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Enzymatic hydrolysates from camel colostrum and milk proteins display inhibitory activity toward a key enzyme related to obesity

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ABSTRACT

This study aimed to investigate the anti-obesity potential of camel colostrum and milk proteins as well as their enzymatic hydrolysates. Camel colostrum and milk proteins were treated using six proteolytic enzymes (pepsin, trypsin, α-chymotrypsin, pancreatin, papain, and pronase). The degree of hydrolysis was measured to verify the degradation of proteins. The *in vitro* anti-obesity activity was evaluated using the pancreatic lipase inhibitory assay. Camel colostrum and milk protein hydrolysates exhibited different degrees of hydrolysis ranging from 17.69 to 43.97%. The protein content varied between 56.08–61.95% and 37.39–41.72% for camel colostrum and milk protein hydrolysates, respectively. The hydrolysates displayed significantly higher anti-obesity activity than the undigested proteins at all tested concentrations ($P < 0.05$). Colostrum protein hydrolysate generated with pancreatin had the highest anti-obesity potential (59.92%). These results suggest that colostrum and milk protein hydrolysates could be used to formulate functional foods and nutraceuticals.

KEYWORDS

camel colostrum, camel milk, enzymatic hydrolysis, anti-obesity activity

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1. INTRODUCTION

Obesity has become a global health concern, with increasing prevalence and associated risks such as diabetes, cardiovascular diseases, and metabolic disorders. An excessive consumption of fat can contribute to the development of obesity. Pancreatic lipase, an enzyme secreted by the pancreas, plays a crucial role in the digestion and absorption of dietary fats (Kumar and Chauhan, 2021). Inhibition of pancreatic lipase has been explored as a therapeutic approach for weight loss and management of obesity by reducing the digestion and absorption of dietary fats (Liu et al., 2020).

Anti-obesity medications, such as Orlistat, work by inhibiting pancreatic lipase activity, thereby reducing the digestion and absorption of dietary fats. This leads to decreased caloric intake from fats and may aid in weight loss efforts. However, Orlistat has several drawbacks including vitamin deficiencies and abdominal pain. Hence, ongoing efforts are underway to identify alternative safe sources (Gil-Rodríguez and Beresford, 2019).

Enzymatic hydrolysis of food proteins has been widely studied for the production of bioactive peptides. Bioactive peptides are short protein fragments that exhibit physiological or functional benefits beyond basic nutrition. They are inactive in the protein source and typically require enzymatic hydrolysis to release their bioactivity (Cruz-Casas et al., 2021). These peptides can exert various bioactive properties, such as antioxidant, antimicrobial, anti-inflammatory, anticancer, and anti-obesity activities, among others (Mudgil et al., 2018; Oussaief et al., 2023).

Colostrum refers to the initial milk secreted by female mammals within the first few days following parturition, prior to the production of regular milk. Camel colostrum is characterised by a higher concentration of protein, ash, oligosaccharides, vitamins, and minerals compared to mature milk. However, the levels of non-protein nitrogen and lactose in camel milk and colostrum are found to be very similar to each other (El-Hatmi et al., 2023). Camel colostrum contains higher concentrations of whey proteins, particularly immunoglobulins G and lactoferrin, than camel milk. The lack of β-lactoglobulin, a whey protein in cow milk associated with allergy in children, is a common characteristic of camel and human colostrum and milk (Jrad et al., 2022).

Considering the high amount and quality of their protein, colostrum and milk may represent a renewable biological resource that can be utilised for the production of protein hydrolysates and bioactive peptides. To the best of our knowledge, the study conducted by Mudgil et al. (2018) is the only one that focused on the effect of enzymatic hydrolysis of camel milk proteins, using alcalase, bromelain, and papain, on their anti-obesity activity. Protein hydrolysates from colostrum have not yet been documented for their anti-obesity activity. Hence, the objective of the current study was to assess the *in vitro* anti-obesity potential of camel colostrum and milk protein as well as their enzymatic hydrolysates.

2. MATERIALS AND METHODS

2.1. Materials

Colostrum was collected within 24 h postpartum from 10 multiparous dromedaries (*Camelus dromedarius*), aged between and 6 and 23 years, belonging to the experimental herd of the

Livestock and Wildlife laboratory (Arid Lands Institute, Medenine, Tunisia). Camel milk was obtained from the same dromedaries in their third month of lactation.

Dromedaries were raised under a semi-intensive management system. Indeed, they were allowed to graze daily for about 8 h, starting early in the morning, on natural pastures. Upon their return to the farm, they were provided with a well-balanced diet comprising a forage mixture containing oat hay, waste dates, and olive pomace, alongside a commercial concentrate containing soy, barley, bran, essential minerals, and vitamins. Water was made available to them twice daily: once in the morning before grazing and again in the evening upon their return from the grazing area.

One sample was collected per animal in the morning (October 2017 – April 2018) by manual milking after discarding the first jets. Fresh camel colostrum and milk were defatted by centrifugation (5,000 × *g*, 30 min, 4 °C), then lyophilised in a freeze dryer (Christ Gamma 1–20, Germany) and kept at −20 °C until analysis.

Pepsin from porcine gastric mucosa (EC 3.4.23.1, 3,000 U mg^{-1}) and pancreatin from porcine pancreas (EC 232-468-9, 5 × U.S.P. specifications) were purchased from Bio Basic (Ontario, Canada). Trypsin from porcine pancreas (EC 3.4.21.4, 1,000 U mg^{-1}), α-chymotrypsin from bovine pancreas (EC 3.4.21.1, 40 U mg^{-1}), papain from *Carica papaya* (EC 3.4.22.2, 3 U mg^{-1}), and pronase from *Streptomyces griseus* (EC 232-909-5, 3.5 U mg^{-1}) were purchased from Sigma-Aldrich, Merck (St. Louis, MO, USA).

2.2. Preparation of camel colostrum and milk protein hydrolysates

Freeze-dried camel skimmed colostrum and milk were dissolved separately in ultrapure water at 2.5% (m/v, on protein basis). The pH of these solutions was adjusted to the optimal value of enzymes: 2 for pepsin, 8 for trypsin, α-chymotrypsin, pancreatin, and pronase, and 6.5 for papain. The hydrolysis of proteins was realised at a ratio of 1:100 (enzyme/substrate). The temperature of the reactions was maintained at 37 °C using a shaking water bath (Daihan Labtech Co.) at 150 r.p.m. After 6 h of hydrolysis, the enzymes were inactivated by heating the samples for 20 min at 85 °C. Then, the hydrolysates were neutralised to pH = 7.0, centrifuged (10,000 × *g*, 15 min, 4 °C), freeze-dried, and kept at −20 °C for further use. Control samples of undigested camel colostrum and milk proteins underwent the same procedure as the hydrolysates but without the addition of enzymes.

2.3. Degree of hydrolysis

The degree of hydrolysis (DH) was determined according to the method of Hoyle and Merritt (1994).

2.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method described by Laemmli and Favre (1973).

2.5. Chemical composition

The protein, moisture, and ash contents of freeze-dried camel skim colostrum and milk as well as their hydrolysates were determined by the standard Association of Official Analytical Chemists methods number 984.13, 927.05 and 942.05, respectively (AOAC, 2000), while the lactose

content was estimated by the method of Dubois et al. (1956). A factor of 6.38 was used to convert the nitrogen value to protein.

2.6. Anti-obesity activity

The anti-obesity activity was estimated by determining the inhibitory activity of pancreatic lipase as described by Gil-Rodrıguez and Beresford (2019) with some modifications. A volume of 500 µL of each sample and 1 mL of Tris–HCl buffer (pH 8.5, 0.1 M) were incubated with 50 µL of a 5 mg mL^{-1} solution of porcine pancreatic lipase (in the buffer Tris–HCl buffer (pH 8.5) for 15 min at 37 °C. After incubation, 50 µL of *p*-nitrophenyl butyrate was added and the mixture was incubated again at 37 °C for 15 min. Then, the absorbance values were read at 405 nm. The control was carried out in the same manner but the sample was replaced by distilled water. The activity was calculated as follows:

$$\text{Pancreatic lipase inhibition} = (\text{Ac}-\text{As})/\text{Ac}\times 100 \quad (1)$$

where Ac and As refer to the absorbance of the control and of the sample, respectively.

2.7. Statistical analysis

Each experiment was performed in triplicate. Data were statistically analysed using XLSTAT (Version 2019). A one-way analysis of variance (ANOVA) was performed, followed by Tukey's test using a probability value of 5%. The relationship between the degree of hydrolysis and the anti-obesity activity of camel colostrum and milk protein hydrolysates was determined by calculating the Pearson correlation coefficient.

3. RESULTS AND DISCUSSION

3.1. Degree of hydrolysis of camel colostrum and milk proteins

Several proteolytic enzymes were used to hydrolyse camel milk and colostrum proteins to assess the functionality of the generated hydrolysates. The proteolysis of camel milk and colostrum proteins was monitored by measuring their degree of hydrolysis (Fig. 1).

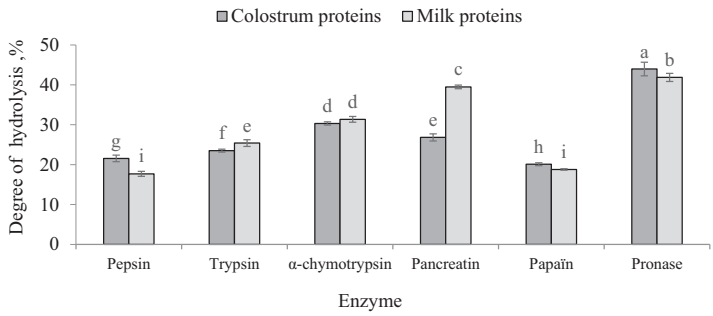

Fig. 1. Degree of hydrolysis of camel colostrum and milk protein

The degree of hydrolysis of protein hydrolysates from camel colostrum and milk reflects the number of cleavage sites available for each enzyme. The degree of hydrolysis varies from 17.69 to 41.86% and from 20.10 to 43.97% for the hydrolysates of milk proteins and colostrum proteins, respectively. Pronase-treated hydrolysates showed the highest degree of hydrolysis for both colostrum and milk protein hydrolysates ($P < 0.05$).

The susceptibility of proteins to enzymatic hydrolysis could be attributed to the difference in the specificity of the enzymes used. Indeed, pronase has a very broad specificity of action toward proteins, because it is formed by a set of proteinases and peptidases from the strain *Streptomyces griseus* (Frackenpohl et al., 2001). Pancreatin is a mixture of enzymes released by the pancreas. It also has a high specificity, but it has a preference for Arg, Lys, Tyr, and Leu (Gonzalez-Rodriguez et al., 1995). Moreover, pepsin preferentially cuts peptide bonds involving aromatic and hydrophobic amino acids. Trypsin hydrolyses peptide bonds where Lys and Arg residues are located on the carboxyl side of the bond, while chymotrypsin cleaves at the C-terminal of Phe, Tyr, Lys, and Trp residues. Papain attacks Arg, Lys, and Phe bonds (Nongonierma and FitzGerald, 2015).

In addition to the enzymatic specificity, the difference in the degrees of hydrolysis could be linked to the type of the substrate used. Indeed, the protein composition of camel milk differs from that of colostrum; colostrum is richer in soluble proteins (immunoglobulins, lactoferrin, α-lactalbumin, and camel serum albumin), while milk is richer in caseins (especially β-casein). Thus, the degree of hydrolysis of these substrates may differ even if they were hydrolysed by the same enzyme, since each protein has well-defined sites of cleavage.

3.2. Assessment of protein hydrolysis via SDS-PAGE

Electrophoretic pattern of camel colostrum and milk proteins before and after enzymatic hydrolysis is presented in Fig. 2. Lactoferrin and camel serum albumin, which have approximately a molecular mass of 75 and 67 kDa, respectively, are present in both camel colostrum and milk. However, these two proteins were more abundant in colostrum (the band was more intense).

Fig. 2. SDS-PAGE of camel colostrum and milk proteins and their enzymatic hydrolysates. M: molecular mass marker; 1: colostrum proteins; 2: milk proteins; 3: pepsin-colostrum hydrolysates; 4: pepsin-milk hydrolysates; 5: trypsin-colostrum hydrolysates; 6: trypsin-milk hydrolysates; 7: α-chymotrypsin-colostrum hydrolysates; 8: α-chymotrypsin-milk hydrolysates; 9: pancreatin-colostrum hydrolysates; 10: pancreatin-milk hydrolysates; 11: papain-colostrum hydrolysates; 12: papain-milk hydrolysates; 13: pronase-colostrum hydrolysates; 14: pronase-milk hydrolysates; Lf: lactoferrin; CSA: camel serum albumin; CN: caseins; and α-La: α-lactalbumin

Camel colostrum showed a richness in immunoglobulins (IgGs) (IgG2 ≈ 45 kDa and IgG3 ≈ 41 kDa). Immunoglobulins were not detected in camel milk probably because their content was very low. Moreover, the bands corresponding to caseins are more abundant in camel milk in comparison with camel colostrum. α-Lactalbumin (α-La), with a molecular weight of approximately 14 kDa, was detected in camel colostrum and milk. SDS-PAGE profiles confirmed the absence of β-lactoglobulin, the main whey protein in cow milk (Jrad et al., 2014).

The electrophoretic profile changed after hydrolysis of the proteins by the different enzymes. In fact, most of the bands disappeared, which confirms that the hydrolysis of milk and colostrum proteins occurred. However, some proteins were partially degraded. Indeed, traces of lactoferrin were observed in the tryptic and chymotrypsic hydrolysates of camel milk proteins and in the chymotrypsic and pancreatic hydrolysates of camel colostrum proteins. Besides, some traces of IgGs were visible on the profile corresponding to peptic, tryptic, and chymotryptic hydrolysates of camel colostrum proteins. Camel serum albumin and caseins were almost degraded in all hydrolysates. Salami et al. (2011) and Jrad et al. (2014) also reported that camel caseins were rapidly degraded by proteolytic enzymes owing to their flexibility (open tertiary structure). α-Lactalbumin resisted the hydrolysis by all enzymes used except pronase. This is explained by the compact globular structure of α-La, which hides its cleavage sites (Salami et al., 2008). These results suggest that the most extensive proteolysis was carried out by pronase, which is in agreement with the results of degree of hydrolysis. The variability of the substrates (colostrum and milk proteins) and the proteolytic enzymes used are the origin of the difference between the electrophoretic profiles obtained.

3.3. Chemical composition of camel colostrum and milk protein and their hydrolysates

The composition of camel milk is more variable than cow milk. Region and season influence significantly the ratio of compounds in camel colostrum and milk (Swelum et al., 2021). The chemical composition of freeze-dried camel skim colostrum and milk and their protein hydrolysates are reported in Table 1. Camel skim colostrum powder contained higher amount of proteins than camel skim milk powder (P <0.05). In fact, camel colostrum differs from camel milk in that it contains high concentrations of whey proteins, particularly immunoglobulins G, which give the newborn immunity (El-Hatmi et al., 2023). Camel milk proteins may be bioactive by themselves or can act as precursors for the formation of bioactive peptides (Swelum et al., 2021). The freeze-dried hydrolysates of camel colostrum and milk were characterised by a protein content ranging from 56.08 to 61.95% and from 37.39 to 41.72%, respectively, and therefore, could be an essential source of proteins. The decrease of protein content after enzymatic hydrolysis could be explained by the production of some peptides having the ability to interact with undigested proteins resulting in an increase in the insoluble protein fraction. It could be also explained by the removal of this insoluble undigested protein fraction by centrifugation after hydrolysis (Gani et al., 2015).

In addition, for all samples, the amount of lactose in the hydrolysates was lower than that in the undigested protein. Generally, similar lactose content was found between hydrolysates having the same substrate (colostrum or milk). A relatively high content of ash was found in both camel colostrum and milk protein hydrolysates, and values obtained were higher than those of undigested proteins; this is probably due to salts from the buffer solution used to solubilise the freeze-dried camel colostrum and milk. Pepsin-treated hydrolysates showed the highest ash

Table 1. Chemical composition of freeze-dried skim camel colostrum and milk and their protein hydrolysates prepared by different proteolytic enzymes

Enzyme	Proteins	Moisture (%)	Protein (%)	Lactose (%)	Ash (%)
None	Colostrum	7.22 ± 0.47^{cde}	65.10 ± 0.37^{a}	21.05 ± 0.84^{d}	6.09 ± 0.12^{h}
	Milk	4.16 ± 0.21^{g}	44.62 ± 0.29^{g}	41.30 ± 0.17^{a}	9.70 ± 0.33^{g}
Pepsin	Colostrum	7.93 ± 0.27^{bc}	56.08 ± 0.10^{f}	19.06 ± 0.02^{ef}	16.27 ± 0.25^{b}
	Milk	6.01 ± 0.46^{f}	37.39 ± 0.25^{k}	38.41 ± 0.34^{bc}	17.23 ± 0.30^{a}
Trypsin	Colostrum	8.72 ± 0.33^{ab}	60.23 ± 0.06^{d}	19.44 ± 1.07^{ef}	12.29 ± 0.18^{e}
	Milk	6.39 ± 0.24^{ef}	39.18 ± 0.04^{j}	39.24 ± 0.27^{bc}	15.44 ± 0.22^{bc}
α-chymotrypsin	Colostrum	8.08 ± 0.03^{abc}	60.57 ± 0.04^{cd}	19.11 ± 0.38^{ef}	12.09 ± 0.42^{e}
	Milk	6.45 ± 0.36^{ef}	40.01 ± 0.16^{i}	39.36 ± 0.22^{bc}	14.46 ± 0.40^{d}
Pancreatin	Colostrum	8.32 ± 0.22^{ab}	61.06 ± 0.11^{c}	18.25 ± 0.27^{f}	12.13 ± 0.17^{e}
	Milk	6.03 ± 0.11^{f}	40.21 ± 0.30^{i}	38.08 ± 0.57^{c}	14.64 ± 0.16^{cd}
Papain	Colostrum	8.83 ± 0.26^{a}	58.80 ± 0.46^{e}	20.10 ± 0.05^{de}	12.25 ± 0.25^{e}
	Milk	6.49 ± 0.16^{def}	38.05 ± 0.09^{k}	39.48 ± 0.33^{b}	15.53 ± 0.56^{b}
Pronase	Colostrum	7.38 ± 0.44^{cd}	61.95 ± 0.07^{b}	19.51 ± 0.47^{ef}	11.01 ± 0.04^{f}
	Milk	6.37 ± 0.26^{ef}	41.72 ± 0.48^{h}	39.05 ± 0.08^{bc}	12.30 ± 0.18^{e}

Averages ± Standard deviation (SD) of three replicates.
[a–k]: Values within the same column with different superscripts differed significantly ($P < 0.05$).

content, which might be due to the addition of NaOH after the proteolysis in order to increase the pH of this hydrolysate from acidic pH (optimum pH for pepsin) to neutral pH.

3.4. Lipase inhibitory activity of camel colostrum and milk protein and their hydrolysates

Pancreatic lipase is responsible for the intestinal digestion of dietary triglycerides. Inhibition of pancreatic lipase decreased the efficiency of fat absorption in the small intestine, resulting in reduced body weight. Therefore, the suppression or delay of fat digestion and absorption by inhibiting lipase activity has been targeted for the development of anti-obesity agents (Abdul Rahman et al., 2017). The percentage of inhibition of pancreatic lipase by camel colostrum and milk proteins and their hydrolysates, at different concentrations, are shown in Fig. 3. The ability to inhibit porcine pancreatic lipase depends on the concentration of the sample used. Indeed, the inhibition of pancreatic lipase increases with increasing concentration of the sample tested. The hydrolysates of camel colostrum and milk showed greater pancreatic lipase inhibitory activity than native proteins for all concentrations tested. Similar results were obtained for camel whey hydrolysates (Jafar et al., 2018). Enzymatic hydrolysis of proteins can expose bioactive peptides, previously hidden in the native protein, with the ability to inhibit the active site of pancreatic lipase. This latter could be bound easily by hydrophobic amino acids due the its lipophilic character (Gil-Rodríguez and Beresford, 2019). At a concentration of 10 mg mL^{-1}, camel colostrum protein hydrolysates obtained by pancreatin had the strongest inhibitory activity of pancreatic lipase (59.92%), while those obtained by pronase had the weakest activity (31.38%). With regard to camel milk protein hydrolysates (10 mg mL^{-1}), those treated by pepsin and pancreatin had the highest pancreatic lipase inhibitory capacity (40.02 and 38.98%, respectively), while those hydrolysed by trypsin had the lowest activity (30.68%). Similarly,

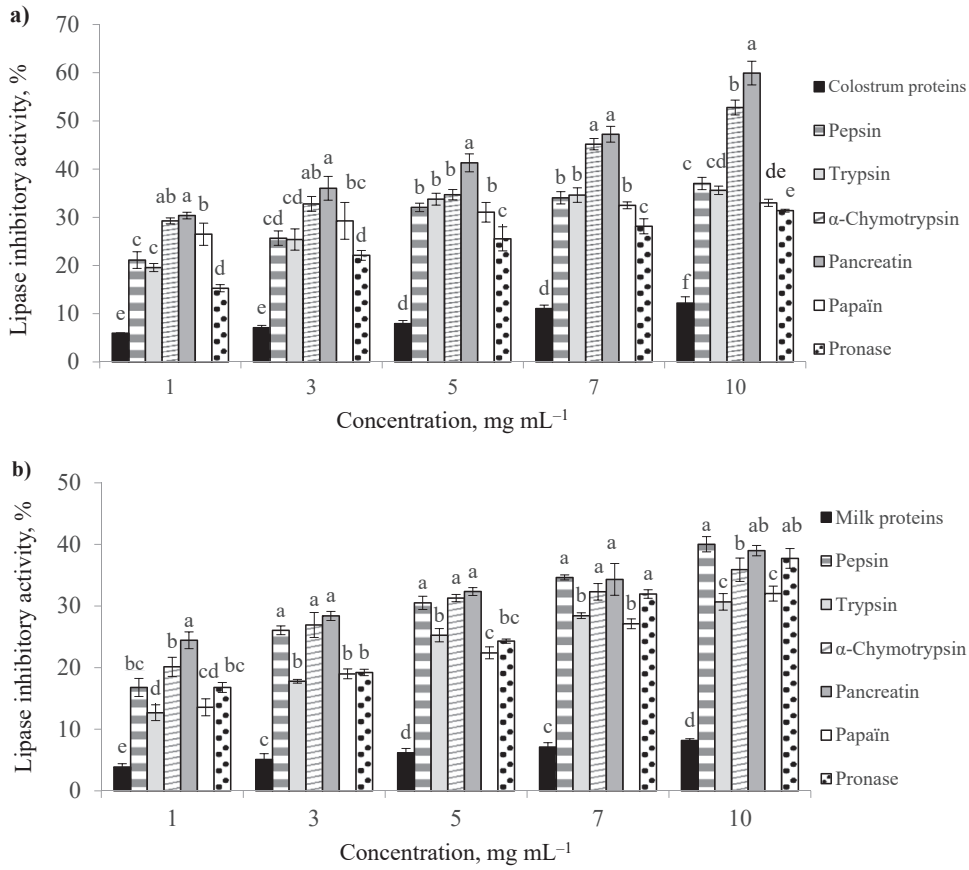

Fig. 3. Lipase inhibitory activity as a function of the concentration: (a) camel colostrum proteins and their hydrolysates; (b) camel milk proteins and their hydrolysates

Oussaief et al. (2023) reported an inhibitory pancreatic lipase activity for camel milk lactoferrin, isolated from camel colostrum, varying between 29.9 and 48.1%. Overall, camel colostrum hydrolysates had a higher pancreatic lipase inhibiting activity than camel milk protein hydrolysates hydrolysed by the same enzymes with the exception of pronase. This was explained by the difference in the protein composition of camel colostrum and milk.

Statistical results revealed no strong correlation between the degree of hydrolysis and the lipase inhibitory activity of camel colostrum protein hydrolysates ($r = -0.82$, $P = 0.746$) as well as camel milk protein hydrolysates ($r = 0.322$, $P = 0.193$). Therefore, the difference in the pancreatic lipase inhibitory activity between the hydrolysates could be associated with the sequence and the hydrophobicity of the peptides generated rather than their molecular mass.

Several authors reported that the biological activities of camel milk, including antioxidant, antimicrobial, anti-hypertensive, antidiabetic as well as anticholesterol activities, increased after enzymatic hydrolysis (Swelum et al., 2021).

To our knowledge, this study reports for the first time the anti-obesity activity of colostrum proteins and their enzymatic hydrolysates.

4. CONCLUSIONS

In this study, several protein hydrolysates were obtained from camel colostrum and milk by different proteolytic enzymes. The degree of protein hydrolysis as well as the chemical composition were evaluated to characterise the resultant protein hydrolysates. The *in vitro* anti-obesity activity of camel colostrum and milk proteins increased after their enzymatic hydrolysis. The difference in the anti-obesity activity among the hydrolysates was mainly due to the type of substrate and the specificity of the enzymes employed. Colostrum protein hydrolysates treated with pancreatin exhibited the strongest anti-obesity activity. Further work is needed to identify potent anti-obesity peptides and to investigate the mechanism of their action.

REFERENCES

Abdul Rahman, H., Saari, N., Abas, F., Ismail, A., Mumtaz, M.W., and Abdul Hamid, A. (2017). Anti-obesity and antioxidant activities of selected medicinal plants and phytochemical profiling of bioactive compounds. *International Journal of Food Properties*, 20(11): 2616–2629.

AOAC. (2000). *Official methods of analysis* (17th ed.). Washington, DC: Association of Official Analytical Chemists.

Cruz-Casas, D.E., Aguilar, C.N., Ascacio-Valdés, J.A., Rodríguez-Herrera, R., Chávez-González, M.L., and Flores-Gallegos, A.C. (2021). Enzymatic hydrolysis and microbial fermentation: the most favorable biotechnological methods for the release of bioactive peptides. *Food Chemistry: Molecular Sciences*, 3: 100047.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.T, and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3): 350–356.

El-Hatmi, H., Oussaief, O., Hammadi, I., Dbara, M., Hammadi, M., Khorchani, T., and Jrad, Z. (2023). Relation between color and chemical composition of dromedary camel colostrum. *Animals*, 13(3): 442.

Frackenpohl, J., Arvidsson, P.I., Schreiber, J.V., and Seebach, D. (2001). The outstanding biological stability of β-and γ-peptides toward proteolytic enzymes: an *in vitro* investigation with fifteen peptidases. *ChemBioChem*, 2(6): 445–455.

Gani, A., Broadway, A.A., Masoodi, F.A., Wani, A.A., Maqsood, S., Ashwar, B.A., Shah, A., Rather, S.A., and Gani, A. (2015). Enzymatic hydrolysis of whey and casein protein-effect on functional, rheological, textural and sensory properties of breads. *Journal of Food Science and Technology*, 52(12): 7697–7709.

Gil-Rodríguez, A.M. and Beresford, T.P. (2019). Lipase inhibitory activity of skim milk fermented with different strains of lactic acid bacteria. *Journal of Functional Foods*, 60: 103413.

Gonzalez-Rodriguez, M.C., Gonzalo, C., San Primitivo, F., and Carmenes, P. (1995). Relationship between somatic cell count and intramammary infection of the half udder in dairy ewes. *Journal of Dairy Science*, 78(12): 2753–2759.

Hoyle, N.T. and Merritt, J.H. (1994). Quality of fish protein hydrolysates from herring (*Clupea harengus*). *Journal of Food Science*, 59(1): 76–79.

Jafar, S., Kamal, H., Mudgil, P., Hassan, H M., and Maqsood, S. (2018). Camel whey protein hydrolysates displayed enhanced cholesteryl esterase and lipase inhibitory, anti-hypertensive and anti-haemolytic properties. *LWT – Food Science and Technology*, 98: 212–218.

Jrad, Z., El Hatmi, H., Adt, I., Girardet, J. M., Cakir-Kiefer, C., Jardin, J., Degraeve, P., Khorchani, T., and Oulahal, N. (2014). Effect of digestive enzymes on antimicrobial, radical scavenging and angiotensin I-converting enzyme inhibitory activities of camel colostrum and milk proteins. *Dairy Science and Technology*, 94: 205–224.

Jrad, Z., Oussaief, O., Khorchani, T., and El-Hatmi, H. (2022). Camel colostrum composition, nutritional value, and nutraceuticals. In: Alhaj, O.A., Faye, B., and Agrawal, R.P. (Eds.), *Research anthology on recent advancements in ethnopharmacology and nutraceuticals*. IGI Global, pp. 902–924.

Kumar, A. and Chauhan, S. (2021). Pancreatic lipase inhibitors: the road voyaged and successes. *Life Sciences*, 271: 119115.

Laemmli, U.K. and Favre, M. (1973). Maturation of the head of bacteriophage T4: I. DNA packaging events. *Journal of Molecular Biology*, 80(4): 575–599.

Liu, T.T., Liu, X.T., Chen, Q.X., and Shi, Y. (2020). Lipase inhibitors for obesity: a review. *Biomedicine and Pharmacotherapy*, 128: 110314.

Mudgil, P., Kamal, H., Yuen, G.C., and Maqsood, S. (2018). Characterization and identification of novel antidiabetic and anti-obesity peptides from camel milk protein hydrolysates. *Food Chemistry*, 259: 46–54.

Nongonierma, A.B. and FitzGerald, R.J. (2015). The scientific evidence for the role of milk protein-derived bioactive peptides in humans: a review. *Journal of Functional Foods*, 17: 640–656.

Oussaief, O., Jrad, Z., Adt, I., Kaddes, K., Khorchani, T., Degraeve, P., and El Hatmi, H. (2023). Antioxidant, lipase and ACE-inhibitory properties of camel lactoferrin and its enzymatic hydrolysates. *International Journal of Dairy Technology*, 76(1): 126–137.

Salami, M., Moosavi-Movahedi, A.A., Moosavi-Movahedi, F., Ehsani, M.R., Yousefi, R., Farhadi, M., Niasari-Naslaji, A., Saboury, A.A., Chobert, J.M., and Haertlé, T. (2011). Biological activity of camel milk casein following enzymatic digestion. *Journal of Dairy Research*, 78(4), 471–478.

Salami, M., Yousefi, R., Ehsani, M.R., Dalgalarrondo, M., Chobert, J.M., Haertlé, T., Razavi, S.H., Saboury, A.A., Niasari-Naslaji, A., and Moosavi-Movahedi, A.A. (2008). Kinetic characterization of hydrolysis of camel and bovine milk proteins by pancreatic enzymes. *International Dairy Journal*, 18(12): 1097–1102.

Swelum, A.A., El-Saadony, M.T., Abdo, M., Ombarak, R.A., Hussein, E.O., Suliman, G., Alhimaidi, A.R., Ammari, A.A., Ba-Awadh, H., Taha, A.E., El-Tarabily, K.A., and Abd El-Hack, M.E. (2021). Nutritional, antimicrobial and medicinal properties of camel's milk: a review. *Saudi Journal of Biological Sciences*, 28: 3126–3136.