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Research Article

NUTRITIONAL, PHYSICOCHEMICAL, AND ANTIDIABETIC PROPERTIES OF PASTEURIZED AND UNPASTEURIZED CAMEL MILK: A SUSTAINABLE ALTERNATIVE FOR DIABETICS

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ABSTRACT

This present study was focused on compared properties of unpasteurized camel milk (UCM) and pasteurized camel milk (PCM) powder. Physicochemical analyses revealed nearly identical parameters, with PCM showing slightly higher fat content and 1.2-fold increase in leucine. Significant variations were observed in true density (1.5-fold), angle of repose (1.16-fold), and porosity (1.26-fold) upon pasteurization. Functional characteristics differed significantly ($p<0.05$) for UCM and PCM. FTIR analysis reflected physico-chemical transformations occurring during pasteurization. Antioxidant activity was found to be potent in UCM (70.93= 10mg/ml), The alpha amylase inhibition activity was found significantly ($p>0.05$) higher in unpasteurized milk (43-67 %) at 10-50 mg/mL concentrations compared to pasteurized milks (16.7-36.7%), respectively. Target gene prediction for bioactive compounds, conducted using PharmMapper, DisGENET, and AutoDock Vina, revealed strong binding of lactoferrin to haptoglobin (-7.4 Kcal/mol) and ceruloplasmin (-7.3 Kcal/mol). Results highlighted the potential of camel milk as a dietary supplement, particularly for diabetic patients.

1.Introduction

The global demand for milk and dairy products is rapidly increasing, with over six billion people consuming these products, predominantly in developing countries. However, the cattle dairy sector faces significant

challenges from climate change, including increased risks of drought, floods, and diseases, which can adversely impact production. Consequently, it is essential to explore alternative, environmentally sustainable, and climate-resilient options. Camel milk and its derivatives present a viable solution, offering a sustainable supply of high-quality proteins (Djenane & Aider, 2024). Camel milk is widely consumed in countries such as India, Sudan, Russia, Ethiopia, Somalia, South Sudan, and Djibouti. Traditionally, it has been used to treat various ailments, including tuberculosis, asthma, dropsy, and jaundice (Swelum *et al*., 2021). Known for its superior digestibility and nutritional value compared to bovine milk, camel milk is increasingly recognized as a valuable alternative for human consumption, particularly in arid and semi-arid regions (Seifu, 2022). This growing recognition has spurred high demand for camel milk in European countries and North America. Often referred to as "white gold," camel milk offers numerous health benefits and significant economic potential. Evidence has shown that camel milk provides therapeutic benefits against various diabetes, hypertension, cancer, inflammatory, and allergic responses (Anwar *et al*., 2022; Khan *et al*., 2022). Among the varied properties, its therapeutic potential against diabetes mellitus has been extensively explored. Diabetes mellitus, characterized by sustained hyperglycemia is due to lack or inadequate insulin secretion by β -cells of the pancreas improper cellular response to insulin, or both. Oral insulin therapy is a common treatment for managing diabetes; however, its efficacy is often reduced due to coagulation in acidic environments, which neutralizes insulin's action (American Diabetes Association, 2009). Recent research indicates that camel milk may serve as a beneficial adjunct to insulin therapy. Reports have shown that camel milk consumption not only lowers the prevalence of diabetes but also mitigates the detrimental effects associated with hyperglycemia and reduces the insulin requirements in type-1 diabetics (Khan *et al*., 2022). The antidiabetic potential of camel milk is mainly attributed to the presence of insulin and/or insulin-like peptides (Anwar *et al*., 2022). Studies have demonstrated that camel milk is both safe and effective in enhancing long-term glycemic control, potentially due to its unique insulin-like proteins and bioactive compounds (AlKurd *et al.,* 2022). Additionally, its unique composition, which includes low cholesterol content and a high concentration of polyunsaturated fatty acids, contributes to its health benefits (Swelum, 2021). Thus, the present research focuses on elucidating the effect of pasteurization on the physicochemical, functional characterization, and in silico mechanisms of camel milk influencing glycemic control and insulin sensitivity. The present study focus was on the explore the identification of specific bioactive compounds and their interactions within the body for the prevention and management of diabetes.

2. Materials and methods

2.1. Materials

2.2.1.Samples

Camel milk unpasteurized and pasteurized was procured from Aadvik Foods & Products Pvt. Ltd., India. All the chemicals used were of high-grade quality and procured from Sigma, Hi-media, and SRL chemicals.

2.2. Physico-chemical properties

2.2.1.Proximate analysis

The proximate analysis such as moisture, protein, fat, crude fiber, and ash content of the pasteurized and unpasteurized camel milk powder were determined as per the official method of AOAC (1990). Total carbohydrates were calculated by the difference method using the equation (1):

Carbohydrate % = 100 – (Protein % + Fat % + Ash % + Moisture %). (1)

2.2.2.Color profile

The color attributes (L∗, a∗, b∗) of the pasteurized and unpasteurized camel milk were recorded using a Hunterlab colorimeter (Konica Minolta Chroma Meter CR-400).

$$WI = \sqrt{(L^*-L0)+(a^*-a0)+(b^*-b0)} \quad (2)$$

Where, L* measures samples' lightness with its value ranging from 0 to 100 for black and white respectively, a* measures redness (+)/greenness (−), b* measures yellowness (+)/blueness (−), while ΔE indicates the total color difference.

2.2.3.Bulk density, true density, tapped density, porosity, angle of repose

The physical properties such as bulk density (ρb), true density (ρt), tapped density (ρtap), and porosity (ε) of pasteurized and unpasteurized milk powder were determined according to the process followed by Sonawane *et al*. (2020). The angle of repose (θ) was determined by measuring the height and diameter of a naturally formed heap of dry powder on a circular plate, following the method described by Kingsly et al. (2006) with minor modifications. The milk powder density and porosity were calculated using below equations (3-6).

ρ_b = weight of Camel milk cylinder (g)/ volume of cylinder (cm^3) (3)

ρ_{tap} = weight of Camel milk powder dipped in toluene(g)/ volume of cylinder (cm^3) (4)

ρ_{tap} = weight of Camel milk powder (g)/ volume of cylinder (cm^3) (5)

$$\varepsilon = (1 - \rho_b/\rho_t) \times 100 \quad (6)$$

2.2.4. Amino Acid Profiling

The amino acid profiling of the pasteurized and unpasteurized camel milk powder was analyzed using a High-Performance Liquid Chromatography (HPLC) system equipped with a Photodiode Array (PDA) detector. Chromatographic separation was achieved on a Zorbax Eclipse C18 AAA column (150 mm × 4.6 mm, 3.5 µm particle size). The mobile phase consisted of 10 mmol K_2HPO_4 and Na2B4O7·10H_2O, adjusted to pH 8.2 with HCl (Mobile Phase A), and a mixture of acetonitrile, methanol, and water in the ratio 45:45:10 (Mobile Phase B). A gradient elution was performed at 0.7 mL/min, 40°C, with a 20 µL injection volume, a 27-minute run time, a 2-minute post-run, and a maximum pressure of 400 bar.

2.2.5. Fatty Acid Profiling

Fatty acid analysis was performed using Gas Chromatograph equipped with a Flame Ionization Detector (GC-FID). The separation was achieved using SP®-2560 capillary GC column (100 m × 0.25 mm, 0.20 µm film thickness). Helium was used as the carrier gas at a flow rate of 0.95 mL/min with a split ratio of 100:1. The injector temperature was set at 250°C, and the column oven temperature was programmed as follows: an initial temperature of 100°C was maintained for 3 minutes, followed by a ramp at 4°C/min to a final temperature of 240°C, which was held for 18 minutes. The detector temperature was set at 280°C, with hydrogen and zero air supplied at flow rates of 40 mL/min and 300 mL/min, respectively. Fatty acid peaks were identified by comparing their retention times with those of a reference standard analyzed under identical condition and area under each peak was calculated for quantitative analysis.

2.3. Functional properties

2.3.1.Fourier transform infrared (FTIR) spectral pattern

The functional groups in unpasteurized and pasteurized camel milk were analyzed using FTIR (Bruker Alpha Laser Class 1). The absorbance of the powdered samples was recorded directly by placing them on the ATR ZnSe crystal, within a wave number range of 4000–400 cm^{-1}, with a spectral resolution of 2 cm^{-1}, by co-adding 64 interferograms. Measurements were conducted under ambient conditions.

2.3.2.Thermal properties

The thermal properties of unpasteurized and pasteurized camel milk samples were assessed using a DSC (200S3, Netzsch, Germany,). The

samples were heated at a rate of 10°C/min over a temperature range of -20 to 200 °C. The onset temperature (T_o), end temperature (T_f), peak temperature (T_p), and enthalpy (ΔH) were determined from the thermograms.

2.3.3.Water absorption index (WAI), water solubility index (WSI), water absorption capacity (WAC), and Oil absorption capacity (OAC)

The water absorption index (WAI), water solubility index (WSI), and oil absorption capacity (OAC) of both unpasteurized and pasteurized camel milk was measured using the procedures outlined by Kataria *et al*. (2022) by using the equations (7-9).

WAI = wt. of sediment after centrifuge (g) – wt. of dry P & UP (g)/ wt. of dry powder (g) (7)

WSI = wt. of soluble powder(g)/ wt. of dry powder used for estimation (g) (8)

OAC = wt. of centrifuged precipitate – wt. of dry powder(g)/ wt. of dry camel milk (g) (9)

2.4. Bioactive properties

2.4.1.DPPH free radical scavenging activity

Free radical scavenging activity of milk sample was determined using standard DPPH (2,2-diphenyl-1-picrylhydrazyl) assay reported by Behrouz *et al*. (2022) with a slight modification. Briefly, 100 µl of extracts (2 to 10 mg/ml) were mixed with 1 ml of methanolic solution of 0.1 mM DPPH. The contents were thoroughly mixed and incubated at room temperature for 30 min in dark. The absorbance was recorded at 517 nm using a UV-Vis spectrophotometer (Shimadzu – UV- 2600). The analysis was performed in triplicates and average value express as results. Percent inhibition was calculated from control using equation (10).

Scavenging activity (%) = (1 – absorbance sample/absorbance control) × 100 (10)

2.4.2.In vitro antidiabetic assay

In vitro antidiabetic potential was determined using α-amylase inhibition assay and non-enzymatic glycosylation of hemoglobin.

2.4.2.1.α-Amylase Inhibition Assay

Alpha amylase inhibition assay was conducted following a method outlined by Karakaya *et al*. (2018) with slight modifications. Initially, 1 mL of alpha-amylase solution (1% w/v in sodium phosphate buffer, pH 6.9) was mixed with the sample (1 mg/mL) and incubated at 37°C for a duration of 5 to 15 min. Thereafter, 1 mL of 1% starch solution (1% w/v in sodium phosphate buffer, pH 6.9) was added and the mixture was incubated for 15 min. Subsequently, 1 mL of 3, 5-dinitro salicylic acid was added, and the mixture was placed in a thermoregulatory water bath at 85°C for 5 to 10 min. The different concentrations (10-50 mg/mL) of prepared were cooled up to room temperature and absorbance was recorded at 540 mm using a UV-visible spectrophotometer (Shimadzu – UV- 2600).

$$\%\ Inhibition = \frac{(Abs\ of\ Control - Abs\ of\ Test) * 100}{Abs\ of Control} \quad (11)$$

2.4.2.2.Non-enzymatic glycosylation of hemoglobin

The non-enzymatic glycosylation of hemoglobin was determined by the method of Hosseini *et al*. (2015).1 mL of the camel milk (1mg/mL) added 1mLof glucose (0.2%), 1ml of hemoglobin (0.06%), and 5µL of gentamicin (0.02%) solutions were prepared in phosphate buffer (0.01 M, pH 7.4). Methanolic extract of camel milk both unpasteurized and pasteurized was weighed and dissolved in DMSO to obtain a stock solution of 500 mg/ml and then 10-50 mg/ml solutions were prepared. 1 ml of each concentration was added to the above mixture. The mixture was incubated in the dark for 72 hrs. at room temperature. The degree of glycosylation of hemoglobin was measured

calorimetrically at 520 nm. Percentage inhibition was calculated by using the equation (12).

$$\%\ Inhibition = \frac{(Abs\ of\ Control - Abs\ of\ Test) * 100}{Abs\ of Control} \quad (12)$$

Where, Abs control is the absorbance of the control reaction containing all reagents without the test sample, Abs sample is the absorbance of the test sample.

2.5. *In-silico* antidiabetic activity

2.5.1. Target prediction

The PubChem database was utilized for obtaining the structural file of the bioactive compound lactoferrin in camel milk, in mol2 format, which was converted to an SDF format file (https://www.ncbi.nlm.nih.gov/pccompound/). The gene targets of the lactoferrin compound were analyzed through the PharmMapper database (Gfeller *et al*., 2014; Wang *et al*., 2017). The targets were matched with the genes linked with diabetes mellitus using the DisGeNET database followed by screening of targets according to their gene-disease association score (≥ 0.1).

2.5.2. Construction of Protein-Protein Interaction (PPI) network and Gene ontology (GO) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis

To build the Protein-Protein Interaction (PPI) network, Cytoscape 3.8.2 was used to explore interactions among the target proteins (Kushwaha and Shakya, 2010; Lee *et al*., 2009). The network construction relied on degrees and pathways associated with the targets. Functional analysis of the targets, investigating molecular functions, cellular components, biological processes, and KEGG enrichment, was done using Cytoscape 3.8.2 and visualized through R Studio.

2.5.3. Molecular Docking

The crystal structure of the targets was obtained from the RCSB database. Compound structures (in mol format) were retrieved from PubChem and then converted to SDF format using Open Babel. Alpha 2-Macroglobulin (AMG), Ceruloplasmin (Cp), and Haptoglobin (Hp) were found to be significantly higher in T1Diabetes Mellitus and T2Diabetes Mellitus (de Paula Silva *et al*., 2021). Each protein was converted to a Protein Data Bank (PDB) ID and then subjected to AutoDock Vina to obtain binding scores, following the method described by Pipaliya *et al*., (2024). An effective binding energy was considered when the binding score of target proteins and compounds was ≥7.00.

3.Results and discussions

3.1. Nutritional, amino acid and fatty acid profiling

The results of nutritional and amino acid profiling of unpasteurized and pasteurized camel milk are depicted in Table (1 a & b). The results showed, pasteurized camel milk revealed nutritional changes in proteins, saturated fats, total carbohydrates, sodium, and calcium content concerning pasteurization (Table 1). The amino acid profiling confers the changes in protein content. The amino acid profiling improved with pasteurization with marked differences observed in arginine (3.42), tyrosine (0.86), methionine (0.112), valine (0.34), and leucine content (8.30). The pasteurization of camel milk resulted in slight but unsignificant changes in fatty acid profile in comparison to the unpasteurized milk, though the change was not significant ($p>0.05$). The fatty acid content in pasteurized milk showed a decreasing trend with an exception for palmitic acid which increased by 1.18%. Earlier studies conducted have demonstrated similar results (Dhahir *et al*., 2021; Narmuratova *et al*., 2006). Interestingly the study conducted by Dhahir *et al*. (2021) compared the effect of pasteurization (65ºC for 30min; 72ºC for min; 80ºC for 15 min) on fatty acid profiling of raw camel milk. This study also indicated that, although some specific fatty acids may be affected by heat, the overall composition and nutritional quality of the fatty acids in camel milk are preserved. The results follow the previous findings wherein Dhahir *et al*. (2020) studied the effect of varied pasteurization

treatments (65°C/30 min, 72°C/5 min, and 80°C/15 min) on camel milk constituents and demonstrated minimal changes in protein content at 65°C/30 min. Unprocessed camel milk has a shelf life of 5 days at 7°C, while pasteurized camel milk, heated to 65°C for 20 minutes and stored at 7°C, lasts for 22 days. Additionally, fresh camel milk can be stored for up to 1 year if frozen. However, with an increase in pasteurization temperature treatment (72°C/5 min), certain changes were reported in the alpha-lactalbumin band (Mwt 15 kDa) and correspondingly the band intensities of lactoferrin (Mwt 84 kDa) and alpha-lactalbumin (Mwt 15 kDa) were altered at 80°C/15min. The fatty acid profiling showed no significant ($p>0.05$) difference in pasteurized and unpasteurized milk including the cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid (CLA), and the lipid peroxidation products. The calcium and sodium content of the camel milk showed noticeable changes upon thermal treatment. The decrease in calcium content corresponds to a decrease in calcium ions during pasteurization and is further probably due to the spray drying of pasteurized camel milk (Deshwal *et al*., 2020; Konuspayeva, 2020). Camels are known for their ability to store water and survive in dehydrated conditions. The dehydration status also influences the calcium content (Yagil and Etzion, 1980). On the contrary sodium content increased from 13.18 to 121.28. The increase is attributed to the concentration of total ash content during freeze (9.66/100g) and spray drying (9.27g/100g) in comparison to unpasteurized camel milk (0.88g/100g) as conferred from earlier study. The iron content also decreased upon pasteurization (8.6%) attributing thermal processing leading to possible iron degradation or interaction with other components in the milk matrix. Similar results have been documented in earlier studies showing a reduction in iron content in camel milk upon freezing (12mg/kg) and spray drying (11mg/kg) (Rakhmatulina *et al*., 2024).

Table 1 (a). Chemical composition of camel milk Pasteurized and Unpasteurized Camel milk

Parameters	PCM	UCM
Calories (Kcal)	59.0±0.65^{a}	59.20±0.45^{a}
Proteins (g)	5.01±0.13^{a}	4.26±0.17^{b}
Total Fat(g)	6.56±0.23^{a}	6.61±0.91^{a}
Saturated fat(g)	4.26±0.81^{a}	2.74±0.81^{b}
Trans Fat(g)	NF	NF
Total Carbohydrates(g)	6.42±1.61^{b}	7.64±1.12^{a}
Sugar(g)	4.28±0.56^{a}	3.84±0.93^{b}
Added Sugar(g)	NF	NF
Cholesterol (mg)	5.08±0.12^{a}	5.01±0.87^{a}
Sodium (mg)	121.28±0.27^{a}	13.18±0.002^{b}
Calcium (mg)	183.26±0.31^{b}	202.2±0.18^{a}

Mean ± standard deviation (n=3), Where, PCM = pasteurized camel milk, UCM= Unpasteurized camel milk, NF=Not found. Means with different superscripts (a, b) depict significant difference ($p<0.05$) between unpasteurized and pasteurized camel milk.

The amino acid profiling confers the changes in protein content. The amino acid profiling improved with pasteurization with marked differences observed in arginine (3.42), tyrosine (0.86), methionine (0.112), valine (0.34), and leucine content (8.30) (Table 1b). The pasteurization of camel milk resulted in slight but unsignificant changes in fatty acid profile in comparison to the unpasteurized milk, though the change was not significant ($p>0.05$).

Table 1 (b). Amino acid (g/100 g protein) and fatty acid (g/100g fatty acid) profiles in pasteurized and unpasteurized camel milk

Parameters	PCM	UCM	Fatty acid (g/100g fatty acids)		UCM	PCM
Histidine	1.254	0.939	Butyric acid	C4:0	0.36±0.21[a]	0.33±0.11[a]
Serine	0.571	0.576	Caproic Acid	C6:0	0.31±0.11[a]	0.27±0.01[a]
Arginine	3.427	2.692	Caprylic Acid	C8:0	0.38±0.03[a]	0.35±0.05[a]
Glycine	0.585	0.551	Capric Acid	C10:0	0.32±0.01[a]	0.31±0.04[a]
Aspartic acid	0.208	0.171	Lauric acid	C12:0	0.96±0.02[a]	0.92±0.07[a]
Glutamic acid	0.706	0.630	Myristic acid	C14:0	10.1±1.12[a]	9.57±1.16[a]
Alanine	0.600	0.520	Myristoleic acid	C14:1	0.80±0.01[a]	0.78±0.11[a]
Proline	0.585	0.584	Palmitic Acid	C16:0	21.87±1.16[a]	22.13±2.10[a]
Lysine	0.364	0.375	Palmitoleic Acid	C16:1	6.15±1.20[a]	5.92±1.17[a]
Tyrosine	0.865	0.746	Stearic Acid	C18:0	17.03±1.67[a]	17.05±1.45[a]
Methionine	0.112	0.064	Eladic Acid	C18:1trans	5.87±1.21[a]	5.33±1.27[a]
Valine	0.349	0.238		C18:1c9	21.86±1.23[a]	21.72±2.01[a]
Isoleucine	0.493	0.403	Oleic Acid	C18:1c11	0.47±0.04[a]	0.45±0.04[a]
Leucine	8.301	6.832	Linoleic acid	C18:2n6	3.77±1.10[a]	3.74±1.10[a]
Phenylalanine	0.548	0.513	Alpha-linoleic acid	C18:3n3	0.61±0.05[a]	0.57±0.02[a]
			Arachidic Acid	C20:1n9	0.05±0.01[a]	0.05±0.03[a]
				C20:4n6	0.20±0.08[a]	0.19±0.01[a]
				C20:5n3 (EPA)	0.02±0.00[a]	0.03±0.00[a]
			Behenic Acid	C22:5n3	0.09±0.00[a]	0.10±0.00[a]
				C22:6n3 (DHA)	0.04±0.00[a]	0.03±0.00[a]

Mean ± standard deviation of three replicates. EPA – Eicopentanoic acid; DHA – Docosahexanoic acid, Mean with different superscripts (a, b) depict significant difference ($p<0.05$) between unpasteurized and pasteurized camel milk.

3.2.Effect of pasteurization on physical properties of pasteurized and unpasteurized milk

The results of physical properties of pasteurized and unpasteurized milk samples are depicted in Table 2a. Results indicate marked difference ($p<0.05$) was observed in true density (0.65, 1.01), angle of repose (37.70°, 43.86°), porosity (76.0, 60.2) of pasteurized and unpasteurized camel milk respectively. The functional properties showed a significant difference ($p<0.05$) between the water solubility index (4.17, 2.73g/100g) and oil absorption capacity (11.40, 12.05) respectively. The chromatic variables (L*, a*, and b*) did not show a significant ($p\geq0.05$) difference between pasteurized and unpasteurized camel milk. However, the total color difference (ΔE) showed a marked difference ($p<0.05$) in both the powders with a lower value (91.0) of unpasteurized than pasteurized camel milk (95.4) (Table 2b).

The color difference is affected by several factors such as the dietary intake of the animal, breeding, parity, and seasonal calving. Moreover, research indicates that processing and storage conditions might lead to the denaturation of specific proteins and modifications in physical structure resulting in color change (Milovanovic *et al*., 2020). Previous researchers have indicated a color difference ΔE of 6.5 between treated and untreated ultra-high temperature (UHT) camel milk, as well as differences of 2.26 between high-pressure treatment and 1.5 between pasteurized camel milk (Ho *et al*., 2022).

Table 2 (a). Physicochemical parameters of Pasteurized and Unpasteurized Camel milk

Camel Milk	Bulk Density (g/ml)	Tapped Density (g/ml)	True Density (g/ml)	Angle of Repose	Porosity	WAC (mL/g)	WAI (g/100g)	WSI (g/100g)	OAC (mL/g)
PCM	0.26±0.02^{a}	0.31±0.026^{a}	0.65±0.22^{b}	37.70^{ob}	76.0^{a}	10.89± 0.47^{a}	1.28±0.49^{a}	4.17±0.23^{a}	11.40±0.20^{b}
UCM	0.24±0.03^{a}	0.29±0.046^{a}	1.01±0.01^{a}	43.86^{oa}	60.2^{b}	10.81± 0.14^{a}	1.13±1.06^{a}	2.73±0.74^{b}	12.05±0.31^{a}

Mean ± standard deviation of three replicates.
Means with different superscripts (a, b) depict significant difference ($p < 0.05$) between unfermented and fermented legume.

Table 2 (b). Color analysis of unpasteurized and pasteurized Camel milk

Sample	L*	a*	b*	ΔE
PCM	96.2±0.16^{a}	-2.3±0.03^{a}	8.80±0.13^{a}	95.4±0.12^{a}
UCM	96.3±0.09^{a}	-2.3±0.01^{a}	9.40±0.16^{a}	91.0±0.56^{b}

Mean ± standard deviation of three replicates.
Means with different superscripts (a, b) depict significant difference ($p < 0.05$) between unfermented and fermented legume.

3.3.Fourier Transform Infrared Spectra (FTIR)

FTIR was used to comprehend the functional groups present in the unpasteurized and pasteurized camel milk as shown in Figure 1. The various zones in the spectra are 800-1200^{cm-1} polysaccharide spectral zone; 1500-1800 $^{cm-1}$ amide I zone depicting the secondary structure of the proteins comprising of α-helix (1650-1664^{cm-1}), β-sheet (161-1640 and 1680-1695^{cm-1}), β-turn (1664-1680^{cm-1}) and random coil (1640-1650^{cm-1}); 1200-1400 $^{cm-1}$– amide III zone (Kataria *et al*., 2022); 2802-2968^{cm-1};1692-1773^{cm-1} related to fat and 3200-3300^{cm-1} depicting moisture content or O-H vibrations (Chu *et al*., 2023); and 1000-1100 $^{cm-1}$ depicting lactose or alkoxy C=O stretching or phosphate groups covalently bound to casein (Al-Thaibani *et al*., 2024). The major differences in the unpasteurized and pasteurized camel milk were observed in the spectra from 1000-3000 $^{cm-1}$. The region lower than 900 $^{cm-1}$ did not show any significant variation. The peaks in the range of 2800-2900 $^{cm-1}$ depicted the H-C=O, C-H stretch of alkenes and aldehydes with medium vibrations. Similar peaks have been reported earlier in the range of 1500-1600 $^{cm-1}$ (originated by C=O, C-H, N-H, amide linkages) and 2830-3000 $^{cm-1}$ (corresponding to milk fat) by Khalid *et al*. (2023). The peaks corresponding to wave numbers greater than 3400^{cm-1}originate from lipoprotein interactions. The peak intensities are more prominent in unpasteurized as compared to pasteurized camel milk. The obtained spectra depicted the impact of pasteurization on the conformation of proteins. The secondary structure of protein exhibited significant variations upon thermal processing. The amide III zone was reduced and the peak shifted to higher wavenumbers. Similarly, the peak observed at 1618 $^{cm-1}$ in unpasteurized camel milk powder shifted to 1642 $^{cm-1}$ implying a reduction in β-sheet structure to random coils upon heating which was observed to agree with Li *et al*. (2020). This modification can alter or destroy antigenic epitopes (protein regions interacting with antibodies to trigger an immune response) thereby reducing the allergenicity of the milk proteins (Acuña-Nelson *et al*., 2024). Slight variation in the peak intensities in the range of 3200-3300 $^{cm-1}$ can be correlated with the differing moisture content of the camel milk samples (Deshwal *et al*., 2020). Additionally, the spectra of 1000-1100 $^{cm-1}$ showed differences which can be explained by the thermal modification in the phosphates bound to casein and lactose. The lipid bands at 1739 and 2922 $^{cm-1}$ in unpasteurized camel milk powder shifted to

higher wavenumbers of 1743 and 2924^{cm-1} respectively, which can be explained as temperature-induced variation in the lipid backbone (Khalid *et al*., 2023).

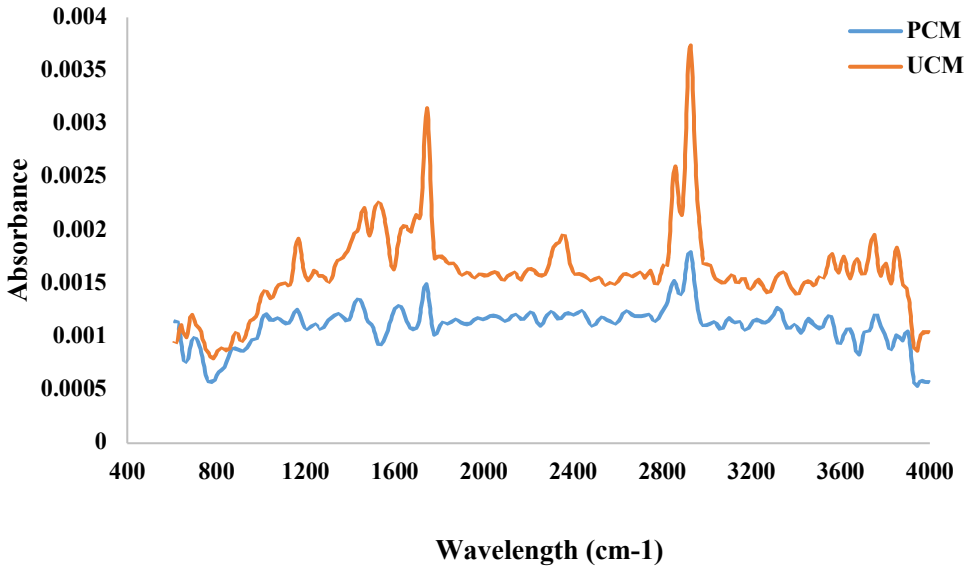


Figure 1. FTIR spectra of pasteurized and unpasteurized camel milk

3.4.Effect of pasteurization on thermal properties of camel milk powder

The specific heat capacities of various thermal changes are measured using differential scanning calorimetry as a function of temperature. The transitions in the DSC thermograms are attributed to protein denaturation associated with changes in structural conformation, lipid polymorphism associated with melting, and thermally induced processes of other macronutrients. Briefly, DSC is used to study different first and second-order transitions such as crystallization, melting, glass transition, and protein denaturation in food matrices. Regardless of pasteurization, camel milk powder exhibited two endothermic peaks between 0 to 140°C. The comparison of the onset (To), peak gelatinization (Tp), endset (Te) temperatures, temperature range (ΔTr), and enthalpy (ΔH) are shown in Table 3.

Table 3. Thermal (DSC) properties of Pasteurized and Unpasteurized Camel milk

Camel Milk	T_p (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_e (°C)	$\Delta T_r = T_o - T_p$ (°C)	ΔH (J/g)
PCM	41.7	39.96	98.3	104.6	106.6	8.3	3325
UCM	51.4	174.6	89.8	98.4	104.4	11.6	2469

Mean ± standard deviation of three replicates, T_o, Onset temperature; T_p, peak gelatinisation temperature; T_e, end temperature; ΔTr, transition temperature range; ΔH heat released per gram sample, PCM , pasteurized camel milk; UCM, unpasteurized camel milk

The peak at a low temperature was attributed to the melting of fat and the peak at a higher temperature indicated the melting of non-fat solids in milk powders (Rahman *et al*., 2012). In the case of peak 1, the unpasteurized and pasteurized milk powders showed Tp at 51.4 and 41.7°C respectively. Moreover, the enthalpy was higher in the case of unpasteurized milk powder (174.6 J/g) in comparison to that of pasteurized milk powder (39.96 J/g). This implied that the fat fractions in unpasteurized milk powder required higher enthalpy for melting. However, pasteurization released these fat fractions leading to a lower peak temperature and enthalpy for fat melting in the pasteurized camel milk powder. Laadhar *et al.* (2006) have reported the presence of a high proportion of high-melting triacylglycerols in camel milk. It was also outlined that the triacylglycerols in the camel milk fat globule membrane have varying crystallization rates thereby influencing the surface activity. However, Sun *et al.* (2018) studied the pasteurized infant formula and reported that this first endothermic peak highlighted the glass transition of lactose. Their findings also showed that peak temperature reduced sharply from 53.6°C in infant formula to 41.4°C in the pasteurized sample.

An interim exothermic transition before the second endothermic peak was also observed in the thermogram which agreed with the findings reported by Sun *et al*. (2018), who attributed this to lactose crystallization. The second peak has been attributed to the changes in the structural conformation of the proteins, mainly casein and whey proteins upon pasteurization (Figure 2). The reduced ΔTr in pasteurized camel milk powder may be attributed to the interactions between proteins and fat globule membrane due to pasteurization pre-processing stages like homogenization (Sun *et al*., 2018). Mizuno *et al*. (2000) suggested that the state of protein secondary structure was the determining factor for its glass transition. Our findings are in good agreement with findings of Rahman *et al*. (2012) who also observed that the transition onset was observed at 77 and 95°C for casein and whey proteins respectively. However, Pugliese *et al*. (2016) have also indicated that many changes such as protein denaturation, lactose crystallization, and Maillard reaction occur in this temperature range. Various authors have extended the temperature range of the thermograms to comprehensively study camel milk. Rahman *et al.* (2012) evaluated the thermal characteristics of freeze-dried camel milk between a higher range of -100 to 300°C and demonstrated that fat melting corresponding to two peaks related to To and Tp at 9.85 and 16.85°C for the first peak and at 33.85 and 40.85°C for the second peak, respectively. The peak temperatures varied for pure camel fat and cream, with the first peak occurring at a lower temperature and the second at a higher temperature. Further, for whole camel milk, a glass transition temperature of 30.95°C was reported. Nevertheless, "structure forming" has been elucidated in dried camel milk at the exothermic transition at 199.85°C. Likewise, the study by Pugliese *et al.* (2016) highlighted the crucial role lactose plays in the thermal behavior of milk powder, as observed through DSC thermograms.

The findings indicate that lactose modifications during processing also become apparent through exothermic peaks above 156°C, suggesting structural and compositional changes. Additionally, the presence of a glass transition followed by exothermic enthalpic recovery helps identify amorphous lactose in the sample. Understanding the lactose behavior is imperative as it can prevent the crystallization of amorphous lactose and control water migration during storage at temperatures below the glass transition value. The insight can optimize the stability and quality of dried milk derivatives.

3.5.Effect of pasteurization on free radical scavenging activity of camel milk

Antioxidants compounds are help in preventing, reducing, or repairing damage caused by reactive oxygen species to biomolecules. The results of DPPH antioxidant activity of the pasteurized and unpasteurized milk samples are shown in Figure 3a. A significant difference ($p<0.05$) was observed in

the DPPH scavenging activity between unpasteurized and pasteurized camel milk extracts. The antioxidant activity was significantly ($p>0.05$) increased with increasing concentration of milk sample in pasteurized and unpasteurized camel milk powder. The significantly higher antioxidant activity (70.93%) was found in unpasteurized camel milk samples at 10 mg/ml concentration, which is highest antioxidant activity as compared to the other concentration range in unpasteurized milk samples and unpasteurized milk samples. The reduction of antioxidant activity in pasteurized are associated with the reducing intensity of total casein (β-CN) and bioactive component presence in the camel milk due to heating process (Hamouda *et al*., 2022). These findings are in line with the previous reports of Padhi and Dwivedi (2022), those reported that the duration of thermal treatment is a major factor influencing DPPH scavenging activity by inducing changes in molecular structure or degradation of heat-labile antioxidants (Padhi and Dwivedi, 2022).

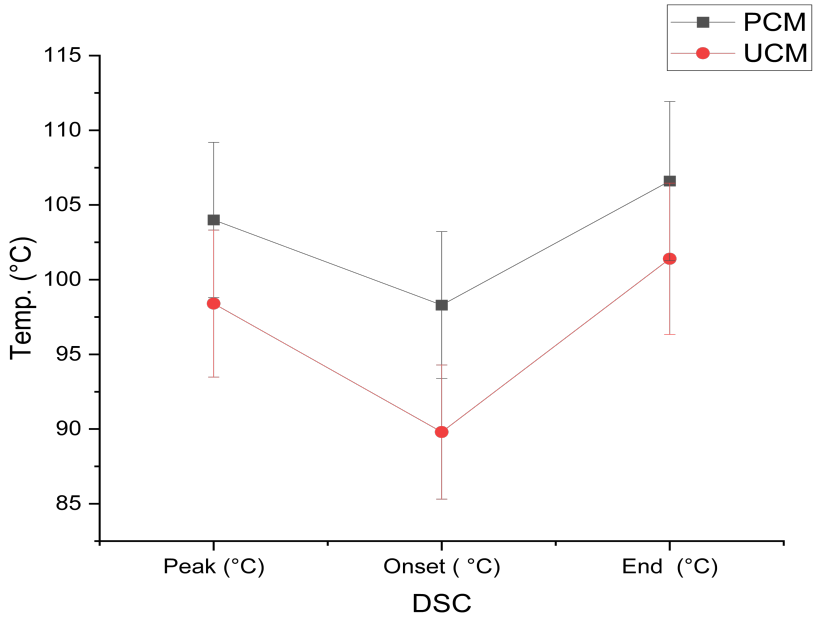
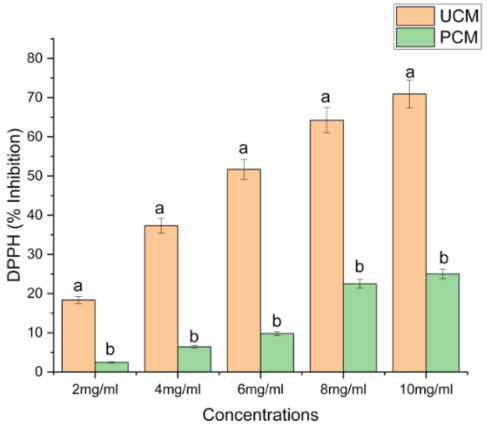
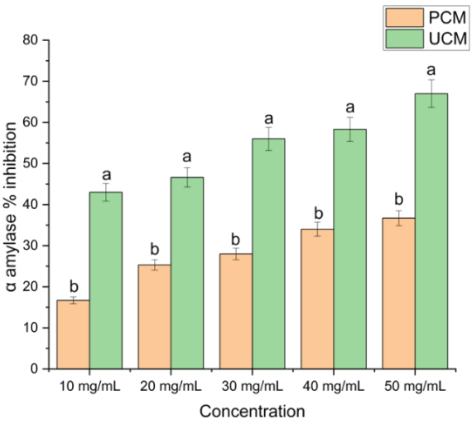


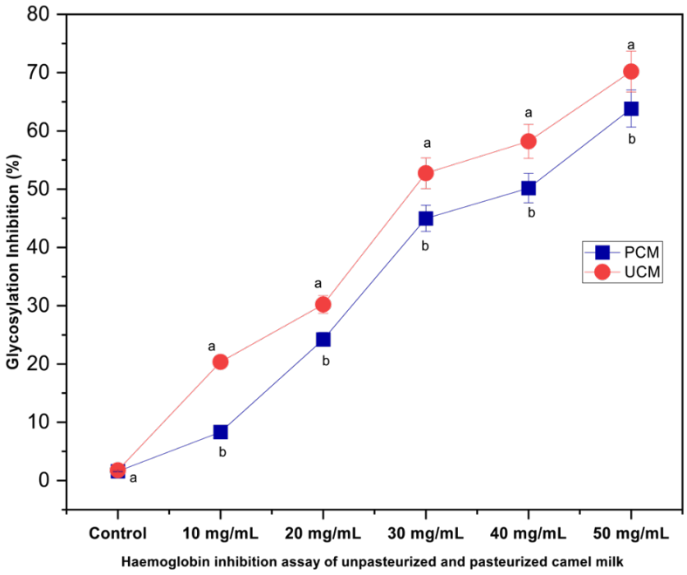
Figure 2. Thermal properties (DSC analysis) of pasteurized and unpasteurized camel milk



3a



3b



3c

Figure 3. DPPH radical scavenging activity (3a), alpha amylase inhibition (3b) and Haemoglobin inhibition assay (3c) of unpasteurized and pasteurized camel milk

3.6.Effect of pasteurization on *in-vitro* antidiabetic potential of camel milk

Camel milk emerges as promising alternative against diabetes and is evident from the present study. Its unique composition, including the presence of insulin-like peptides (Insulin-like Growth Factor 1, Factor 2, Relaxin, and Insulin-like peptide improves glycemic control. The α-amylase inhibitory assay of the pasteurized and unpasteurized milk samples is showed in Figure 3b. The results demonstrated that the methanolic extract of camel milk unpasteurized possessed significant higher inhibitory potential as compared to pasteurized milk sample.

The inhibitory activity was significantly increased with increasing concentration of sample in both pasteurized and unpasteurized milk but the significantly lower found in pasteurized milk (16.7-36.7%) due to eliminated the favorable insulinogenic effects of camel milk (DiGiacomo *et al*., 2022). The highest alpha amylase inhibition activity was found in unpasteurized milk samples at 50 mg/mL concentration (67%) followed by 40 mg/mL (58.3%), respectively. Whereas, the lowest was found in pasteurized milk samples at 10 mg/mL concentration (16.7%), which is lowest alpha amylase activity in both the samples. This might be possible due to thermal degradation of bioactive components, alpha amylase inhibitors and other enzymes. These compounds may help in the control the level of blood sugar. The previous researchers Ayyash *et al*. (2018) and Shukla *et al*. (2023) were reported that bioactive compounds and alpha amylase inhibitor found in the milk are very sensitive to the heat treatment (Gholamhosseinpour *et al*., 2020).

3.7.Effect of pasteurization on Haemoglobin inhibition assay of camel milk

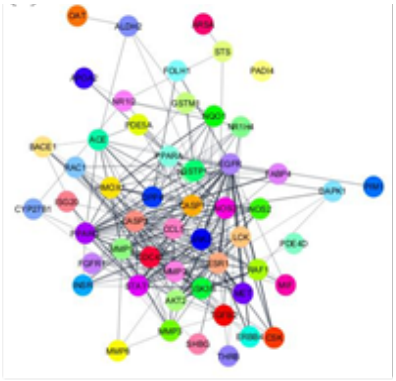
The degree of non-enzymatic hemoglobin glycosylation by camel milk was used to determine its antidiabetic potential. The percentage inhibition of glycosylation was found to be dose-dependent as the concentration of the camel milk increased, the formation of glucose-hemoglobin complexes decreased, leading to a rise in free hemoglobin thereby inhibiting the formation of glycosylated hemoglobin thus pointing towards concentration-dependent reaction. The percentage of inhibition at the concentrations of 10-50 mg/ml by the unpasteurized and

pasteurized camel milk extract showed a concentration-dependent reduction as depicted in Figure 3c. The pasteurized and unpasteurized camel milk showed similar activity of inhibition, which shows that the pasteurization process does not affect the degree of non-enzymatic hemoglobin glycosylation which suggests that the bioactive components responsible for inhibiting glycosylation remain stable during pasteurization (Wilson *et al*., 2007).

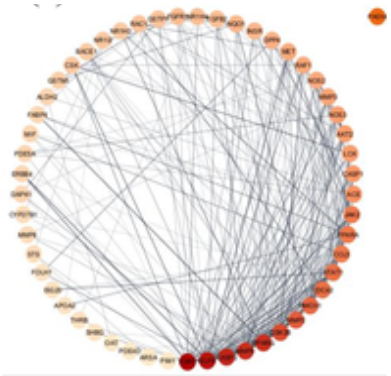
3.8.*In-silico* studies

*3.8.1.*Identification of compound target proteins, DM target genes, and PPI network construction

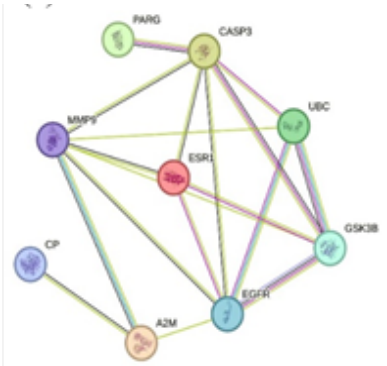
A total of 300 gene targets of selected lactoferrin compounds were predicted by PharmMapper and were matched with proteins related to diabetes mellitus, retrieved from the DisGeNET database, and screened according to the gene-disease association score (Table S1). A total of 53 genes with scores more than 0.1 were screened, and the PPI network was constructed with these selected 53 targets (Figure 4a). The node color for proteins such as ESR1, EGFR, CASP3, MMP9, PPARG, GSK3B, MMP-2, MOX-1, ODC42, and STAT1 are shown comparatively darker, indicating a higher degree of contribution in the disease network (Figure 4b). In-silico analysis illustrated the biological mechanism of lactoferrin by analyzing the protein targets that are regulated directly by lactoferrin through PPI analysis which retrieved the major protein targets that can be targeted to ameliorate diabetes progression.



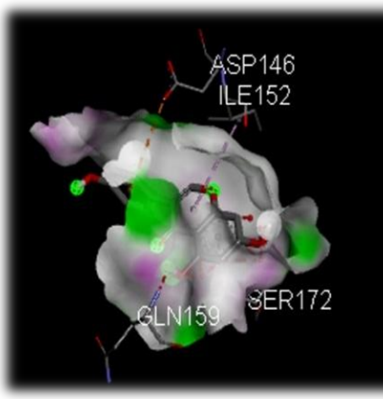
4a



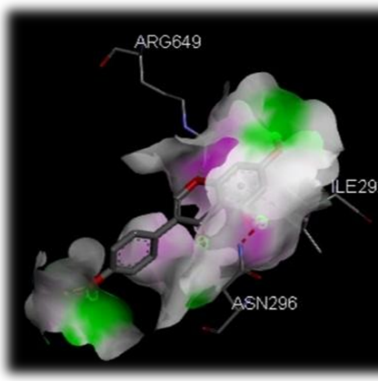
4b



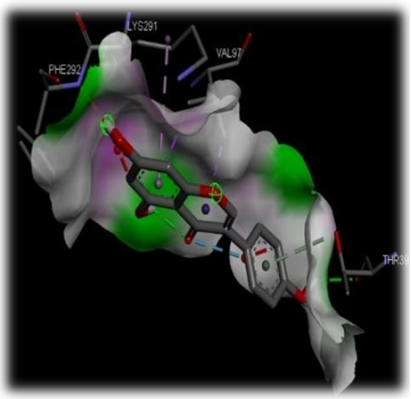
4c



Alpha 2-macroglobulin



Ceruloplasmin
4d



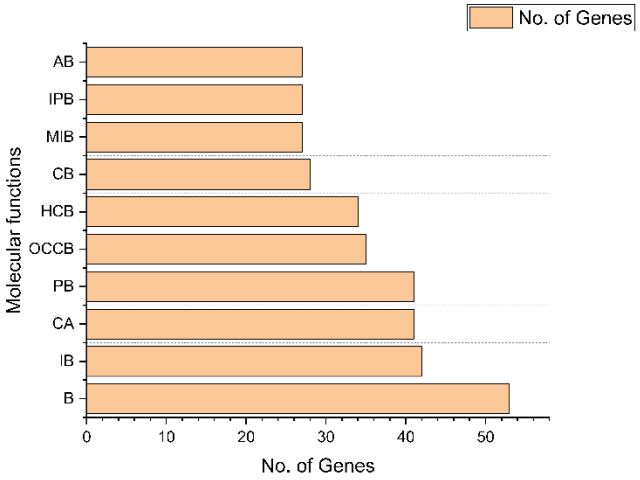
Haptoglobin

Figure 4. Identification of compound target proteins, DM target genes, and PPI network construction

The node color for proteins such as ESR1, EGFR, CASP3, MMP9, PPARG, GSK3B, MMP-2, MOX-1, ODC42, and STAT1 are shown comparatively darker, indicating a higher degree of contribution in the disease network (Figure 4b). *In-silico* analysis illustrated the biological mechanism of lactoferrin by analyzing the protein targets that are regulated directly by lactoferrin through PPI analysis which retrieved the major protein targets that can be targeted to ameliorate diabetes progression.

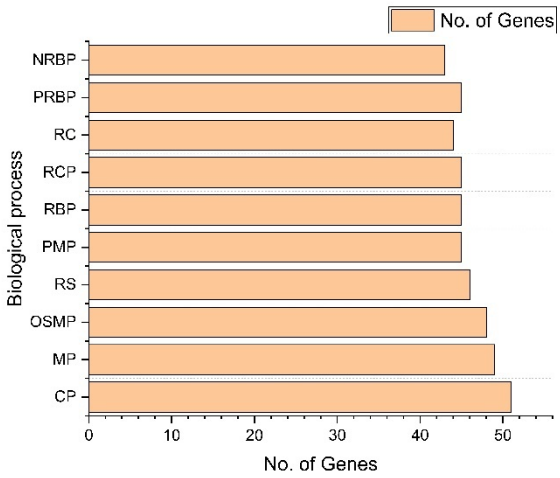
3.8.2.GO and KEGG analysis

GO enrichment analyses of the selected 53 targets were analyzed by Cytoscape, including biological processes, cell components, and molecular function (Figure 5 (a), (b), (c)). Out of 723 GO entries, 86 entries are related to molecular function (Table S2), 627 entries are concerning biological processes (Table S3), and 10 cell components (Table S4) entries were recorded. Out of all these entries involved the top 10 in each category, genes were selected based on p-value and no. of genes, and the graph was plotted. Further, to establish the association of target proteins with the biological pathways, a target pathway network was established using Cytoscape. KEGG analysis revealed 115 pathways (Table S5) and related 53 protein targets were screened according to the significant p-value. These include metabolic pathways, MAPK signaling pathways and cancer pathways, AGE-RAGE signaling pathways, and PI3K-Akt signaling pathways (Figure 5d). Hence the pathway analyses suggest that targeting proteins can regulate the different metabolic pathways, particularly MAPK signaling pathways, AGE-RAGE signaling pathways, and PI3K-Akt signaling pathways that are involved in diabetes progression.



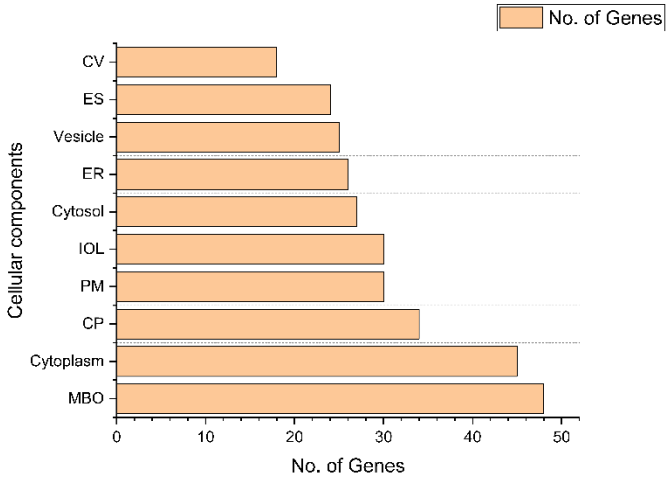
a

Where, B= Binding, IB = Ion binding, CA = Catalytic activity, PB = Protein binding, OCCB = Organic cyclic compound binding, HCB = Heterocyclic compound binding, CB = Cation binding, MIB = Metal ion binding, IPB = Identical protein binding, AB = Anion binding



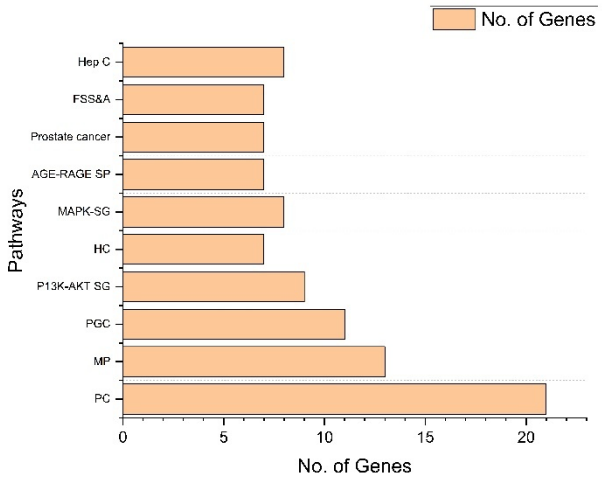
b

Where, CP = Cellular process, MP = Metabolic process, OSMP = Organic substance metabolic process, RS = Response to stimulus, PMP = Primary metabolic process, RBP = Regulations of biological process, RCP = Regulations of cellular process, RC = Response to chemical, BRBP = Positive regulation of biological process, NRBP = Negative regulation of biological process



c

Where, BBO = Membrane bounded organelle, CP = Cell periphery, PM = Plasma membrane, IOL = Intracellular organelle lumen, ER = Extracellular region, ES = Extracellular space, CV = Cytoplasmic vesicle



d

Where, PC = Pathways in cancer, MP = Metabolic pathway, PGC = Proteoglycans in cancer, P13K-AKT SP = P13K-Akt signaling pathway, HC = Hepatocellular carcinoma, MAPK- SG = MAPK signaling pathway, AGE-RAGE SP = AGE-RAGE signaling pathway, FSS&A = Fluid shear stress and atherosclerosis, Hep C= Hepatitis C

Figure 5. GO analysis of the 53 target proteins identified involves examining their associations with (a) molecular functions, (b) biological processes and (c) cellular components, and molecular functions. This analysis aims to characterize how these proteins contribute to various biological functions and processes within the cell; (d) Histogram: KEGG analysis of potential target pathways associated with diabetes progression

3.8.3.Molecular docking

Lactoferrin in camel milk plays a crucial role in promoting health due to its potent antimicrobial, antiviral, and anti-inflammatory properties (de Paula Silva *et al*., 2021). With a higher concentration in camel's milk, Lactoferrin helps boost the immune system by inhibiting the growth of harmful bacteria, viruses, and fungi through its ability to bind iron, which pathogens need for survival (Berlutti *et al*., 2011). It also reduces inflammation, supports gut health by fostering beneficial bacteria, and serves as a natural defense against chronic diseases and infections. The therapeutic potential of lactoferrin in camel milk has garnered attention for treating conditions such as diabetes and autoimmune disorders, making it a valuable functional food with broad health benefits (Swelum *et al*., 2021). Earlier studies have documented significantly elevated levels of Alpha 2-Macroglobulin (AMG), Ceruloplasmin (Cp), and Haptoglobin (Hp) in both Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) (de Paula Silva *et al*., 2021). These proteins are upregulated in response to diabetic conditions, indicating the underlying pathophysiological changes. Therefore, lactoferrin a bioactive compound present in camel milk has been evaluated for its interaction with these upregulated proteins.

Docking studies reveal that lactoferrin exhibits a strong binding affinity for haptoglobin, with a binding energy of -7.4 kcal/mol, suggesting a robust interaction (Table 4). The binding affinity with ceruloplasmin is slightly lower at -7.3 kcal/mol but still significant. The interactions were further analyzed using Discovery Studio, which allowed visualization of hydrogen bonding and the involved amino acid residues. These visualizations (Figure 4 (d)) provide insight into

the specific molecular interactions between lactoferrin and the target proteins, highlighting potential mechanisms through which lactoferrin may influence diabetic pathology. The findings suggest that lactoferrin could play a role in modulating the levels of these key proteins, potentially contributing to its therapeutic effects in diabetes management. Hence, molecular docking suggested that lactoferrin can bind to the proteins and may suppress its expression.

Table 4. Binding studies of Lactoferrin from Camel milk towards differentially regulated protein in Diabetes Mellitus

Mode	Affinity (Kcal/mol)	Distance from RMSD l.b.	Best mode RMSD u.b.	PDB-ID	PubChem
1	-7.4	0.00	0.00	4XOL (Haptoglobin)	
1	-6.7	0.00	0.00	2P9R (Alpha-2-macroglobulin)	Lactoferrin-ID: 5280961
1	-7.3	0.00	0.00	4ENZ (Ceruloplasmin)	

RMSD: Root mean square deviation

The results demonstrated that lactoferrin interacted strongly with haptoglobin with the best Gibb's free energy. Reports documented that the haptoglobin phenotype has been identified as an independent risk factor for cardiovascular disease in individuals with diabetes. Therefore, targeting haptoglobin via lactoferrin in camel milk may help reduce diabetic complications. The mechanistic action of lactoferrin in camel milk in silicon studies illustrates its protein targets involved in diabetes pathogenesis. Docking studies indicated its interaction with haptoglobin that maintains homeostasis during diabetes pathogenesis. Thus, camel milk stands out as a viable solution with significant economic and therapeutic potential.

4.Conclusion

This study highlights the impact of pasteurization on the physicochemical and functional properties of camel milk powder. While pasteurized camel milk exhibited slightly higher fat content and a 1.2-fold increase in leucine, nevertheless, significantly altered structural properties such as true density, angle of repose, and porosity. Functionally, unpasteurized camel milk demonstrated superior antioxidant activity and significantly higher alpha-amylase inhibition, indicating a stronger antidiabetic potential. Molecular docking analysis revealed strong binding interactions of lactoferrin with haptoglobin and ceruloplasmin, reinforcing the bioactive potential of camel milk. These findings suggest that camel milk, particularly in its unpasteurized form, could serve as a valuable functional food for managing oxidative stress and diabetes. Further research is needed to explore its sensory attributes and validate flavor changes induced by pasteurization.

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