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Research Article

Exploring the Functional Potential of Breast Milk Protein Hydrolysates: Antiglycation, Antioxidant, Metal Chelation, and Lipid Peroxidation Activities

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ABSTRACT

The formation of advanced glycation end products (AGEs) is a pivotal factor in the development of various age-related and diabetes-associated pathophysiologies, including but not limited to arthritis, Alzheimer's disease, atherosclerosis, and cataracts. Consequently, the prospect of inhibiting AGE formation emerges as a viable strategy to prevent or halt the advancement of diabetic complications. In the scientific literature, there is still a reluctance to produce bioactive peptides demonstrating antiglycation activity from breast milk. The breast milk protein was hydrolyzed using trypsin for 240 min. The antiglycation, metal chelating activity, lipid peroxidation activity, and antioxidant activity of the peptides in the hydrolysates obtained after hydrolysis of human milk with trypsin enzyme were performed. The peptide diversity obtained after hydrolysis was determined by RP-HPLC. The breast milk hydrolysate demonstrated significant antiglycation activity (IC50: 312.8 ± 12.1 µg/mL), antioxidant activity (61.8±4.58 mM AAE/µg peptide), metal chelation activity (24.4%/µg peptide). The hydrolysate effectively inhibited lipid peroxidation (30.5±0.12%) compared to Trolox (51.2±0.3%). These findings highlight the potential of breast milk protein hydrolysates as a source of bioactive peptides with diverse health benefits. The present study offers valuable insights into utilizing human milk peptides as novel functional food components.

1. Introduction

Recent studies have focused on the nutritional value and health effects of foods. Therefore, In addition to their nutritional effects, functional foods have emerged, which are foods that have health-protective, corrective, and/or disease risk-reducing effects depending on one or more effective components, and these effects have been scientifically and clinically proven. Therefore, natural resources, additives, and new technologies are needed to make foods functional. In recent years, research on physiologically bioactive proteins and peptides derived from foods has gained importance [1].

Peptides are derived from plant or animal proteins that have regulatory functions in normal and adequate nutrition. These peptides with biological activity are obtained from foods such as milk, eggs, meat, fish, soy, and wheat. However, to date, these peptides have mostly been isolated from dairy products. They are encoded in the protein and are inactive. Inactive bioactive peptides are activated either by the action of proteolytic enzymes of the digestive tract, starter culture proteolytic enzymes, or exogenously added proteolytic enzymes [2].

Bioactive peptides derived from various protein sources with a notable emphasis on milk proteins, encompass a wide array of biological attributes. These include antidiabetic, anticancer, opioid, antimicrobial, and antioxidant properties There have been many biochemical studies on cow, goat, sheep, and camel milk [3]. However, the studies on breast milk are still limited. Protein glycation arises through spontaneous interactions between protein amino groups and sugar carbonyl groups. This process leads to structural

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alterations in proteins, contributing to various significant conditions including aging-related ailments, neuropathy, nephropathy, retinopathy, and Alzheimer's disease [4]. Researchers investigate glycation inhibition as a potential strategy. In the literature, while the antiglycation activity of plant-origin products has been studied, there has been no study regarding the isolation of deglycation peptides from milk and dairy-like products.

Free radicals and reactive oxygen species (ROS) play a pivotal role in initiating and advancing conditions such as cancer, diabetes, and degenerative disorders like Alzheimer's and Parkinson's disease [5]. Managing oxidative stress is essential to impede the progression of degenerative illnesses and decelerate disease growth. The antioxidants present in breast milk collaborate synergistically to effectively neutralize free radicals and aid in their removal within a newborn's body. In literature, there are many studies on the antioxidant activity of human milk protein, but on research metal chelating activity and lipid peroxidation activity is still scarce. Zarban et al [6] reported that colostrum milk was more potent (50.4 ± 19.7%) in reducing stable DPPH radicals as opposed to transitional and mature milk. Churchill et al [7] determined that human milk obtained from 60 breastfeeding women at month postpartum had antioxidant activity (IC50 = 3.3 µg /mL) by the oxygen radical absorbance capacity (ORAC) assay.

Breast milk is dominated by whey protein. Whey protein contains a large proportion of α-lactalbumin and lactoferrin. Breast milk lacks lactoglobulin, which causes antigenic reactions. Breast milk is rich in protective proteins such as IgA, lactoferrin, and lysozyme, which protect the baby from infections and are more abundant in colostrum. Whey and casein proteins are easily digestible and high-quality proteins with the highest biological value. While the whey/casein ratio in breast milk is 60/40, this ratio is 20/80 in cow and goat milk. β-caseins are the main proteins in breast and goat milk [8, 9].
Breast milk is known to contain peptides and these components have been actively researched for more than half a century. Some peptides released from breast milk have bioactive properties such as antimicrobial, opioid, immunomodulatory, antihypertensive, antioxidant, antithrombotic, angiotensin-converting enzyme (ACE) and dipeptidyl peptidase IV inhibitory activities [10]. As the targets of these peptides are discovered, it will be possible to develop diagnostics, new strategies, and new products to monitor these processes. In literature, there is still a scare to produce bioactive peptides from breast milk. Therefore, the current study aimed to explore breast milk for potential bioactive peptides that have a pharmacological effect on diabetes and its related complications.

The study focused on the extraction of potential bioactive peptides from the hydrolysates of breast milk protein. The obtained hydrolysates were tested for some biological activities related to Alzheimer’s and related diseases such as antiglycation, metal chelating activity, lipid peroxidation activity, and lipid peroxidation activity.

2. Materials and Methods

2.1. Chemicals

Trypsin, Bicinchoninic acid (BCA) reagent, bovine serum albümin, picrylsulphonic acid ((TNBS) reagent, L-leucine, Gly-Pro-pNA ((Bachem AG, Bubendorf, Switzerland), P-nitroanilide, neocuproine, L-ascorbic acid, trifluoroacetic acid (TFA), acetonitrile (ACN), murexide linoleic acid, thiobarbituric acid and Ethylenediaminetetraacetic acid (EDTA) were provided by Sigma Aldrich (St. Louis, USA).

2.2. Preparation of human milk protein

To determine the levels of antiglycation effect, metal chelation, lipid peroxidation, and antioxidant efficiency in breast milk. The breast milk was collected from a 42-year-old participant nursing her second child. The mother in the study pumped a single portion of milk (40 mL). The breast milk samples were stored at -80°C for subsequent analysis. Thawing of the breast milk samples was carried out gradually, and the milk fat layer was removed by centrifuging at 3000 x g for 15 min at 4°C. The defatted supernatant was pipetted and utilized for the analysis.

2.3. Enzymatic hydrolysis of breast milk protein

To increase the hydrolysis efficiency in peptide extraction by enzymatic hydrolysis, optimization studies of the Enzyme/Substrate ratio (E/S) and the appropriate incubation time for hydrolysis were carried out. In the enzymatic hydrolysis process, optimization of the enzyme-substrate ratio is important to achieve a high degree of hydrolysis. For enzymatic hydrolysis with trypsin (1 U/mg protein), five different sets of experiments were prepared with E/S ratios of 1:5, 1:10, 1:20, 1:30, and 1:40.The experimental sets were incubated at 40 °C for 170 rpm. The samples incubated with enzyme were taken at 0, 1, 2, 3, 4, 5, and 6 h and kept in a boiling water bath for 15 min for denaturation of enzyme and non-hydrolyzable proteins. After denaturation, the samples were centrifuged at +4 °C for 15 min at 10000 rpm. The hydrolysates were stored at -20 ˚C for further use**.**

2.4. Determination of hydrolysis degree

The hydrolysis degree of hydrolysates was determined as the ratio of the amount of α-amino acid released during the hydrolysis to the α-amino acid in the control sample [11]. Properly samples (15µL) were mixed with 45 µL of 0.21M phosphate buffer (pH 8.2) and 45 µL of 0.01% TNBS. The mixtures were then incubated at 50 ˚C for 60 min. Then, the reaction was terminated by adding 90 µL of 0.1 N HCl. The absorbance was measured at 340 nm. (Thermo Scientific Multiskan FC Microplate reader, Massachusetts, U.S.A). A standard curve was plotted using the leucine standard. DH calculated as the following equation:

$$DH\% = \frac{h}{htot} * 100$$

h: free amino group of the hydrolyzed samples − free amino group of the unhydrolyzed sample
$htot$: total number of peptide bonds per protein equivalent.

2.5. Determination of peptide concentration

The quantification of peptide content in the protein fractions derived from breast milk digestion was conducted following the BCA protocol outlined by Smith et al [12]. Absorbance measurements were taken at 562 nm using a 96-well Microplate Reader. The bovine serum albumin was used as a standard.

2.6. Determination of antiglycation activity

Stock solutions of BSA (10 mg/mL) and D-glucose (500 mM) were prepared separately in potassium phosphate buffer (pH 7.4), each containing 0.02% (w/v) sodium azide. These solutions were then mixed in equal volumes (1.0 mL) and incubated at 60°C for 24 h. To assess the antiglycation activities of breast milk hydrolysate, 100 µL of the samples were added to an in vitro glycation model system consisting of glucose-BSA. Following the incubation, fluorescence measurements were taken using an excitation wavelength of 370 nm and an emission wavelength of 440 nm. All the samples were examined three times for accuracy. Aminoguanidine was used as a reference antiglycation compound and is set at 100% effectiveness. The results were calculated using the formula: Percentage of inhibition $[(Acontrol - Asample/Acontrol)]x100$

2.7. Determination of cupric reducing antioxidant capacity

The antioxidant activity of hydrolysate was determined using the CUPRAC method which is known for its advantages in aqueous or buffered samples [13]. Equal volumes (25 µL) each of copper (II) chloride solution (10 mM), Neocuproine (7.5 mM in ethanol), and ammonium acetate (NH_2Ac) buffer solution (1.0 M, pH 7.0) were mixed in a microplate well and incubated at 37 ˚C for 30 min. The absorbance was measured at 450 nm using a 96-well Microplate Reader (Thermo Scientific, Multiskan Sky, Waltham, Massachusetts, USA. Ascorbic acid was used as the reference standard and the results were defined as ascorbic acid equivalent per microgram of peptide (AAE/ µg peptide).

2.8. Determination of copper-chelating activity

The metal-chelating activity of breast milk protein hydrolysates was estimated with the

copper chelating method with some modifications [14]. The copper II sulfate solution was prepared using 30 mM pH 5.5 hydroxylamine buffer containing 10 mM KCl. 50 µL of diluted samples, Cu_2SO_4 (3mM), and 25 µL murexide (1mM) were mixed well and incubated at room temperature for 3 min. The control group was performed in the absence of murexide. The absorbance was measured at 462 and 530 nm. EDTA is used as a positive control. The results were calculated using the Cu_2SO_4 standard curve (0.025-0.125 mM) with the following equation; % Chelating Activity = [(Ccontrol –Csample) / Ccontrol] x 100.

2.9. Determination of Linolenic acid peroxidation with TRABS

The lipid peroxidation inhibition activity was determined according to a previously reported procedure [15]. Breast milk and hydrolysates were mixed with 0.255 mL Tris-HCl solution, 0.25 mL linoleic acid solution, 0.05 mL $FeSO_4$, and 0.05 mL ascorbic acid. The mixture was incubated at 37 °C for 30 min. After which 1 mL of mixture was removed and added with 1 mL thiobarbituric acid (1.0 % (w/v)). After heating the mixture at 95°C for 10 min, a 200 µL sample was transferred to a 96-well clear microplate, and the absorbance was recorded at 532 nm.

2.10. Peptide separation by reverse-phase high-performance liquid chromatography (RP-HPLC)

The separation of breast milk peptide fractions was performed by reverse-phase high-performance liquid chromatography (RP-HPLC) (Agilent 1260 Infinity series (Agilent Technologies, Santa Clara, CA, USA)). A Supelco C18 column 250 × 4.6 mm × 5 µm, 300 Å pore size (Nucleosil Rp-C17, Supelco, Germany) was used. The sample was diluted in 1.0 mL of the initial phase (A: B 95:5 (A: 0.1% (v/v) TFA in water, B: 0.1 % (v/v) TFA in ACN), and filtered through a 0.45 µm cellulose acetate filter (Sterlitech Corp. WA, USA). The mixture (20 µL) was then injected into a column (40°C) using a linear gradient of B [0–2 min (5%B) 2–20 min (60 %B), 20–23 min (20% B), 23–33 min (5% B)] under a flow rate of 1 mL/min steps consisted of an elution gradient. The peptides were detected at 215 nm and 280 nm.

2.11. Statistics

Bioactivity analyses were performed in triplicates and results are presented as mean ± SD.

3. Results and Discussion

To determine and improve the hydrolysis efficiency of breast milk by trypsin enzyme, optimization studies were carried out by trying different E/S ratios and hydrolysis times. Determination of the E/S ratio is the most important parameter to be optimized in the study of hydrolysis efficiency. Hydrolysis efficiency against different E/S ratios 1:5, 1:10, 1:20, 1:30, and 1:40 are given in Figure 1.

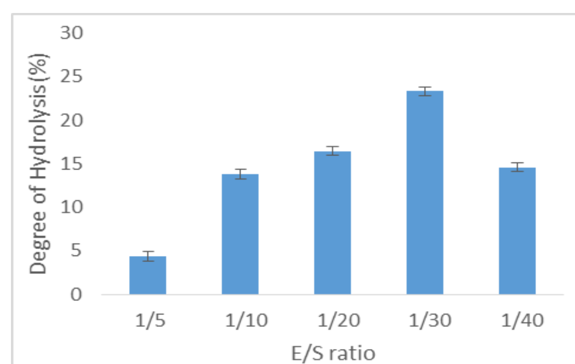
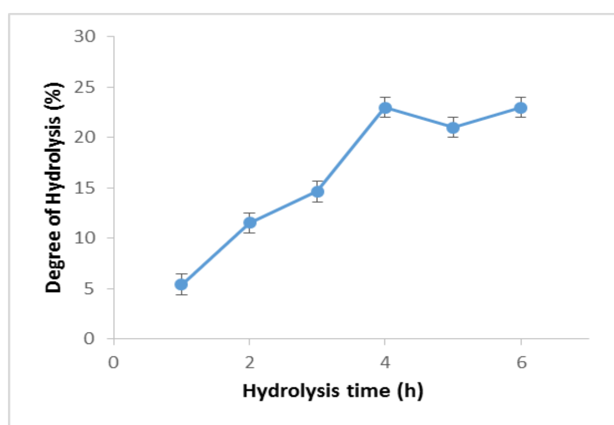


Figure 1. Hydrolysis efficiency of samples with trypsin at different E/S ratios

In general, a linear increase is observed up to an E/S ratio of 1:30. However, it is observed that the degree of hydrolysis slows down when this ratio is increased. In this context, it is concluded that increasing substrate concentration during the hydrolysis process leads to increased product inhibition of the enzyme, particularly above a certain substrate concentration. Therefore, the most suitable E/S ratio was determined as 1:30 for the preparation of trypsin hydrolysates with breast milk with a hydrolysis degree of (23.3±0.25 %).

In the next step, the time-dependent variation of hydrolysis efficiency was analyzed. It was observed that the highest hydrolysis value of hydrolysates from breast milk was observed at 240 min (22.92±0.61 %) reaction time (Figure. 2).



.**Figure 2.** Hydrolysis efficiency of samples with trypsin at different E/S ratios

Hydrolysis degree for trypsin was reported in between 8.78-11.23% of DH% (hydrolyzed for 0.5-5 h) [16]. The hydrolysis values of protein hydrolysates from breast milk increased rapidly initially and then remained constant with increasing hydrolysis time. The decrease in hydrolysis rate could be attributed to a lower count of peptide bonds, causing proteases and their substrates to reach a point of saturation. Kamau and Lu [17] reported that whey protein hydrolysates reached the highest hydrolysis level after 4 h of hydrolysis with trypsin enzyme (21.79%). Jrad et al [18] pointed out that with the hydrolysis of camel milk with pepsin and pancreatin enzymes, the highest hydrolysis value (19 %) was determined. The results of our experiment are similar to the results of other studies.

Protein glycation is a non-enzymatic procedure that occurs slowly under normal body conditions. Recent research highlights certain natural amino acids, dipeptides, and oligopeptides with antiglycation properties. Notably, the tripeptide glutathione γ-glutamylcysteine derivatives and aminoguanidine are successful inhibitors of AGE. Additionally, dipeptides featuring histidine (carnosine, homocarnosine, and anserine) and dipeptides containing tryptophan (NW and QW) are recognized for their ability to curb protein glycation [19]. In this study, antiglycation efficiency with the IC_{50} value of the breast milk hydrolysate was calculated to be 312.8 ±12.1 µg/mL which was higher than the antiglycation efficiency of aminoguanidine (IC_{50}: 629.7 ±45.8 µg/mL) used as control (Figure 3).

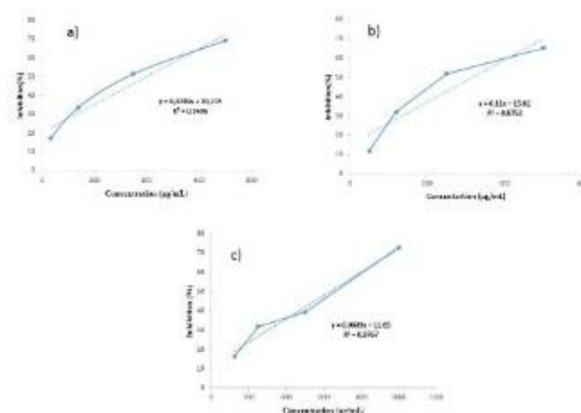


Figure 3. Curves and linear regressions of AGE inhibition activities of (a) the breast milk (b) the breast milk hydrolysate (c) aminoguanidine

Moreover, oxidative stress is significant in the aging process, diabetes complications, and different neurological issues in the human body, like Alzheimer's and Parkinson's diseases. Hence antioxidants are crucial in stopping tissue damage caused by harmful molecules. Lately, scientists have been studying proteins in food as potential sources of special compounds called peptides these peptides have multiple benefits for health and can help protect against oxidative stress [20]. Numerous artificial antioxidants are currently utilized and have demonstrated significant antioxidant capabilities. However, there are concerns that these synthetic compounds might cause varying degrees of harm to the body [21]. Therefore, investigations have been dedicated to uncovering and harnessing antioxidant peptides derived from natural sources like plants, dairy products, fish, and others. This is being explored as an alternative to relying solely on synthetic molecules.

The antioxidant, linolenic acid peroxidation, and metal chelating activities of the hydrolysates prepared with skimmed breast milk and enzymatic hydrolysis are given in Table 1. In the present study, the breast milk hydrolysate (61.8± 4.58 mM AAE/µg peptide) has higher antioxidant activity compared to the skimmed breast milk (4.61±0.65 mM AAE/µg peptide). The antioxidant activity of ascorbic acid used as the control was 9.21 ± 0.22 mM AAE/µg peptide. Results have shown that the hydrolysates from colostrum breast milk protein were found to be more effective than the pure positive control ascorbic acid. In this context, certain amino

acids, such as cysteine, histidine, tryptophan, lysine, arginine, leucine, valine, ß-hydroxy tryptophan, and their derivatives, are likely to be present in protein and peptide structures that contribute to antioxidant effects.

Therefore, the antioxidant activity observed in breast milk hydrolysates can be attributed to the presence of these hydrophobic amino acids and other related compounds. [22]. The results obtained are in line with the information given in the literature. Studies have shown that the oxidant/antioxidant status of breast milk varies according to the stage of lactation and higher antioxidant properties have been reported in colostrum compared to mature milk [23].

Miloudi et al. [24] identified at least two new antioxidative peptides derived from human casein by pepsin and pancreatin. The main casein fraction in human milk was found to prevent oxidative stress in infant guinea pigs receiving total parenteral nutrition. A study conducted by Oveisi et al. [25]. The total antioxidant activity in human breast milk was significantly higher than in formula milk by using the ferric reducing antioxidant power assay (FRAP) method. These findings imply that hydrolysates from breast milk protein could potentially include peptides capable of stopping or ending the chain reaction of radicals. They achieve this by capturing the free radicals and producing a stable end product [26].

Table 1. Antioxidant, metal chelating, and linolenic acid oxidation activities of breast milk and protein hydrolysates.

Samples and Positive Controls	Antioxidant (mMAAE/µg peptide)	Metal chelating (%/µg peptide)	Linolenic acid peroxidation (%/ug peptide)
Breast milk	61.8±4.6	24.4±2.1	30.5±0.1
Hydrolysate	4.61±0.6	1.72±0.1	7.2 ±0.06
EDTA	-	2.19±0.7	-
Trolox	-	-	51.2±0.3
Ascorbic acid	9.21 ±0.2	-	-

The results of metal chelation activity, which is supportive of antioxidant activity, are given in Table 1. The metal chelating ability of breast milk protein hydrolysate was found to be 24.4 %/µg peptide compared to 2.19±0.73 %/µg peptide that was obtained by EDTA. Since there was no previous study on metal chelation in breast milk. Narmuratava et al. [27] determined that lactoferrin purified from Kazakh milk (72.84 ± 1.24 %) showed antioxidant activity with its capture mechanism, reducing capacity, and divalent pro-oxidant metal chelation ability. Ovine casein isolates hydrolyzed with protease P7 for 0.5 h were observed to chelate 83.3% of Fe^{2+} chelating as compared to control hydrolysates (0 h; 76%) [28].

Furthermore, the hydrolysate demonstrates not only the ability to chelate metals but also significant potential as an antioxidant. When the chelation activity of the hydrolysis samples is examined, the highest metal chelation activity is observed in the hydrolysate sample as in the antioxidant activity. These findings support each other. Chelation activity with metals is generally realized through amino acids such as Tyr, Met, His, Lys, Arg, and Trp, which can be interpreted as the peptides in the hydrolysate that are rich in these amino acids [29].

Lipid peroxidation harms cell membranes, disrupting their function by altering the lipid structure. Free radicals damage enzymes and cellular components, and end-product aldehydes induce cell damage. Studies suggest that consuming peroxidized food can lead to heart disease, cancer, premature aging, and increased risks of tumors and atherosclerosis [30]. In the present study, the thiobarbituric acid-reactive substance (TBARS) assay was evaluated for the determination of the lipid peroxidation activity of breast milk and hydrolysate.

As seen in Table 1, the lipid peroxidation effect of the hydrolysate samples obtained by enzymatic hydrolysis was not as strong as the antioxidant effect of ascorbic acid when the lipid peroxidation effect of breast milk was compared with Trolox (51.2±0.3%) which is considered as a control and has a good peroxide effect. On the other hand, the lipid peroxidation activity of milk protein hydrolysate (30.5±0.12%) prepared by trypsin hydrolysis was significantly higher. The high peptide yield of breast milk after trypsin hydrolysis showed an effective peroxidation

activity in terms of peptide diversity compared to unhydrolyzed breast milk. Peña-Ramos and Xiong [31] found that soy protein isolate and its hydrolysates can reduce lipid damage by 28-65% using the TBARS method. Carillo et al. [32] discovered that native casein hydrolysate obtained from whole milk can decrease lipid damage by 33.33% to 55.55%. Tagliazucchi et al. [33] discovered that the amino acids phenylalanine and histidine played an important role in the lipid peroxidation inhibitory activity of the peptides. Peptides derived from αs1-casein have free radical scavenging activity. Therefore, they inhibit enzymatic and non-enzymatic lipid peroxidation.

RP-HPLC was used for the separation of peptides in protein hydrolysates. For this purpose, the hydrolysates were first concentrated due to the low amount of peptides in the hydrolysates. Dry preparations were prepared by dissolving in appropriate media for the determination of peptide complexity and biological activities after hydrolysis in RP-HPLC. Chromatograms of breast milk before and after hydrolysis are given in Figure 4**.** The peptide diversity of the hydrolysate obtained from the chromatograms of the hydrolysis of breast milk is seen. Within the scope of this study, only the biological activities of the hydrolysates obtained were targeted. However, the peptides in the hydrolysate should be purified according to the relevant biological activity, characterization and structure analysis of the peptides in the hydrolysate and their potential for use should be investigated.

4. Conclusion

Bioactive milk peptides have been the subject of functional foods commercially due to their potential health benefits. So far, the physiological effects of antihypertensive, mineral-binding, and anti-cariogenic peptides have been studied mostly. More studies are needed to utilize food-derived bioactive peptides in the prevention and treatment of chronic diseases. More peptide-based products are expected to be developed to manage various risk factors of metabolic syndrome such as hypertension, serum lipid levels, glucose balance, and body mass index. Further experimental studies on natural dietary bioactive peptides should be focused on the reduction of cardiovascular diseases, Alzheimer's, and mood control issues in the current global upward trend.

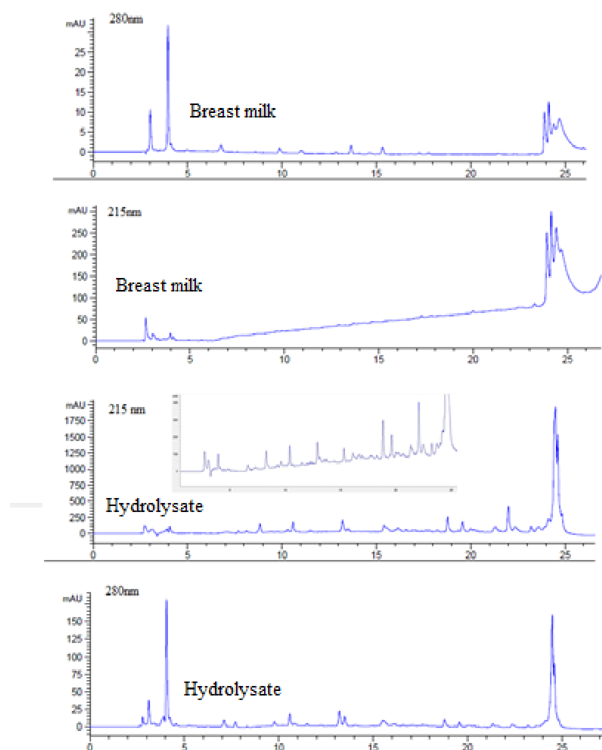


Figure 1. Chromatogram of breast milk protein without/with trypsin enzyme; chromatogram of breast milk after incubation with the enzyme for 4 h. Column: nucleosil RP-C18; Elution A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile; gradient: 0-2 min 5% B, 2-20 min 60% B, 20-23 min 20%, 23-33 min 5% B; flow rate 1.0 mL/min; temperature 40°C; UV: 280nm and 215nm.

In this context, many of the milk peptides with antihypertensive, antioxidative, and opioid properties may be worth further investigation. The current study explored the potential of breast milk for bioactive peptides through trypsin hydrolysis and analysis of different biochemical activities such as antioxidant, metal chelation, deglycation activity, and lipid peroxidation. In this context, studies with antiglycation activity in peptide structure from natural sources are limited and these are mostly studies. As far as we know, this paper is the first to describe hydrolysates with antiglycation and lipid peroxidation activity that were prepared from the trypsin enzymatic breakdown of breast milk protein. Therefore, the determination of bioactivities activity in the hydrolysis of breast milk protein will shed light on future studies. The hydrolysates breast milk

could be included in novel functional food blends aimed at Alzheimer's and related diseases.

Article Information Form

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Authors' Contribution
1st designed and performed the experiments, collected data from the results, directed and managed the study, and wrote the manuscript.

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The Declaration of Ethics Committee Approval
This study does not require ethics committee permission or any special permission.

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References

[1] A. Jakubczyk, M. Karaś, K. Rybczyńska-Tkaczyk, E. Zielińska, D. Zieliński, "Current trends of bioactive peptides—New sources and therapeutic effect", Foods., vol .9, pp. 846, 2020.

[2] M. Akbarian, A. Khani, S. Eghbalpour, V. N. Uversky, "Bioactive peptides: Synthesis, sources, applications, and proposed mechanisms of action", International Journal of Molecular Sciences, vol. 23, pp. 1445, 2022.

[3] S. Guha, H. Sharma, G. K. Deshwal, & P. S. Rao, "A comprehensive review on bioactive peptides derived from milk and milk products of minor dairy species", Food Production, Processing, and Nutrition, vol.3, pp.1-21, 2021.

[4] N. Barić, "Role of advanced glycation end products (AGEs) on the reactive oxygen species (ROS) generation in Alzheimer's disease amyloid plaque", Glycative Stress Res, vol. 2, pp.140-55, 2015.

[5] R. Aluko, "Bioactive Peptides". In: Functional Foods and Nutraceuticals. Food Science Text Series. Springer, New York, NY; 2012.

[6] A. Zarban, F. Taheri, T. Chahkandi, G. Sharifzadeh, M. Khorashadizadeh, "Antioxidant and Radical Scavenging Activity of Human Colostrum, Transitional and Mature Milk", Journal of Clinical Biochemistry and Nutrition, vol. 45 pp.150-154, 2009.

[7] M. Churchill, H. Zawawi, I. Elisia, M. Seider, R. Noseworthy, A. Thompson, & S. Grant, "The Antioxidant Capacity of Breast Milk and Plasma of Women with or without Gestational Diabetes Mellitus", Antioxidants (Basel), vol.12, pp.842, 2023.

[8] O. Ballard, A. L. Morrow, "Human milk composition: Nutrients and bioactive factors", Pediatric Clinics, vol. 60, pp. 49-74, 2013.

[9] F. Muñoz-Salinas, H. M. Andrade-Montemayor, K. De la Torre-Carbot, M. Á. Duarte-Vázquez, J. C. Silva-Jarquin, "Comparative Analysis of the Protein Composition of Goat Milk from French Alpine, Nubian, and Creole Breeds and Holstein Friesian Cow Milk: Implications for Early Infant Nutrition", Animals, vol. 12, pp.2236, 2022.

[10] Y. Wada, B. Lönnerdal, "Bioactive peptides derived from human milk proteins—mechanisms of action", The Journal of Nutritional Biochemistry, vol. 25, pp. 503-514, 2014.

[11] P. M. Nielsen, D. Petersen, C. J. J. O. F. S. Dambmann, "Improved method for determining food protein degree of hydrolysis", Journal of Food Science, vol.66, pp. 642-646, 2001.

[12] P. K. Smith, R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano & D. C. Klenk, "Measurement of protein using bicinchoninic acid" Analytical Biochemistry, vol. 150, pp.76–85, 1985.

[13] R. Apak, K. Güçlü, M. Özyürek, S. E. N. Karademir, M. Altun, "Total antioxidant capacity assay of human serum using copper (II)-neocuproine as chromogenic oxidant: the CUPRAC method", Free Radical Research, vol. 39, pp. 949-961, 2005.

[14] M. Karamac, "Fe [II], Cu [II] and Zn [II] chelating activity of buckwheat and buckwheat groats tannin fractions", Polish Journal of Food and Nutrition Sciences, vol. 57, pp. 357-362, 2007.

[15] Q. Li, L. Wei, J. Zhang, G. Gu, Z. Guo, "Significantly enhanced antioxidant activity of chitosan through chemical modification with coumarins", Polymer Chemistry, vol. 10, pp. 1480-1488, 2019.

[16] R. K. C. Jeewanthi, M. H. Kim, N.K. Lee, Y. C. Yoon, H. D. Paik, "Peptide analysis and the bioactivity of whey protein hydrolysates from cheese whey with several enzymes", Korean Journal for Food Science of Animal Resources, vol. 37, pp. 62-70, 2017.

[17] S. M. Kamau, R. R. Lu, "The effect of enzymes and hydrolysis conditions on the degree of hydrolysis and DPPH radical scavenging activity of whey protein hydrolysates", Current Research in Dairy Sciences, vol. 3, pp. 25-35, 2011.

[18] Z. Jrad, J. M. Girardet, I. Adt, N. Oulahal, P. Degraeve, T. Khorchani, H. El Hatmi, "Antioxidant activity of camel milk casein before and after in vitro simulated enzymatic digestion", Mljekarstvo, vol. 64, pp. 287–294, 2014.

[19] Q. Song, J. Liu, L. Dong, X. Wang, X. Zhang, "Novel advances in inhibiting advanced glycation end product formation using natural compounds", Biomedicine & Pharmacotherapy, vol. 140, pp. 111750, 2021.

[20] Q. Wu, Z. Guo, Z. Zhou, M. Jin, Q. Li, X. Zhou, "Recent advances in bioactive peptides from cereal-derived Foodstuffs", International Journal of Food Sciences and Nutrition, vol.73, pp. 875–888, 2022.

[21] X. Xu, A. Liu, S. Hu, I. Ares, M. R. Martínez-Larrañaga, X. Wang, M. Martinez, A. Anadón, M. A. Martínez, "Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action." Food Chemistry, vol. 353, pp. 129488, 2021.

[22] H. R. Ibrahim, H. Isono, T. Miyata, "Potential antioxidant bioactive peptides from camel milk proteins", Animal Nutrition, vol. 4, pp. 273–280, 2018.

[23] L. T. C. Santiago, N. A. D. Freitas, J. D. D. Meira Junior, J. E. Corrente, V. G. Paula, D. C. Damasceno, L. M. S. de Souza Rugolo, "Oxidative status in colostrum and mature breast milk related to gestational age and fetal growth" The Journal of

Maternal-Fetal & Neonatal Medicine, vol. 36, pp. 2183763, 2023.

[24] K. Miloudi, A. Tsopmo, J. K. Friel, T. Rouleau, B. Comte, J. C. Lavoie, "Hexapeptides from human milk prevent the induction of oxidative stress from parenteral nutrition in the newborn guinea pig", Pediatric Research, vol. 71, pp. 675-681, 2012.

[25] M. R. Oveisi, N. Sadeghi, B. Jannat, M. Hajimahmoodi, F. Jannat, F. MokhtariNasab, "Human breast milk provides better antioxidant capacity than infant formula" Iranian Journal of Pharmaceutical Research, vol. 9, pp. 445-449, 2010.

[26] O. Power, P. Jakeman, R. J. FitzGerald, "Antioxidative peptides: Enzymatic production, in vitro and in vivo antioxidant activity and potential applications of milk-derived antioxidative peptides", Amino Acids, vol. 44, pp. 797-820, 2013.

[27] Z. Narmuratova, F. Hentati, J. M. Girardet, M. Narmuratova, C. Cakir-Kiefer, "Equine lactoferrin: Antioxidant properties related to divalent metal chelation", LWT. 2022; 161: 113426.

[28] A. P. F. Corrêa, D. J. Daroit, J. Coelho, S. M. Meira, F. C. Lopes, J. Segalin, P. H. Rissoet, A. Brandelli, "Antioxidant, antihypertensive and antimicrobial properties of ovine milk caseinate hydrolyzed with a microbial protease", Journal of the Science of Food and Agriculture, vol. 91, pp. 2247-2254, 2011.

[29] G. J. Kontoghiorghes, C. N. Kontoghiorghe, "Iron and chelation in biochemistry and medicine: new approaches to controlling iron metabolism and treating related diseases", Cells, vol. 9, pp. 1456, 2020.

[30] J. A. D. De Leon, C. R. Borges, "Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay", Journal of Visualized Experiments, vol. 159, pp. e61122, 2020.

[31] E. A. Peña-Ramos, Y. L. Xiong, "Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties", Meat Science, vol. 64, pp. 259–263, 2003.

[32] W. Carrillo, X. Guzmán, E.Vilcacundo, "Native and heated hydrolysates of milk proteins and their capacity to inhibit lipid peroxidation in the zebrafish larvae model", Foods, vol. 6, pp. 81, 2017.

[33] D. Tagliazucchi, A. Helal, E. Verzelloni, A. Conte, "Bovine milk antioxidant properties: Effect of in vitro digestion and identification of antioxidant compounds", Dairy Science & Technology, vol. 96, pp. 657-676, 2016.