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Effect of biopreservatives on microbial, physico-chemical and sensory properties of Cheddar cheese

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ABSTRACT

This study aimed to develop a Cheddar cheese using biological preservatives as substitutes for chemical preservatives. Nisin, lysozyme and lactic acid bacteria (*Lactobacillus rhamnosus*) were used as biological preservatives while $NaNO_3$ was used as a chemical preservative. Physico-chemical properties of cheese such as pH, titratable acidity, texture and moisture were measured during four weeks of ripening storage at 16 °C. Sensory properties of cheese were evaluated with the help of untrained sensory panel using a five point hedonic scale and total lactic acid bacteria, yeast and mold and coliform counts were also determined. In nisin incorporated samples, mean sensory scores for flavor, texture, odor and overall acceptability were higher than other cheese samples ($p < 0.05$). Lactic acid bacteria incorporated cheese samples demonstrated the highest acidity and texture hardness development compared to all other samples throughout the storage. Total lactic acid bacteria counts of nisin incorporated samples and control was not significantly different throughout the storage and was within the range of 8.13 ± 0.02–8.59 ± 0.01 log cfu/g. No coliform counts were recorded in any sample and yeast and mold counts were also within the generally acceptable limits during storage. Thus, biopreservatives such as nisin could be used to preserve the Cheddar cheese effectively without affecting its quality characteristics.

1. Introduction

Cheddar is one of the most popular cheese varieties in the world due to its characteristic flavor and texture and is classified as a hard to semi-hard cheese (Møller, Rattray, Bredie, Høier & Ardö, 2013). Although natural cheeses have a reasonable shelf life, quality can be affected by many factors such as undesirable microorganisms, exposure to heat, oxygen and light and enzymatic reactions. These changes in quality cause degradation or formation of active flavor compounds and reduce the consumer acceptability of the product (Asensio, Grosso & Juliani, 2015) and also increase the risk of food borne illnesses. Therefore, effective preservation techniques should be employed during food manufacturing. Nowadays food preservation is often made with the use of permitted chemical preservatives, among which benzoic and sorbic acids, and their respective sodium, potassium and calcium salts, are widely used (Tfouni & Toledo, 2002). In particular, sodium salts are commonly used in cheese manufacturing. In rare cases, these chemical preservatives may adversely affect the health of consumers. For example, the development of allergic reactions to benzoates, such as urticaria, non-immunological contact urticaria and asthma, has been reported in humans (Tfouni & Toledo, 2002). At present there is a higher consumer demand for natural and minimally processed foods, because consumers are becoming more concerned over the chemical preservatives in food products. Thus, use of natural treatments capable of assuring the quality and safety of foods are attracting interest of the industry (Sobrino-López & Martín-Belloso, 2008).

The bacteriocin nisin, produced by strains of *Lactococcs lactis,* is Generally Regarded As Safe (GRAS) and the world health organization has approved its use as a food additive. Nisin has received particular attention in the food industry due to its large inhibitory effect against a wide variety of Gram-positive organisms including certain spoilage and pathogenic bacteria such as *Staphylococcus aureus, Listeria monocytogenes* and *Clostridium botulinum* (Benech, Kheadr, Lacroix & Fliss, 2003). In addition, nisin is the only

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bacteriocin authorized for wide applications in the food industry and many recent studies have shown the successful application of nisin against pathogenic microbes in many different kinds of cheeses (Camembert, Manchego, Cheddar, Vidiago, Gouda, Cottage cheeses), demonstrating 1–3.5 log of viability loss for many pathogenic microorganisms (Aly, Floury, Piot, Lortal & Jeanson, 2012).

Lysozyme is a lytic enzyme found in many natural food systems such as milk and eggs, and it is used in cheese production to prevent the growth of lactate-fermenting and gas-forming *Clostridia* spp. The antimicrobial ability of lysozyme enhances the shelf life of cheese (Sinigaglia, Bevilacqua, Corbo, Pati & Del Nobile, 2008; Conte, Brescia & Del Nobile, 2011). According to Sinigaglia et al. (2008) the shelf life of mozzarella cheese could be prolonged by dissolving lysozyme and the disodium salt of EDTA (Na_2-EDTA) in the packaging brine. Na_2-EDTA and lysozyme were effective in inhibiting the growth of spoilage microorganisms such as coliforms and *Pseudomonas* spp. without affecting the starter culture lactic acid bacteria in mozzarella cheese. Lactic acid bacteria (LAB) occur naturally in many food systems and have a long history of safe use in fermented foods, thus classed as GRAS. Other than their use in food fermentation, they also possess a great potential for extended use in preservation of cheese. The antimicrobial activity of LAB has been credited to their ability in producing antimicrobial substances such as organic acids including lactic acid, hydrogen peroxide, reuterin, diacetyl and bacteriocins (Cheong et al., 2014). Antibacterial activities of LAB against common pathogenic and spoilage bacteria have been well documented in the literature (Ammor, Tauveron, Dufour & Chevallier, 2006; Wong et al., 2015) while Cheong et al. (2014) recently showed that LAB isolated from different fruits and vegetables can exhibit antifungal activity against a number of common cheese spoilage molds including *Penicilium commune* in cheese.

To the best of our knowledge, only few studies have focused on comparative evaluation of the efficacy of chemical and biopreservatives on the quality and the shelf life of Cheddar cheese. Further, incorporation of biopreservatives such as LAB and nisin influences the biochemical and textural properties, and these properties determine the sensory acceptability of resultant Cheddar cheese (Sallami, Kheadr, Fliss & Vuillemard, 2004). In this context, the aim of this study was to compare the storage stability of Cheddar cheese produced by using different chemical ($NaNO_3$) and biopreservatives (nisin, lysozyme and non-starter LAB) in terms of physico-chemical, sensory and microbial properties of the product during 4 weeks of ripening storage at 16 °C.

2. Materials and methods

2.1. Cheddar cheese manufacture

Fresh cow's milk (40 L) was obtained from the Livestock Field Station of the University of Peradeniya and transported to the Dairy Processing Unit at the Faculty of Agriculture, University of Peradeniya, Sri Lanka. The milk was standardized and pasteurized (Tessa NMRV053, Israel) at 72 °C for 15 seconds. According to the manufacturer's instructions, the starter culture containing a blend of *Lactococcus lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis, Lactobacillus helvetics and Streptococcus thermophiles* (kindly provided by CHR Hansen Division, J.L. Morison Son & Jones (Ceylon) PLC, Sri Lanka) were added and milk was allowed to ripen for 45 min at 32 °C. The mixture was divided into 5 equal portions containing approximately 8 L each and different preservatives were added (wt/v) as follows: (1) 0.2% $NaNO_3$, (2) 0.05% nisin, (3) 0.03% lysozyme, (4) 0.02% cultures of LAB containing *Lactobacillus rhamnosus*. The preservatives were provided by CHR Hansen Division, J.L. Morison Son & Jones (Ceylon) PLC, Sri Lanka. The fifth portion was considered as the control (without any added preservatives). The each portion of curd was then subjected to renneting (0.04% wt/wt), draining, stirring, cheddaring and salting. The cheese was further pressed manually in a cheese mold for 2–3 min and stored at 16 °C.

2.2. Physico-chemical properties

According to the Association of Official Analytical Chemist (AOAC, 1995), dry matter percentage (oven drying method), pH (using a digital pH meter, Eutech, USA), titratable acidity (by titrating with 1 M NaOH solution in the presence of 2 drops of phenolphthalein) and also texture hardness (using Instron machine, M-2239, England) of cheese samples were measured in triplicates in weekly intervals during 4 weeks of storage at 16 °C ($n=3$). Texture hardness in cheese was measured as described by Kumari, Ranadheera, Prasanna, Senevirathne, and Vidanarachchi (2015) with some modifications. Force needed to destruct cheese sample (force per area) was measured in N by using cheese samples (3 cm × 3 cm × 1 cm sized pieces) and a 38 mm in diameter flat base cylinder with a constant speed of 150 mm/min was thrust into the sample in order to measure the texture hardness. Protein (Kjeldhal method) contents of cheese samples were also measured at the end of the storage. Fat (Gerber method), protein (Kjeldhal method) and pH of cow's milk were also measured at the beginning of the experiment (AOAC, 1995) ($n=3$).

2.3. Microbial analysis

MRS agar (Oxoid, United Kingdom) was used to enumerate total lactic acid bacteria in cheese samples during storage. 1.0 g of cheese sample was serially diluted in peptone water (Oxoid, United Kingdom) solution followed by pour plating. The plates were incubated at 37 °C for 18 h anaerobically (Anarogen, Oxoid, United Kingdom). Yeast and mold counts were estimated by serial dilution and pour plating on potato dextrose agar (Oxoid, United Kingdom) followed by 3–5 days incubation at 25 °C. Similarly, coliform counts were assessed on violet red bile agar (Oxoid, United Kingdom) (37 °C for 18 h incubation) ($n=3$).

2.4. Sensory evaluation

A sensory evaluation test was conducted to evaluate the color, odor, texture, flavor and overall acceptability of cheese samples at week 3 of storage with slight modifications to Lawless and Heymann (2010). In brief, the sensory attributes were evaluated through a 5 point hedonic scale (5- like very much to 1-dislike very much) with 30 untrained panelists (students and staff members of the Faculty of Agriculture, University of Peradeniya, Sri Lanka). During the sensory evaluation procedure, all the samples were presented in uniform plastic plates coded with random three digit numbers in regular size and shape cubes ($\sim 5 \times 5 \times 3$ cm^3) under normal white fluorescent illumination. The sensory evaluation procedure was approved by the Department of Animal and Food Sciences, Rajarata University of Sri Lanka.

2.4.1. Data analysis

Parametric data were analyzed using ANOVA in SAS statistical software (Version 9.0, SAS Institute Inc., USA). Data from sensory evaluation were analyzed using Friedman non-parametric test. A p value <0.05 was considered statistically significant for all analysis.