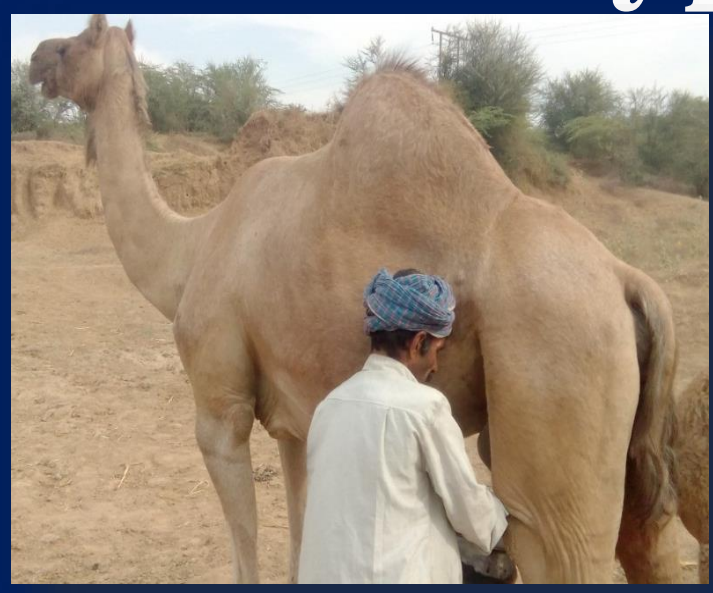


Antihypertensive peptides derived from fermented Camel milk by proteolytic Lactobacilli (*in silico* & *in vitro* study)

Divyangkumar Solanki, Subrota Hati, Amar Sakure and JB Prajapati
Anand Agricultural University, Gujarat, India.

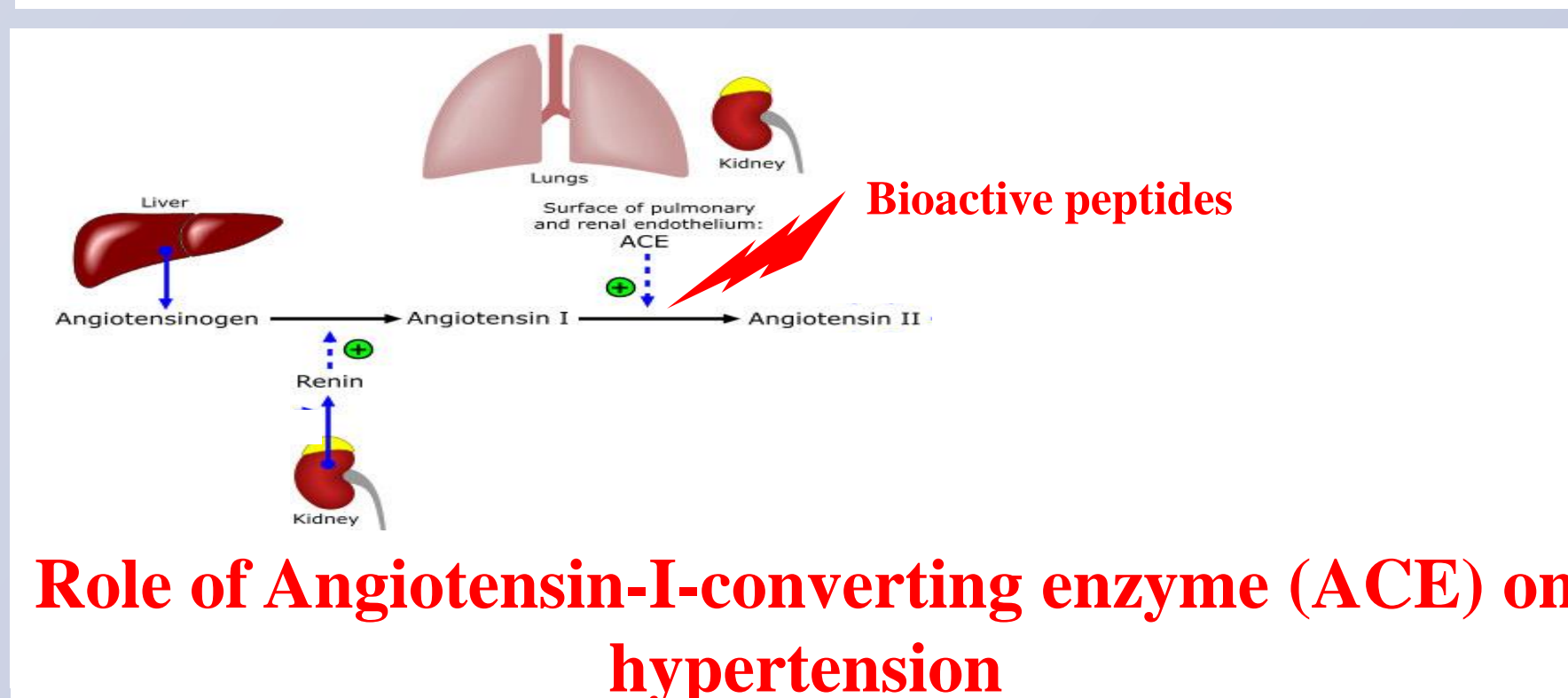


Introduction

- ✓ Camel milk is known for different health attributes like anti-diabetic, antimicrobial and antioxidant etc.
- ✓ Fermented camel milk is known as probiotic with unique antibodies and medicinal properties.
- ✓ Biologically active peptides are generated during milk fermentation by proteolytic Lactic cultures.
- ✓ ACE-inhibitory peptides produced by fermentation of milk are reported to reduce hypertension without any side effects.
- ✓ Present study was designed to purify and characterize the novel ACE-inhibitory peptides from fermented camel milk (*Camelus dromedarius*) using potent proteolytic lactic cultures.

Objectives

- ✓ To evaluate the PepX and ACE-inhibitory activity of lactic cultures.
- ✓ To optimise the growth conditions for the production of peptides.
- ✓ To determine the relative proteolytic activity of lactic cultures.
- ✓ To purify and characterize the novel ACE-inhibitory peptides from fermented camel milk.



Role of Angiotensin-I-converting enzyme (ACE) on hypertension

Methods

- ✓ PepX activity was carried out following Donkor *et al.* (2007).
- ✓ ACE-inhibitory was determined according to Hati *et al.* (2015).
- ✓ Growth conditions (i.e., inoculation rate and incubation period) for the production of peptides were optimized according to o-phthaldialdehyde (OPA) method (Donkor *et al.* 2007).
- ✓ Relative proteolytic activity was carried out following Vasiljevic and Jelen (2002).
- ✓ Di/Ti peptidase activity was also determined (Donkor *et al.* 2007).
- ✓ Peptide extraction from freeze dried fermented camel milk powder according to the method of Gibbs *et al.* (2004).
- ✓ Fractionation of peptides through RP-HPLC analysis (Rodriguez-Figueroa *et al.* 2012).
- ✓ Purification of peptides through SDS-PAGE and 2D gel electrophoresis [Carrasco-Castilla *et al.* (2012); Yang *et al.* (2014)].
- ✓ Amino acid characterization of ACE-inhibitory peptides through RP-LC/MS and database matching (Tagliazucchi *et al.* 2016).

Results

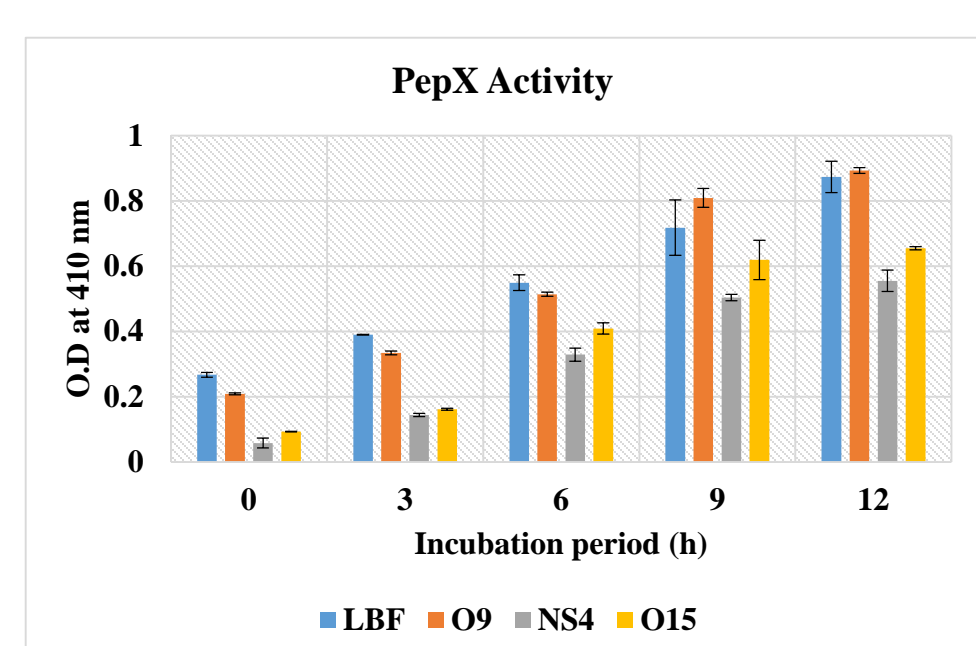


Figure 1- PepX activity of lactic cultures

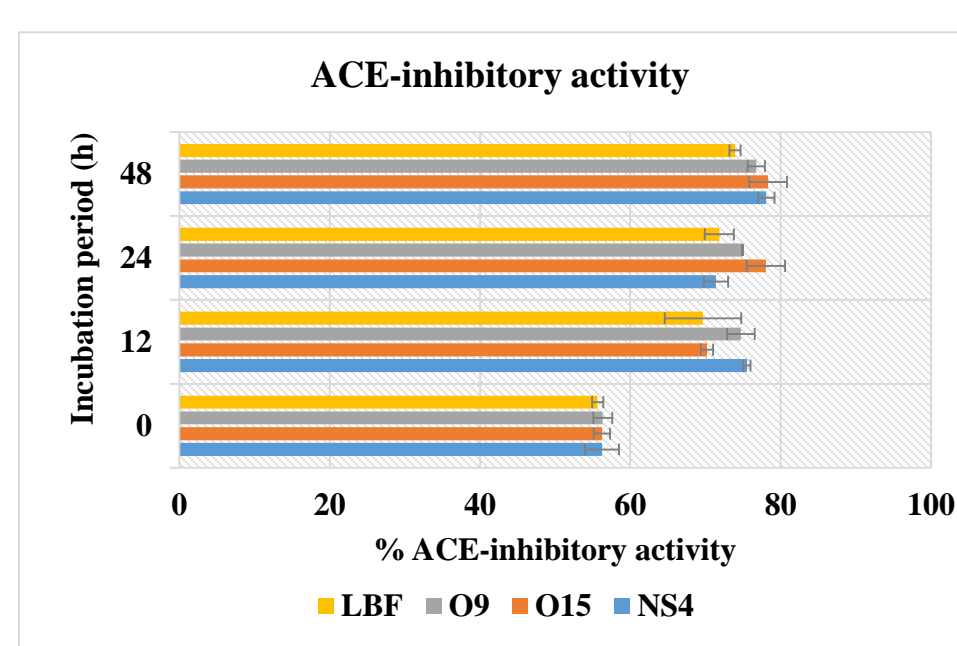


Figure 2- ACE-inhibitory activity of lactic cultures

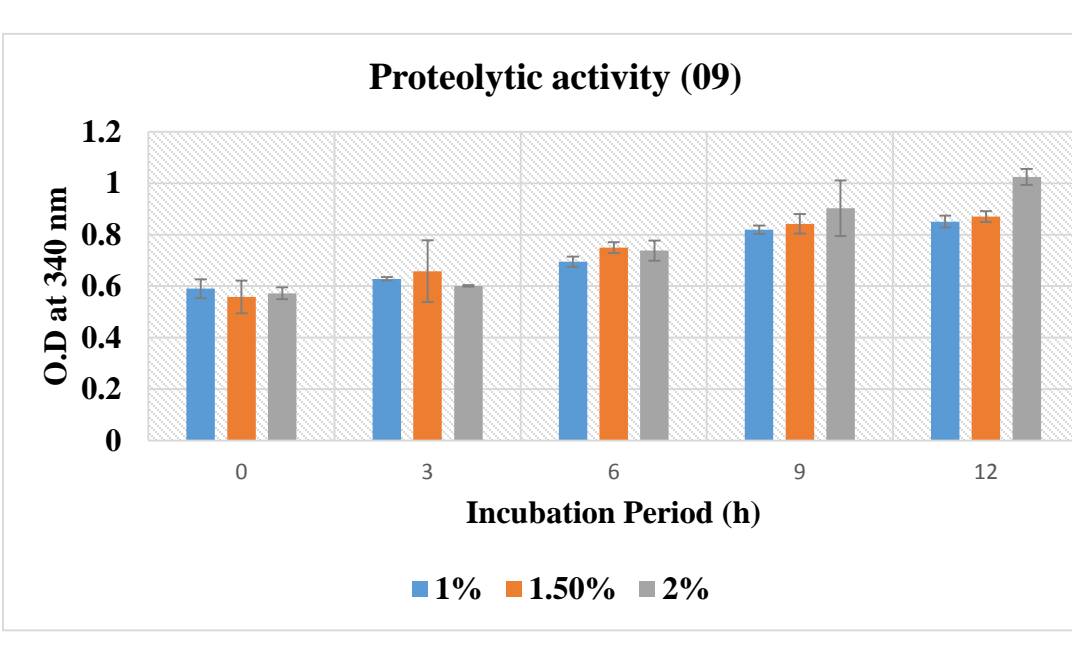


Figure 3- Proteolytic activity of O9 under optimized growth conditions

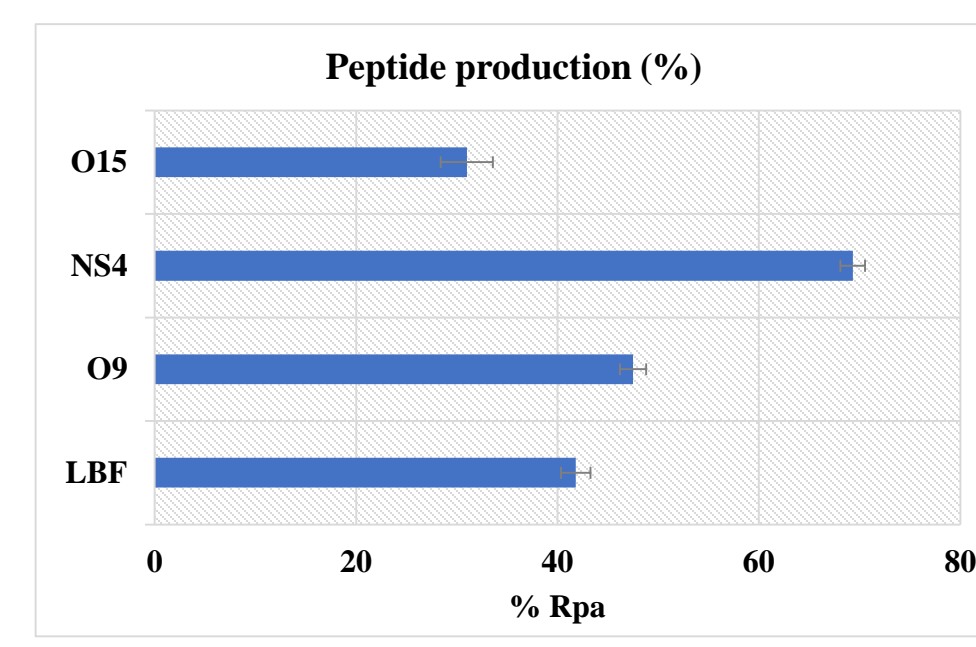


Figure 4- Relative proteolytic activity under optimized growth conditions

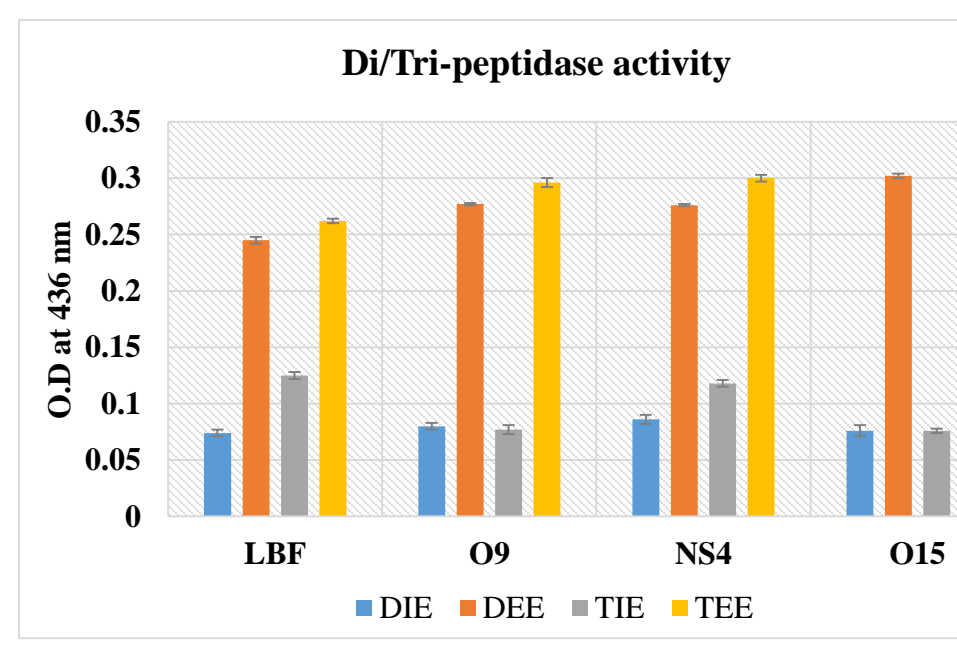


Figure 5- Di/Tri-peptidase activity of lactic cultures

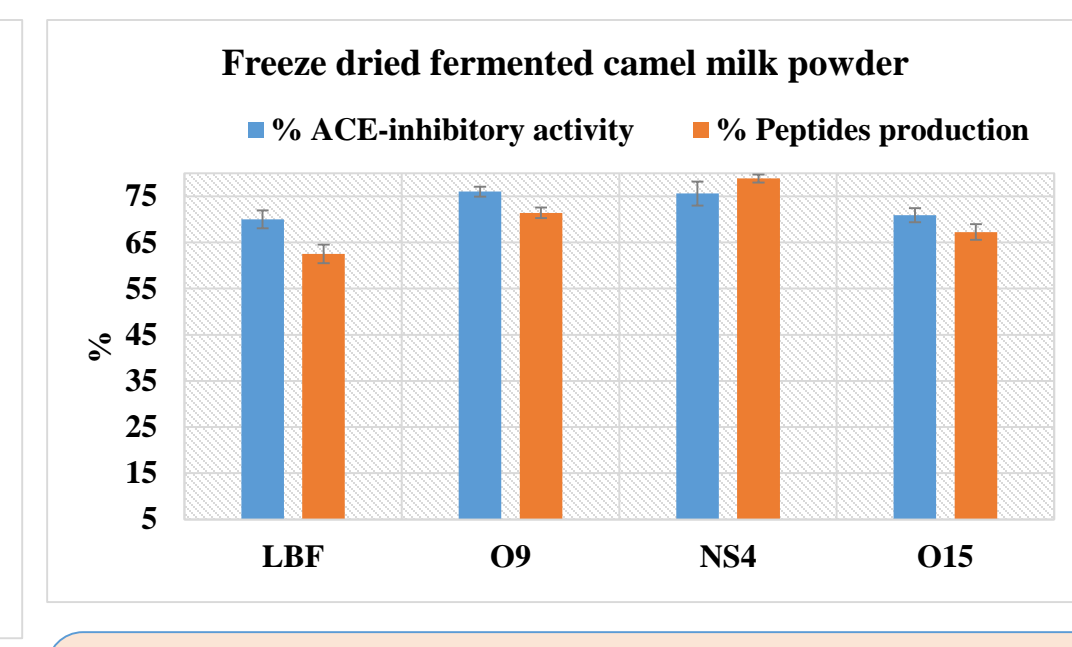


Figure 6- ACE-inhibitory activity and peptide production from freeze dried fermented camel milk powder under optimized growth conditions

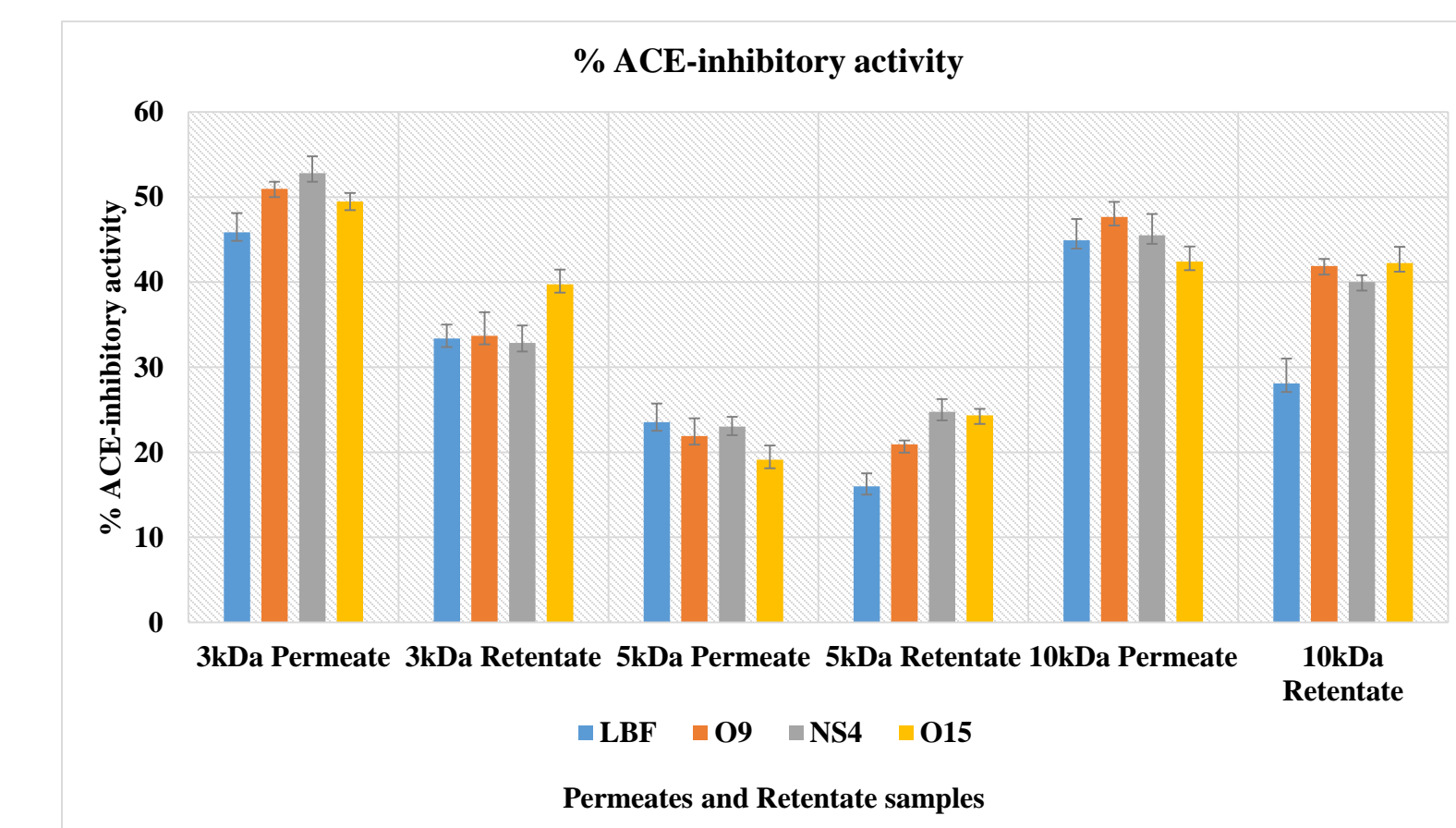


Figure 7- ACE-inhibitory activity of retentate and permeates of 3 kDa, 5 kDa and 10 kDa after fractionation under optimized growth conditions

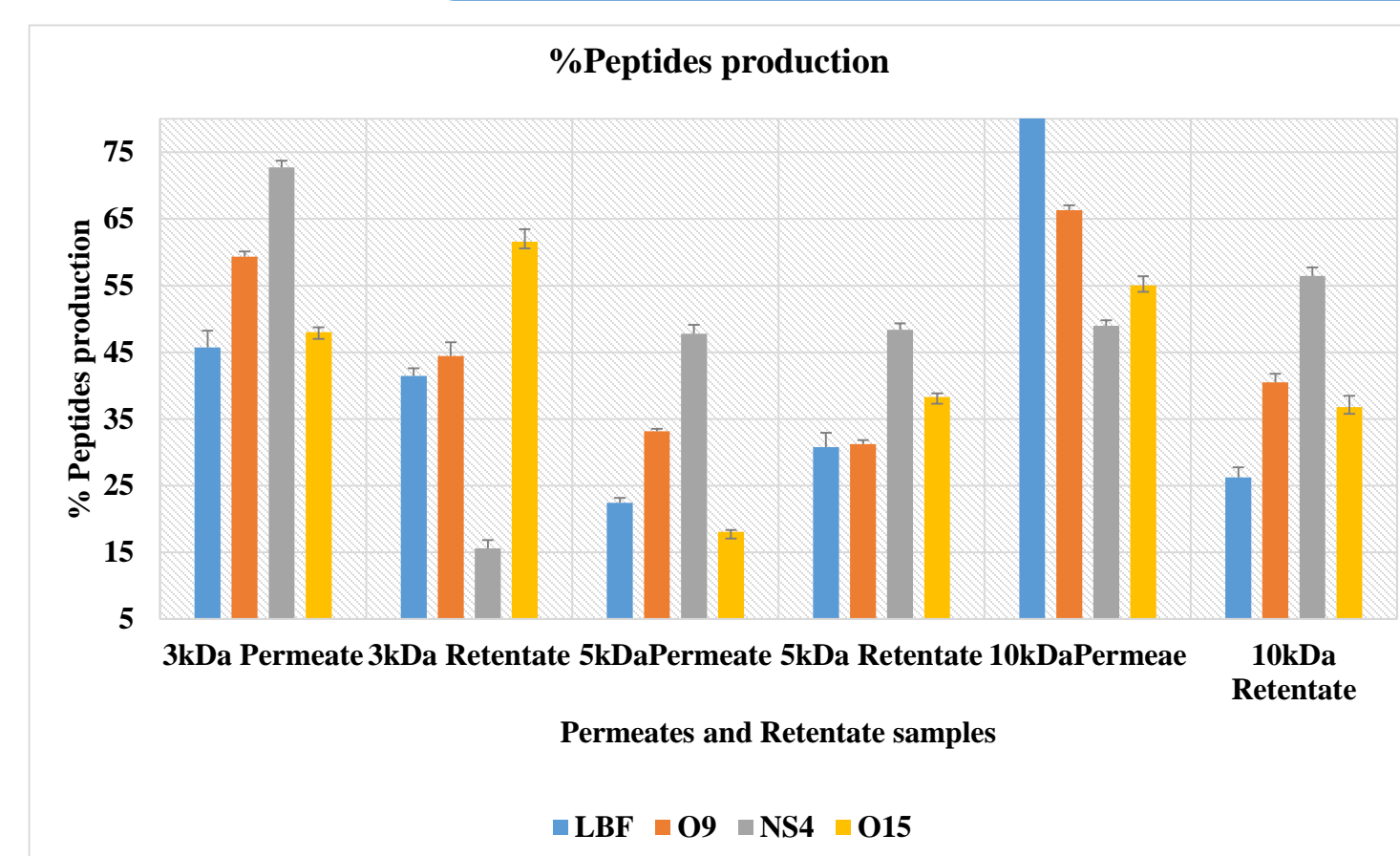


Figure 8- %peptide production of retentate and permeates of 3 kDa, 5 kDa and 10 kDa after fractionation under optimized growth conditions

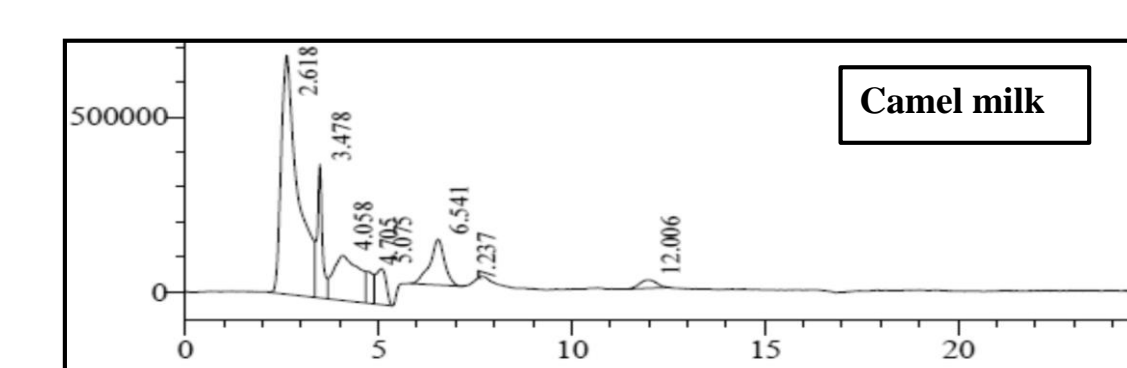


Figure 9- RP-HPLC chromatogram of Unfermented camel milk

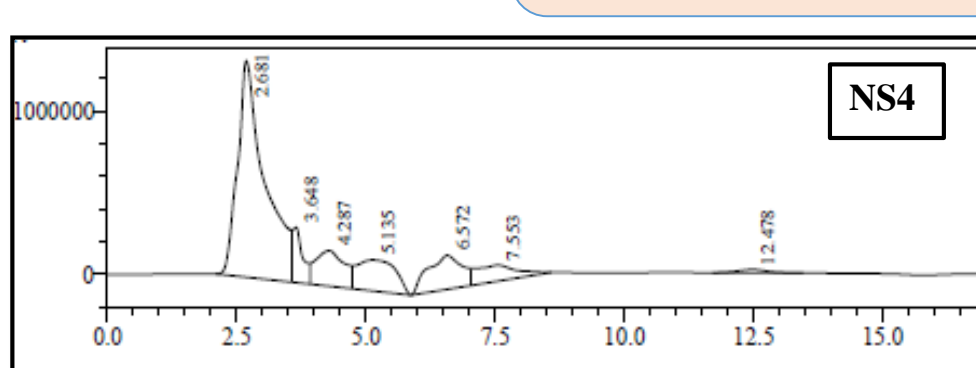


Figure 10- RP-HPLC chromatogram of NS4 fermented camel milk

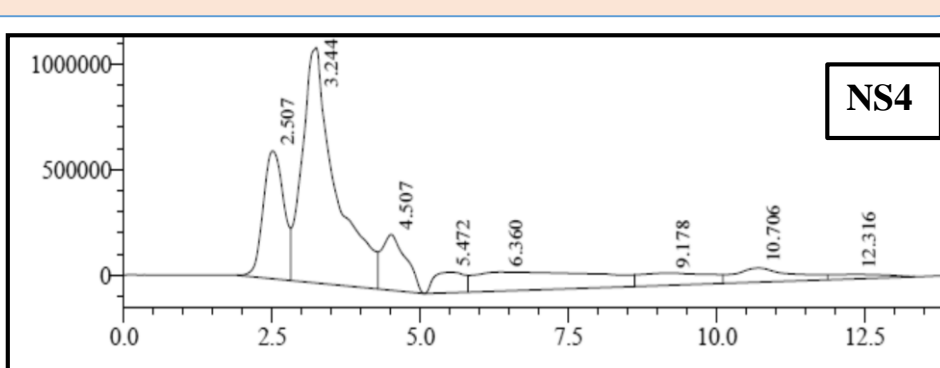


Figure 11- RP-HPLC chromatogram of 10 kDa permeate of fermented camel milk under optimized growth conditions

Results

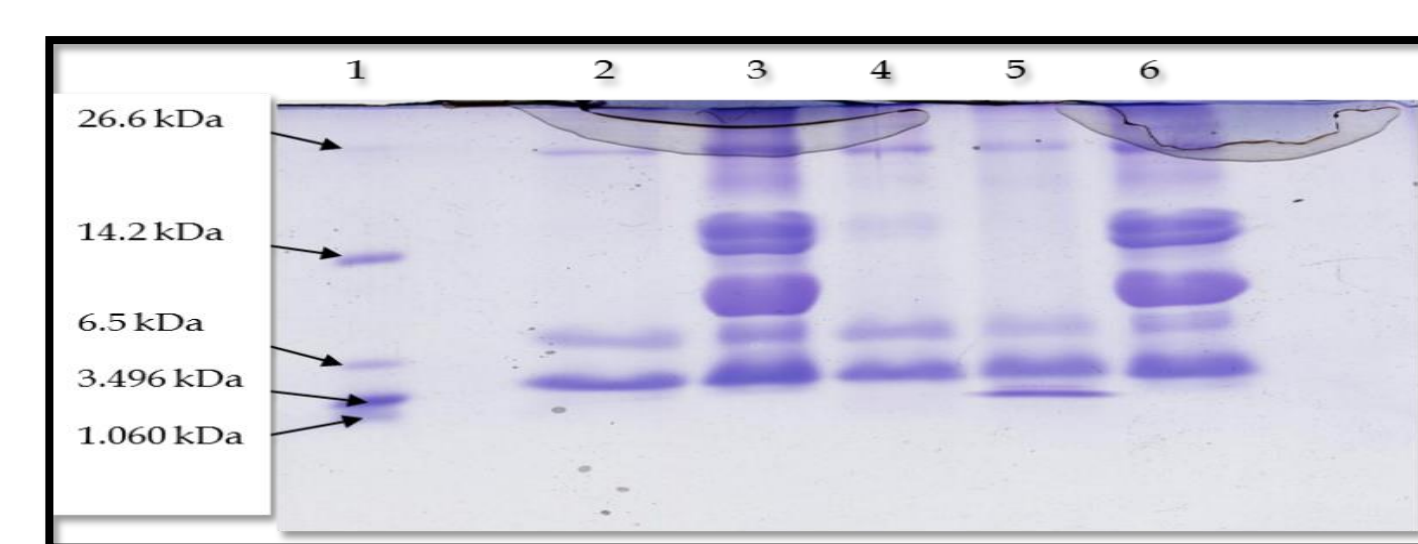


Figure 12- Protein profile of fermented camel milk revealed by SDS-PAGE. Lane 1-Marker; lane 2-LBF; lane 3-NS4; lane 4-O9; lane 5-O15; lane 6-unfermented camel milk (C).

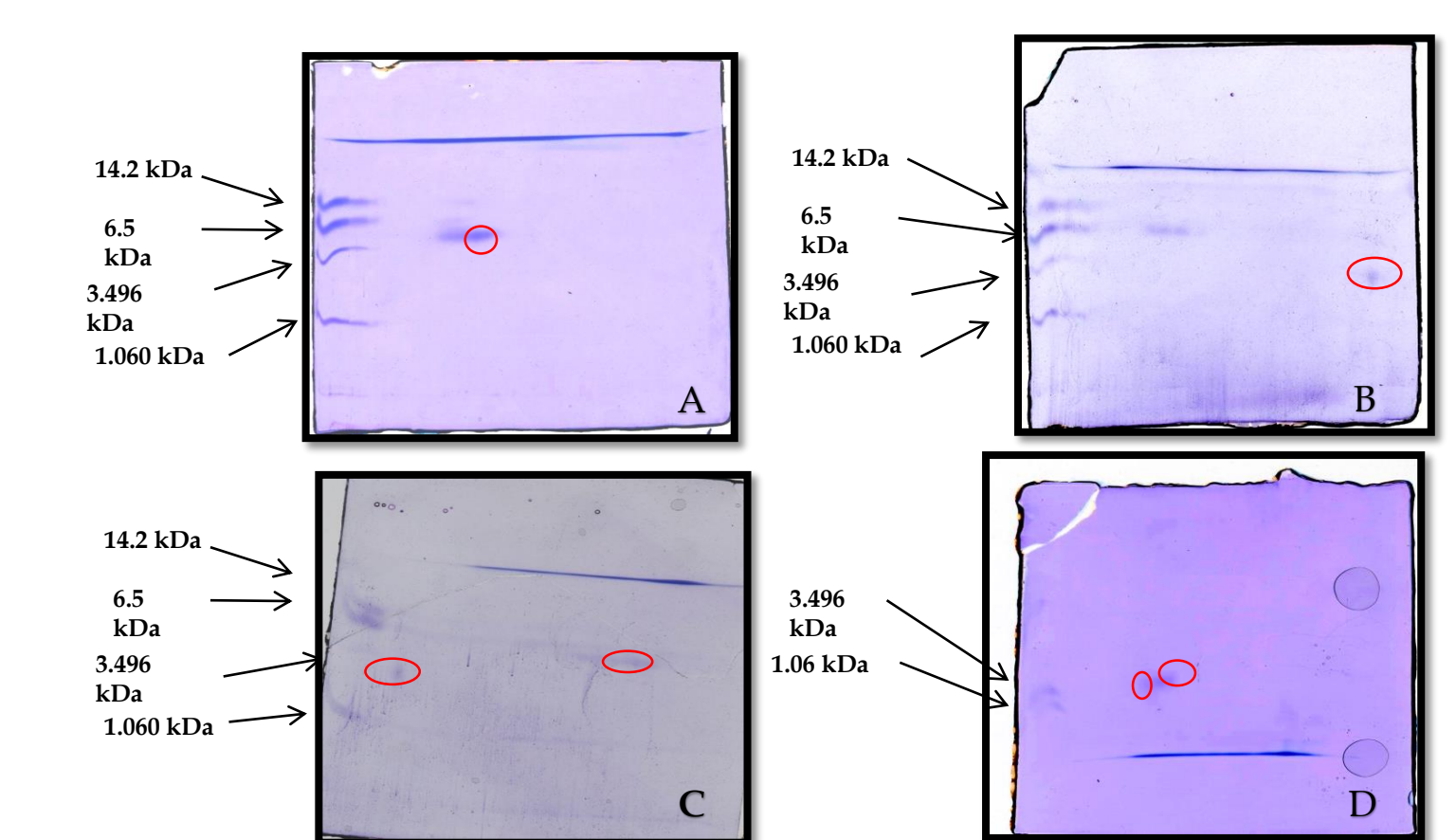


Figure 13- Two dimensional Gel Electrophoresis of 3 kDa permeate samples A: O15, B: O9, C: NS4, D: LBF

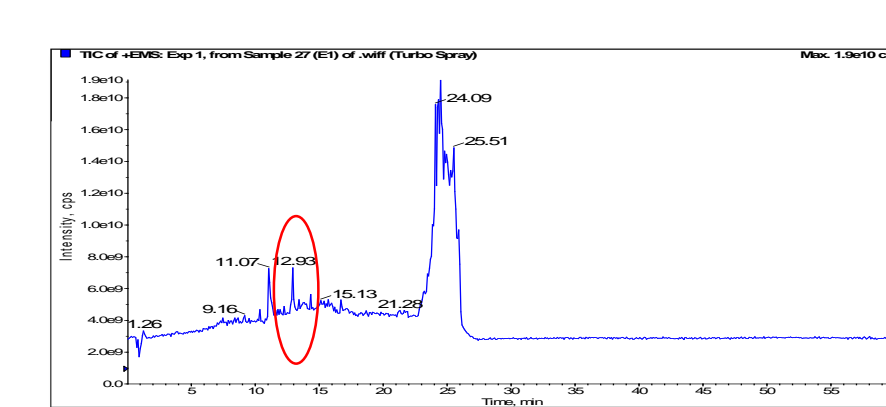


Figure 14- The Total ion chromatogram of fraction E1 of lactic culture LBF generated by EMS to EPI scan in LC-MS. It was identified as QSAPGNEAIPP.

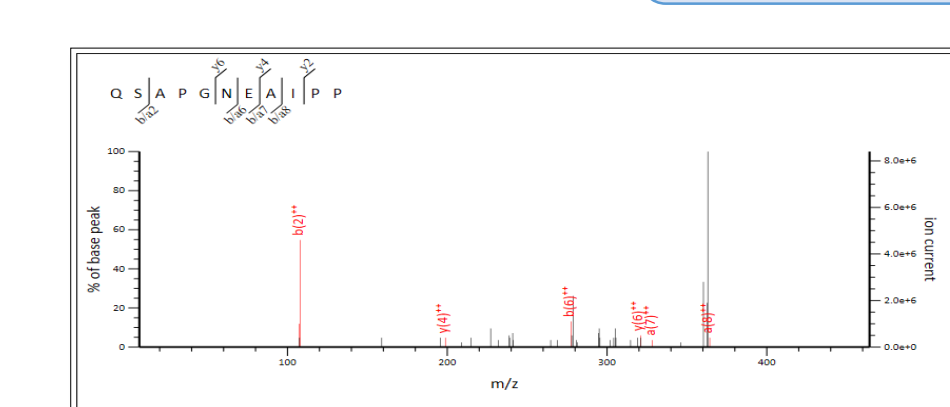


Figure 15- MS/MS spectrum of fraction E1 inspected in MASCOT database.

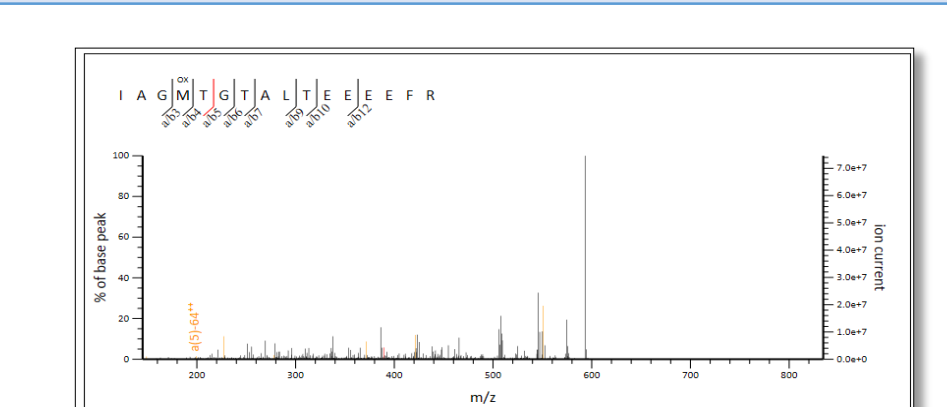


Figure 16- MS/MS spectrum of trypsin digested protein spot of O9 inspected in MASCOT database.

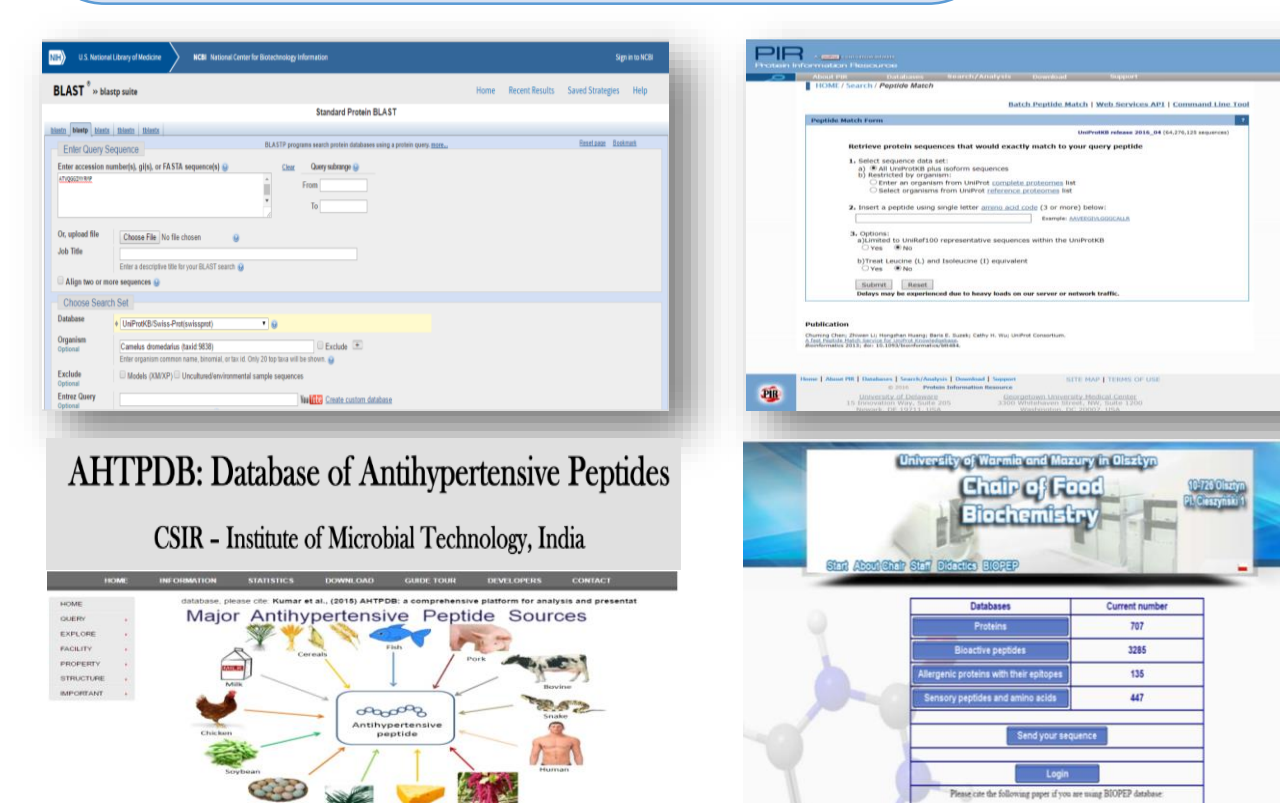


Figure 17- Blast p tool (NCBI) and PIR database to confirm peptides belongs to camel milk protein. AHTPDB and BIOPEP database to confirm antihypertensive activity (ACE-inhibitory activity) of novel peptides derived from fermented camel milk.

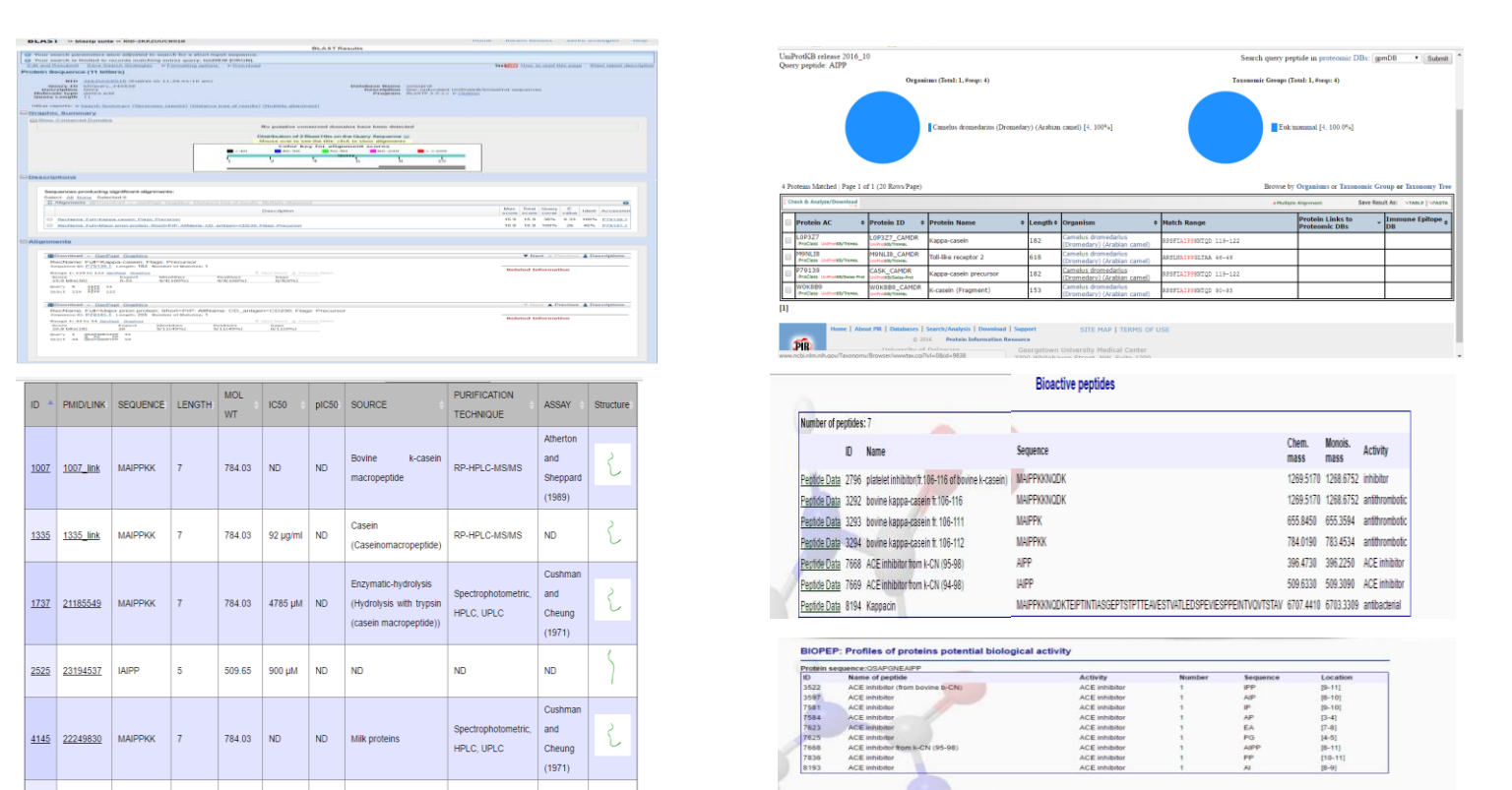


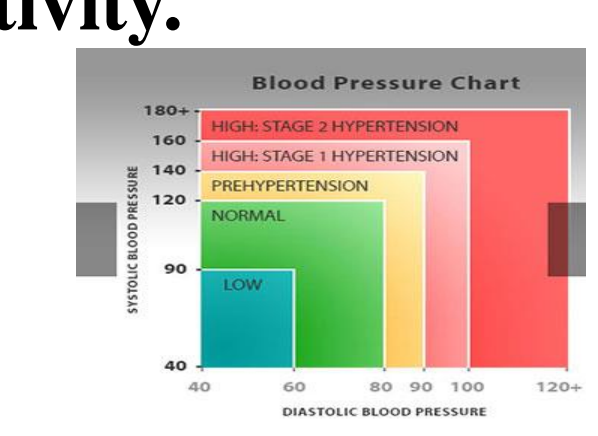
Figure 18- Results indicating that the peptides belong from camel milk proteins and it have ACE-inhibitory activity (Blast p, PIR, AHTPDB, BIOPEP)

Discussion

- ✓ PepX activity of lactic cultures observed due to the presence of proline specific activity of lactic cultures to ferment milk proteins in to peptides.
- ✓ ACE-inhibitory activity of all the lactic cultures found to increase significantly with incubation period.
- ✓ Inoculation rate of 2% (v/v) and incubation period of 12h were optimized through OPA method.
- ✓ Relative proteolytic activity was the measure of peptides production of lactic culture in fermented milk.
- ✓ 3 and 10 kDa permeate of all the lactic cultures exhibited higher ACE-inhibitory and Peptide production.
- ✓ Presence of lower molecular weight protein bands in SDS-PAGE was due to the proteolytic activity.
- ✓ Trypsin digestion followed by RP-LC/MS analysis of O9 lactic culture (protein spot) showed the presence of novel peptide (IAGMTGTALTEEEFR) in fermented camel milk.
- ✓ Novel ACE-inhibitory peptides were identified through homology search in NCBI and PIR databases. Biological property was confirmed through the search in AHTPDB and BIOPEP databases.

Conclusion

- ✓ NS4 and O9 could be used for the production of ACE-inhibitory peptides from fermented camel milk.
- ✓ Fermented camel milk could be explored as novel fermented product having anti-hypertensive activity.



Key Message

- ✓ ACE-inhibitory peptides rich functional fermented camel milk could be prepared using potent proteolytic lactic cultures.

