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Camel milk protein hydrolysates with improved technofunctional properties and enhanced antioxidant potential in in vitro and in food model systems

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ABSTRACT

Camel milk protein hydrolysates (CMPH) were generated using proteolytic enzymes, such as alcalase, bromelain, and papain, to explore the effect on the technofunctional properties and antioxidant potential under in vitro and in real food model systems. Characterization of the CMPH via degree of hydrolysis, sodium dodecyl sulfate-PAGE, and HPLC revealed that different proteins in camel milk underwent degradation at different degrees after enzymatic hydrolysis using 3 different enzymes for 2, 4, and 6 h, with papain displaying the highest degradation. Technofunctional properties, such as emulsifying activity index, surface hydrophobicity, and protein solubility, were higher in CMPH than unhydrolyzed camel milk proteins. However, the water and fat absorption capacity were lower in CMPH compared with unhydrolyzed camel milk proteins. Antioxidant properties as assessed by 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activities and metal-chelating activity were enhanced after hydrolysis, in contrast to ferric-reducing antioxidant power which showed a decrease after hydrolysis. The CMPH were also tested in real food model systems for their potential to inhibit lipid peroxidation in fish mince and grape seed oil-in-water emulsion, and we found that papain-produced hydrolysate displayed higher inhibition than alcalase- and bromelain-produced hydrolysates. Therefore, the CMPH demonstrated effective antioxidant potential in vitro as well as in real food systems and showed enhanced functional properties, which guarantees their potential applications in functional foods. The present study is one of few reports available on CMPH being explored in vitro as well as in real food model systems.

Key words: camel milk, hydrolysates, proteases, functional properties, bioactivities

INTRODUCTION

Bioactive peptides and protein hydrolysates are frequently added as an active ingredient in the formulation of functional foods due to their high nutritional values and role in promoting health and preventing diseases. Bioactive peptides are obtained upon hydrolysis of proteins by digestive, microbial, or plant proteases that release low-molecular weight bioactive peptides. Bioactive peptides obtained from milk have been reported to exert various health-related activities such as antioxidant, antimicrobial, anticancerous, antihypertensive, and opioid activity, mineral binding, and growth stimulation (Kilara and Panyam, 2003) Moreover, protein hydrolysates and peptides prepared from food proteins have emerged as a new source of natural antioxidants exerting effective metal ion (Fe^{2+}/Cu^{2+}) chelating and radical scavenging activities and lipid peroxidation inhibitory capacity, which increase their potential to be used as functional food additives. For example, sunflower protein (Megías et al., 2004), milk protein (Suetsuna et al., 2000), soybean protein (Gibbs et al., 2004), egg-white albumin (Miguel et al., 2005), and pacific hake protein (Cinq-Mars et al., 2008) have been known to possess antioxidant properties upon hydrolysis. Hydrolysates from bovine milk proteins have been widely studied for technofunctional and various bioactive properties, including antioxidative activities. However, the technofunctional properties of proteins from nonbovine sources, particularly camel milk, are scarce. In fact, the search for novel protein hydrolysates has increased considerably in the last decade, which demands exploration of new sources of protein hydrolysates and biologically active peptides.

Camel milk differs from bovine milk in its composition and protein content and structure, and therefore is expected to possess functional and bioactive properties different than bovine milk. Composition of camel milk proteins has well been studied. Camel milk lacks β-LG and is richer in β-CN, α-LA, and serum albumin than bovine milk (El-Agamy, 2009) Caseins constitutes 75 to 80% of total proteins of camel milk, which are highly susceptible to proteolysis. The bioactivities and functional properties of camel milk proteins can be

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further enhanced via generation of shorter bioactive peptides through enzymatic hydrolysis (Kumar et al., 2016d). Recently, camel milk casein has demonstrated an increase in antioxidant properties after enzymatic hydrolysis (Salami et al., 2011; Kumar et al., 2016c). It was also reported that camel milk protein hydrolysates (**CMPH**) possess higher antioxidant and antimicrobial activities as compared with bovine milk upon limited hydrolysis by alcalase, papain, chymotrypsin, and trypsin (Salami et al., 2010; Kumar et al., 2016a).

Studies on antioxidant potential and technofunctional properties of CMPH in the in vitro and in real food model systems are currently very limited and demand further exploration to gain more insight into the properties and characterization of camel milk protein hydrolysate (Kumar et al., 2016b). Therefore, the present study was carried out to generate hydrolysates from camel milk using food-grade proteolytic enzymes (alcalase, bromelain, and papain) and to explore the effect of enzymatic hydrolysis on the functional properties, antioxidative activity, and inhibition of lipid oxidation in different food model systems.

MATERIALS AND METHODS

Chemicals and Reagents

We purchased 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (**ABTS**), 2,2-diphenyl-1-picrylhydrazyl (**DPPH**), 2,4,6-tripyridyl-s-triazine, *o*-phthalaldehyde (**OPA**), trifluoroacetic acid (**TFA**), bromophenol blue (**BPB**), ferrozine, glacial acetic acid, and thiobarbituric acid from Sigma Aldrich (St. Louis, MO). Other chemicals, such as ferric chloride and sodium acetate, were procured from BDH Middle East (Dubai, United Arab Emirates). All reagents for electrophoresis were purchased from Bio-Rad (Richmond, CA).

Camel Milk and Preparation of CMPH

Raw milk from 3 different camels (*Camelus dromedarius*) of 1 breed was procured from a local farm in Al-Ain, United Arab Emirates, and skimmed by centrifugation at 2,326 × *g*, 20°C, for 20 min (Beckman Coulter, Allegra X-30R). Milk from 3 different camels were prepared separately in the further steps and served as replicates for each treatment. One portion of skimmed camel milk was kept as control, whereas other portions were adjusted to pH 8 for alcalase and pH 7 for bromelain and papain using a digital type pH meter (Starter 3100, Ohaus, Parsippany, NJ). These 3 enzymes were selected based on their high specificity toward milk proteins and long history of their application for production of bioactive peptides from food proteins. Protein hydrolysis was carried out at 50°C under constant stirring in a water bath at an enzyme-to-substrate (protein) ratio of 1:100 (wt/wt) for up to 6 h. Samples (10 mL) were taken every 2 h, boiled for 10 min to deactivate the enzyme, stored at 4°C, and analyzed by electrophoresis and HPLC within 24 h to monitor the hydrolysis pattern with respect to time. Upon completion of hydrolysis for 6 h, enzymes were deactivated by keeping the samples in water bath adjusted to 100°C for 10 min. The hydrolysates were centrifuged at 10,000 × *g* at 4°C for 15 min, supernatants were collected, freeze-dried using a Telstar freeze dryer (Telstar Life Science Solutions, Bristol, PA) at −80°C, 10,000 Pa pressure, and stored at −20°C for further analysis.

Characterization of CMPH

Degree of Hydrolysis. Degree of hydrolysis (**DH**) was analyzed using the OPA method described by Nielsen et al. (2001) with a few modifications. The OPA reagent was freshly prepared by mixing 25 mL of sodium tetraborate buffer (100 m*M*; pH 9.3), 2.5 mL of SDS (20%, wt/wt), 40 mg of OPA (dissolved in 1 mL of methanol), and 100 μL of β-mercaptoethanol. Final volume was raised to 50 mL with milli-Q water (Elix-10, Millipore, Molsheim, France). Small aliquots (100 μL) of the samples were added directly to cuvette containing 1 mL of OPA reagent and mixed gently for 5 s. The absorbance was measured at 340 nm using a Nova-Spec-II Spectrophotometer (Pharmacia, Pfizer, New York, NY) after 2 min of incubation in the dark at room temperature.

Degree of hydrolysis was determined using DH (%) = $h/h_{tot} \times 100$, where, h_{tot} is the total number of peptide bonds per protein equivalent and h is the number of hydrolyzed bonds. The number of hydrolyzed bonds was determined using $h = (SerineNH_2 - \beta)/\alpha$, where α, β, and h_{tot} values were 1.039, 0.383, and 8.2 mEq/g of protein, respectively (Nielsen et al., 2001).

Characterization of CMPH by SDS-PAGE. Skimmed camel milk and CMPH were characterized by SDS-PAGE following the method described by Laemmli (1970), with slight modification under reducing conditions on a 12.5% resolving gel and 4% stacking gel using the Mini Protean III apparatus (gel size = 7 cm × 8 cm × 0.75 mm; Bio-Rad). Each sample was incubated with the sample buffer at 1:1 ratio (12% glycerol, 1.2% SDS, 5.4% b-mercaptoethanol, bromophenol blue) at 100°C for 3 min and then loaded onto the gel. Pure bovine milk proteins (Sigma Aldrich) of known molecular weights were used as standard. The gels were visualized and images were captured using