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Dual functional properties of enzymatically hydrolyzed dromedary milk proteins with improved antioxidant and antibacterial activity

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ABSTRACT

Dromedary milk has a proven outstanding history of being a nutrient-rich food. Camel milk possesses both medicinal and technological advantages, including antioxidant, antibacterial, and antiparasitic activities. The present investigation is proposed to assess the antibacterial ability and free radical scavenging capacity against Gram-negative bacteria of various enzyme-digested skimmed camel's milk (SCM) using papain, pepsin, and trypsin at four hydrolysis times at one-hour intervals. An antibacterial growth assay was conducted to detect the antibacterial capacity of the generated various enzymatic camel milk hydrolysates. The results showed that the antibacterial activity of unhydrolyzed intact SCM displayed mild effect against *Escherichia coli* and no effect against *Salmonella enteritidis*. Pepsin-treated SCM hydrolysates after 3h hydrolysis significantly enhanced SCM antimicrobial activity against *E. coli*, and after 2h, hydrolysis produces SCM hydrolysates against *Salmonella enteritidis*. Pepsin and trypsin SCM-treated hydrolysates after 2h hydrolysis could completely abolish the survival growth rate of *Salmonella enteritidis.* Additionally, hydrolysates treated with papain SCM after 3 and 4 hours of hydrolysis showed a 100% ability to kill *Salmonella enteritidis*. Both DPPH method and ABTS assays were employed to evaluate antioxidants. Our findings demonstrated that all SCM hydrolysates gradually acquired strong antioxidant capacity after four hours of hydrolysis, with trypsin-treated hydrolysates having the highest values. The current work offered a great role of skimmed camel's milk hydrolysates and the possibilities for using these hydrolysates as food natural preservatives.

1. INTRODUCTION

Microbial contamination is a critical food safety issue as it leads to the spread of food poisoning. Many foodborne diseases and outbreaks are documented due to pathogens, viruses, and protozoa contamination (Bintsis, 2018). Bacterial foodborne illnesses continue to seriously impact public health, the global economy, and society. World Health Organization emphasizing the need to measure the burden and incidence of foodborne illnesses and promote policies that control and limit foodborne diseases through prioritizing food safety measures as a public health issue (WHO, 2015; Pires *et al.*, 2021).

Salmonellosis is one of the most reported foodborne diseases leading to gastroenteritis symptoms, abdominal cramps, headache, bloody diarrhea, myalgia, fever, nausea, and vomiting (Ehuwa *et al.,* 2021). Food ingestion items associated with salmonella spp, primarily from livestock such as chickens, pigs, egg products, and milk products, are associated with salmonellosis food poisoning. Among the avenues for transmission pathways are the bad hygienic practice as improper hand sanitation and exposure to infected animals (Munck *et al.,* 2020). According to cross sectional study, showing high prevalence of *Salmonella enterica,* it has antibiotic resistance from food items, water and human (Sabeq et al., 2022). Additionally, some pathogenic strains of *Escherichia coli*, considered serious foodborne pathogens, can infect the gastrointestinal tract and cause life-threatening sickness and acute diarrhea (Croxen *et al.,* 2013;Yang *et al.,* 2017). Therefore, novel natural antimicrobials are urgently needed to reduce bacterial infections in food. The ability of food's derived components to reduce the chance of human food poisoning is widely acknowledged. In place of synthetic chemical preservatives, functional foods, and nutraceuticals are now used, particularly in preventing foodborne illness and acting as natural preservatives (Kris-Etherton *et al.*, 2002).

Dietary proteins can modulate the body's physiological processes and act as good sources of amino acids. Some of the peptides that are encoded in original parent food protein sequences have acquired bioactive biological properties. These bioactive peptides serve as principles for nutraceuticals or functional foods because of their potential to have positive health effects (Chakrabarti*et al.,* 2018). It is possible to designate various food products or components as functional smart foods or nutraceuticals with health

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benefits by adding various bioactive peptides derived from food (Hartmann and Meisel, 2007).

Many food proteins are hydrolyzed to produce bioactive peptides, which have a variety of biological effects, including antibacterial, antihypertensive, antioxidant, antiviral and antiparasitic activities. Several bioactive peptides can be generated from a variety of protein forms, such as meat, milk, and egg products, as well as marine, animal, and plant biomasses. Enzymatic hydrolysis is the most prevalent technique being researched for the production of bioactive peptides due to its generally recognized as safe (GRAS) (Marciniak *et al.,* 2018). Utilizing commercial enzymes derived from various sources is known as "enzymatic hydrolysis" (Toldrá *et al.,* 2020). Protein enzymatic hydrolysis uses mild temperature and pH settings, commercial selective enzymes, and a dearth of secondary by-products (Cruz-Casas *et al.,* 2021). Furthermore, the hydrolysis process using enzymes is quick and simple to inactivate the reaction, and if optimized, it can generate large quantities of high-quality bioactive peptides. Peptide structural characteristics, including amino acid arrangement, sequence, hydrophobicity, chain length, and net charge, play a significant impact in determining the peptide's biological activity (Maestri e*t al.,* 2019).

Dromedary milk protein possesses a variety of bioactive qualities, including impacts on the immune system, antioxidant protection, cancer-fighting abilities, and antibacterial, antiviral, and hypoglycemic, ovicidal capabilities (Gader and Alhaider, 2016;Behrouz *et al.*, 2022; Awad *et al.,* 2023). Additionally, camel's milk bioactive peptides availability for technologically functional uses in food systems has been recently increased (Al-Shamsi *et al.*, 2018).

Camel's milk proteins are diverse types of proteins with varied properties in structure and characteristics. They are divided into two main categories according to their solubility whey protein and caseins, which comprise 26.9% and 73.1% of total proteins, respectively (Lajnaf *et al.,* 2020).Recently, Camel milk hydrolysates derived from skimmed camel milk (SCM) has made by enzymatic procedures display more noticeable functional bioactive properties as compared to intact camel milk proteins (AliRedha *et al.*, 2022).

The antioxidant activity of camel milk proteins is due to their functional properties. Reactive oxygen species is a component of oxidative stress that has been related to several diseases, (Collins, 2005). Many research papers claimed camel milk has antioxidant properties, but these investigations were focused on bioactive components derived from fermented camel's milk products (Ayyash *et al.,* 2018).

So, there is an urgent need for more information on the comprehensive examination of camel protein hydrolysates' bioactive functional properties against various foodborne pathogens. In addition, it would be highly fruitful to assess camel milk protein hydrolysates from enzymes (papain, pepsin, and trypsin) for their antioxidant capacities and antibacterial against foodborne pathogens (*Salmonella enteritidis*, and *Escherichia coli*).

2. MATERIALS AND METHODS

2.1 Materials

Raw camel's milk (estimated 4.55% fat and 3.42% total protein) was procured from the open grazing land in Ras Sedr, South Sinai Governorate, Egypt. After collecting two litters from healthy female *Camelus dromedarius,* a local breed, samples were kept in sterile plastic bottles and on the ice bag till reach to the laboratory. Papain, pepsin, and trypsin were received from Yanaye Bio-Technology Co., Ltd. (Shanghai, China). Mueller Hinton broth (MHB) and Tris-HCl buffer were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2, 2′-azinobis (3- ethylbenzthiazoline-6-sulphonic acid) (ABTS) and 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich Chemical Co., India.

2.2 Skimmed camel milk hydrolysates

Raw camel's milk was skimmed (SCM) via centrifugation at 4700 x g for 10 min, at 4°C, using a refrigerated centrifuge (Thermo Scientific USA, central laboratory, faculty of veterinary medicine) till reached fat 1% after skimming. Enzymatic hydrolysis will be performed using digestive enzymes papain, pepsin, and trypsin at their optimal conditions at pH 6.0, 3.0, and 8.0, respectively, as shown in flow chart fig. (1). The protein substrate was dissolved in the appropriate buffer at 10 mg/ml. The enzyme had been dissolved at an enzyme-to-substrate ratio of 1:200 (w/w). After a 4-hour reaction, samples were heated in a boiling water bath for 10 minutes to inactivate the enzyme. The various SCM hydrolysis products were centrifuged at 4000 g for 15 minutes, and the supernatant was dialyzed, lyophilized, and then stored at 4 °C (Al-Shamsi *et al.,* 2018).

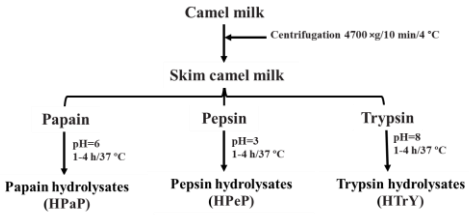

Fig. 1 Flowchart of skimmed camel milk hydrolysate

2.3 Antibacterial activity

Skimmed camel milk protein hydrolysates were tested for antibacterial activity as reported by Shavandi *et al.* (2017) and Esmaeilpour *et al.* (2017) method with slight modifications by bacterial count. Two indicator Gram-negative common pathogenic bacterial strains, *Salmonella enteritidis,* and *Escherichia coli*, were grown overnight in Mueller Hinton broth (MHB) medium at 37°C and working cultures were generated by diluting to roughly 4.0 $\log_{10}$cfu/ml using sterile MHB medium. The antimicrobial activity was assessed by monitoring the growth of indicator microorganisms in the presence or absence of 1000 µg/ml of different SCM hydrolysates after 2h of incubation at 37°C. The bacterial or colony forming unit (cfu) on nutrient agar was obtained by incubation at 37ºC for 18 h. The bacterial growth rate is represented as $\log_{10}$cfu/ml, and killing power was also calculated. All assays were performed in triplicate. Killing power = ($\log_{10}$ control - $\log_{10}$test).

2.4 Antioxidant activity

2.4.1 DPPH radical scavenging activity

The radical scavenging activity against DPPH was carried out in accordance El-Hadary and Ramadan (2019) with some changes using specific dissolving buffer. Briefly, DPPH stock solution prepared (0.004% w/v) in ethyl alcohol was used for the assay. The ethanolic group of DPPH was used as a control, and samples of various skimmed milk hydrolysates produced from different enzyme digestion were dissolved in the alcohol. One milliliter of samples was mixed with DPPH solution (1ml). After vigorously shaking the mixture and storing it at room temperature for 30 minutes in the dark, the absorbance (Abs) of the DPPH radical was

measured by spectrophotometry at 517 nm (UV-1800 spectrophotometer, TOMOS, Italy).
According to the equation to measure the free radical scavenging activity.

$$\text{RSA } \% = \frac{[\text{Abs } Blank - \text{Abs } Test]}{\text{Abs } Blank} \text{x } 100$$

2.4.2 ABTS radical scavenging activity
Following the method outlined by Hernández-Ledesma et al. (2007), radical scavenging activity was measured against 2, 2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS). The stock was prepared and stored in the dark at room temperature for 16h before use. After that, ethanol was added to the solution to dilute it and produce a wavelength absorbance of 0.706 at 734 nm. Samples of various skim milk hydrolysates produced from different enzyme digestion were mixed well with (0.9 mL) of the ABTS solution and then measured at 734 nm after 15 minutes of incubation at room temperature. In the blank solution, distilled water was utilized in place of the sample. The reduction % was calculated using the reaction absorbance at 734 nm by the following equation:

$$\text{Inhibition } \% = \frac{[\text{Absorbance } Control - \text{Absorbance } Sample]}{\text{Absorbance } Control} \text{x } 100$$

2.5 Statistical analysis
The statistical analysis results were provided as mean value of three repeated trials, standard error (±), and a P value of 0.05 was utilized for statistical evaluation using the MS Excel program (Spss, 2007).

3. RESULTS

The SCM antimicrobial efficacy and its different enzymatic hydrolysates, at various hydrolysis times, on the bacterial growth survival and inhibition rate against Gram-negative bacteria *E. coli* and *Salmonella enteritidis* were observed, and the mean values were recorded in $\log_{10}$ cfu/ml as illustrated in figures (2-5) and table (1).

3.1 Antibacterial activity of SCM against tested Gram-negative foodborne pathogens
Intact undigested SCM at a concentration of 1000 µg/ml displayed minor antibacterial activity on *E. coli* as in fig. (2A), while intact undigested SCM showed no inhibitory antibacterial activity against *Salmonella enteritidis* in comparison to the control groups (Ctrl). As indicated in Figures (2 to 5A, B). It was observed that intact unhydrolyzed SCM could inhibit the *E. coli* growth rate to 6.04±0.16 $\log_{10}$cfu/ml, whereas the Ctrl group reached 8.48±0.17 $\log_{10}$cfu/ml, with killing power 2.43±0.16 $\log_{10}$cfu/ml as showed in Fg. (5. A). On the other hand, in the case of *Salmonella enteritidis*, intact SCM showed antibacterial resistance with no killing capacity during the antibacterial assay against intact SCM, as demonstrated in Fig. (5. B).

3.2 Antibacterial activity of papain camel milk hydrolysates
The effect of papain skim camel milk hydrolysates (HPaP) at different hydrolysis times (1-4h) at protein content 1000µg/ml against tested indicator Gram-negative bacteria was shown in Fig. (2). We discovered the antibacterial efficacy of HPaP was hydrolysis time rate dependent as illustrated in fig. 2 (A, B). We observed that the inhibitory growth rate at the first hour of hydrolysis was 6.41±0.19 $\log_{10}$cfu/ml, and it increased with increasing hydrolysis time rate at 4h with a mean value of 4.76± 0.17 $\log_{10}$cfu/ml. Furthermore, the hydrolysis time rate showed a great significant difference against *Salmonella enteritidis*, with skimmed camel milk displaying 100% killing power with a killing capacity of 7.22±0.13 $\log_{10}$cfu/ml after enzymatic hydrolysis with papain (3 and 4h), as shown in Figs. (2B) and (5B).

3.3 Antibacterial activity of pepsin camel milk hydrolysates
Figure (3A) has been showed the efficacy of pepsin hydrolysis concerning the antibacterial activity of SCM. We discovered pepsin hydrolysate (HPeP) after 3h hydrolysis performing a higher bactericidal activity against *E. coli* with a growth survival rate of 3.31± 0.16 $\log_{10}$cfu/ml in comparison to the Ctrl group, which reached 8.48 ±0.17 $\log_{10}$cfu/ml during the antibacterial assay. Conversely, pepsin hydrolysate (HPeP) after 2h hydrolysis could completely abolish *Salmonella enteritidis* survival growth rate with killing power 100%, as illustrated in Fig. (3B).

3.4 Antibacterial activity of trypsin camel milk hydrolysates
Figure (4) depicts the impact of trypsin hydrolysis for four hours (HTrY) on the antibacterial efficacy of skim camel milk. It was worth noting that HTrY, after 2h hydrolysis, acquired potent antibacterial activity compared to intact SCM and Ctrl group. HTrY after 2h hydrolysis could inhibit *E. coli* survival growth rate to 5.07±0.16 $\log_{10}$cfu/ml. In contrast, it reached 6.4± 0.16 and 8.4 ±0.17 $\log_{10}$cfu/ml in SCM and Ctrl groups, respectively, as reported in fig. (4A). At the same time, HTrY after 2h hydrolysis could completely inhibit *Salmonella enteritidis* survival growth rate with 100% killing capacity as recorded in fig. (4B).

3.5 Antioxidant activity of camel milk hydrolysates
The skim milk hydrolysates using different digestive enzymes (papain, pepsin, and trypsin) were estimated for the radical scavenging antioxidant activity using DPPH and ABTS assay (Fig. 6). It was observed that all various enzymatic digestion for SCM using papain, pepsin, and trypsin exhibited higher antioxidant activity than intact unhydrolyzed SCM. By increasing hydrolysis time, we found that the antioxidant activity gradually improved, as illustrated in Fig. (6, red arrow) in DPPH and ABTS assay.
Skim camel milk hydrolysates generated by trypsin showed higher antioxidant properties in the DPPH and ABTS assays, followed by skim camel milk hydrolysates produced by papain and pepsin, respectively. As a result, the DPPH activity in HTrY after 1h hydrolysis increased 3 times the DPPH activity of intact SCM with mean values of 32.33% and 10.33%, respectively, and with prolonged hydrolysis rate; we found HTrY after 4h hydrolysis, we reported that DPPH radical scavenging activity enhanced six times the value of SCM antioxidant activity with mean 61.76%. Similarly, HTrY acquired the highest antioxidant activity in the ABTS assay than intact SCM.

Table (1) The antibacterial of camel milk hydrolysates using (papain ,pepsin and trypsin) enzymes at different hydrolysis rates.

Strain/Enzyme	*Escherichia coli*				*Salmonella enteritidis*			
Hydrolysis rate (hours)	1h	2h	3h	4h	1h	2h	3h	4h
Papain hydrolysates (HPaP)	6.41±0.19a	5.20±0.19b	5.70±0.16b	4.67±0.17c	6.50±0.20a	6.43±0.23a	ND	ND
Pepsin hydrolysates (HPeP)	5.47±0.16a	5.42±0.12a	3.31±0.06b	5.43±0.12a	3.88±0.07b	ND	3.54±0.13a	3.41±0.07a
Trypsin hydrolysates (HTrY)	5.53±0.17b	5.07±0.16b	6.71±0.16a	6.20±0.12a	3.65±0.18c	ND	3.43±0.17c	3.44±0.18c

The antibacterial activity estimated against indicators Gram negative food borne pathogens were the camel milk hydrolysates at specific concentration (1000 ug/ml) incubated with indicators bacteria at 37 ºC/2h. The control *E. coli* reach 8.48±0.17 and control *Sal. enteritidis* reach 7.21±0.12 to after incubation. The antibacterial activity estimated as mean $\log_{10}$ cfu/ml.
ND: Non detected, no bacterial colonies appear during the antibacterial assay.

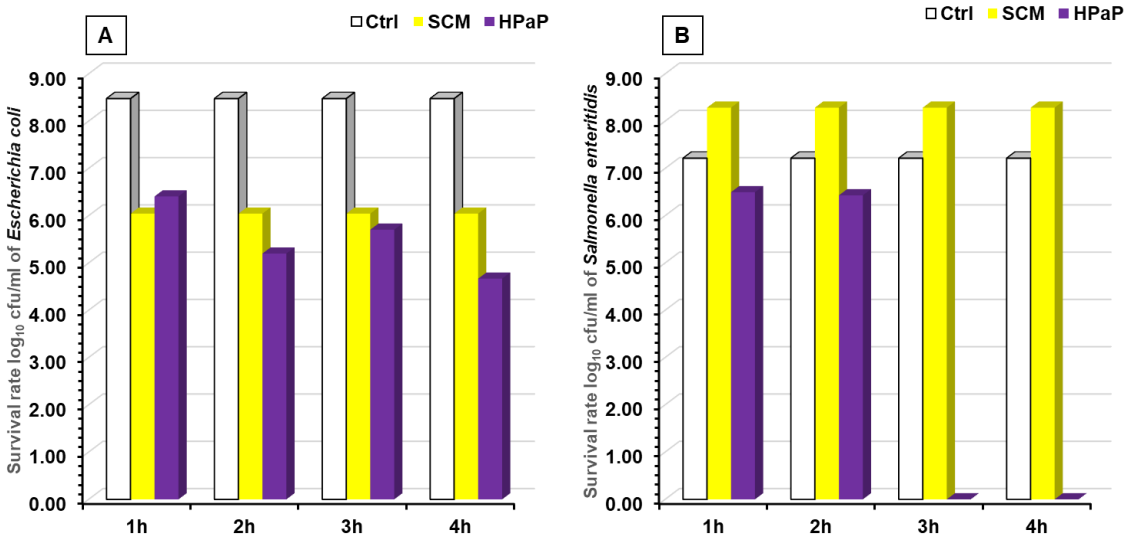

Fig. (2) The antibacterial of skim camel milk hydrolysates using papain enzyme (HPaP) at different hydrolysis rate at specific concentration (1000 µg/ml). The antibacterial activity estimated against indicators Gram negative food borne pathogens where the skim camel milk hydrolysates by papain at specific concentration (1000 µg/ml) incubated with indicators bacteria at 37 °C/2h in comparison with intact skim camel milk (SCM) . The antibacterial activity is estimated as mean log cfu_{10}/ml.

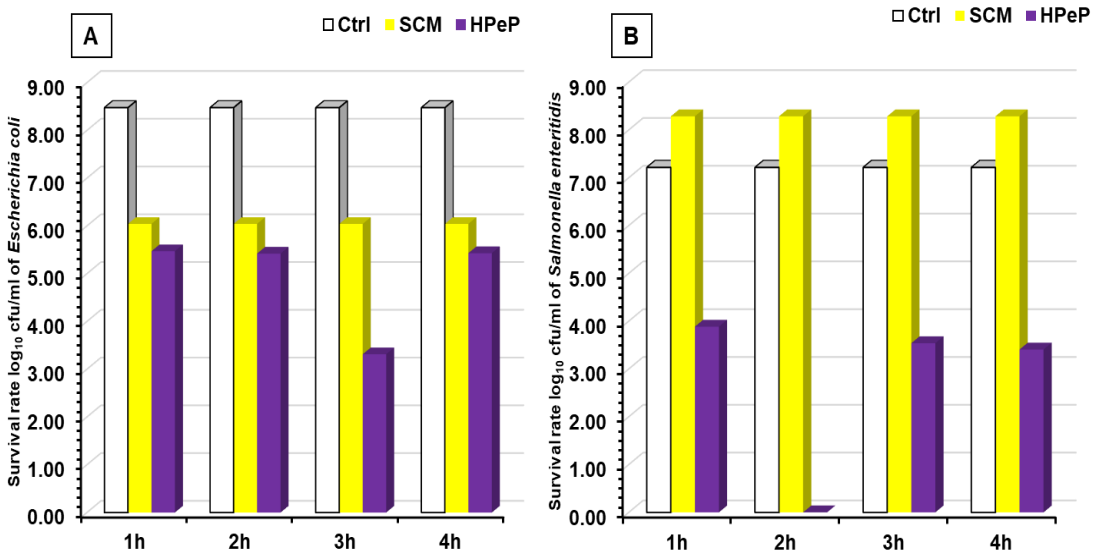

Fig. (3) The antibacterial of skim camel milk hydrolysates using pepsin enzyme (HPeP) at different hydrolysis rate at specific concentration (1000 µg/ml). The antibacterial activity estimated against indicators Gram negative food borne pathogens where the skim camel milk hydrolysates by pepsin at specific concentration (1000 µg/ml) incubated with indicators bacteria at 37 °C/2h in comparison with intact skim camel milk (SCM) . The antibacterial activity estimated as mean log_{10} cfu/ml.

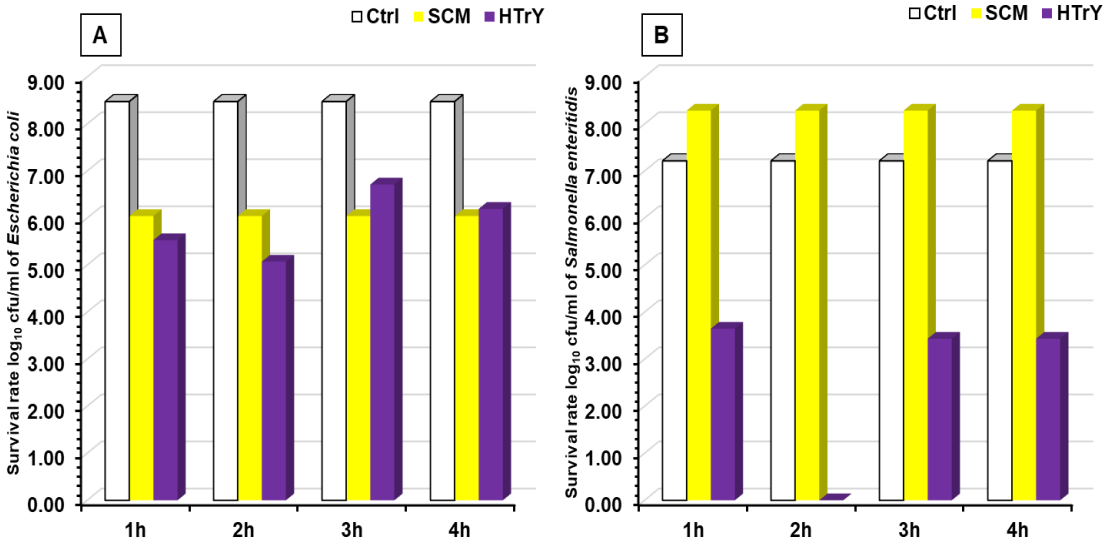

Fig. (4) The antibacterial of skim camel milk hydrolysates using trypsin enzyme (HtrY) at different hydrolysis rate at specific concentration (1000 µg/ml). The antibacterial activity estimated against indicators Gram negative food borne pathogens where the skim camel milk hydrolysates by trypsin at specific concentration (1000 µg/ml) incubated with indicators bacteria at 37 °C/2h in comparison with intact skim camel milk (SCM) . The antibacterial activity estimated as mean log_{10} cfu/ml.

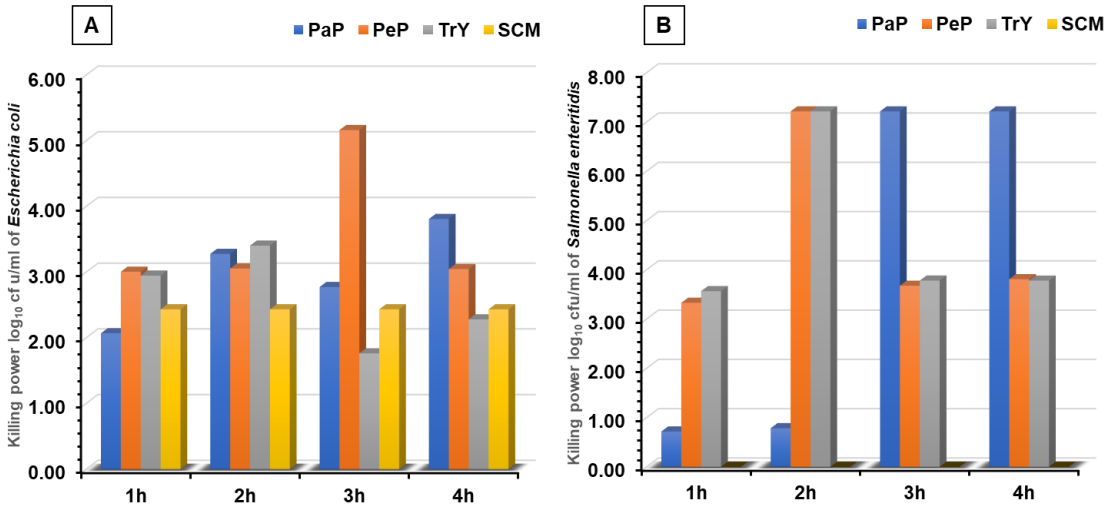

Fig. (5) The killing power of skim camel milk hydrolysates using different digestive enzymes enzyme (papain, pepsin, trypsin) at different hydrolysis rate at specific concentration (1000 µg/ml). The antibacterial activity estimated against indicators Gram negative food borne pathogens where the skim camel milk hydrolysates at specific concentration (1000 µg/ml) incubated with indicators bacteria at 37 °C/2h in comparison with intact skim camel milk (SCM) . The killing power activity estimated as mean log cfu_{10}/ml.

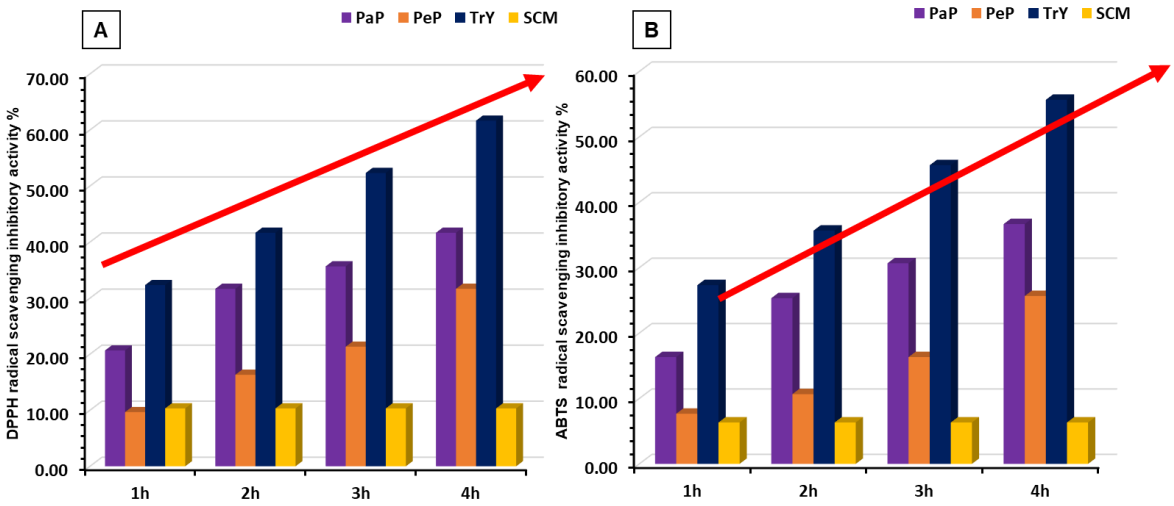

Fig. (6) The antioxidant activity of skim camel milk hydrolysates using different digestive enzymes enzyme (papain, pepsin, trypsin) at different hydrolysis rate at specific concentration (1000 µg/ml). The antioxidant activity is estimated using DPPH assay and ABTS assay.

4. DISCUSSION

Through consuming contaminated foods, pathogenic microorganisms, particularly bacteria, are the primary common cause of various food poisonings that carry a moderate to high risk of illness and a higher mortality rate (Fu *et al.*, 2016). Synthetic preservatives like synthetic chemicals and antimicrobials have largely been used to limit food deterioration and pathogenic bacteria (Calo *et al.*, 2015; Zheng *et al.*, 2013). However, these chemicals' use is restricted because of their unfavorable characteristics, such as teratogenicity, acute toxicity, and carcinogenicity (Calo *et al.*, 2015). Therefore, the current research aimed to find natural antibacterial compounds that might be extracted from camel milk and employed as natural preservatives.

Compared to bovine milk, camel milk is characterized by more pronounced inhibitory systems (El Agamy *et al.*, 1992). Notably, dromedary milk contains twice as much Lysozyme and lactoferrins as bovine milk (Kappeler*et al.*, 1999). Additionally, Bioactive functional peptides and proteins included in camel's milk have various biological effects that could account for the antibacterial impact of skimmed camel milk against the growth rate of *E. coli* (Korhonen and Pihlanto, 2003)

On the contrary, the Gram-negative bacteria in the form of *Salmonella enteritidis*, which could enter the colon and translocate in the gastrointestinal epithelium (Eng *et al.*, 2015). *Escherichia coli* has a high pathogenicity level for the host it infects. The current results showed that intact SCM had slight antibacterial activity against *E.coli* and *Salmonella enteritidis*, which came in agreement with that of Benkerroum *et al.*(2004), who claimed that camel's milk has bacteriostatic properties against *E. coli* and *Listeria monocytogenes*. High levels of antimicrobial proteins, like Lysozyme, lactoferrin, lacto-peroxidase, and short peptidoglycan recognition protein, present in camel milk may cause this inhibitory effect (Benkerroum *et al.*,2004). Although Gram-negative bacteria acquired an extra barrier outer membrane covering the peptidoglycan (Ramachandran, 2014).

The antibacterial activity and antioxidant capacity of the peptide fragments produced by the hydrolysis of dromedary skim milk proteins were evaluated in the current study using a variety of digestive enzymes. Generally, the various SCM hydrolysates exhibited more potent antibacterial activity than intact SCM, This result agreed with Kumar *et al.* (2016), who claimed that camel milk's antibacterial and antioxidant properties were enhanced by enzymatic hydrolysis.

Current result showed that intact SCM has no killing power, where the SCM generated hydrolysates at different hydrolysis times acquired potent antibacterial activity as in Fig. (5). It was suggested that the hydrophobicity was acquired for peptides generated from papain than from pepsin and trypsin at different hours of enzymatic hydrolysis. Due to the cationic charge of the produced SCM hydrophobic peptides and their initial contact with the microbial membrane, these peptides primarily target anionic microbial membranes (Teixeira *et al.*,2012; Oñate-Garzón *et al.*, 2017). The hydrophobic amino acids are then introduced into the hydrophobic core of the membrane, causing the phospholipids to become more disordered and the barrier membrane function to be lost (Teixeira*et al.*, 2012).In order to achieve insertion into the microbial membrane, various peptide structural characteristics, such as hydrophobicity, amphipathicity, and secondary structure, are crucial(Lee *et al.*, 2016). Such structural qualities can alter the peptide's physicochemical properties and antibacterial effectiveness (Giangaspero*et al.*, 2001;Torrent *et al.*, 2011).

During the 4h of hydrolysis, the antioxidant capacity was increased, indicating that SCM contains various cleavage and cutting sites for the used enzymes; the same increase was observed during the first 2h of hydrolysis (Oussaief *et al.*, 2020). As the hydrophobic areas buried within the parent protein are revealed during enzymatic proteolysis, alterations in protein globular shape may result. It is understood that more hydrophobic amino acids are exposed following proteolytic digestion due to the catalytic selectivity of the enzymes that cleave the peptide bonds produced by these amino acid residues. Due to enzyme differences specificity, the various digestive enzymes utilized in this study were able to have a variety of effects on the surface hydrophobicity, leading to diverse proteolytic patterns and the exposure of more hydrophobic amino groups, including phenylalanine, tyrosine, tryptophan, and leucine amino acids (Al-Shamsi *et al.*, 2018). Trypsin-treated SCM hydrolysates showed the highest antioxidant values, reflecting the highest degree of hydrophobicity, the main reason for higher antioxidant activity as in Fig. (6).

However, pepsin-treated SCM after 3h hydrolysis could inhibit bacterial growth. Papain SCM hydrolysates could completely inhibit *Salmonella enteritidis* after 3 and 4h hydrolysis, pepsin SCM hydrolysates after 2h hydrolysis, and trypsin-treated SCM after 2h hydrolysis as in Fig. (6). Conversely, all SCM enzymatic-treated hydrolysates gradually acquired potent antioxidant activity with prolonged hydrolysis time as in Fig. (6). It was suggested for future studies to apply camel milk enzymatic hydrolysates generated by papain, pepsin, and trypsin as a natural preservative in food.

5. CONCLUSIONS

Most of the medicinal value of camel milk is attributed to its biological qualities, such as its antibacterial activity. In this study, after being in vitro hydrolyzed by papain, pepsin, and trypsin, skim camel's antibacterial activity was detected against *E. coli* and *Salmonella enteritidis*. These preliminary results confirmed the inhibitory activity of powerful camel's milk protein hydrolysates produced against various Gram-negative bacteria (*E. coli* and *Salmonella enteritidis*). The papain hydrolysates demonstrated pronounced antibacterial activity at different times of hydrolysis compared to that produced from pepsin and trypsin hydrolysis. The SCM hydrolysates showed a gradual increase in radical scavenging activity after 4 hours of hydrolysis. The results demonstrated that skimmed camel milk protein hydrolysates can be tuned and manipulated to achieve specific functionalities, opening up a wide range of potential applications as new antimicrobial agents in food. Therefore, additional research is required to identify the antibacterial peptides and explore the potential for their use as natural preservative in food models.

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