Bioactive peptides in Dairy products*

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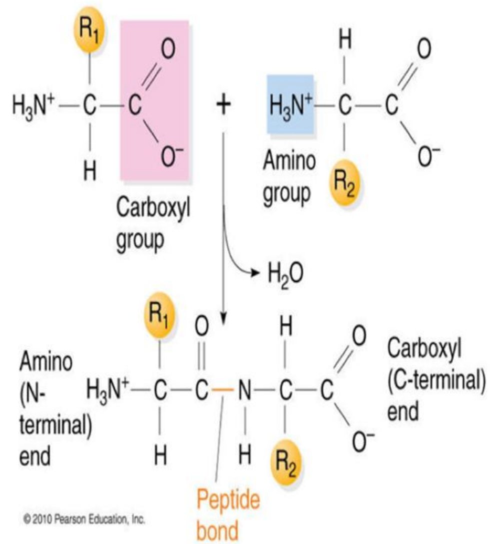
***No Conflict of Interest for this presentation**

Bioactive Peptides

Proteins in foods do not only serve as nutrients but also perform physiochemical roles that promote various health benefits.
(Froetschel, 1996)

Bioactive peptides (BAPs) are organic substances formed by amino acids joined by covalent bonds also known as amide or peptide bonds.
(Walther & Sieber, 2011)

Bioactive peptides are structurally short chained form 2-20 amino acids residues and molecular weight <6000 kDa.
(Hartmann & Meisel, 2007)



Peptide Bond between Amino Acid

Cont…

Bioactive peptides are specific protein fragments that have a positive impact on the functioning or conditions of living beings, thereby improving their health.

(Korhonen & Pihlanto, 2006)

Bioactive peptides are inactive within the sequence of the parent protein, but after the released by enzymatic hydrolysis, exert various physiological functions.

(Park, 2009)

The bioactivity and functionality of peptides depend on the amino acid composition, sequences, and molecular masses. (Lassoued *et al.*, 2015)

Bioactive peptides are known for their ability to inhibit protein-protein interactions.

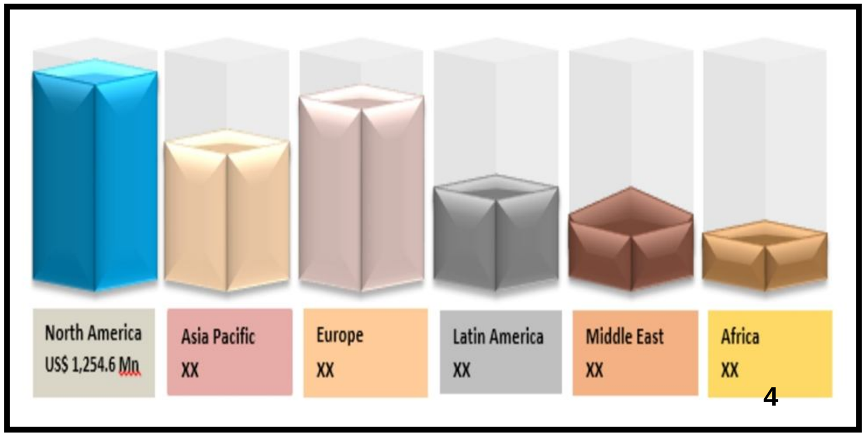
More than 1500 different BAPs have been reported in a database named 'BIOPEP'.

(Singh *et al*., 2014)

Market status………..

According to coherent market insight, 2019 the global bioactive peptide market size was valued at US$ 3,265.2 Mn in 2017, and is expected to exhibit a CAGR of 9.4% over the forecast period (2018–2026).

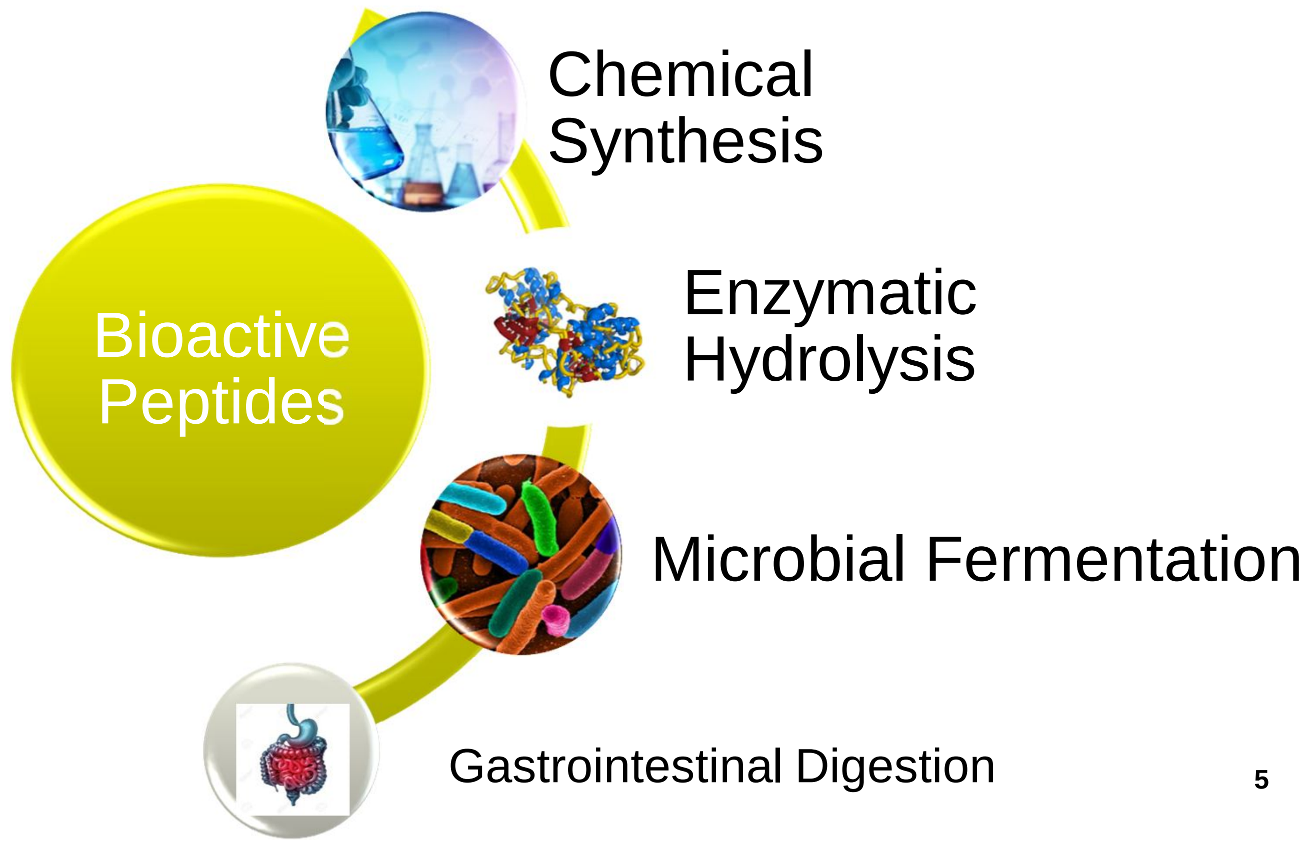
North America dominating the global bioactive peptides market in 2017 reported 35.9% market share in terms of revenue.



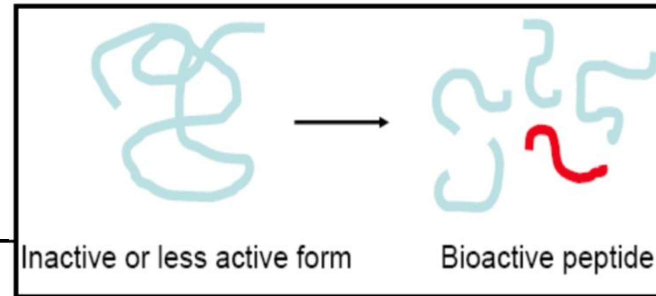
Global bioactive peptide market value by region

(https://www.coherentmarketinsights.com/market-insight/bioactive-peptide-market-3018)

Synthesis of Bioactive Peptides



Milk protein



- Milk proteins exert a wide range of nutritional, functional and biological activities
- Physiologically active peptides are derived from milk proteins
- These peptides are inactive within the sequence of the parent protein molecule
- Released during gastric digestion or food processing (e.g. renneting or fermentation of milk)
- Bioactive peptides usually contain 3 – 20 amino acid residues per molecule
- Many bioactive peptides possess multi-functional properties
- At present, milk proteins are the most important source of bioactive peptides.

- A variety of naturally formed bioactive peptides have been found in fermented dairy products, such as yoghurt, dahi, probiotic dairy foods and cheese

- Bioactive peptides are released upon fermentation of milk using different live proteolytic microorganisms or proteolytic enzymes derived from such microorganisms.

- Fermentation of milk with certain dairy starters, peptides with various bioactivities can be formed and detected in an active form even in the final product i.e. Fermented milk and cheese

- They can be absorbed and reach peripheral organs

Bioactivity of milk peptides depend on

- Amino acid sequence of protein substrate
- Pre-treatment
- Enzyme specificity
- Hydrolysis condition (pH, Temp)
- Enzyme inactivation
- Separation
- Purification

Korhonen and pihlanto, 2006

More than 60 peptides approved by US Food and Drug Administration (FDA) as a medicines on the market and this is expected to grow significantly with approximately 140 peptide drugs currently in clinical trials and more than 500 therapeutic peptides in preclinical development.

Fosgerau and Hoffmann, 2015

Bioactive peptides released from milk proteins by proteolytic enzymes of different microorganisms

Microorganism	Precursor protein	Peptide sequence	Bioactivity
Lactobacillus helveticus and Saccharomyces cerevisiae	β-casein, α-casein	Val-Pro-Pro, Ile-Pro-Pro	ACE inhibitor, Antihypertensive
***Lactobacillus* GG enzymes + pepsin and trypsin**	β-casein, αs1 casein	Tyr-Pro-Phe-Pro, Ala-Val-Pro-Tyr-Pro--Gln-Arg, Thr-Thr-Met-Pro-Leu-Trp	opioid, ACE inhibitor, immunostimulating
***Lb. helveticus* CP90 proteinase**	β-casein	Lys-Val-Leu-Pro-Val-Pro-(Glu)	ACE inhibitor
***Lb. helveticus* CPN 4**	whey proteins	Tyr-Pro	ACE inhibitor
***Lb. delbrueckii* ssp. *bulgaricus* IFO13953**	α-casein	Ala-Arg-His-Pro-His-Pro-His-Leu--Ser-Phe-Met	Antioxidative
***Lb. rhamnosus* + hydrolysis with pepsin and Corolase PP**	β-casein	Asp-Lys-Ile-His-Pro-Phe, Tyr-Gln-Glu-Pro-Val-Leu, Val-Lys-Glu-Ala-Met-Ala-Pro-Lys	ACE inhibitor Antioxidative
Lb. delbrueckii* ssp. *bulgaricus	β-casein	Ser-Lys-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile	ACE inhibitor

Dziuba & Dziuba, 2014

Work done at Anand

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Considering the potential of *Lactobacillus rhamnosus* for producing Angiotensin I-Converting Enzyme (ACE) inhibitory peptides in fermented camel milk (Indian breed)

Divyang Solanki, Subrota Hati*

J Food Sci Technol
https://doi.org/10.1007/s13197-022-05357-9

ORIGINAL ARTICLE

Characterization of Angiotensin I-Converting Enzyme (ACE) inhibitory peptides produced in fermented camel milk (Indian breed) by *Lactobacillus acidophilus* NCDC-15

Divyang Solanki[1] · Amar Sakure[2] · Sangeeta Prakash[3] · Subrota Hati[1]

Purification and characterization of ACE-inhibitory peptides derived from fermented Camel milk

Int J Pept Res Ther
DOI 10.1007/s10989-017-9577-5

In Silico and _In vitro_ Analysis of Novel Angiotensin I-Converting Enzyme (ACE) inhibitory Bioactive Peptides Derived from Fermented Camel Milk (_Camelus dromedarius_)

Divyangkumar Solanki[1] · Subrota Hati[1] · Amar Sakure[1]

Lactic cultures used for ACE-inhibitory activity

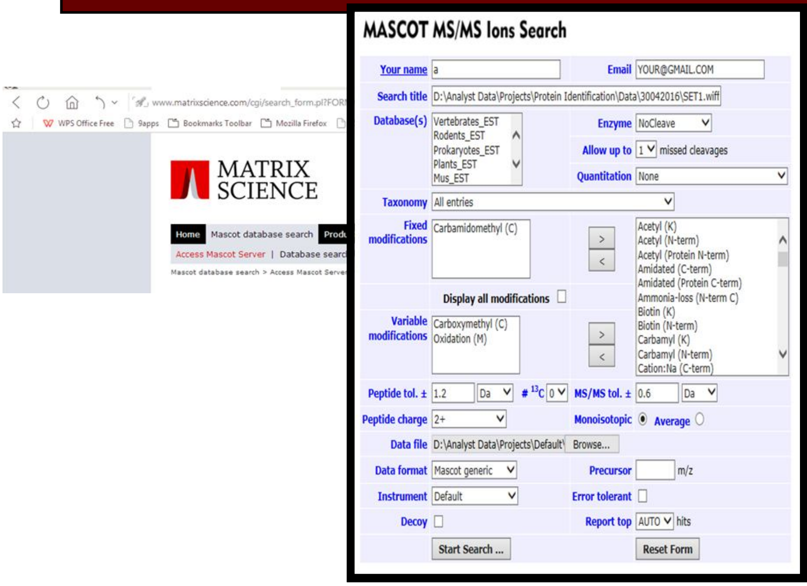
Sr. No	Culture Name	Source of Isolation	Selective Media	Growth Conditions
1	*L. rhamnosus* MTCC 5945 **(NS4)**	Shrikhand	MRS Agar	37^0C for 24h
2	*L. acidophilus* NCDC **(298)**	NDRI, Karnal	MRS Agar	37^0C for 24h
3	*L. helveticus* MTCC 5463 **(V3)**	Human vagina	MRS Agar	37^0C for 24h
4	*L. acidophilus* NCDC (**015**)	NDRI, Karnal	MRS Agar	37^0C for 24h
5	*L. rhamnosus* MTCC 5946 **(NS6)**	Shrikhand	MRS Agar	37^0C for 24h
6	*S. thermophilus* MTCC 5460 **(MD2)**	Market dahi	M17 Agar	42^0C for 24h
7	*L. bulgaricus* NCDC (**09**)	NDRI, Karnal	MRS Agar	37^0C for 24h
8	*Lactococcus lactis* subsp. *lactis* MTCC 25066 **(NK6)**	Dahi	M17 Agar	30^0C for 24h
9	*L. fermentum* TDS030603 **(LBF)**	Dahi	MRS Agar	37^0C for 24h

ACE-inhibitory activity of lactic cultures incubated at 37°C up to 48h

Treatment (T)	Period (Time in hours)*			
	0	12	24	48
	% ACE-inhibitory activity			
NS4	56.25±2.25	75.50±0.50	71.38±1.61	78.09±1.10
298	55.74±0.22	67.55±1.50	74.93±0.94	68.41±1.28
NS6	56.05±0.54	70.83±1.04	72.69±1.60	75.75±0.25
015	56.22±1.07	70.19±0.80	78.022±2.55	78.33±2.51
V3	50.99±1.38	52±2.0	63.18±3.20	68.56±4.55
NK6	56.11±2.45	77.08±1.29	83.27±3.74	85.30±1.13
MD2	55.16±0.76	69.35±3.08	81.86±1.06	84.45±1.52
09	56.34±1.24	74.71±1.83	74.93±0.059	76.75±1.14
LBF	55.66±0.73	69.66±5.08	71.84±1.94	73.93±0.74

Peptide content (%) produced by selected lactic cultures under optimized growth conditions (10kDa Permeate and Retentate)

Lactic cultures	Peptide production (%) (10kDa Permeate)	Peptide production (%) (10kDa Retentate)
LBF	65.52±2.723[a]	26.22±1.538[d]
09	59.29±0.761[b]	40.50±1.291[b]
NS4	48.98±0.818[c]	56.44±1.296[a]
015	55.04±1.376[d]	36.79±1.684[c]

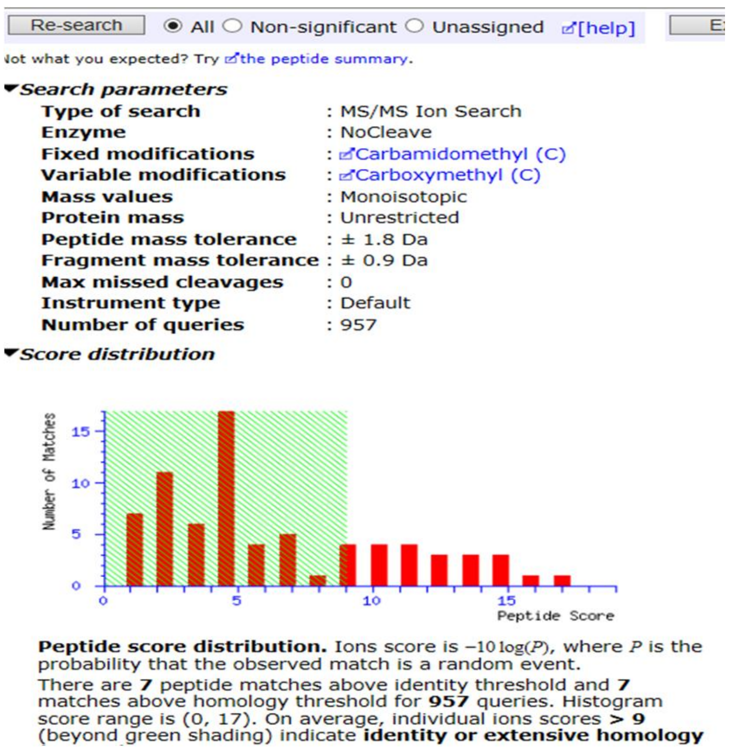


http://www.matrixscience.com/cgi/search_form.pl?FORMVER=2&SEARCH=MIS

- Peptides were matched in MASCOT (online server)
- Peptide with RED BOLD fashion with significant score was confirmed.

✓**ATVQGGIMYRMPV**

(Figueroa *et al.*, 2011)

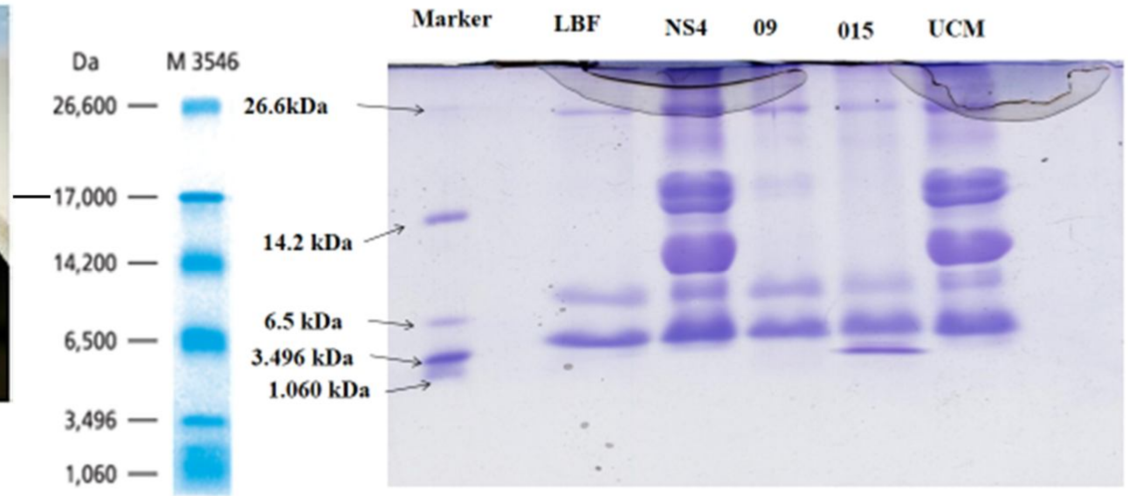


Confirmation of peptides sequence in camel milk proteins was performed using Peptide Match

❑http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome

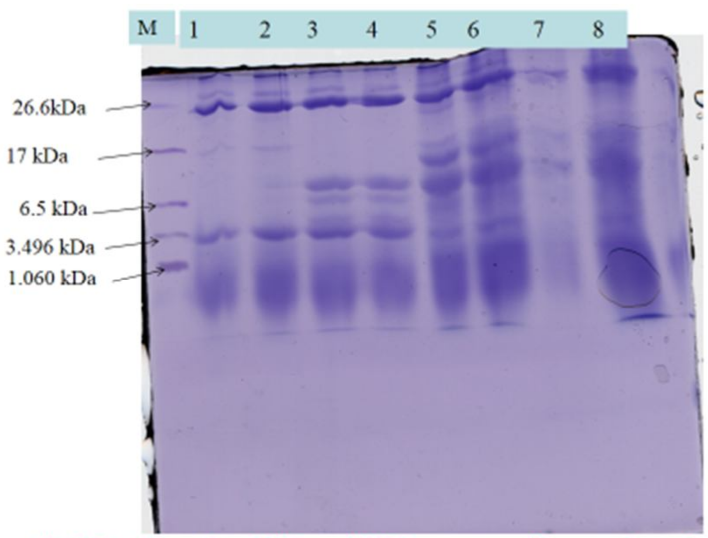
❑(http://research.bioinformatics.udel.edu/peptidematch/index.jsp).

(Tagliazucchi *et al.*, 2016)



Here, UCM= unfermented camel milk

Fig.15 Protein profile of fermented and camel milk reveled by SDS-PAGE.



M=MARKER
1) 015 3kDa permeate
2) 09
3) LBF
4) NS4
5) 015 10kDa permeate
6) 09
7) LBF
8) NS4

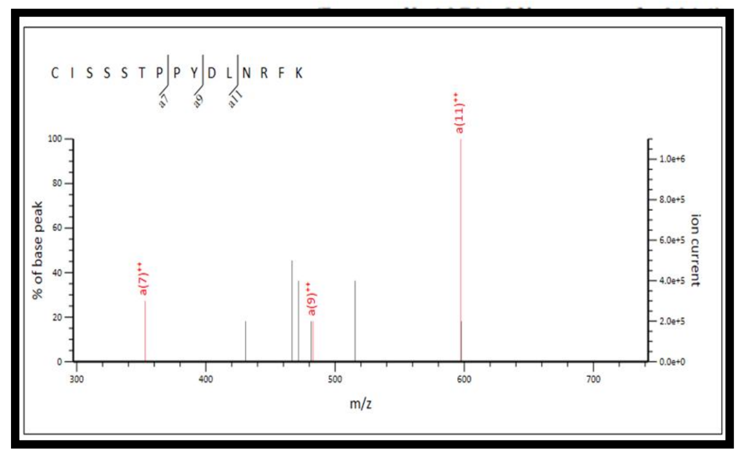


Fig.16 Protein profile of 3kDa and 10kDa permeate of fermented camel milk reveled by SDS-PAGE.

(Laemmli, 1970; Olivares *et al.*, 2014)

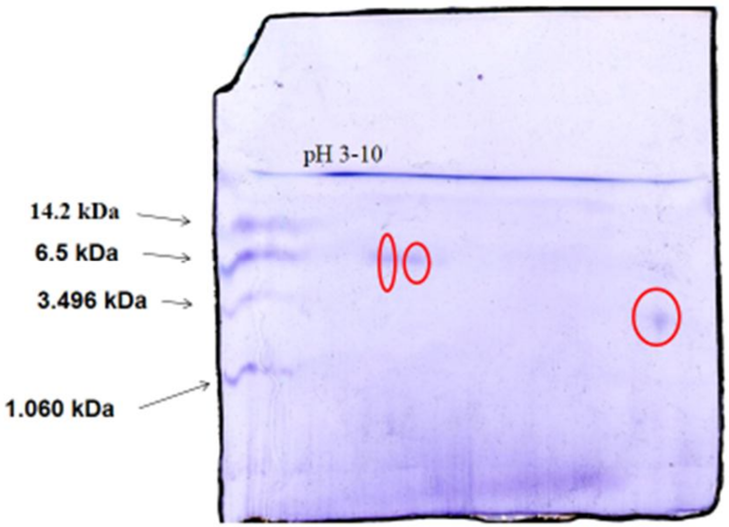


Fig.18- 2D gel electrophoresis of 3 Kda permeate 09

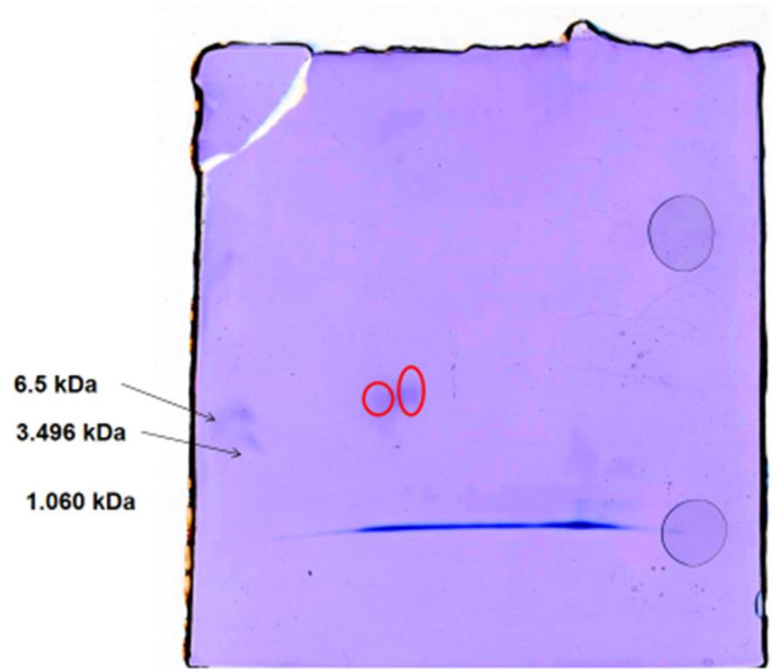


Fig.20- 2D gel electrophoresis of 3 Kda permeate LBF

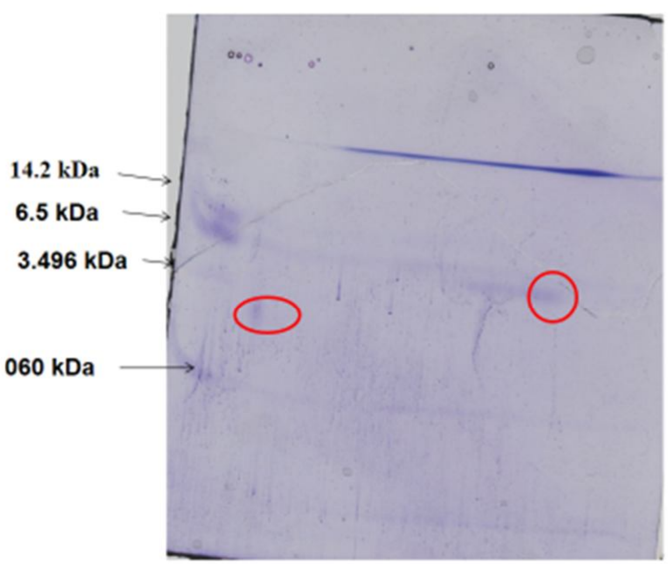
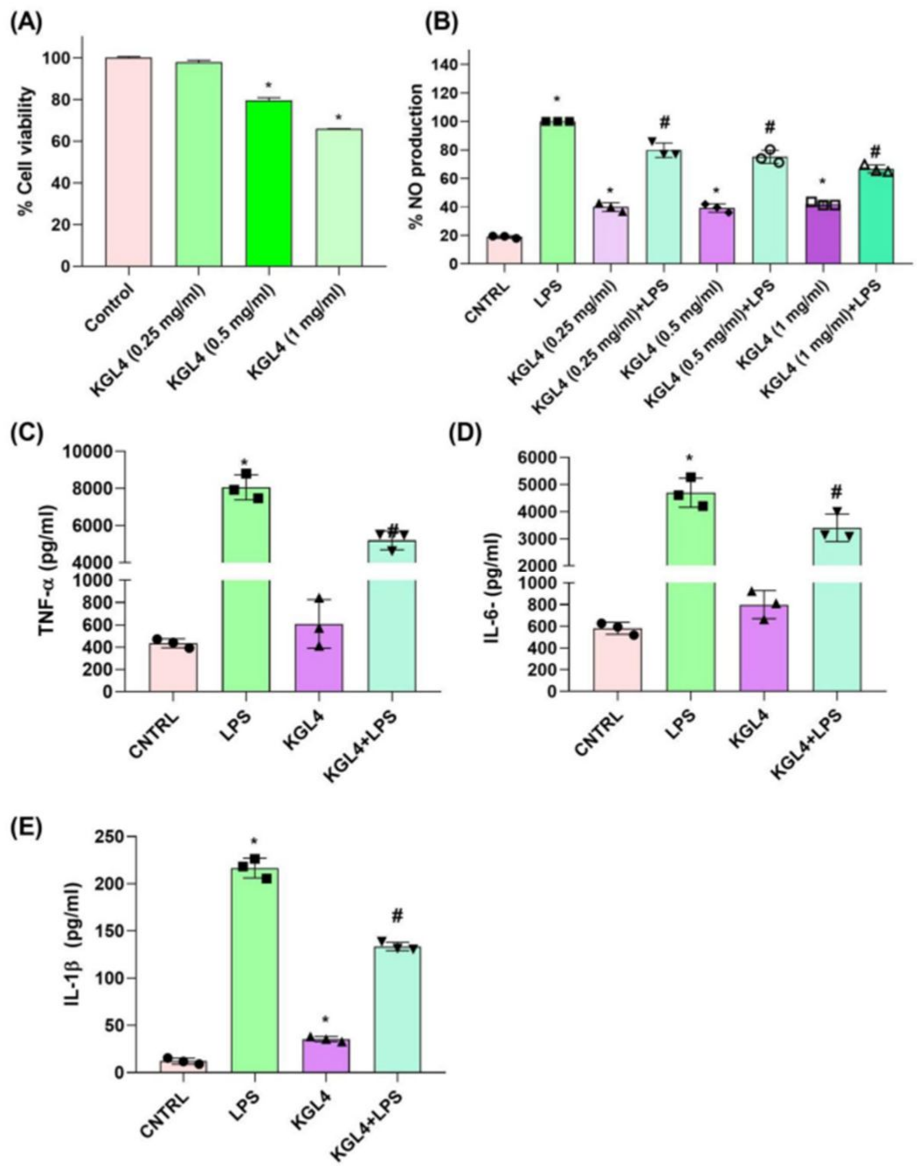


Fig.19- 2D gel electrophoresis of 3 Kda permeate NS4

During amino acid profiling, 015, LBF, NS4 and 09 produced peptides with amino acid sequences GPPYQPLVPR, CISSSTPPYDLNRFK, VCNYVSWIK and MDTIEPVSVACIS respectively from Beta-casein f(225-229); Beta-casein f(31-34); Alpha S_1- Casein f(190-193) and Alpha-lactalbumin f(42-43) in camel milk protein database (NCBI).



Effect of the Fermented camel milk with KGL4 on A) Viability of RAW 264.7 macrophage cells, (B) Dose dependency in NO production (C) TNF-α (D) IL-6 (E) IL-1β measured in supernatants of LPS-stimulated RAW 264.7 macrophage cells. Data are presented as mean±SEM; n=5 in (A), n=3 in (B), n=3 in (C-E) and evaluated by one-way ANOVA followed by Tukey's post hoc test. *p

(Patel et al. 2021)

International Journal of Peptide Research and Therapeutics
https://doi.org/10.1007/s10989-019-09902-7

Purification and Production of Novel Angiotensin I-Converting Enzyme (ACE) Inhibitory Bioactive Peptides Derived from Fermented Goat Milk

Heena Parmar[1] · Subrota Hati[1] · Gauravkumar Panchal[1] · Amar A. Sakure[2]

LAB cultures used in the study

***L. rhamnosus* (NK2) (KR080695)**
***L. casei* (NK9) (KR732325)**
***L. paracasei* (M16) (KU366368)**
***L. fermentum* TDS030603 (MTCC 25067) (LF)**
***L. fermentum* (M5) (KU366365)**

- Fresh goat milk of Indian Surti breed (*Capra aegagrus hircus*) procured and filtered than heated at 90 °C for 10–15 min. Then stored at 5 ± 1 °C.
- Pure cultures were inoculated at the rate of 2% and incubated at 37 °C for 0, 6, 12, 24 and 48 h.

Parmar *et al.*, 2019

LWT - Food Science and Technology xxx (xxxx) xxxx

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Characterization and production of novel antioxidative peptides derived from fermented goat milk by L. fermentum

Gauravkumar Panchal[a], Subrota Hati[a,*], Amar Sakure[b]

- Goat milk (Surti breed, India) collected and sterilized at 121 °C for 15 min and stored at 5±2 °C.
- Inoculating M4 (*L. fermentum*) culture at the rate of 2% and incubated for 0, 12, 24, 36 and 48 h at 37 °C.

Antioxidant activity of Fermented got milk

- ABTS assay (2, 2-Azino-bis (3-ethylbenzothaizoline 6-sulfonic acid) assay)
- Hydroxyl free radical scavenging assay
- Superoxide free radical scavenging assay

Panchal *et al.*, 2019

J Food Sci Technol
https://doi.org/10.1007/s13197-021-05282-3

ORIGINAL ARTICLE

Significance of whey protein hydrolysate on anti-oxidative, ACE-inhibitory and anti-inflammatory activities and release of peptides with biofunctionality: an in vitro and in silico approach

Chaudhari Hiralben Mansinhbhai[1] · Amar Sakure[2] · Ruchika Maurya[3,4] · Mahendra Bishnoi[3] · Kanthi Kiran Kondepudi[3] · Sujit Das[5] · Subrota Hati[1]

Int J Pept Res Ther (2018) 24:441–453
DOI 10.1007/s10989-017-9630-4

In Vitro and In Silico Analysis of Novel ACE-Inhibitory Bioactive Peptides Derived from Fermented Goat Milk

Heena Parmar[1] · Subrota Hati[1] · Amar Sakure[2]

International Journal of Peptide Research and Therapeutics
https://doi.org/10.1007/s10989-019-09902-7

Purification and Production of Novel Angiotensin I-Converting Enzyme (ACE) Inhibitory Bioactive Peptides Derived from Fermented Goat Milk

Heena Parmar[1] · Subrota Hati[1] · Gauravkumar Panchal[1] · Amar A. Sakure[2]

Food Bioscience 47 (2022) 101666

Contents lists available at ScienceDirect

Food Bioscience

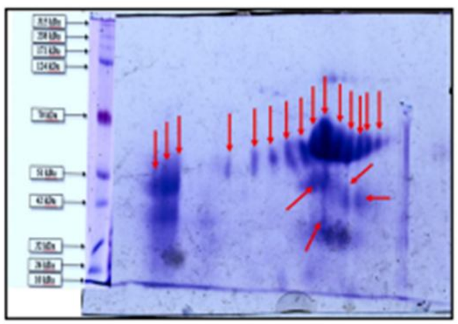
journal homepage: www.elsevier.com/locate/fbio

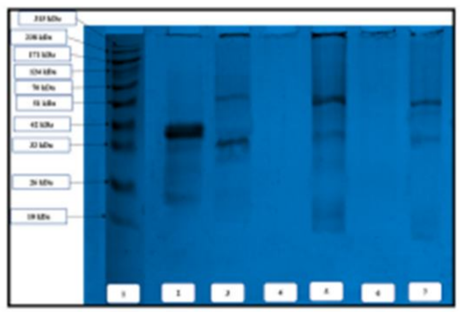
Antioxidative, antimicrobial and anti-inflammatory activities and release of ultra-filtered antioxidative and antimicrobial peptides during fermentation of sheep milk: *In-vitro*, *in-silico* and molecular interaction studies

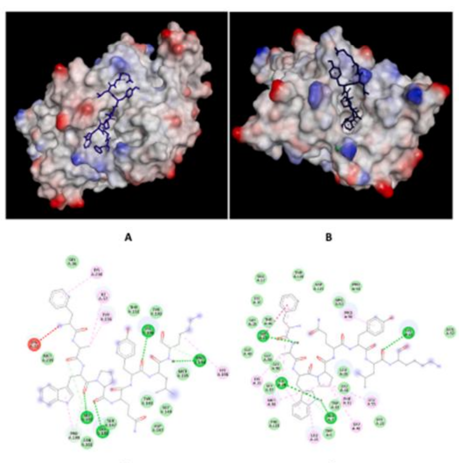
Jodhani Keyur Ashokbhai [a], Bethsheba Basaiawmoit [b], Sujit Das [b], Amar Sakure [c], Ruchika Maurya [d,e], Mahendra Bishnoi [e], Kanthi Kiran Kondepudi [e], Srichandan Padhi [f], Amit Kumar Rai [f], Zhenbin Liu [g], Subrota Hatia [a,*]

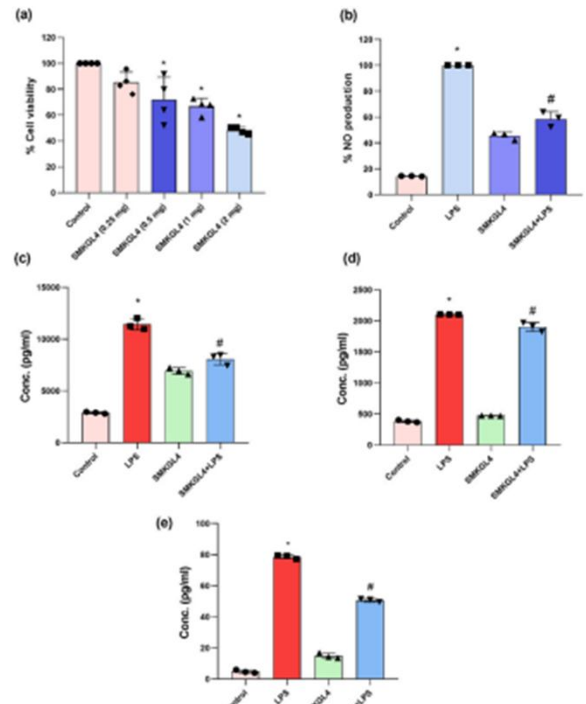
Table 4
All sequences found in study of fermentation of sheep milk produced by KGL4.

Culture	2D-PAGE Sequence	Score of peptide ranker	Charge	Toxicity	Molecular weight
KGL4	ITMPLW	0.59823	0.00	Non-toxic	1051.31
	FAWPQYLK	0.525769	1.00	Non-toxic	1034.31
	HKEMPFPK	0.859678	1.50	Non-toxic	760.05
	LDQWLCEK	0.906686	−1.00	Non-toxic	1052.35
	KADEKKFW	0.616444	1.00	Non-toxic	1013.33









Food Bioscience 44 (2021) 101404

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

ELSEVIER

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Exploring potentials of antioxidative, anti-inflammatory activities and production of bioactive peptides in lactic fermented camel milk

Patel Dharmisthaben [a], Bethsheba Basaiawmoit [b], Amar Sakure [c], Sujit Das [b], Ruchika Maurya [d,e], Mahendra Bishnoi [e], Kanthi Kiran Kondepudi [e], Subrota Hati [a,*]

JOURNAL OF THE AMERICAN COLLEGE OF NUTRITION
https://doi.org/10.1080/07315724.2021.1983485

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Significance of *Lactobacillus fermentum* on Antioxidative and Anti-Inflammatory Activities and Ultrafiltration Peptide Fractions as Potential Sources of Antioxidative Peptides from Fermented Camel Milk (Indian Breed)

Dharmisthaben Patel[a], Amar Sakure[b], Dikshita Lodha[c], Bethsheba Basaiawmoit[d], Ruchika Maurya[e,f], Sujit Das[d], Mahendra Bishnoi[f], Kanthi Kiran Kondepudi[f] and Subrota Hati[a]

Int J Pept Res Ther (2018) 24:87–98
DOI 10.1007/s10989-017-9596-2

CrossMark

Influence of Whey Protein Concentrate on the Production of Antibacterial Peptides Derived from Fermented Milk by Lactic Acid Bacteria

Subrota Hati[1] · Nikita Patel[1] · Amar Sakure[2] · Surajit Mandal[3]

J Food Sci Technol
https://doi.org/10.1007/s13197-021-05243-w

ORIGINAL ARTICLE

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Peptidomic profiling of fermented goat milk: considering the fermentation-time dependent proteolysis by *Lactobacillus* and characterization of novel peptides with Antioxidative activity

Gauravkumar Panchal[1] · Amar Sakure[2] · Subrota Hati[1]

Received: 4 May 2021 | Revised: 22 July 2021 | Accepted: 22 September 2021
DOI: 10.1111/jfpp.15992

ORIGINAL ARTICLE

Journal of Food Processing and Preservation ifst WILEY

Production and characterization of antioxidative peptides during lactic fermentation of goat milk

Gaurav Kumar Panchal[1] | Sujit Das[2] | Amar Sakure[3] | Brij Pal Singh[4] |
Subrota Hati[1]

Current Trends and Applications of Food Derived Antihypertensive Peptides for the Management of Cardiovascular Disease.

Shukla P[1], Chopda K[1], Sakure A[2], Hati S[1]

Author information

Protein and Peptide Letters, 06 Jan 2022,
DOI: 10.2174/0929866529666220106100225 PMID: 34994309

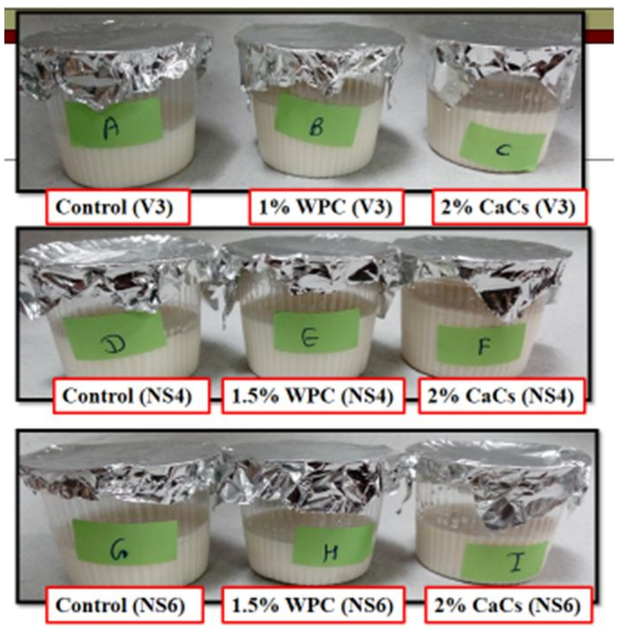
Indian J Dairy Sci 70(5):533-540(2017)

RESEARCH ARTICLE

Significance of WPC and calcium caseinate on shelf-life study and textural profiles of dahi

Rekha S Patel, Subrota Hati, BM Mehta and Smitha B

Process optimization for the preparation of ACE inhibitory peptides rich dahi



RMM REVIEWS IN MEDICAL MICROBIOLOGY

BACTERIOLOGY

Production of antihypertensive (angiotensin I-converting enzyme inhibitory) peptides derived from fermented milk supplemented with WPC70 and Calcium caseinate by *Lactobacillus* cultures

Patel, Rekha; Hati, Subrota **Author Information**

Reviews in Medical Microbiology: January 2018 - Volume 29 - Issue 1 - p 30-40
doi: 10.1097/MRM.0000000000000119

Conclusion

Bioactive peptides offer a new means to promote health and can provide health benefits beyond their basic nutritional role.

There is a tremendous global interest in promoting the use of food proteins/peptides as novel alternatives for present pharmaceutical therapeutics in the treatment and prevention of high blood pressure and other life style diseases.

Milk peptides are the most studied bioactive peptides and various health beneficial properties have also been studied.

Thanks…..for listening