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Inhibitory properties of camel whey protein hydrolysates toward liver cancer cells, dipeptidyl peptidase-IV, and inflammation

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ABSTRACT

This report describes an investigation of camel whey protein hydrolysates (CWPH) produced by gastric and pancreatic enzymes for their in vitro antidiabetic, anticancer, and anti-inflammatory properties. Degree of hydrolysis (DH) ranged from 8.54 to 47.53%, with hydrolysates generated using chymotrypsin for 6 h displaying the highest DH. Reverse phase-HPLC analysis showed that α-lactalbumin underwent complete degradation, with no intact α-lactalbumin detected in CWPH. The CWPH displayed enhanced antidiabetic activity compared with intact whey proteins; with pepsin- and chymotrypsin-generated CWPH displaying greater inhibition of dipeptidyl peptidase IV (DPP-IV), α-glucosidase, and α-amylase compared with trypsin-generated CWPH. The highest antiproliferative effect was observed for CWPH generated by chymotrypsin for 3 h, with only 4.5 to 6.5% viable liver cancer cells (HepG2) remaining when tested at concentrations from 400 to 1,000 μg/mL. The highest anti-inflammatory activity was manifested by CWPH generated by pepsin at 6-h hydrolysis. We report enhanced antiproliferative, antidiabetic, and anti-inflammatory activities upon hydrolysis of camel whey proteins, indicating their potential utilization as bioactive and functional ingredients.
Key words: camel whey protein, antiproliferation, antidiabetic, anti-inflammation, functional food

INTRODUCTION

Milk from the one-humped camel (*Camelus dromedarius*) is widely consumed in arid regions of Asia and Africa. Physiologically, camel milk is easily digested compared with bovine milk; it forms a soft curd in the human stomach, making it suitable as an infant food. The lack of β-LG in the camel whey makes it suitable for those who are allergic to cow milk (El-Agamy et al., 2009). In addition to its nutritional benefits, camel milk has been used for its medicinal properties, including immunomodulatory, antimicrobial, anticancer, and antidiabetic activities (Abdel Gader and Alhaider, 2016). The therapeutic properties of camel milk against diseases such as diabetes and cancer have been widely studied and reviewed (Agrawal et al., 2007). Among the proteins in the milk, whey proteins are believed to have the highest quality in term of the presence of high content of α-LA, lactophorin, immunoglobulins (IgG), and lactoferrin, even when compared with proteins such as egg, casein, beef, or soy (Lucas, 1999). Camel whey contains greater contents of antimicrobial factors such as lysozyme, lactoferrin, and immunoglobulins compared with bovine milk (El-Agamy et al., 1996). In addition, camel whey contains several immunomodulatory proteins (serum albumin, α-LA, lactophorin, and peptidoglycan) that are naturally present or that are part of the primary sequence of whey proteins.

The past decade has seen growing interest in the formulation of functional foods that are not only health-promoting but also effective in reducing the risk of many diseases. Recently, peptides derived from camel milk protein and their potential health-related bioactive properties have been the focus for researchers. Previous studies indicate that camel milk has beneficial effects on treatment of wounds because it enhances wound healing and increases immune cell proliferation and the chemotaxis state of experimental animals (Badr, 2013). It is increasingly recognized worldwide that camel milk consumption helps in prevention and control of diabetes (Agrawal et al., 2007). Camel milk has been demonstrated to be beneficial in reducing the dose of insulin needed to induce glycemic control and increases glycosylate hemoglobin, anti-insulin antibodies, and urinary albumin excretion, and lowers body mass index (Mohamadet al., 2009).

Studies on several human cancer cell lines have shown that milk-derived peptides may act to regulate cell growth, differentiation, and apoptosis. Milk-derived peptides showed interesting chemopreventive effects. In particular, bovine lactoferrin (a whey protein) and buffalo milk waste whey have been demonstrated to inhibit

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the proliferation of breast cancer (MDA-MB-231) and affect the viability of H-Caco-2 cells in in vitro models, respectively (Damiens et al., 1999; De Simone et al., 2011).

So far, studies on camel milk protein hydrolysates have emphasized the antioxidant (Kumar et al., 2016; Salami et al., 2008), antidiabetic (Nongonierma et al., 2017), angiotensin-converting enzyme (ACE)-inhibitory (Moslehishad et al., 2013), and antibacterial activities (Kumar et al., 2016). However, camel whey proteins and their hydrolysates have not received attention with respect to their antidiabetic, anti-inflammatory, and anticancer properties. Exploring camel whey proteins following hydrolysis with gastric and pancreatic enzymes might reveal novel information about their health-related bioactive properties. Therefore, the aim of this work was to investigate the use of camel whey protein hydrolysates (**CWPH**) as antidiabetic, anticancer, and anti-inflammatory agents upon hydrolysis with gastric and pancreatic enzymes.

MATERIALS AND METHODS

Chemicals and Reagents

Pepsin from porcine gastric mucosa (EC 3.4.23.1; 800–2,500 units/mg of protein), trypsin acetylated from bovine pancreas [EC 3.4.21.4; ≥40 Na-benzoyl-L-arginine ethyl ester (**BAEE**) units/mg of protein], α-chymotrypsin from bovine pancreas (EC 3.4.21.1; ≥40 units/mg of protein), α-glucosidase from *Saccharomyces cerevisiae* (type I, lyophilized powder ≥10 units/mg of protein), α-amylase from porcine pancreas (type VI-B ≥5 units/mg of solid), dipeptidyl peptidase IV (**DPP-IV**; expressed recombinantly in baculovirus-infected Sf9 cells, ≥10 unit/mg of protein), and all solvents and chemicals used were purchased from Sigma-Aldrich (St. Louis, MO) and were of analytical grade. One BAEE unit will produce a ΔA253 of 0.001 per min at pH 7.6 at 25°C using BAEE as substrate. Reaction volume = 3.2 mL (1 cm light path).

Milk Samples

Raw camel milk from 3 healthy camels of the same breed (*Camelus dromedarius*) was procured from Al Ain Dairy farm (Al Ain, United Arab Emirates). Milk was pooled to be used as a single composite (representative) sample for further processing (e.g., separation of whey protein and production of CWPH) described below. Camels were reared under semi-intensive conditions and the feeding regimen was approximately the same for all camels on the farm. Milk samples were immediately refrigerated after collection and transferred to the laboratory in the chilled conditions.

Preparation of Camel Whey and Production of CWPH

Camel milk was skimmed by centrifugation at 2,326 × *g* for 20 min at 10°C. The skimmed milk was then subjected to acid precipitation at pH 4.6 by the addition of 1 *N* HCl, followed by centrifugation at 2,326 × *g* for 10 min at 4°C to separate the whey from caseins. Centrifugation was repeated 2 times to allow efficient separation of whey from casein. Whey samples were then frozen at −20°C for further experiments and used within 3 d. Prepared camel whey protein was adjusted to 3.0% (wt/vol) by adding ultra-pure water and was divided into 3 batches of 150 mL for each enzymatic treatment with gastric and pancreatic enzymes (pepsin, trypsin, and α-chymotrypsin). The pH of each whey group was adjusted to the optimum for each enzyme (pH 2 for pepsin using 1 *N* HCl; pH 8 for trypsin and chymotrypsin using 1 *M* NaOH). Briefly, to achieve an enzyme:substrate ratio of 1:100, the calculated weight of each enzyme (based on unit activity/mg of enzyme) for 3% protein in the whey sample was initially dissolved in 5 mL of whey sample (at optimum pH) and then transferred to the whole volume (150 mL). Each lot of enzyme-added whey was then further divided into 6 tubes of 25 mL each to represent 3 replicates at each time interval. The hydrolysis of whey protein was then carried out in different batches at the optimal temperature for each enzyme (37°C for pepsin; 55°C for trypsin and chymotrypsin) in a water bath under constant agitation of 100 rpm (752A model, Fisatom, São Paulo, Brazil). The samples were incubated for 3 and 6 h of hydrolysis for each enzyme and then the enzymes were deactivated at 95°C for 10 min. The samples were centrifuged (10,000 × *g*, 15 min, 4°C) and supernatant was collected and stored at −20°C for further analysis which was carried out within 1 wk. The resulting CWPH were designated **P3**, **T3**, **C3**, **P6**, **T6**, and **C6** for hydrolysates generated by pepsin, trypsin, and chymotrypsin after 3 and 6 h of hydrolysis, respectively.

Characterization of CWPH

Degree of Hydrolysis. Degree of hydrolysis (**DH**) was analyzed using the *o*-phthaldialdehyde (**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. The final volume was made up to 50 mL with ultra-pure water obtained with a Milli-Q equipment (Elix-10, Millipore, Molsheim, France). A small aliquot (100 μL) of each sample was added directly to a cuvette containing 1 mL of OPA reagent