsi leaves extract ([Abbas and Osman, 1998](#); [Sivakumar, 2017](#)), these changes was limited by the addition of antibacterial aqueous extract of tulsi leaves at a concentration of 0.25–1% in raw milk because of phenolic compounds content in extracts. In the same route, [Osman et al. \(2013\)](#) used 11 s soybean protein subunit in milk preservation for 30 days and found the soybean 11S subunit exerted considerable antibacterial actions.

In the present study, the milk supplementation with antimicrobial peptides was significantly reduced the bacterial load to an acceptable level ([Sivakumar and Dhanalakshmi, 2016](#)). The antimicrobial mechanism was explained by various models, which occur when peptides attach to the cell membrane of bacteria and it completely disrupts the cell membrane integrity ([Berglund et al., 2015](#)). The carpet model, toroidal pore model, brave straw model and aggregate model are well-studied models of antimicrobial peptides ([Stempel et al., 2015](#); [Zhao et al., 2015](#)).

Color is an indicator of milk quality, freshness, and food safety. Light reflects by dispersed fat granules and proteins cause milk color, besides natural milk pigments such as riboflavin and carotenoids ([Nozière et al., 2006](#); [Solah et al., 2007](#)). Bacterial growth affects the sensory properties of milk, make color, texture, odor, and taste were unacceptable commercially ([Ahmed and Abdellatif, 2013](#); [Samet-Bali et al., 2013](#)), because of sensory

evaluation of food products is an essential indicator of potential consumer preference, the addition of bioactive peptides was enhanced the color parameters and sensorial traits of milk and that agree with [Bakr et al. \(2015\)](#); [Cottica et al. \(2015\)](#).

5. Conclusion

Microbial contamination and chemical deterioration are the main problems that affected milk and milk products after milking and during handling. Therefore, the need to find suitable preservatives to extend the lifetime of milk handling and maintain its quality. Natural additives, especially bioactive peptides, are a new trend to achieve this equation. The bioactive peptides in this work have considerable antioxidant and antimicrobial activity. The present study was confirmed that the supplementation of raw milk with HPI, DPI, PSP, and PKH as a natural preservative significantly maintained the shelf life of milk after milking and during handling besides enhancing its quality, where the addition of the peptide reduced the microbial load to an acceptable level, and that decrease the sugar decay in milk, consequently, the titratable acidity decreased and that extend the shelf life of milk. The M-PSP showed the highest color and sensory scores compared with the control. This preservation method encourages dairy farming to produce much milk with longer shelf life, which is a prerequisite for increased manufacture of high-quality milk.

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Declaration of Competing Interest

All authors declare that they do not have any conflicts of interest that could inappropriately influence this manuscript.

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