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In vitro antidiabetic and antihypercholesterolemic activities of camel milk protein hydrolysates derived upon simulated gastrointestinal digestion of milk from different camel breeds

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ABSTRACT

Milk protein hydrolysates derived from 4 camel breeds (Pakistani, Saheli, Hozami, and Omani) were evaluated for in vitro inhibition of antidiabetic enzymatic markers (dipeptidyl peptidase IV and α-amylase) and antihypercholesterolemic enzymatic markers (pancreatic lipase and cholesterol esterase). Milk samples were subjected to in vitro simulated gastric (SGD) and gastrointestinal digestion (SGID) conditions. In comparison with intact milk proteins, the SGD-derived milk protein hydrolysates showed enhanced inhibition of α-amylase, dipeptidyl peptidase IV, pancreatic lipase, and cholesterol esterase as reflected by lower half-maximal inhibitory concentration values. Overall, milk protein hydrolysates derived from the milk of Hozami and Omani camel breeds displayed higher inhibition of different enzymatic markers compared with milk protein hydrolysates from Pakistani and Saheli breeds. In vitro SGD and SGID processes significantly increased the bioactive properties of milk from all camel breeds. Milk protein hydrolysates from different camel breeds showed significant variations for inhibition of antidiabetic and antihypercholesterolemic enzymatic markers, suggesting the importance of breed selection for production of bioactive peptides. However, further studies on identifying the peptides generated upon SGD and SGID of milk from different camel breeds are needed.

Key words: camel milk, milk protein hydrolysates, antidiabetic, antihypercholesterolemic, simulated gastrointestinal digestion, enzyme inhibition

INTRODUCTION

Camels (*Camelus dromedarius*) are a common type of livestock in the arid regions of the Asian and African continents, and camel milk (**CM**) is considered an attractive alternative to bovine milk owing to its unique health-promoting properties (Maqsood et al., 2019). Camel milk lacks β-LG, a major allergen in bovine milk, which makes it desirable for the development of hypoallergenic infant formulas (Mudgil et al., 2022c). Moreover, the lack of β-LG also contributes to the enhanced digestibility of CM in the human gastrointestinal (**GI**) tract (Khalesi et al., 2017). Camel milk is considered a good source of sodium, potassium, calcium, magnesium, minerals, and vitamins such as vitamins C and B_9 (Rezaei et al., 2020). The bioactive peptides derived from CM proteins have been shown to have a broad range of nutraceutical and bioactive properties, such as antioxidant, antidiabetic, antiobesity, antihypertension, antiinflammation, anticancer, and antibacterial activities (Salami et al., 2010; Abdel-Hamid et al., 2016, 2020; Kumar et al., 2016; Alhaj, 2017; Kamal et al., 2018; Mudgil et al., 2019a,b, 2022b,d; Nongonierma et al., 2019; Ashraf et al., 2021; Baba et al., 2021a,b,c).

The protein composition of milk varies between different breeds of camel, which might directly influence the peptides (in terms of composition and sequence) generated upon consumption and thus the bioactivity associated with them. This variability was demonstrated by analyzing the antioxidant and angiotensin-converting enzyme inhibitory activities of in vitro digested milk proteins obtained from the milk of different camel breeds (Maqsood et al., 2019). Such variation in the milk proteins could be responsible for the production of novel bioactive peptides with enhanced biological, nutraceutical, and pharmacological properties associated with the milk of different camel breeds. Maqsood et al.

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(2019) explored differences in the functional properties of CM obtained from different breeds. They reported significant differences in the thermal behavior, protein solubility, emulsifying activity index, capacities of foaming, water absorption, and fat absorption of milk obtained from different camel breeds, as well as from cows.

Camel milk-derived protein hydrolysates with high bioactive potential can be used for various applications, including in the pharmaceutical and nutraceutical industries to produce health supplements and in the food industry to formulate functional foods. Diabetes mellitus and hypercholesteremia are 2 common chronic diseases that are on the rise in developed countries and have significant adverse effects on people's lives. Both are associated with cardiovascular disease and obesity. Furthermore, in addition to the health risk posed by obesity, psychological issues, such as mental disorders and social discrimination, and physical inability are possible problems (Ali Redha et al., 2022). Thus, exploring functional foods with antidiabetes and antihypercholesteremia properties is important to contribute to human health and well-being.

Most studies on functional foods have focused on deriving bioactive peptides through the use of specific food-grade enzymes or fermentative bacteria. In recent years, in vitro simulated gastrointestinal digestion (**SGID**) has drawn the attention of researchers because it can mimic how the biochemical conditions of the GI tract affect the bioactivity of proteins and peptides (David et al., 2019). A good amount of research has been carried out on the antidiabetic properties of enzymatic protein hydrolysates from CM (Mudgil et al., 2018, 2021; Nongonierma et al., 2018; Baba et al., 2021c; Kilari et al., 2021), but no studies have compared the antidiabetic and antihypercholesterolemic effects of protein hydrolysates derived from the milk of different camel breeds. Furthermore, no investigation has been done regarding how various proteolytic enzymes present in the GI tract might positively or negatively affect the bioactive properties of the hydrolysates after their consumption (Nongonierma et al., 2017). Thus, investigating the effect of in vitro digestion on the bioactive properties of generated protein hydrolysates is necessary before claims can be made for their health-related advantageous effects and before using them as functional food ingredients.

The current research focuses on evaluating the in vitro capability of CM hydrolysates to inhibit (1) α-amylase and dipeptidyl peptidase IV (**DPP-IV**) enzymes in relation to their antidiabetic potential and (2) pancreatic lipase (**PL**) and pancreatic cholesterol esterase (**CE**) in relation to their antihypercholesterolemic potential. Hydrolysates were derived from the milk of 4 different breeds (Pakistani, Saheli, Hozami, and Omani; major CM-producing breeds used in the United Arab Emirates) upon simulated digestion through gastric and GI phases.

MATERIALS AND METHODS

Chemicals and Reagents

Pancreatic α-amylase, human recombinant DPP-IV, lipase from porcine pancreas (type VI-S, ≥20,000 U/mg of protein), CE from porcine pancreas (~35 U/mg), *p*-nitrophenyl-α-D-maltohexaoside, gly-pro-*p*-nitroanilide hydrochloride, and *p*-nitrophenyl butyrate were purchased from Sigma Aldrich. All the chemicals, solvents, and standards used for SDS-PAGE were purchased from Bio-Rad and those for HPLC were purchased from Sigma Aldrich and were of analytical grade.

Milk Samples

Raw CM was obtained from 4 different camel breeds (Pakistani, Saheli, Hozami, and Omani) from local dairy farms in Al-Ain, United Arab Emirates. Three healthy camels were selected from each breed to obtain CM samples. All of the collected milk samples were instantly refrigerated and transported to the laboratory under chilled conditions. Samples were procured from the local camel farmers, and the researchers were not directly involved in sample collection or milking. Therefore, Institutional Animal Care and Use Committee approval was not required.

SGID of Milk from Different Camel Breeds

In vitro SGID was performed for all 4 milk samples according to the methodology described by Walsh et al. (2004) and Nongonierma et al. (2018). First, the collected milk samples in triplicate were defatted twice using centrifugation at 4,255 × *g* at 10°C for 30 min (Beckman Coulter, Allegra X-30R). The skim milk samples were then subjected to simulated gastric digestion (**SGD**) and SGID. Sequentially, pepsin [enzyme: substrate (E:S) 2.5% (wt/wt); 37°C for 2 h at pH 2.0] was used for SGD, and chymotrypsin and trypsin [E:S 1% (wt/wt); 37°C for 3 h] were used for intestinal digestion. The enzymes were then heat inactivated at 90°C for 15 min. One batch of skim milk samples was collected after SGD, and another batch was continued to complete SGID. All the digested samples were centrifuged at 15,000 × *g* for 15 min at 4°C, and the supernatant was collected and stored at −20°C for use in the in vitro enzyme inhibition assays within 2 d.

Characterization of CM Protein Hydrolysates

Degree of Hydrolysis. The *o*-phthaldialdehyde (**OPA**) method was employed for the measurement of free amino nitrogen released upon hydrolysis, using the methodology of Mudgil et al. (2022c). Briefly, hydrolysate samples were mixed with freshly prepared OPA reagent (25 mL of 100 m*M* sodium borate buffer at pH 8.3; 2.5 mL of SDS 20%; OPA, 40 mg/mL in methanol; 100 mL of β-mercaptoethanol; and deionized water to reach a total volume of 50 mL), and spectrophotometric measurements were obtained at 340 nm in a microplate reader. The degree of hydrolysis (**DH%**) was calculated using the formula described by Nielsen et al. (2001):

$$\mathrm{DH\%} = \left(\frac{h}{h_{\mathrm{tot}}}\right) \times 100, \qquad [1]$$

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 $h = (\text{serine } NH_2 - \beta)/\alpha$, where α, β, and h_{tot} values were 1.039, 0.383, and 8.2 mEq/g of protein, respectively (Nielsen et al., 2001).

SDS-PAGE. Milk protein hydrolysates (**MPH**) obtained upon hydrolysis of milk from all 4 breeds were characterized by SDS-PAGE as described by Mudgil et al. (2022a). Briefly, samples were incubated with sample buffer (12% glycerol, 1.2% SDS, 5.4% β-mercaptoethanol, and bromophenol blue) at 100°C for 3 min and then loaded onto a 12% resolving gel and 4% stacking gel. The separation was carried out under reducing conditions, using a Mini Protean III apparatus (Bio-Rad, gel size 7 cm × 8 cm × 1 mm).

Peptide Profiling Using Reverse-Phase Ultra-Performance Liquid Chromatography. Intact milk proteins and MPH obtained upon hydrolysis of milk from all 4 breeds were subjected to characterization using reverse-phase ultra-performance liquid chromatography (**RP-UPLC**), following the method described by Fisayo Ajayi et al. (2021). Briefly, samples were mixed with buffer A (0.1% trifluoroacetic acid in HPLC grade water) at a ratio of 1:1 (vol/vol). Thereafter, samples were vortexed for 5 min and then filtered using 0.45-μm syringe filters. Separation of the peptides and individual milk proteins was carried out at 22°C, using an Acquity UPLC BEH Shield RP18 column (2.1 mm × 100 mm, 1.7 μm i.d.). The flow rate was set at 0.3 mL/min, and peptide separation was done using a linear gradient solvent B (0.05% trifluoroacetic acid in 60% acetonitrile) from 0 to 80% over 100 min. Eluted fractions of peptides and proteins were monitored using a photodiode array detector (Dionex Ultimate 3000 RS) equipped with the HPLC system at a wavelength of 215 nm.

Antidiabetic Assays Via Inhibition of α-Amylase and DPP-IV. The effectiveness of various CM hydrolysates for inhibiting α-amylase and DPP-IV activity was evaluated according to the methodology described by Mudgil et al. (2019c) and Mudgil et al. (2021), respectively. The percentage of enzyme inhibition was calculated according to the following equation:

$$\%\text{Enzyme inhibition} = \left[1 - \left(\frac{X - Y}{A - B}\right)\right] \times 100, \qquad [2]$$

where A is the control, B is the control blank, X is the sample, and Y is the sample blank. These parameters respectively refer to the absorbance values of each reaction well with enzyme, substrate, and buffer; substrate and buffer; test sample, enzyme, substrate, and buffer; and sample, substrate, and buffer. A plot of the percentage of inhibition as a function of the sample's concentration (mg/mL on a protein-equivalent basis) was constructed using GraphPad Prism 7 (GraphPad Software) to determine the α-amylase half-maximal inhibitory concentration ($\mathbf{IC_{50}}$) values.

Antihypercholesterolemic Activity Assay Via Inhibition of PL and Pancreatic CE Activity. The inhibitory activity of all the samples against PL and pancreatic CE was evaluated using the methodology described by Mudgil et al. (2022d). Briefly, in a 96-well microplate, 50 μL of the test sample was mixed with 20 μL of PL solution (0.2 U/mL) or 20 μL of CE solution (10 μg/mL) and 25 μL of *p*-nitrophenyl butyrate (substrate) in sodium phosphate. The total volume of the reaction solution was adjusted to 150 μL, using 100 m*M* sodium chloride buffer (pH 7.3). After incubation of the microplate at 37°C for 30 min, the absorbance of the released *p*-nitrophenyl was recorded at a wavelength of 405 nm in a microplate reader. A control reaction was also performed (with no milk or hydrolysate samples) to determine 100% enzymatic activity and to eliminate background absorbances produced from the test samples. The percentage of enzyme inhibition was calculated according to Equation 2, and IC_{50} was calculated as described above for antidiabetic assays.

Statistical Analysis

Three batches of MPH from the milk of 4 different camel breeds were generated. Each analysis was performed in triplicate (n = 3). All the collected data were subjected to 1-way ANOVA by using SPSS 24.0

software (SPSS Inc.). Tukey's new multiple-range test was used to separate significant treatment means at a significance level of 0.05.

RESULTS AND DISCUSSION

Characterization of CM Peptides: Degree of Hydrolysis

The DH% of CM samples was compared based on camel breed. Simulated digestion was significantly effective in converting intact CM proteins into hydrolysates as shown in Figure 1. Significantly different amounts of hydrolysates were produced in both stages, SGD and SGID, as reflected by significant differences in the DH%. Following SGD, the DH% of the Omani breed sample was significantly greater ($P < 0.05$) than that of other breeds (~20%), suggesting the formation of the highest amount of hydrolysates from Omani CM in comparison with CM from other breeds during the SGD stage. Upon SGID, the Omani and Pakistani breed samples showed the highest DH% (22–23%) with a significant difference compared with other breeds ($P < 0.05$). The Hozami breed sample showed the lowest DH% (~15%), suggesting the formation of the lowest amount of hydrolysates upon the completion of simulated digestion. This outcome could suggest that the intact proteins of CM from the Hozami breed are more resistant to hydrolysis than those from the other breeds. The results related to the DH% upon SGID are comparable to those obtained by Al-Shamsi et al. (2018), who reported that degradation of milk protein by 3 proteolytic enzymes (alcalase, bromelain, and papain) yielded hydrolysates with DH% of 15.5, 23.8, and 39.6%, respectively. The results are, however, contrary to those obtained by Tagliazucchi et al. (2016), who found that hydrolysis of CM proteins by pancreatic enzymes yielded CM digesta with a DH% value of 69.6%. However, the results are comparatively similar to those obtained by Salami et al. (2011), Mudgil et al. (2021), and Mudgil et al. (2022d), whereby SGID of CM caseins yielded hydrolysates with DH% values of 20 to 30%. To the best of our knowledge, our study is the first to evaluate the comparative digestibility of CM proteins and DH% for milk obtained from different camel breeds.

Electrophoretic Protein Profile

Electrophoretic protein profiling was performed to qualitatively evaluate the hydrolysis of intact CM proteins and the effectiveness of simulated digestion. The intact proteins α-LA, α-CN, β-CN, κ-CN, and serum albumin were detected in all the CM samples (Figure 2). Regardless of camel breed, all milk samples showed similar behavior toward SGD and SGID processes. Intact proteins detected in undigested samples were not observed in the digested samples and were converted into peptides. Upon SGID, no traces of intact proteins were observed in the electrophoretic protein profile, suggesting the effectiveness of simulated digestion to hydrolyze the intact proteins into peptides. The effectiveness of simulated digestion in producing hydrolysates from intact CM proteins has also been previously shown by electrophoretic protein profiling reported by Maqsood et al. (2019) and Mudgil et al. (2022d).

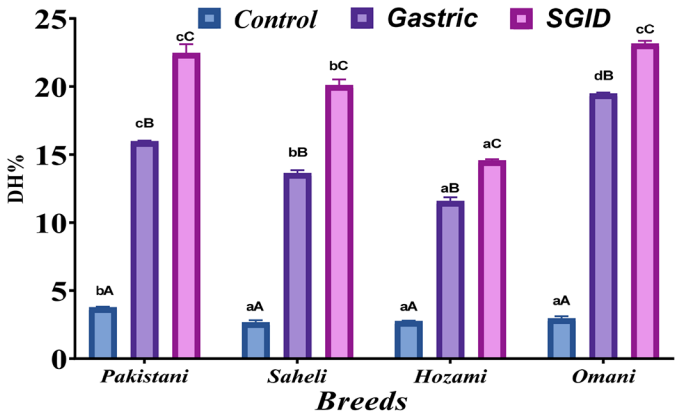

Figure 1. Degree of hydrolysis (DH%) of milk from different camel breeds subjected to simulated gastric digestion and simulated gastrointestinal digestion (SGID). Different uppercase letters within each breed show a significant difference at different stages of simulated digestion, and different lowercase letters show a significant ($P < 0.05$) difference between the breeds at the same stage of digestion. Values are represented as mean ± SD (n = 3).

Peptide Profile from RP-UPLC

An RP-UPLC analysis was performed to further study the hydrolysis of intact proteins upon SGD and SGID. As shown in Figure 3, the intact proteins of all the CM samples were eluted between 60 and 80 min, with a similar composition in all samples. Upon SGD, the majority of the intact proteins were hydrolyzed, and a wide range of peptides of different compositions were formed and eluted between 0 and 65 min. Further hydrolysis by SGID caused shorter peptides to hydrolyze further, likely into amino acids. Thus, very weak intensity peaks were observed between 0 and 35 min of elution time. These results were notable in Saheli and Pakistani breed samples, while Omani and Hozami breed samples showed slightly greater variation and a higher number of shorter peptides during an elution time of 0 to 35 min. These differences could reflect variation in the composition of hydrolysates derived from milk of different camel breeds upon digestion.

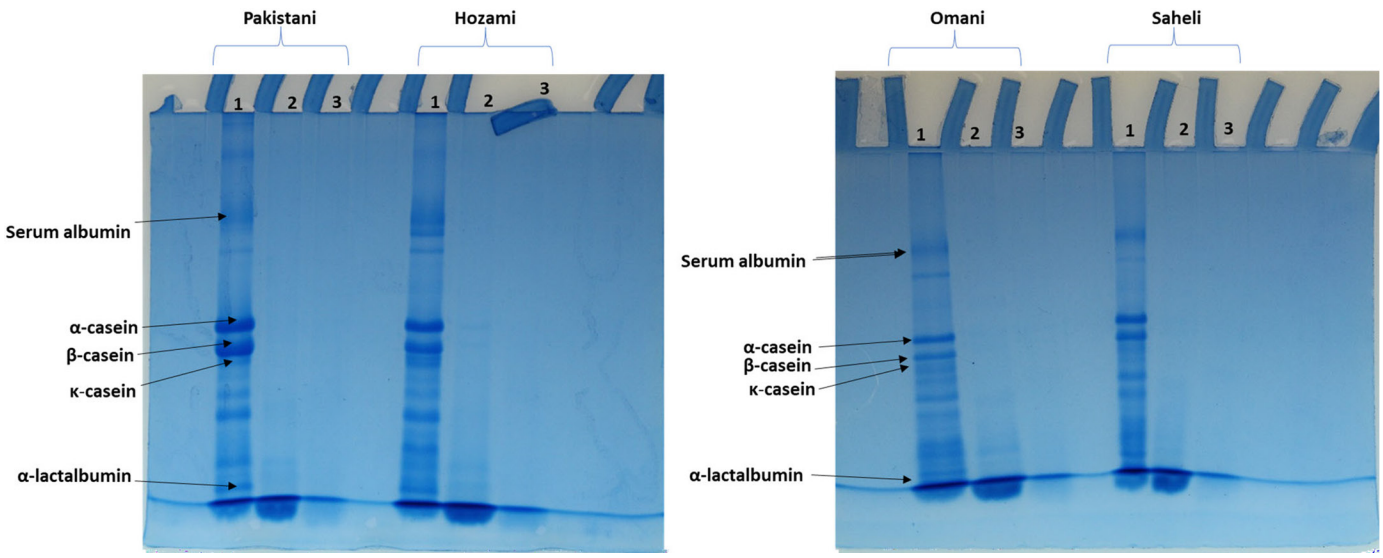

Figure 2. Electrophoretic protein profile of milk proteins from 4 breeds of camel subjected to simulated gastric (SGD) and simulated gastrointestinal digestion (SGID). Lane 1: Undigested milk samples; lane 2: SGD samples; lane 3: SGID samples.

Nevertheless, samples from all breed showed intense peaks and an expected wide range of peptide composition, with elution times ranging from 40 to 65 min. Again, the chromatographic results provided deeper insight into the protein hydrolysis of simulated digestion. Previously reported results have also revealed the productiveness of simulated digestion in deriving hydrolysates from intact CM proteins (Salami et al., 2011; Tagliazucchi et al., 2018; Mudgil et al., 2021, 2022d).

Antidiabetic Activity

The antidiabetic activity associated with milk from different camel breeds and the SGD and SGID CM hydrolysates were compared with samples derived from bovine milk (Supplemental Table S1; https://data.mendeley.com/datasets/57263str7j; Mudgil et al., 2023). The antidiabetic activity was evaluated by studying the inhibitory activity of the MPH produced through SGD and SGID against α-amylase (Figure 4) and DPP-IV (Figure 5). α-Amylase is responsible for the hydrolysis of starch into smaller oligosaccharides that are subsequently hydrolyzed by α-glucosidases into glucose units (Ali Redha et al., 2018). Inhibition of α-amylase delays the breakdown of carbohydrates and thus regulates blood glucose by decreasing the speed at which glucose is released into the blood. The inhibition of DPP-IV decreases blood glucose by regulating the degradation of glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide (Nongonierma et al., 2019). This degradation negatively influences the insulinotropic activity of the peptides and thus causes a rise in insulin secretion during the postprandial phase (Nongonierma et al., 2019). Camel milk has strong potential for use as an alternative therapy in the management of type 2 diabetes due to its effect in controlling postprandial glucose spikes, and this effect may be due to various bioactive peptides released during digestion of the milk (Agrawal et al., 2013; Kilari et al., 2021).

Milk from different camel breeds showed different α-amylase inhibitory potentials. Undigested CM from the Hozami breed had the highest α-amylase inhibitory activity (IC_{50} value = 0.62 ± 0.018 mg/mL on a protein-equivalent basis), followed by CM from the Saheli breed (IC_{50} value = 0.73 ± 0.05 mg/mL), CM from the Omani breed (IC_{50} value = 0.92 ± 0.027 mg/mL), and CM from the Pakistani breed (IC_{50} value = 1.17 ± 0.034 mg/mL; Figure 4). Upon SGD, all the MPH showed significantly higher α-amylase inhibition in comparison with undigested samples ($P < 0.05$; Figure 4). Nevertheless, the hydrolysates produced by SGID showed lower α-amylase inhibition in comparison with those produced by SGD (Figure 4). This outcome suggests that SGD conditions favor the formation of α-amylase inhibitor peptides more than SGID conditions. Extensive hydrolysis during the intestinal phase might have degraded some of the potential peptides, resulting in lower α-amylase inhibition. Overall, undigested samples of CM showed lower α-amylase inhibition activity in comparison with digested samples, suggesting that protein hydrolysis via SGD and SGID released bioactive peptides that possessed a greater ability to inhibit α-amylase. Among the camel breeds, milk samples from the Hozami and Omani breeds that

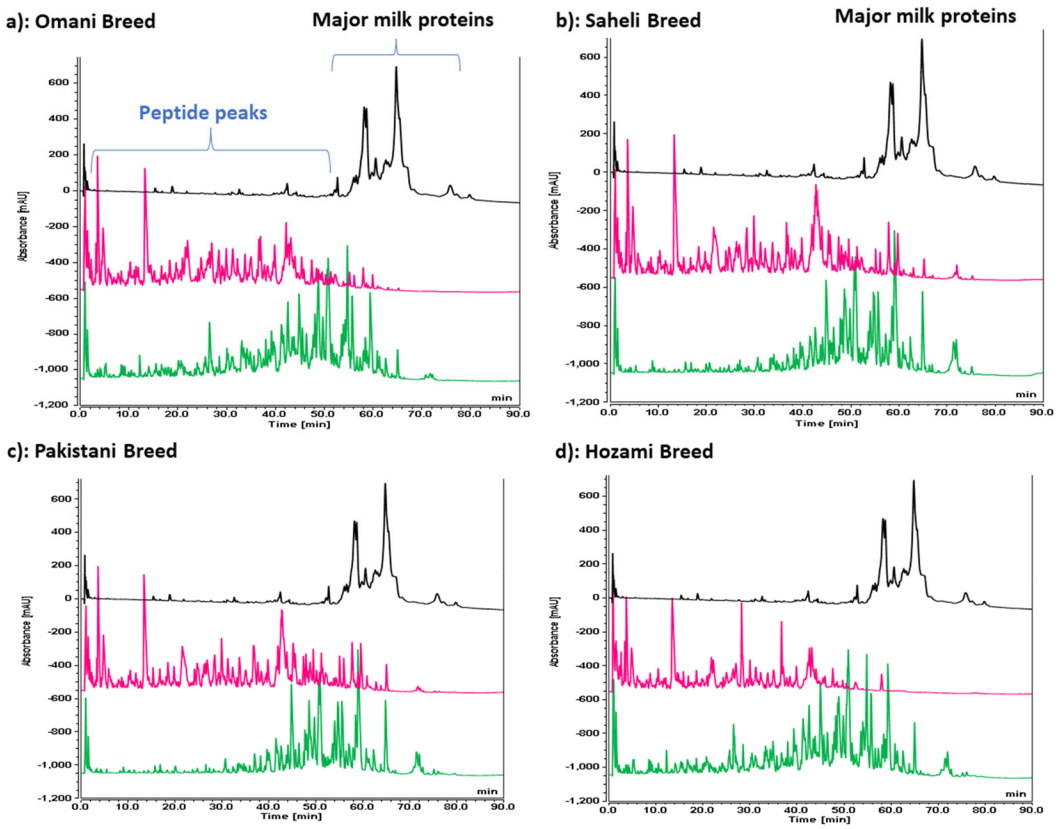

Figure 3. Reverse-phase ultra-performance liquid chromatography peptide profile of milk proteins from Omani (a), Saheli (b), Pakistani (c), and Hozami (d) breeds of camel subjected to simulated gastric (SGD) and simulated gastrointestinal digestion (SGID). The black chromatogram refers to major milk proteins, the pink chromatogram refers to peptides derived after SGD, and the green chromatogram refers to peptides derived after SGID.

were subjected to SGD showed the greatest α-amylase inhibitory activity, with an IC_{50} value of 0.05 ± 0.001 and 0.08 ± 0.002 mg/mL on a protein-equivalent basis, respectively. These samples could potentially be further analyzed to identify the peptide sequences present in them. Previous studies have also reported that whole CM hydrolysates generated via alcalase, bromelain, and papain for 3, 6, and 9 h displayed increased efficiency to inhibit α-amylase activity (Mudgil et al., 2018). Peptides KDLWDDFKGL and MPSKPPLL were anticipated to be 2 potent α-amylase inhibitory peptides derived from CM protein hydrolysates obtained through the use of alcalase, bromelain, and papain (Mudgil et al., 2018). Moreover, Mudgil et al. (2021) recently reported that pronase-E-derived camel and bovine casein protein hydrolysate obtained upon SGID displayed enhanced α-amylase inhibitory activity compared with undigested camel casein hydrolysates. The alcalase-derived bovine casein hydrolysate showed the highest α-amylase inhibitory activity (IC_{50} values of 0.58 and 0.64 mg/mL on a protein-equivalent basis) followed by alcalase-generated camel casein hydrolysates (IC_{50} values of 0.66, 0.74, and 0.74 mg/mL; Ashraf et al., 2021). These α-amylase inhibitory IC_{50} values are within a similar range of those reported in the current study for SGD and SGID hydrolysates from CM breeds (1.05 to 0.05 mg/mL). Baba et al. (2021c) recently also reported an increase in the α-amylase inhibitory potential of hydrolysates from camel whey produced by gastric digestion using pepsin, with α-amylase-inhibitory IC_{50} values ranging from 0.29 to 3.69 mg/mL on a protein-equivalent basis. A study by Baba et al. (2021c) identified 3 novel peptides (i.e., CCGM, MFE) as more potent α-glucosidase inhibitory and FCCLGPVPP as α-amylase inhibitory peptide.

Among the undigested samples, CM from the Hozami breed again had the highest DPP-IV-inhibiting activity (IC_{50} = 0.82 ± 0.002 mg/mL on a protein-equivalent basis), followed by CM from the Saheli breed (IC_{50} = 0.97 ± 0.085 mg/mL), the Omani breed (IC_{50} = 1.43 ± 0.033 mg/mL), and the Pakistani breed (IC_{50} = 1.64 ± 0.002 mg/mL; Figure 5). Subjecting the CM samples to SGD and SGID produced protein hydrolysates with significantly greater DPP-IV inhibition activity in

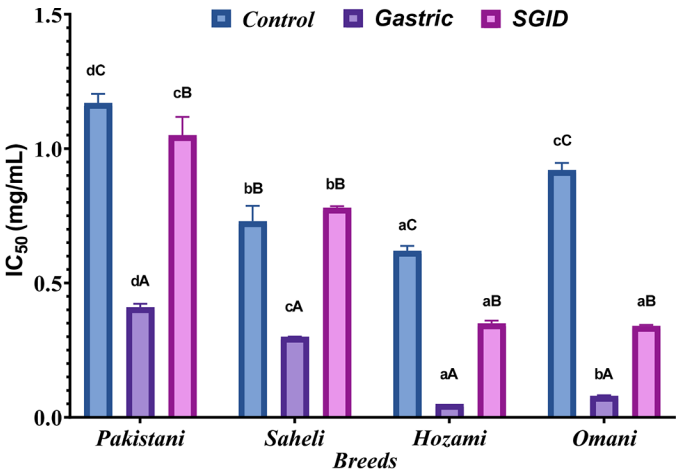

Figure 4. Effect of in vitro simulated gastric digestion and simulated gastrointestinal digestion (SGID) of milk from Pakistani, Saheli, Hozami, and Omani breeds of camel on half-maximal inhibitory concentration (IC_{50}) of α-amylase. Values are mean ± SD (n = 3). IC_{50} = concentration inducing 50% inhibition of α-amylase expressed in milligrams of protein equivalent per milliliter (mg/mL). Different uppercase letters within each breed show a significant difference at different stages of simulated digestion, and different lowercase letters show a significant ($P < 0.05$) difference between the breeds at the same stage of digestion.

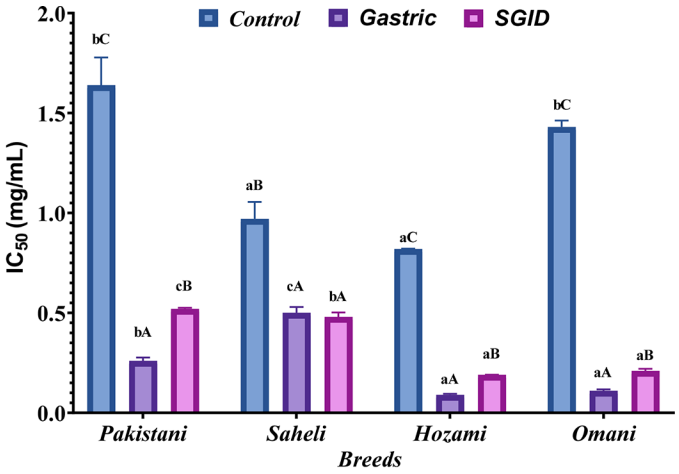

Figure 5. Effect of in vitro simulated gastric digestion and simulated gastrointestinal digestion (SGID) of milk from Pakistani, Saheli, Hozami, and Omani breeds of camel on half-maximal inhibitory concentration (IC_{50}) of dipeptidyl peptidase IV (DPP-IV). Values are mean ± SD (n = 3). IC_{50} = concentration inducing 50% inhibition of DPP-IV expressed in milligrams of protein equivalent per milliliter (mg/mL). Different uppercase letters within each breed show a significant difference at different stages of simulated digestion, and different lowercase letters show a significant ($P < 0.05$) difference between the breeds at the same stage of digestion.

contrast to the intact proteins ($P < 0.05$). The hydrolysates produced by SGID showed lower DPP-IV inhibition in comparison with SGD-derived hydrolysates from the Pakistani, Hozami, and Omani breeds; however, SGID hydrolysate from the Saheli breed showed higher DPP-IV inhibitory activity compared with SGD hydrolysates. The greatest DPP-IV inhibitory activity was demonstrated by SGD hydrolysates of the Hozami breed (IC_{50} = 0.09 ± 0.005 mg/mL on a protein-equivalent basis) followed by those of the Omani breed (IC_{50} = 0.11 ± 0.008 mg/mL; Figure 5).

Camel whey protein digested with pepsin has previously been reported to have elevated DPP-IV inhibitory activity compared with intact camel whey proteins (Ashraf et al., 2021). Similarly, hydrolysis of camel whey proteins by gastric and pancreatic enzymes resulted in enhanced DPP-IV inhibitory activity (3- to 4-fold) compared with intact whey proteins (Kamal et al., 2018). Furthermore, strong DPP-IV inhibition by CM protein hydrolysates derived using alcalase and papain have been reported, with IC_{50} values ranging from 0.09 ± 0.01 to 0.46 ± 0.04 mg/mL, respectively (Mudgil et al., 2018). The DPP-IV inhibitory IC_{50} values reported in the current study are in a similar range to the values reported by Mudgil et al. (2018) and Nongonierma et al. (2017) for CM protein hydrolysates, by Ashraf et al. (2021) and Nongonierma et al. (2019) for camel whey protein hydrolysates, and by Mudgil et al. (2021) for camel casein protein hydrolysates. The peptides LAHKPL and ILDKEGIDY derived from CM α-LA and VPV, YPI, and VPF from CM β-CN by SGID have been reported to be effective DPP-IV inhibitory peptides (Nongonierma et al., 2019). In a different study, Nongonierma et al. (2018) reported LPVP and MPVQA as 2 CM protein-derived peptides with significant DPP-IV inhibition activity. The findings of the current study agree with the findings of Mudgil et al. (2021), who explored the antidiabetic activity of protein hydrolysates generated by SGID of camel casein proteins. In addition, they reported FLWPEYGAL as a potent α-amylase inhibitory peptide, while HLPGRG, QNVLPLH, and PLMLP were efficient in inhibiting the activity of DPP-IV. In fact, the same study also reported the antidiabetic activity of CM protein hydrolysates via the inhibition of α-glucosidase activity. These results suggest that CM proteins from different breeds could release potent bioactive peptides following SGD and SGID that are highly effective in inhibiting enzymes such as α-amylase and DPP-IV that play a vital role in diabetes. The findings of Tagliazucchi et al. (2018) showed that the DPP-IV inhibitory activity of in vitro SGID hydrolysates of cow, camel, sheep, and goat milk proteins varied, with cow milk-derived hydrolysate exhibiting higher inhibition compared with the hydrolysates derived from the milk of the other 3 species (Tagliazucchi et al., 2018). Camel whey protein hydrolysates derived by SGID have also shown enhanced DPP-IV inhibitory potency (Nongonierma

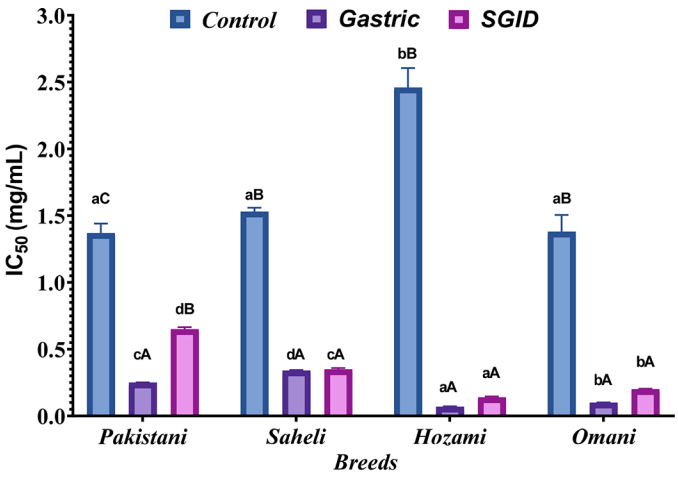

Figure 6. Effect of in vitro simulated gastric digestion and simulated gastrointestinal digestion (SGID) of milk from Pakistani, Saheli, Hozami, and Omani breeds of camel on half-maximal inhibitory concentration (IC_{50}) of pancreatic lipase (PL). Values are mean ± SD (n = 3). IC_{50} = concentration inducing 50% inhibition of PL expressed in milligrams of protein equivalent per milliliter (mg/mL). Different uppercase letters within each breed show a significant ($P < 0.05$) difference at different stages of simulated digestion, and different lowercase letters show a significant difference between the breeds at the same stage of digestion.

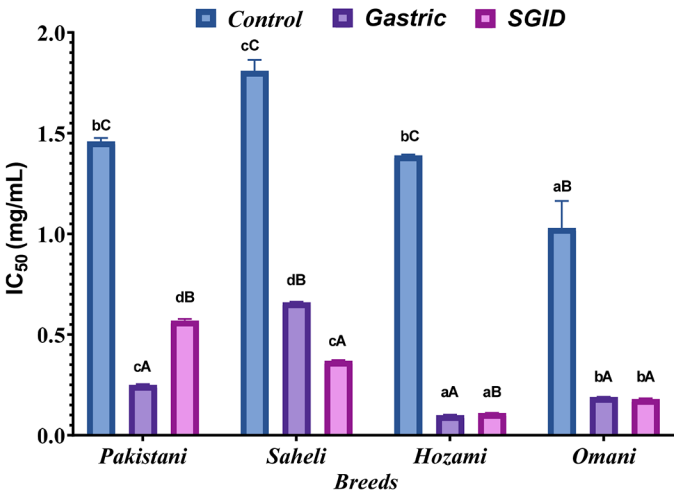

Figure 7. Effect of in vitro simulated gastric digestion and simulated gastrointestinal digestion (SGID) of milk from Pakistani, Saheli, Hozami, and Omani breeds of camel on half-maximal inhibitory concentration (IC_{50}) of cholesterol esterase (CE). Values are mean ± SD (n = 3). IC_{50} = concentration inducing 50% inhibition of CE expressed in milligrams of protein equivalent per milliliter (mg/mL). Different uppercase letters within each breed show a significant ($P < 0.05$) difference at different stages of simulated digestion, and different lowercase letters show a significant difference between the breeds at the same stage of digestion.

et al., 2018). Simulated digestion of milk proteins was previously documented to increase DPP-IV inhibitory activity, with Cermeño et al. (2018) suggested that digestion of casein proteins by simulated GI enzymes could potentially release peptides with enhanced DPP-IV inhibitory activity.

Camel milk is generally known for its antidiabetic effects, although the mechanisms are not currently known. Based on recent studies exploring α-amylase, DPP-IV, and to some extent α-glucosidase and the inhibitory potential of hydrolysates produced from different protein fractions of CM, the antidiabetic mechanism for CM proteins may be linked to the inhibition of key metabolic enzymes involved in diabetes progression (Tagliazucchi et al., 2018; Mudgil et al., 2019a,c, 2021; Nongonierma et al., 2019; Ashraf et al., 2021). The findings from our study indicate that levels of α-amylase and DPP-IV inhibitory activities varied according to camel breed, with milk from Hozami and Omani camel breeds showing higher antidiabetic properties than that of the other 2 breeds analyzed. Further studies based on proteomic and peptidomic analysis are required to provide deeper insights into an interbreed variation for the antidiabetic potential of CM.

Antihypercholesterolemic Activity

The antihypercholesterolemic potential of CM hydrolysates from 4 major breeds was evaluated in the current study by assessing their inhibitory activity against PL (Figure 6) and CE (Figure 7) after SGD and SGID. In addition, a comparative analysis with the values obtained for bovine milk-derived samples after SGD and SGID is shown in Supplemental Table S1. Pancreatic lipase is a vital enzyme involved in the breakdown process and absorption of triglycerides; therefore, inhibition of PL activity has become a typical approach for controlling hypercholesteremia and obesity (Noorolahi et al., 2020). Hypercholesterolemia can also be managed by regulating the activity of CE, which is directly involved in controlling plasma cholesterol levels. Cholesterol esterase can catalyze the hydrolysis of sterol esters into their sterol and fatty acid components, which can easily undergo absorption (Williams et al., 2020). Various synthetic compounds or drugs (statins, niacin, ezetimibe, and fibrates) are used as inhibitors for enzymes involved in hypercholesteremia, such as PL and CE (Baba et al., 2021b). However, long-term use of these drugs is known to have adverse metabolic effects, such as myopathy and GI tract disorders (Srivastava and Apovian, 2018). Therefore, safer and more sustainable options are being explored as adjunct therapy.

The highest PL inhibition was achieved by gastric hydrolysates derived from the milk of Hozami (IC_{50} = 0.07 ± 0.002 mg/mL on a protein-equivalent basis) and Omani breeds (IC_{50} = 0.10 ± 0.001 mg/mL). When milk protein samples were further subjected to com-

plete SGID, no enhancement of PL inhibitory activity was observed for MPH from different camel breeds (Figure 6). Upon their transit through SGD and SGID, all camel MPH showed significantly greater PL inhibitory activity in comparison with CM intact proteins. Very few studies have been reported on the potential of MPH to inhibit PL and CE.

Recently, camel whey protein hydrolysates generated using gastric and pancreatic enzymes were reported for their PL inhibitory activity (Jafar et al., 2018). Mudgil et al. (2018) also evaluated the potential of CM protein hydrolysates against PL. They found that hydrolysates generated by papain for 3 h showed the strongest PL inhibition, with an IC_{50} of 0.025 ± 0.0005 mg/mL on a protein-equivalent basis. The same study predicted that the peptides FCLPLPLLK and KFQWGY could significantly bind to 3 main active sites of PL: Ser153, Phe216, and His264. Furthermore, a recent study demonstrated that camel casein hydrolysates showed stronger PL inhibition compared with cow casein hydrolysates, following enzymatic hydrolysis of the proteins using the enzymes alcalase and pronase E (Mudgil et al., 2022d). Upon SGID, significant enhancement in the inhibition of PL was demonstrated for both cow and casein hydrolysates. The 6 peptides MMML, FDML, HLPGRG, AAGF, MSNYF, and FLWPEYGAL were predicted to be the most potent PL inhibitory peptides from cow and camel casein hydrolysates (Nongonierma et al., 2018). The peptide LP derived from camel casein-derived protein hydrolysates was also effective in inhibiting PL activity.

Inhibition of CE was another approach considered to examine the antihypercholesterolemic activity of MPH from the milk of different breeds of camel. Among the undigested milk samples, milk protein from the Omani breed of camel showed higher CE inhibitory activity as depicted by the lowest IC_{50} value (1.03 mg/mL on a protein-equivalent basis), which was significantly lower than other undigested milk samples ($P < 0.05$; Figure 7). Among the MPH generated upon SGD and SGID, the Hozami camel breed showed the greatest CE inhibitory activity (IC_{50} = 0.10 ± 0.002 and 0.11 ± 0.001 mg/mL, respectively). The CM hydrolysates from the Omani breed generated upon SGD and SGID (Figure 7) also showed promising CE inhibitory activity (0.19 ± 0.001 and 0.1 8 ± 0.003 mg/mL, respectively), which did not differ significantly from CE inhibition of MPH from the Hozami camel breed. Interestingly, the MPH generated by SGD and SGID of milk from the Hozami and Omani camel breeds produced the most potent CE inhibition (IC_{50} values ranging from 0.10 to 0.19 mg/mL on a protein-equivalent basis) as well as PL inhibition (IC_{50} values ranging from 0.07 to 0.20 mg/mL). The CE inhibitory IC_{50} value for SGD and SGID CM hydrolysates in this study ranged from 0.10 to 0.91 mg/mL on a protein-equivalent basis, which was within the range of previously reported CE inhibitory values for CM protein hydrolysates (0.091–0.032 mg/mL) by Mudgil et al. (2019a), camel whey protein hydrolysates (0.240–1.243 mg/mL) by Baba et al. (2021b), and camel casein hydrolysates (0.3–0.63 mg/mL) by Mudgil et al. (2022d). Protein hydrolysates derived from CM have previously been reported to have significant CE inhibition activity, with IC_{50} values ranging from 32.43 ± 0.60 to 91.10 ± 3.20 μg/mL (Mudgil et al., 2019b). In a recent study on camel whey protein hydrolysates generated upon pepsin digestion, the peptide FCCLGPVPP was predicted to be a potent CE inhibitor and PAGNFLPPVAAAPVM, MLPLMLPFTMGY, and LRFPL were predicted to be potent PL inhibitory peptides (Baba et al., 2021b). Whereas, another recent study reported the peptide LP, obtained from both camel and cow casein protein hydrolysates, to be a potent CE-inhibiting peptide (Mudgil et al., 2022d). Overall, MPH from different camel breeds, and especially from Hozami and Omani breeds, demonstrated potent inhibitory activity of PL and CE following SGD and SGID. These findings were similar to a previous study in which camel casein-derived hydrolysates showed higher inhibition of PL (IC_{50} = 0.4–2.67 mg/mL on a protein-equivalent basis) and CE (IC_{50} = 0.3–0.63 mg/mL) compared with the cow casein hydrolysates (for PL, IC_{50} = 0.5–7.03; for CE, IC_{50} = 0.52 mg/mL; Mudgil et al., 2022d).

CONCLUSIONS

Protein hydrolysates derived from milk from different camel breeds upon SGD and SGID displayed significant inhibitory activities toward enzymes α-amylase and DPP-IV, which are involved in diabetes, and PL and CE, which are involved in hypercholesteremia. Overall, the SGD and SGID MPH from camel breeds proved to be more potent inhibitors of different enzymes investigated in this study, indicating hydrolysis of CM can produce bioactive peptides with significant antidiabetic and antihypercholesterolemic activities. Different camel breeds could produce milk with different protein content and variable composition, which might influence the potential bioactive peptides that are released from CM upon digestion. Among the 4 camel breeds considered in this study, the Hozami and Omani breeds produced milk that showed the greatest bioactive properties upon SGD and SGID. The inhibitory potential of these hydrolysates was greater than those derived from the milk of other breeds, as shown by the lower IC_{50} values for the inhibition of α-amylase, DPP-IV, PL, and CE. In future research on CM, it would be more

efficient to focus on Hozami and Omani breeds and further explore their milk protein content.

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