LWT - Food Science and Technology 69 (2016) 161–168

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

The effect of savoury plants, fermented with lactic acid bacteria, on the microbiological contamination, quality, and acceptability of unripened curd cheese

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ARTICLE INFO

Article history:
Received 7 September 2015
Received in revised form
6 January 2016
Accepted 10 January 2016
Available online 13 January 2016

Keywords:
Lactic acid bacteria
Unripened curd cheese
Satureja montana L.
Rhaponticum carthamoides (CD.) Iljin
Biogenic amines

ABSTRACT

Five bacteriocins producing lactic acid bacteria (LAB) strains were selected to carry out the fermentation of the plants called *Satureja montana* L. (*Sm*) and *Rhaponticum carthamoides* (CD.) Iljin *(Rc)*. The bioproducts obtained were applied in the production of unripened curd cheese. The total amount of LAB in plant bioproducts ranged from 7.46 to 9.42 $\log_{10}$ CFU/g and depended on the LAB strain and the plant species used. To reduce the possibility of biased evaluation, the acceptability of the products was assessed by using Face Reader software. *Pediococcus pentosaceus* KTU05-8 strain was more adaptable in the media of both plants (*Sm* and *Rc*) under both fermentation conditions: traditional submerged fermentation and solid state fermentation. The fermentation of *Sm* and *Rc* plants with LAB significantly reduced the amount of spores of mesophilic bacteria, enterobacteria, yeast and fungi. The addition of these bioproducts to unripened curd cheese reduced pH and increased the total titratable acidity, the content of volatile compounds (thymol, carvacrol, *p*-cymene) and the acceptability of the cheese. In comparison to the results obtained after the addition of non-fermented *Sm*, *Lactobacillus sakei* fermented bioproducts decreased the content of biogenic amines in cheese.

1. Introduction

The consumers demand safer food without synthetic preservatives, colourants or flavours. Bio-preservation means the use of microorganisms or their metabolites in food production in order to extend shelf life of foods and enhance food safety (Ross, Morgan, & Hill, 2002). Fresh unripened curd cheese (acid curd cheeses, tvorogs, cottage cheeses) is a traditional Russian cottage cheese, which is also consumed in the neighbouring countries, including Lithuania. Short shelf-life is considered the greatest fault of fresh curd cheeses (Jasińska, Harabin, & Dmytrow, 2014). Since the lactic acid bacteria (LAB) play a key role in food fermentation, they not only contribute to the development of the desired sensory properties in the final product, but also ensure its microbiological safety. Therefore LAB with antimicrobial properties can be used in cheese production as natural preservatives to extend the shelf-life of the product (Coelho, Silva, Ribeiro, Dapkevicius, & Rosa, 2014). Currently, much of the research is based on the application of bacteriocins producing LAB to control of the growth of pathogenic bacteria in food (Coelho et al., 2014). LAB produce lactic acid, which occurs naturally in two optical isomers: D(−) and L(+)-lactic acids. L(+) lactic acid is used in the food industry in many ways, because the human body can only assimilate this form of lactic acid, whereas D(−) lactic acid is toxic to humans (Vijayakumar, Aravindan, & Viruthagiri, 2008) and therefore its use should be controlled. Among the most frequently consumed food products, cheese is most closely associated with biogenic amines (BAs) after fish (Rodriguez, Carneiro, Feijó, Conte, & Mano, 2014). Several

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http://dx.doi.org/10.1016/j.lwt.2016.01.027
0023-6438/

factors influence the BAs content in cheese: cheese production conditions, time of ripening and storage, milk pasteurization and starter culture (Standarová et al., 2010). BAs production by LAB may be controlled at various levels during food fermentation (Linares, Martin, Ladero, Alvarez, & Fernandes, 2011). Therefore the selection of LAB, which is able to decrease the content of BAs, is of outstanding importance.

Natural food additives deriving from herbs and spices have been recognized and used in food preservation for centuries (Park et al., 2013). A recent research study on spices and aromatic herbs suggests that natural spices and aromatic herbs can provide better tastes to food and help to preserve food longer than artificial flavourings (Libran et al., 2013). Because of the presence of high content of thymol and carvacrol in *Satureja* species, its attractive aroma and simple cultivation, these plants are used as a flavouring substance in many foods (Cavar, Šolić, & Maksimović, 2013). *Rhaponticum carthamoides* is considered to be highly promising in developing new classes of biologically active food additives (Buděšínský, Vokáč, Harmatha, & Cvačka, 2008). One of the modern approaches to improve the safety and functional value of manufactured food products is the application of combined antimicrobial properties of savoury plants and bacteriocins producing LAB in food production.

The aim of the study is to evaluate the impact of *Satureja montana* L. and *R. carthamoides* (CD.) Iljin bioproducts fermented with *Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7 and *P. pentosaceus* KTU05-8 strains, used to produce BLIS, on curd cheese quality and safety parameters.

2. Materials & methods

2.1. Plant material

S. montana L. family Lamiaceae Lindl. (*Sm*) and *R. carthamoides* family Asteraceae (*Rc*) were grown in an experimental field located in the Kaunas Botanical Garden of Vytautas Magnus University (Kaunas, Lithuania) in 2013. The upper part of plants was collected during the flowering vegetative phase, the plants were dried until the moisture level was approximately 7% and stored in the dark at ambient temperature. Before starting the experiment, the plants were ground in a laboratory-scale impact mill (Bühler-Miag, Brunswick, Germany) to particles with a size of 0.5–2.0 mm.

2.2. The microorganisms used for fermentation

L. sakei KTU05-6, *P. acidilactici* KTU05-7 and *P. pentosaceus* KTU05-8 strains, previously isolated from spontaneous Lithuanian rye sourdoughs and selected due to their preceding ability to inhibit undesirable bacteria (Cizeikiene, Juodeikiene, Paskevicius, & Bartkiene, 2013), were obtained from the culture collection of Kaunas University of Technology. The LAB were cultured at temperatures of 30; 32 and 35 °C respectively, for 24 h in MRS broth (CM0359, Oxoid Ltd, Hampshire, UK) and used in further experiments.

2.3. The preparation of fermented LAB bioproducts

Ground plant samples (*Sm* and *Rc*) were weighed in glass beakers covered with aluminium foil. The samples were autoclaved at the temperature of 121 °C for 10 min and soaked in sterile water. Two types of fermented LAB bioproducts were prepared: I - bioproducts containing 50% of water (solid state fermentation (SSF)); and II - bioproducts containing 70% of water (traditional submerged fermentation (SMF)). 2% of a fresh LAB culture suspension was added and mixed under aseptic conditions. The prepared plant samples were fermented for 48 h at the temperature that is optimal for certain LAB. The prepared fermented *Sm* and *Rc* plant bioproducts were used for the analysis and for the production of unripened curd cheese.

2.4. Microbiological analysis

The viability of LAB was evaluated in fermented bioproducts. LAB counts were determined on MRS agar (Liofilchem, Roseto degli Abruzzi, Teramo, Italy) using standard plate count techniques. The plates were incubated at the temperature of 30 °C for 72 h under anaerobic conditions (using atmosphere generation system AnaeroGen, Oxoid). The number of bacteria spores in non-fermented *Sm* and *Rc* plants, as well as in plant bioproducts, were determined on Plate Count Agar (CM0325, Oxoid, UK) after incubation at the temperature of 30 °C for 72 h. Before their inoculation on agar plates, the diluted samples were heated at the temperature of 80 °C for 10 min to remove the vegetative bacteria cells and evaluate the amount of spores. Enterobacteria were determined on Violet Red Bile glucose Agar (VRBA) (Liofilchem, Italy) after the incubation at the temperature of 37 °C for 24 h. The yeasts and fungi were determined on chloramphenicol agar (CM0549, Oxoid, UK) after a five-day incubation at the temperature of 25 °C. All experiments were carried out in triplicate and the number of microorganisms was expressed as $\log_{10}$ of colony-forming units per gram ($\log_{10}$ CFU/g).

The antimicrobial activity of essential oils extracted from plant-based bioproducts and non-fermented *Sm* and *Rc* plants were tested by applying an agar diffusion assay method as described by Bartkiene et al. (2015). The indicator strains (*Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas fluorescens* biovar. III, and *P. fluorescens* biovar. V obtained from the Institute of Botany of Nature Research Centre) for antimicrobial activity determination were propagated in a nutritional medium (per 1 L used: 5 g peptone, 1.5 g meat and yeast extract and 5 g NaCl) at 37 °C. The antimicrobial activities against the indicator microorganisms were determined by measuring the inhibition zones (mm). The extraction of essential oils from *Sm* and *Rc* plants and their bioproducts was carried out by using the supercritical carbon dioxide (99.9% purity, AGA) extraction, according to Juodeikiene et al. (2013). For supercritical fluid extraction, an HP 7680 T (Hewlett Packard, Palo Alto, CA, USA) apparatus was used.

2.5. Preparation of curd cheese with fermented bioproducts

Raw cow milk was collected from a local farm (Mažeikiai, Lithuania). The curd cheese was made from 10 L of raw cow milk. The raw milk, intended for the production of unripened cheese (fresh cheese), was pasteurised at 72–73 °C for 15–20 s, then it was cooled to 30 ± 2 °C. Next, 3% w/vol of fermented LAB bioproducts were added. After spontaneous coagulation of the milk, the curd was mixed and gently cut into cubes of 100 g. Then the cubes of curd were drained placed in nylon containers and pressed for 12 h and held in 4 °C. In addition, curd cheese samples with non-fermented plants were prepared. Samples of curd cheese without fermented bioproducts were analysed as control samples. Curd cheese samples for chemical and microbiological analysis were collected after the manufacturing process, which lasted for 12 h.

2.6. Determination of pH, total titratable acidity (TTA) and content of D(−)/L(+)-lactic acid isomers

The pH values of fermented *Sm* and *Rc* bioproducts were measured and recorded with a pH electrode (PP-15, Sartorius, Goettingen, Germany). Cheese slurry was prepared by blending