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Biogenic amine production by *Lactococcus lactis* subsp. *cremoris* strains in the model system of Dutch-type cheese

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ABSTRACT

The aim of this study was to compare the biogenic amine production of two starter strains of *Lactococcus lactis* subsp. *cremoris* (strains from the Culture Collection of Dairy Microorganisms – CCDM 824 and CCDM 946) with decarboxylase positive activity in a model system of Dutch-type cheese during a 90-day ripening period at 10 °C. During ripening, biogenic amine and free amino acid content, microbiological characteristics and proximate chemical properties were observed. By the end of the ripening period, the putrescine content in both samples with the addition of the biogenic amine producing strain almost evened out and the concentration of putrescine was >800 mg/kg. The amount of tyramine in the cheeses with the addition of the strain of CCDM 824 approached the limit of 400 mg/kg by the end of ripening. In the cheeses with the addition of the strain of CCDM 946 it even exceeded 500 mg/kg. In the control samples, the amount of biogenic amines was insignificant.

1. Introduction

An integral part of the cheese production technology is the addition of starter cultures. Thanks to their metabolism, a series of biochemical reactions affecting the final properties of cheeses occurs during cheese ripening. Such reactions include, in particular, the metabolism of lactose producing lactic acid and its subsequent decomposition, decomposition of proteins to amino acids and their subsequent reactions or lipolysis. Frequently used mesophilic dairy cultures during the production of natural Dutch-type cheese include the representatives of *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *lactis* biovar *diacetylactis* and *L. lactis* subsp. *cremoris* (Fox, McSweeney, Cogan, & Guinee, 2004; Walstra, Wouters, & Geurts, 2006). At the beginning of cheese ripening, lactic acid bacteria are predominant microbiota (within the first 24 h after the inoculation of milk their numbers in cheeses range from 10^7 to 10^9 CFU/mL). However, the storage time leads to their lysis and releasing of intracellular enzymes (e.g. peptidases) at the same time (Adams & Nout, 2001; Fox et al., 2004; McSweeney, 2004). The role of proteolytic enzymes is the decomposition of caseins leading to the formation of peptides with a shorter chain. The proteolysis usually proceeds even further, which leads to the production of free amino acids. The emerging peptides and free amino acids can significantly contribute to the specific final taste (Fox, Guinee, Cogan, & McSweeney, 2000; Vandenberghe, Choucharina, De Ketelaere, De Baerdemaeker, & Claes, 2014). Free amino acids may enter a number of other reactions producing significant sensorially active substances (e.g. deamination to give α-ketoacids, conversion of amino acids to aldehydes, ketones, esters, alcohols or carboxylic acids) (McSweeney, 2004; McSweeney & Sousa, 2000; Pachlová et al., 2011; Pachlová et al., 2013; Pachlová, Bunka, Flasarová, Válková, & Bunková, 2012).

From a toxicological point of view, significant reactions of free amino acids include their decarboxylation leading to the formation of biogenic amines (BA) caused by the action of microorganisms with positive decarboxylase activity. In low concentrations, biogenic amines occur naturally in all animals, plants and microorganisms (where they perform many important functions such as stabilisers of macromolecules, precursors of hormones or local

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tissue hormones) (Adams & Nout, 2001; Fuquay, Fox, & McSweeney, 2011; Nout, 1994). On the other hand, the consumption of higher concentrations of biogenic amines may have a negative impact on the consumer's health due to their effect on neurotransmitters, changes in perception, smooth muscle contractions or a negative effect on blood pressure (Dadáková, Křížek, & Pelikánová, 2009). Under normal conditions, the human body is protected against the intoxication by biogenic amines by means of detoxification mechanism (mainly by mono- and diaminooxidases). However, the effectiveness of the detoxification mechanism decreases with the increasing age of the human and it can be inhibited by alcohol or drugs (mainly psychopharmaceuticals or other drugs containing aminooxidase inhibitors) (Önal, 2007). Ripening cheeses represent a suitable environment for the formation of higher concentrations of biogenic amines by both starter and non-starter microorganisms (Adams & Nout, 2001; Buňková et al., 2009; Fuquay et al., 2011; Nout, 1994). At present, there is no legislation in the European Union to limit the content of biogenic amines in cheeses. Therefore, the cheese producers are not forced to deal with the problems concerning high concentrations of biogenic amines in cheeses despite the fact that numerous studies proved significant decarboxylase-positive activity in many lactic acid bacteria threatening the consumers' health (Adams & Nout, 2001; Buňková, Buňka, Pollaková, Podešvová, & Dráb, 2011; Buňková et al., 2009, 2010; Santos, Souza, Cerqueira, & Gloria, 2003). Recent studies mainly deal with the investigation of kinetics of the biogenic amine production under optimum conditions in a model growth medium (Buňková et al., 2009; Buňková et al., 2011; Santos et al., 2003). The ability of microorganisms to produce biogenic amines in a real environment of the foodstuff (e. g. cheese) can be completely different. The different biogenic amine production can be significantly influenced by the environmental conditions of the real system of foodstuffs (dry matter and NaCl content, pH of the system). It is therefore necessary to observe the abilities of microorganisms to produce biogenic amines not only under the conditions *in vitro*, but also in a real system of foodstuffs.

The aim of this study was to observe the development of the biogenic amine content and other quality parameters (free amino acid content and microbiological characteristics) of the model Dutch-type cheeses individually inoculated with two biogenic amine (BA) producing strains of *L. lactis* subsp. *cremoris* CCDM 824 and CCDM 946 during the ripening period (90 days at 10 ± 1 °C). Both strains are commonly used as a starter culture in dairy production, especially cheese production (Buňková et al., 2011), and their activity could contribute to the increase in biogenic amine content during cheese ripening. At the same time, the above-mentioned parameters were compared with the control samples without the addition of the BA producing strains of lactococci. In the future, the description of kinetics of the development of biogenic amine content affected by the selected strains may enable us to find tools for the development of such dairy cultures which will eliminate or minimise the production of biogenic amines.

2. Material and methods

Two types of model batches with the addition of the BA producing starter strain were produced: (i) a batch of cheeses with the addition of the BA producing strain of *L. lactis* subsp. *cremoris* CCDM 824 (hereafter L_{824}) and (ii) a batch of cheeses with the addition of the BA producing strain of *L. lactis* subsp. *cremoris* CCDM 946 (hereafter L_{946}). Both of these BA producing strains were obtained from the Culture Collection of Dairy Microorganisms (Laktoflora®, Prague, Czech Republic) as isolates from natural cheeses and their BA production was proved (Buňková et al., 2009). The taxonomy of both strains was confirmed by 16S RNA sequencing. These strains of *L. lactis* subsp. *lactis* are commonly used as part of primary starter cultures (Buňková et al., 2010, 2011) to produce a large diversity of cheeses and also fermented milks. Furthermore, control samples without the addition of the BA producing strains (hereafter "C") were also made.

2.1. Bulk starter preparation

For the preparation of the bulk starter for the control production (control cheese group C – without the addition of the BA producing strains), a commercial lyophilised mesophilic culture (Laktoflora®, Milcom, Prague, Czech Republic) containing *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar *diacetylactis* was used. In our previous work, we found out that the above-mentioned commercial culture did not produce a significant amount of biogenic amines (unpublished data). For the preparation of the bulk starter, heat-treated milk (30 min at a temperature of 100 ± 1 °C) was used. One hundred millilitres of thus prepared heat-treated milk after cooling (at a temperature of 25 ± 1 °C) was inoculated with 0.3 g of the lyophilised commercial mesophilic culture. The resulting mixture was incubated at a temperature of 25 ± 1 °C for 20 h.

For each model batch of cheeses (with the addition of the BA producing strain), two bulk starters were prepared separately: (i) a basic bulk starter of 50 mL volume (containing the above-mentioned commercial mesophilic culture of Laktoflora®), and (ii) a second bulk starter containing BA producing lactococci of 50 mL volume. The basic bulk starter was prepared by mixing 50 mL of heat-treated milk and 0.15 g of the lyophilised commercial culture and was incubated at a temperature of 25 ± 1 °C for 20 h. The second bulk starter (containing the BA producing strain of CCDM 824 or CCDM 946) was prepared by mixing 50 mL of heat-treated milk and 5 mL of broth inoculated with the appropriate BA producing strain (CCDM 824 or CCDM 946) and was incubated at a temperature of 25 ± 1 °C for 20 h. The broth used for the preparation of the bulk starter was prepared according to Buňková et al. (2011) – 5 mL of a culture medium M17 broth (Merck, New Jersey, USA) with the addition of 0.2% (w/v) precursors of biogenic amines (histidine, tyrosine, lysine, ornithine and arginine; Sigma–Aldrich, St. Louis, USA) was inoculated with 25 μl of an overnight culture (10^7–10^8 CFU/mL) and incubated at a temperature of 30 ± 1 °C for 20 h. The bulk starters after the incubation (both the basic one and also the one containing the BA producing strains) and the broths for the preparation of the bulk starters with the BA producing strains (including the overnight culture) were analysed to determine biogenic amine content. Also, microbiological analyses were performed (see Sections 2.4 and 2.6).

2.2. Cheese production

All three types of natural cheese samples: (i) the control samples (C – without the addition of the BA producing strains), (ii) the products with the addition of the BA producing strain of *L. lactis* subsp. *cremoris* CCDM 824 (L_{824}) and (iii) the products with the addition of the BA producing strain of *L. lactis* subsp. *cremoris* CCDM 946 (L_{946}) were produced in technological laboratories at the Department of Food Technology (Tomas Bata University in Zlín, Czech Republic). For the production of one batch of cheeses, 20 L of cow milk with 2.5% fat content (pasteurised at 75 °C for 30 s; FT75 pasteuriser, Armfield, Ringwood, UK) were used, from which 24 cheese blocks weighing 90 ± 3 g were produced. Three batches were produced for each of the three types of cheese (9 batches in total).

After the pasteurisation, the milk for cheese production was tempered to 32 ± 1 °C (cheese vat MSKD-1, Driml, Brno, Czech