A comparative study on production and characterization of antihypertensive and anti-diabetic peptides from buffalo (*Bubalus bubalis*) and camel milk (*camelus dromedaries*) fermented with *Limosilactobacillus fermentum* and *Saccharomyces cerevisiae*

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Introduction

- Milk is an essential food in the diet of the majority of the world's residents, and due to its significant nutritional benefits, it is often recognized as a complete diet. Protein, fat, carbohydrates, vitamins, minerals are all present in sufficient amounts in milk.
- Milk-borne bioactive peptides have a variety of health benefits, including antipyretic, anti-carcinogenic, anti-microbial, anti-hypertensive, immunomodulatory etc.
- The present study was designed for a comparative assessment of fermented buffalo and camel milk with ACE-inhibitory and anti-diabetic properties and the release of bioactive peptides using Lactobacilli and yeast culture

Objectives

- To evaluate the ACE inhibitory and anti-diabetic activities of buffalo and camel milk fermented using (KGL4) in combination with (WBS2A)
- To optimize the production of peptides in fermented buffalo and camel milk
- To purify and characterize the ACE inhibitory and anti-diabetic peptides produced from fermented buffalo and camel milk

Methods

- ACE inhibitory was carried out according to the method described by Hati *et al*. (2015)
- α-amylase inhibition activity was evaluated by following the method described by Ademiluyi & Oboh (2013) & Telagari & Hullatti (2015). α-glucosidase inhibition activity was evaluated by following the method described by Yamaki & Mori (2006) and Shai *et al*. (2011). Pancreatic lipase inhibitory activity was carried out by following the method described by Kurihara *et al*. (2003) & Sergent *et al*. (2012)
- Proteolytic activity was measured by O-phthalaldehyde method (Quantitative method) described by Hati *et al*. (2015) and Solanki *et al*. (2017)
- Purification of peptides through SDS-PAGE and 2D gel electrophoresis (Laemmli, 1970; Carrasco-Castilla *et al*., 2012; Yang *et al*., 2014)
- Characterization of ACE inhibitory and anti-diabetic peptides through RP-LC/MS and database matching (Parmar, 2017)
- Fourier Transform-Infrared Spectroscopy (FTIR) Evaluations according to the procedure described by Leon-Lopez *et al*. (2020)
- MTT assay was carried out according to Khare *et al*. (2020)

Results

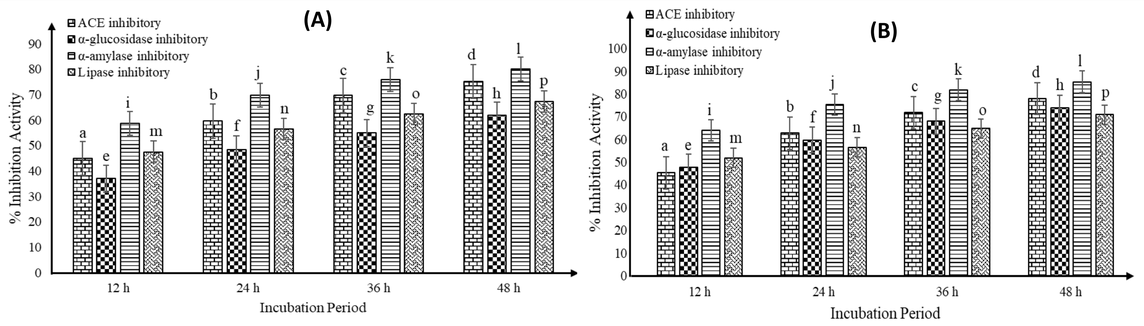

Figure 1 :- ACE-inhibitory & anti-diabetic activity (%) of fermented buffalo milk (A) & camel milk (B)

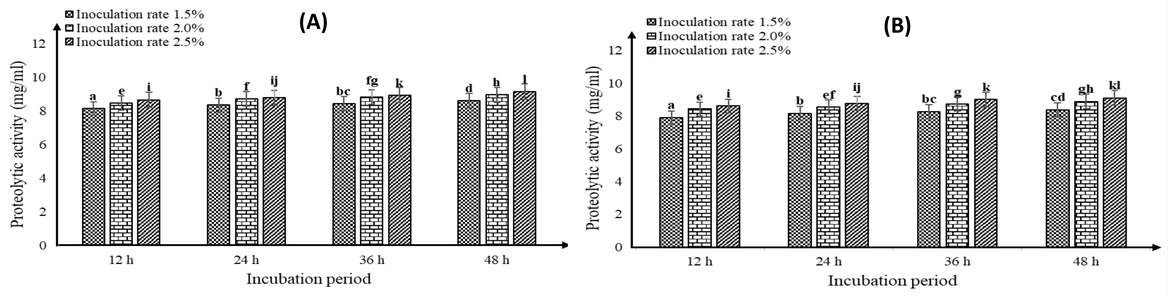

Figure 2 :- Effect of inoculation rates & incubation period on proteolytic activity of fermented A) buffalo & B) camel milk

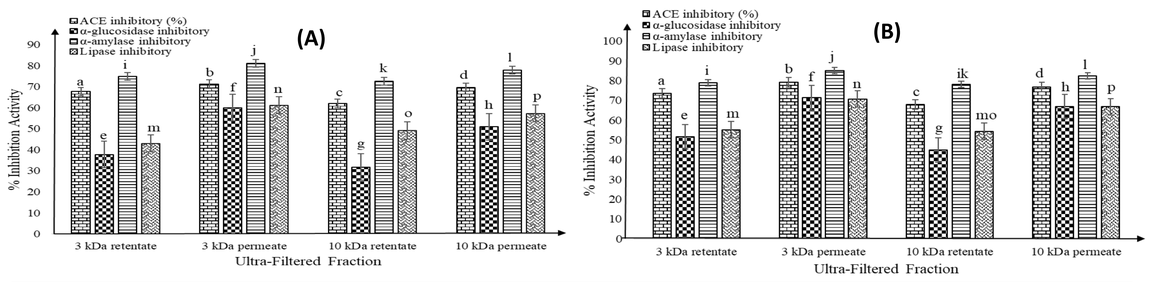

Figure 3 :- ACE-inhibitory and anti-diabetic activities of ultra-filtered fractions (3 kDa and 10 kDa permeate and retentate) from fermented (A) buffalo and (B) camel milk

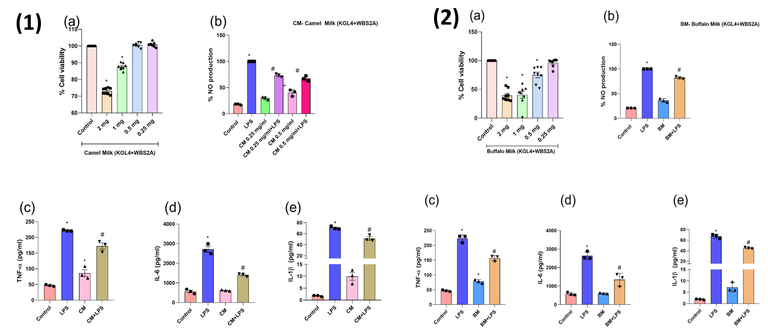

Figure 8 :- Effect of the fermented 1) buffalo milk and 2) camel milk on (A) Cell viability (MTT assay) of murine macrophages RAW 264.7 cells, (B) Nitric oxide productions (C) TNF-α; (D) IL-6; and (E) IL-1β measured in the supernatants of LPS-stimulated (RAW 264.7) cells

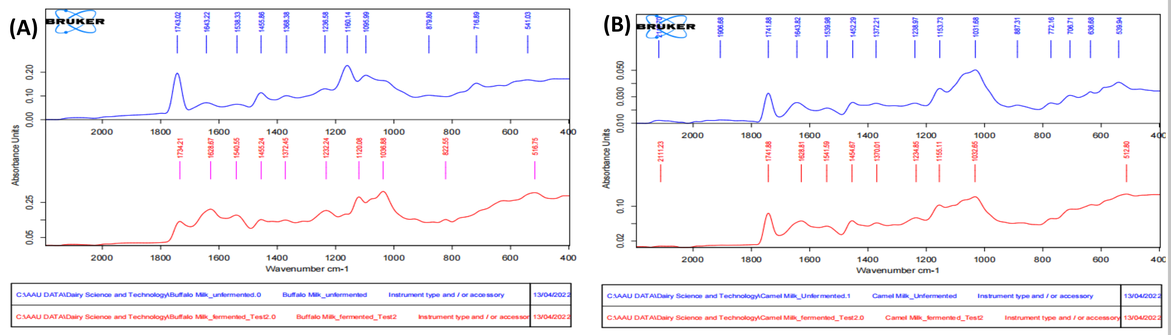

Figure 9 :– FTIR spectra of unfermented (Blue) & fermented (Orange) (A) Buffalo and (B) Camel milk using KGL4+WBS2A culture

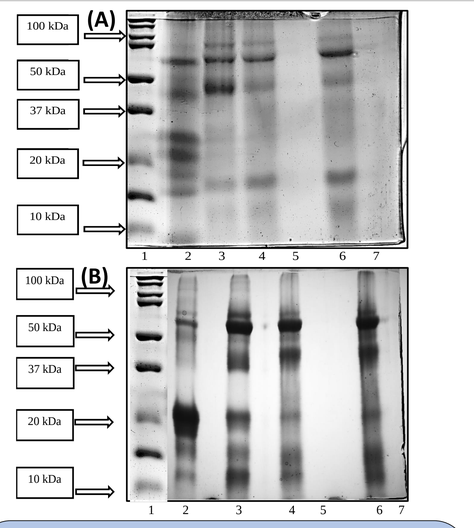

Figure 4 :- Protein profile of fermented A) Buffalo milk & B) Camel milk revealed by SDS-PAGE (1: Protein ladder, 2: Unfermented milk, 3: Fermented milk, 4: 3 kDa Retentate, 5: 3 kDa Permeate, 6: 10 kDa Retentate, 7: 10 kDa Permeate)

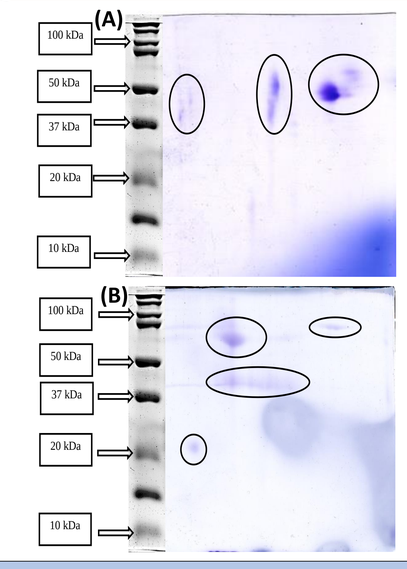

Figure 5 :- 2D Gel Electrophoresis of fermented A) Buffalo milk & B) Camel milk

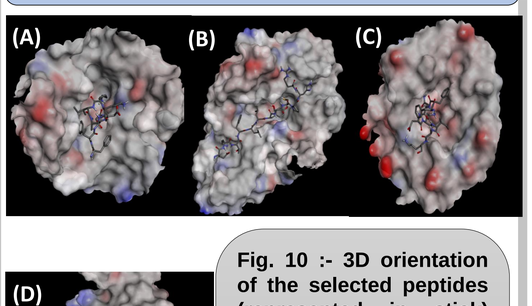

Fig. 10 :- 3D orientation of the selected peptides (represented in stick) FFNDKIAK & EKTFLLYSCPHR, respectively in the active pockets of human pancreatic amylase (A & B) & maltase glucoamylase (C & D)

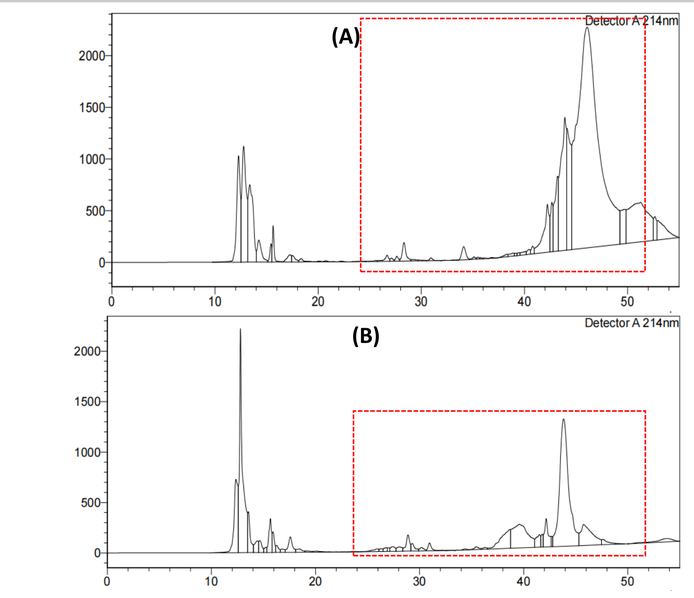

Figure 6 :- RP-HPLC chromatogram of Unfermented A) Buffalo & B) Camel milk

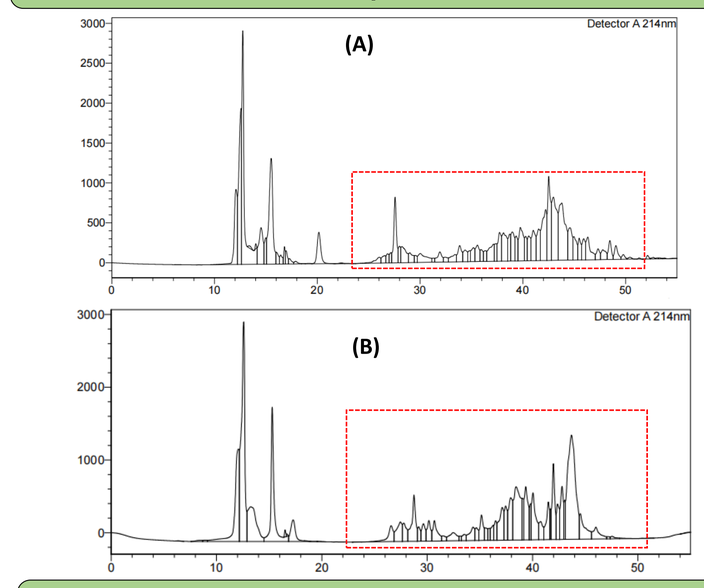

Figure 7 :- RP-HPLC chromatogram of fermented A) Buffalo & B) Camel milk

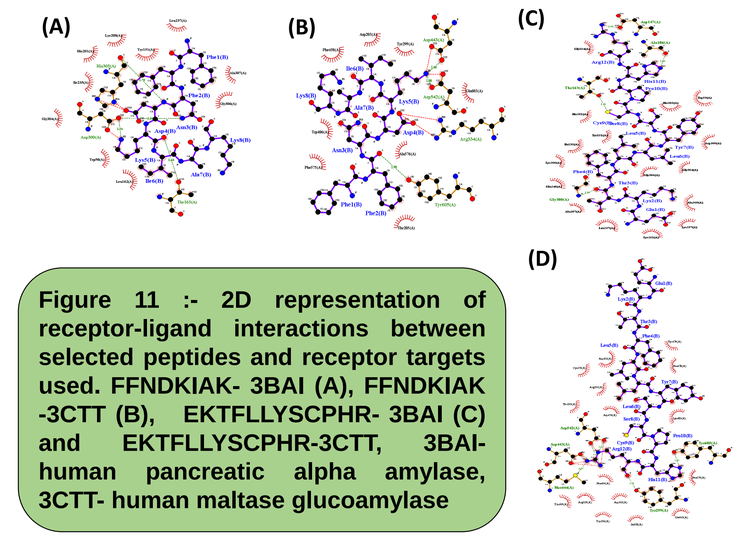

Figure 11 :- 2D representation of receptor-ligand interactions between selected peptides and receptor targets used. FFNDKIAK- 3BAI (A), FFNDKIAK -3CTT (B), EKTFLLYSCPHR- 3BAI (C) and EKTFLLYSCPHR-3CTT, 3BAI- human pancreatic alpha amylase, 3CTT- human maltase glucoamylase

Discussion

- ACE inhibitory and anti-diabetic activity of buffalo milk and camel milk fermented using *Limosilactobacillus fermentum* (KGL4) in combination with *Saccharomyces cerevisiae* (WBS2A) found to increase significantly with incubation periods.
- Inoculation rate of 2.5% (v/v) and incubation period of 48 h were optimized through OPA method.
- Unfermented buffalo and camel milk SDS-PAGE study shown additional protein bands than fermented buffalo & camel milk which shows high proteolytic activity of cultures in both milk have during fermentation.
- Peptide sequences with high peptide ranking scores (>0.450) were found in 2D-PAGE of fermented buffalo and camel milk i.e., SCQAQPTTMTR, EMPFPK, TTMPLW, HPHPHLSFMAIPPK, FFNDKIAK, ALPMHIR, IPAVFK, LDQWLCEK, and AVPYPQR from the fermented buffalo milk, and TDVMPQWW, EKTFLLYSCPHR, SSHPYLEQLY, IDSGLYLGSNYITAIR, and FDEFLSQSCAPGSDPR from the fermented camel milk.

Conclusion

- ACE inhibitory and anti-diabetic activity (α-glucosidase, α-amylase & lipase inhibitory activity) were found higher in fermented camel milk compared to fermented buffalo milk
- Buffalo and camel milk Fermented using KGL4+WBS2A can be used as a functional food with good ACE inhibitory and anti-diabetic activity

Key Message

Limosilactobacillus fermentum (KGL4) and *Saccharomyces cerevisiae* (WBS2A) fermented buffalo and camel milk produced bioactive peptides may help to manage hypertension and diabetes.