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Effects of Fermentation and Storage on Bioactive Activities in Milks and Yoghurts

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Abstract

Fermentation of milk enhances its nutritional value through improved bioavailability of nutrients and production of bioactive substances which have biological functions. The goals of this research were to study the effect of fermentation and storage on antioxidant and antimicrobial activities in buffalo, goat and cow milks and yoghurts. Samples of buffalo, goat, cow milks and their yoghurts during their fermentation and storage were determined for proximate analysis and bioactive activities including antioxidant activities of DPPH, ABTS and reducing power assays, and antimicrobial activities against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium* and *Escherichia coli*. Results showed that buffalo, cow and goat yoghurts had antioxidant activities in all assays and their activities significantly increased during their fermentation. Pasteurization did not affect the antioxidant activities. The activities of all yoghurts remained unchanged after a storage time of 21 days at 4°C. For the antimicrobial activities, only yoghurts from buffalo, cow and goat milks had the activities, while all milks did not show any activities. However, buffalo yoghurt could inhibit only Gram positive strains (*S. aureus* and *B. cereus*), while goat and cow yoghurt inhibited all tested strains. A chemical responsible for antimicrobial activities in yoghurts was lactic acid formed by lactic acid bacteria. However, bioactive peptides produced by protein digestion during milk fermentation by lactic acid bacteria could not be ruled out for antioxidant and antimicrobial activities present. The antimicrobial activities of all yoghurts remained constant during their storage. It is concluded that all yoghurts would retain milk nutrition and bioactive functions during their storage in a refrigerator and may be served as a functional food with benefits from those activities for consumers.

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Keywords: Antioxidant activity; Antimicrobial activity; Milk; Yoghurt; Fermentation; Storage

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Nomenclature

LAB	Lactic acid bacteria
DPPH	2,2-Diphenyl-1-picrylhydrazil
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid

1. Introduction

Bioactive peptides are short chains of amino acids that are produced during gastrointestinal digestion or food processing. The peptides have showed a wide range of biological activities such as anti-hypertension, anti-oxidant, anti-microbial, anti-angiotensin converting enzyme (anti-ACE) and anti-carcinogenic activities[1]. More recently, a great interest has been focused on peptides that can lower the blood pressure in hypertensive patients, since hypertension is a disease that is increasing at high rates, especially in developed countries[2]. Sources of bioactive peptides are found to be milk and milk products. Fermentation of milk enhances its nutritional value through improved bioavailability of nutrients and production of substances which have a biological function[3,4]. Fermented dairy products, in addition to providing both energy and nutrients, are the excellent sources of bioactive peptides. They provide numerous peptides with bioactive properties and form lactic acid and flavor compounds during fermentation and storage[5]. A large number of oligopeptides are generated by casein degradation by extracellular proteases from microbial cells. Consequently, amino acids and small peptides are generated by further breakdown by intracellular peptidases[6,7]. The proteolytic activities result in the release of bioactive peptides from specific amino acid sequences within the parent proteins and they can provide physiological benefits[8,9,10]. The size of bioactive peptides may vary from 2 to 20 amino acid residues with their activities depending on their amino acid sequence and composition[3].

Lactic acid bacteria (LAB) are commonly used to ferment milk into yoghurt and other fermented milk products. The types of LAB usually used in the dairy industries are thermophilic and mesophilic strains of *Streptococcus*, *Lactococcus*, and *Lactobacillus* species[6]. During fermentation of milk, the cell wall associated proteinase of LAB hydrolyses caseins into large peptides which are taken up into their cells, then broken down by intracellular peptidases resulting in a range of bioactive peptides showing, for example, hypertensive or angiotensin-I-converting enzyme (ACE)-inhibitory activity[6].

Cow milk and its products have been studied for bioactive peptide activities. Based on FAO Statistics (2009), cow milk is the most important milk among the different types of milk produced and its production has been growing at the rate of about 6.9% annually. The percentage proportions of milk production are as follow: cow milk (73.4%), goat milk (12.7%), buffalo milk (8.9%) and sheep milk (5.0%). Among the countries, spectacular annual growth rates for milk production have been recorded in Thailand (24.1%) and Indonesia (13.4%). Consumers drink goat or buffalo milk less than cow milk because of a problem in taste. However, in recent years, the trend of goat and buffalo milk consumption is increasing due to consumers' awareness of their health and nutrition. Some people are also sufferering from cow milk allergy and digestive problems. In addition, goat and buffalo milk products are available commercially worldwide. Unfortunately, no studies have been done to investigate bioactive activities in such products. The goals of this work were to determine bioactive activities of cow, buffalo and goat milk and their products and to study the effect of fermentation and storage on bioactive activities of the milk products.

2. Methods

2.1 Samples and Sample Preparation

Raw and processed milk including their yoghurts made from cow, goat and buffalo were collected from local farms. In addition, their yoghurt samples during fermentation and during storage for 21 days at 4°C were collected. Samples were prepared by centrifugation at 6,000 g, 4°C for 15 minutes. The supernatant (water soluble peptide extract) was kept and determined for bioactive activities.

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