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

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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. 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 milk 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 continuous 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 their 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) [11]. Fermented dairy products, such as yoghurt, contain a wide spectrum of natural bioactive peptides (Fitzgerald 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 Gobetti 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) [9].

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 aims to study the effect of fermentation on antioxidant activity and physical-chemical properties of milk from Vechur and Kasargod Dwarf cattle on refrigerated storage.

2. Materials and methods

2.1 Collection 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°C 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 at 102 ± 2°C until constant mass., the protein content was determined by Kjeldahl method and the fat content was determined by Kjeldahl 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) [13]. 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-picrylhydrazyl (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-Denn et al. , 2001 [2] and Hussain et al., 2009 [10] both reported similar findings. DH represents the percentage of peptide bonds cleaved. The degree 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

<table>
<thead>
<tr>
<th></th>
<th>0th day</th>
<th>7th day</th>
<th>14th day</th>
<th>1st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>A 0.23 ± 0.585a</td>
<td>0.77 ± 0.057ab</td>
<td>4.57 ± 0.152c</td>
<td>0.47 ± 0.152c</td>
</tr>
<tr>
<td></td>
<td>B 5.21 ± 0.608a</td>
<td>4.63 ± 0.208ab</td>
<td>4.45 ± 0.251c</td>
<td>0.43 ± 0.251c</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td>A 0.86 ± 0.172a</td>
<td>0.86 ± 0.170a</td>
<td>1.04 ± 0.004bc</td>
<td>1.06 ± 0.004bc</td>
</tr>
<tr>
<td></td>
<td>B 14.60±0.650ab</td>
<td>14.87±0.550ab</td>
<td>5.71±0.685ab</td>
<td>16.25±0.252ab</td>
</tr>
<tr>
<td>Total solid (%)</td>
<td>A 16.81±0.501ab</td>
<td>17.34±0.550ab</td>
<td>18.46±0.497bc</td>
<td>18.99±0.367bc</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>A 3.85 ± 0.297ab</td>
<td>3.91 ± 0.305ab</td>
<td>3.92 ± 0.440ab</td>
<td>4.10 ± 0.443ab</td>
</tr>
<tr>
<td></td>
<td>B 5.03 ± 0.270a</td>
<td>5.23±0.487ab</td>
<td>5.79±0.301bc</td>
<td>6.04 ± 0.467bc</td>
</tr>
</tbody>
</table>
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, 83.42±4.23 per cent and 89.51± 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) [1] 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