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Pooja JS
M.Sc. Scholar, Department of Veterinary Biochemistry, Kerala Veterinary and Animal Sciences University, Kerala, India

Rachana CR
Assistant Professor, Department of Dairy Chemistry, Kerala Veterinary and Animal Sciences University, Kerala, India

Corresponding Author:
Pooja JS
M.Sc. Scholar, Department Of Veterinary Biochemistry, Kerala Veterinary and Animal Sciences University, Kerala, India

Effect of fermentation on antioxidant activity of milk from Vechur and Kasargod Dwarf cattle

Pooja JS and Rachana CR

Abstract
Consumption of foods high in natural antioxidants improves an organism's antioxidant status by protecting it from oxidative stress and damage. Bioactive peptides produced by lactic acid bacteria in natural sources such as dairy foods have gained interest as a potential source of biotherapeutic peptides. Thus, this study was conducted to evaluate the effect of fermentation on antioxidant activity of milk from Vechur and Kasargod Dwarf cattle during storage at 5 °C for 21 days. Water-soluble crude peptide extracts were prepared by high-speed centrifugation. The water-soluble peptides from Kasargod Dwarf and Vechur cattle by fermentation showed maximum antioxidant activity on 21st day of storage. The antioxidant activity of fermented milk samples was increased with increasing storage period. This increase in antioxidant activity may be due to continous slow release of peptides from different types of casein after fermentation which possess antioxidant effect.

Keywords: *Vechur cattle, kasargod dwarf, antioxidant activity, bioactive peptides,* water-soluble peptides

1. Introduction
India has the world's largest cattle population, and Vechur (*Bos indicus*) is one of Kerala's indigenous cattle breeds. It is the world's smallest cow (nearly 90cm tall). It also has low feed requirements and a high level of disease resistance. Vechur cow milk contains a high concentration of small fat globules and saturated fatty acids.
As a result, it is appropriate for infants and the elderly. This milk has medicinal properties and is used in the Ayurvedic medical system. (Ravi *et al.*, 2006) [14].
The Kasargod Dwarf is another type of dwarf cattle breed found in Kerala. They are well known for its superior milking ability and mineral-rich milk Moreover, the milk of Kasargod Dwarf contains high amounts of α-2 casein proteins, which makes it a beneficial for diabetics and hypertensive patients (Anu *et al.* 2018) [1].
Fermented dairy products, such as yoghurt, contain a wide spectrum of natural bioactive peptides (Fitzerald and Murray, 2006) [4]. The amount and activity of bioactive peptides produced from fermentation are dependent on several factors including the type of starter cultures used, product type, fermentation time, and storage conditions (Korhonen, 2009) [11].
Bioactive peptides are protein fragments that have a favourable effect on physiological processes or circumstances, and may influence health. They influence numerous biological processes including neurological, hormonal, gastrointestinal, and nutritional responses. They range from two to twenty amino acids and many have multifunctional properties. Antibacterial properties, antioxidant activities (Pihlanto-Leppala, 2000) [13], mineral binding (Lorenzen and Meisel, 2005) [12], and ACE inhibitory activities are some of the health benefits of these peptides (Yamamoto and Takano, 1999 and Gobbetti *et al.*, 2004) [16, 6].
The creation of extremely reactive oxidation compounds owing to electron acceptability or another natural mechanism in the body is known as reactive oxygen species (ROS), ROS can harm the body's inherent cells or organs. As a result, antioxidants are critical for preventing ROS-induced cell damage. Lactic acid bacteria, in general, are responsible for the release of bioactive peptides from milk during fermentation, demonstrating antioxidant properties (Gjorgievski *et al.*, 2014) [5].
Milk and milk products have high antioxidant activity due to sulfur-containing amino acids, phosphate, vitamins A, E, carotenoid, zinc, selenium, enzyme systems such as superoxide dismutase, catalase, glutathione peroxidase, milk oligosaccharides, and peptides, which are produced during fermentation Imran *et al.* (2019) [10]. Some synthetic antioxidants have been shown to pose a potential risk *in vivo*.

As a result, natural antioxidant compounds are extremely valuable.

The Present work aim to study the effect of fermentation on antioxidant activity and Physico-chemical properties of milk from Vechur and Kasargod Dwarf cattle on refrigerated storage.

2. Materials and methods

2.1Collection of Milk

Fresh, pooled, whole milk from Vechur and Kasargod Dwarf cattle were collected separately from the animals maintained at Vechur Conservation unit, KVASU, Mannuthy.

2.2 Preparation of Sample

The milk samples from both the breeds were heated separately at 80°C for 5 min to kill the microorganisms and to denature the indigenous enzymes of milk. The heated samples were cooled immediately to 42 °C and then inoculated with yoghurt culture (*Streptococcus thermophilus and Lactobacillus bulgaricus* 1:1). Inoculated milk was packed in sterilised plastic cups and incubated for 4-5 hours at 42°C.The samples were then stored at 5 °C ± 2 ºC for storage studies for 21 days of storage. The analysis was carried out at 0th day (after 4 to 5 hours), 7th day, 14th day and 21st day.

2.3 Physico-chemical analysis

Proximate composition of yoghurt samples was analyzed: About 100 g of prepared yoghurt was blended, and the pH was determined using a digital pH meter, ash content was measured by dry ash method, total solids of the yogurt was determined by drying at102 ± 2°C until constant mass., the protein content was determined by Kjeldahl method and the fat content was determined by Gerber method, adopting ISI procedure (ISI, 1981) [9].

2.4 Preparation of Water-soluble Peptide Extracts

Water-soluble peptide extracts (WSPE) were prepared from fermented samples using high-speed centrifugation, as described by Sah *et al*. (2016) [15]. The samples were centrifuged at 16,099 × g using a Remi High-performance centrifuge at 4°C for 30 min.

2.5 Determination of Degree of Hydrolysis

The degree of hydrolysis (DH) was determined according to Hoyle and Merritt, (1994) [7].

DH (%) = [Soluble protein (mg) / Total protein (mg)] × 100

2.6 Measurement of Antioxidant Activity

The antioxidant activity was evaluated by using 2, 2 Diphenyl-1-picryl-1-hydrazyl (DPPH) radical assays described by Zou *et al.*, 2014[17]. An amount of 0.1mL of the test samples were added into 2.3 mL of anhydrous ethanol. After they had been mixed vigorously, 1.6ml of 60 µL/mL DPPH solution was added. The redox reaction was carried out at room temperature for 20 min, and the free radical scavenging activities of samples were quantified by determining the change of absorbance at 517 nm. The control solution contained the same amount of blank nanoliposome and 3.9 mL of anhydrous ethanol. DPPH radical scavenging activity was evaluated by using the equation:

Radical scavenging activity (%)
= (A – B) / (A-C) × 100%

Where
'A' is the absorbance of control after reaction,
'B' is the absorbance of samples after the reaction and
'C' is the absorbance of the blank solution.

2.7 Statistical Analysis

All experiments were carried out in triplicates and the mean values were tabulated. Differences between samples with respect to bioactivity were tested using one-way analysis of variance (GLM procedure), and means of samples were compared using Duncan's Multiple Range test (SPSS software, version 23).

3. Results and discussions

Table 1 shows the chemical composition of yoghurt samples under cold storage. TS content ranged 14.6–16.81% in 0th day samples and significantly increased ($p<0.05$) during storage, up to 16.25–18.98%. The same trend was observed for total protein and fat contents. The increase in the total solids contents, fat content, total protein content and ash content in the samples during storage may be attributed due to the relative reduction of moisture content in the samples. The TA increased insignificantly ($p<0.05$) during storage. Decreasing pH values is the result of lactose fermentation by associative growth of two thermophilic, homo-fermentative lactic acid bacteria, *St. thermophilus*, and *L. bulgaricus*. Moreover, the action of the bacterial enzyme beta-galactosidase is not completely stopped by the storage of fermented milk which causes a decrease in the pH and lactose content and increases the titratable acidity. Durdevic-Denin *et al.*, 2001[2] and Hussain *et al.*, 2009[8] both reported similar findings. DH represents the percentage of peptide bonds cleaved. The degrees of protein hydrolysis as shown in Table 2, were 49.22 and 35.11% for fermented milk from Kasargod Dwarf and Vechur cattle on 0th day respectively; they increased at the end of cold storage to reach 77.11 and 69.83

Table 1: Physicochemical properties of fermented milk made from milk of Kasargod Dwarf (A) and Vechur cattle (B) during storage at 5 °C for 21 days

		0th day	7th day	14th day	1st day
pH	A	.23 ± 0.585^{a}	.77± 0.057^{ab}	4.57 ± 0.152^{c}	.47 ± 0.152^{c}
	B	5.21 ± 0.608 ^{a}	4.63 ± 0.208^{ab}	4.45 ± 0.251^{c}	.43 ± 0.251^{c}
Titratable acidity (%)	A	0.86± 0.172^{a}	.89±0.262^{a}	1.03± 0.053^{a}	1.06 ± 0.063^{a}
	B	0.87 ± 0.170^{a}	1.04 ± 0.004^{b}	1.06± 0.017^{b}	1.10 ± 0.045^{b}
Total solid (%)	A	14.60±0.650^{ab}	14.87±0.556^{b}	5.71±0.685^{bc}	16.25±0.252^{c}
	B	16.81±0.501^{ab}	17.34±0.550^{b}	18.46±0.497^{c}	18.99±0.367^{c}
Fat (%)	A	3.85 ± 0.297^{ab}	3.91± 0.305^{ab}	3.92 ± 0.440^{ab}	4.10 ± 0.443^{b}
	B	5.03 ± 0.270^{a}	5.23±0.487^{ab}	5.79±0.301^{bc}	6.04 ± 0.467^{c}

Total protein (%)	A	4.77 ± 0.230^{ab}	5.38± 0.678^{b}	5.46± 0.670^{b}	5.63 ± 0.692^{b}
	B	4.54 ± 0.125^{a}	5.10 ± 0.120^{a}	5.20 ± 0.544^{a}	5.25 ± 0.557^{a}
Ash (%)	A	.91±0.069^{ab}	0.95±0.055^{b}	0.98±0.071^{b}	1.04± 0.039^{b}
	B	0.92 ± 0.16^{ab}	1.01 ± 0.11^{ab}	1.04 ± 0.18^{ab}	1.10 ± 0.20^{b}

Table 2: Degree of hydrolysis of fermented milk samples made from milk of Kasargod Dwarf (A) and Vechur cattle (B) during storage at 5°C

	Sample	TH DAY	TH DAY	4TH DAY	1ST DAY
Degree of hydrolysis (%)	A	9.22	5.46	0.36	77.11
	B	35.11	46.34	61.26	69.83

Table 3: Antioxidant activity of fermented milk samples made from milk of Kasargod Dwarf (A) and Vechur cattle (B) during storage at 5°C

	Samples	0th day	7th day	14th day	21st day
Antioxidant activity (%)	A	4.2 ± 2.63^{a}	61.8 ± 4.32^{b}	3.425±4.23^{c}	89.513±3.03^{d}
	B	50.73± 2.98^{a}	62.02±2.01^{b}	71.25± 1.54^{c}	80.98 ± 3.28^{d}

The antioxidant activity of fermented milks of Kasargod Dwarf and Vechur cattle were analysed during storage at refrigerated temperature of 5± 2°C for 21 days at an interval of 7 days. The data are depicted in Table 3. From the Table 3 it was observed that antioxidant activity in fermented milk made from Kasargod Dwarf milk after four hours of incubation (0th day) was 54.2± 2.63 per cent which was further increased to 61.8± 4.32 per cent, 83.425± 4.23 per cent and 89.513± 3.03 per cent after 7, 14 and 21 days of storage respectively. Similarly, antioxidant activity in fermented milk made from Vechur cattle milk after four hours of incubation (0th day) was 50.73± 2.98 per cent which was further increased to 62.02± 2.01 per cent, 71.25± 1.54 per cent and 80.98± 3.28 per cent after 7, 14 and 21 days of storage respectively. Data also revealed that the antioxidant activity of fermented milk samples was increased with increasing storage period. This increase in antioxidant activity may be due to continous slow release of peptides after fermentation which possess antioxidant effect.

The antioxidant activity was increased insignificantly from 50.73 per cent, 54.2 per cent to 89.51 per cent, 80.98 per cent at 21st days after fermentation for Kasargod Dwarf and Vechur cattle respectively. Oxidizing compounds can cause damage to proteins, lipids, or DNA. These damages are related to the development of various diseases and aging. Antioxidant peptides present in dietary proteins can limit oxidative damage, both in food and in the oxidation of body cells when they are ingested in the diet. There are many milks derived peptides from casein and whey proteins with antioxidant activity. Antioxidant peptides derived from milk are formed from 5 to 11 hydrophobic amino acids, including proline, histidine, tyrosine, and tryptophan, in sequence, that are widely distributed among the caseins, which can work by eliminating or preventing the formation of radicals as well as inhibiting enzymatic and non-enzymatic lipid peroxidation. Increased antioxidant activities in fermented milks may be due to bioactive (antioxidative) peptides released during protein digestion by bacterial fermentation. A number of bioactive peptides have been identified in milk proteins in an encrypted form, stored as propeptides or mature C-terminal peptides that are only released upon proteolysis. Peptides generated in milk digestion may act as electron donors reacting with free radicals to form more stable products. As per Farvin *et al.* (2010) [3] who reported lactic acid bacteria produce metabolic compounds acting as scavengers or degraded products of milk proteins acting as hydroxyl radicals. Reductones formed during fermentation in fermented milk could react with free radicals to stabilise and terminate radical chain reactions. Yoghurt had higher oxidative stability than milk because microorganisms could produce antioxidant peptides that act as electron donors. They reacted with free radicals and reduced radical scavenging activity.

4. Conclusion

From this study, it can be concluded that fermentation of the milk from Kasargod dwarf and Vechur cattle by yoghurt starter culture releases a large number of bioactive peptides and amino acids. These bioactive peptides showed increase in antioxidant activity during storage at 5°C for 21 days. The results are suggestive of higher therapeutic potential of fermented milk produced from milk of native cattle varieties. Further studies are needed to isolate the antioxidant components in Vechur cattle and Kasargod Dwarf fermented milk and utilize its therapeutic potential in pharmaceutical formulations.

5. Acknowledgement

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6. Conflict of interest

The authors declare that they have no conflict of interest.

7. References

1. Anu B, Aravindakshan TV, Radhika G, Jinty S. Whole exome sequencing analysis of native dwarf cattle genetic groups of kerala by next generation DNA sequencing. Journal of Pharmaceutical Innovation. 2018;7:131-135.
2. Durdevic-Denin J, Macej O, Jovanovic S. The influence of dairy matter and heat treatment on the viscosity of set-style yoghurt produced from reconstituted skim milk power. Journal of Agricultural science. 2001;46:123-135.
3. Farvin SKH, Baron CP, Nielsen NS, J Otte, Jacobsen C. Antioxidant activity of yoghurt peptides:2-characterisation of peptide fractions. Food Chemistry. 2010;123:1090-1097.
4. Fitzgeraldv RJ, Murray BA. Bioactive peptides and lactic fermentations. International Journal of Dairy Technology. 2006;59:118-125.
5. Gjorgievski N, Tomovska J, Dimitrovska G, Makarijoski M, Shariati MA. Determination of the antioxidant activity in yoghurt. Journal of Hygienic Engineering and Design. 2014;8:88-92.
6. Gobbetti M, Minervini F, Rizzello CG. Angiotensin-I-converting enzyme inhibitory and antimicrobial bioactive

peptides. International Journal of Dairy Technology. 2004;57:173-188.
7. Hoyl NT, Merritt JH. Quality of fish protein hydrolysates from herring (*Clupea harengus*). Journal of Food Science. 1994;59:76-79.
8. Hussain A, Rashid MH, Perveen R, Ashraf M. Purification, kinetic and thermodynamic characterization of soluble acid invertase from sugarcane (*Saccharum officinarum* L.). Plant Physiology and Biochemistry. 2009;47:188-194.
9. IS: (SP: 18). ISI Handbook of Food Analysis. Part XI: Dairy Products. Indian Standard Institution, New Delhi. 1981.
10. Imran TK, Nadeem M, Imran M, Ullah R, Ajmal M, Jaspal MH. Antioxidant properties of milk and dairy products: A comprehensive review of the current knowledge. Lipids in Health and Disease. 2019;18:1-13.
11. Korhonen H, Pihlanto A. Bioactive peptides: production and functionality. International. Dairy Journal. 2006;16:945-960.
12. Lorenzen PC, Meisel H. Influence of trysin action in yoghurt milk on the release of caseino-phospho peptide rich fractions and physical properties of the fermented products. International Journal of Dairy Technology. 2005;58:119-124.
13. Pihlanto-Leppälä A. Bioactive peptides derived from bovine whey proteins: opioid and ace-inhibitory peptides. Trends in Food Science and Technology. 2000;11:347-356.
14. Ravi P, Porteen K, Kandeepan G. Vechur Cattle: A Miniature Breed of India. Cow Journal. 2006;8:2-4.
15. Sah BNP, Vasiljevic T, McKechnie S, Donkor O. Antibacterial and antiproliferative peptides in synbiotic yoghurt- Release and stability during refrigerated storage. Journal of Dairy Science. 2016;99:4233-4242.
16. Yamamoto N, Maeno M, Takano T. Purification and characterization of an antihypertensive peptides from a yoghurt-like product fermented by Lactobacillus helveticus CPN4. International Journal of Dairy Science. 1999;82:1388-1393.
17. Zou L, Wei Liu, Wei-lin Liu, Rui-hong Liang, Ti Li, Cheng-mei Liu, *et al*. Characterization and bioavailability of tea polyphenol nanoliposome prepared by combining an ethanol injection method with dynamic high pressure microfluidizatiom. Journal of Agriculture and Food Chemistry. 2014;62:934-941.