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Aroma development in semi-hard reduced-fat cheese inoculated with *Lactobacillus paracasei* strains with different aminotransferase profiles

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Abstract

Reduced-fat, semi-hard round-eyed cheese was made from bovine milk with a mesophilic DL-starter and three *Lactobacillus paracasei* subsp. *paracasei* single-strain adjuncts with different aminotransferase (AT) activity profiles. The pilot-plant procedure was not influenced by the adjuncts, and similar cheese was made from all vats. The growth of each adjunct to dominate the cheese was confirmed by using DNA fingerprinting. Flavour profiles were different for cheeses made with the different *Lactobacillus* strains. Use of the adjunct CHCC 4256 significantly increased the content of flavour compounds that were produced from the branched-chain amino acids (BcAAs: Leu, Ile and Val) and Asp. These cheeses also had superior sensory characteristics as they tasted aromatic and sweet without bitterness. The adjunct CHCC 4256 did not have glutamate dehydrogenase activity, or the highest activity on BcAA, but showed typical AT activity, with a similar activity on Leu, Phe and Asp substrates.

Keywords: Cheese; *Lactobacillus paracasei*; Aminotransferase; Aroma formation; Glutamate dehydrogenase; Amino acid conversion

1. Introduction

Reduced-fat, semi-hard cheese with rather high moisture content often fails to produce a balanced intense flavour (Ardö, 1997). It is, however, possible to achieve enhanced flavour development in low-fat cheese by using heat-treated *Lactobacillus helveticus* with high and broad aminopeptidase activity, which more than doubles the total amount of amino acids during early cheese ripening (Ardö, Larsson, Lindmark Månsson, & Hedenberg, 1989). The composition of individual amino acids may change on addition of *Lb. helveticus* in a similar way as it does in full fat semi-hard cheese during long-time ripening (Ardö, Thage, & Madsen, 2002). Non-starter and adjunct lactobacilli that grow at high numbers in cheese during ripening have been shown to play an important role in flavour formation, especially for long-ripened cheese varieties (Peterson & Marshall, 1990; Fox, McSweeney, & Lynch, 1998; Antonsson, Molin, & Ardö, 2003). The composition of amino acids in a cheese is the result of the synergistic activities of the starter and non-starter bacteria and may reflect activities of several different enzymes, such as aminopeptidases, aminotransferases (ATs), asparaginase, serine hydratase, arginine iminase, lyases, dehydrogenases and carboxylases (Ardö et al., 2002).

Lactic acid bacteria (LAB) have the potential to generate a variety of aroma compounds from different amino acids (Urbach, 1993). Aroma compounds from Leu, such as 3-methylbutanal (cheesy, chocolate, malt), 3-methylbutanoic acid (cheesy, sweaty), and from Phe, such as phenylacetaldehyde (floral) are some of the key odour compounds in hard and semi-hard cheese

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varieties (Curioni & Bosset, 2002). It is known that catabolism of Met generates methanethiol (boiled cabbage) and several other sulphuric compounds (Grill, Patton, & Cone, 1967), and recently it was shown that acetoin and diacetyl (butter) might be produced by *Lactobacillus* strains from the oxaloacetate produced as a result of AT activity on Asp (Kieronczyk, Skeie, Langsrud, Le Bars, & Yvon, 2004).

Cheese starter *Lactococcus* strains have been shown to express several enzymes that contribute to flavour development in cheese by catabolising amino acids (Yvon & Rijnen, 2001). The first step in these pathways is performed by ATs: specific AT enzymes are used by LAB for catabolising branched-chain (BcAAs: Leu, Ile and Val), aromatic (ArAA: Phe, Tyr and Trp) and sulphur-containing (Met) amino acids. Recently, the importance of BcAT and ArAT activity was demonstrated in connection with possible cheese flavour formation by *Lactococcus lactis* (Rijnen et al., 2003). It was shown that use of a double AT *araT bcaT* depleted mutant of *L. lactis* completely prevented the formation of aroma compounds from all ArAA, BcAA and Met.

Lactobacillus paracasei is the dominant species of non-starter LAB (NSLAB) in several semi-hard cheese varieties, and produces AT enzymes with specificities similar to those of the starter *Lactococcus*, but with a large variation in activities and specificities among strains (Hansen, Houlberg, & Ardö, 2001; Williams, Noble, & Banks, 2001; Kieronczyk, Skeie, Langsrud, & Yvon, 2003; Liu, Holland, & Crow, 2003; Thage, 2003; Thage, Houlberg, & Ardö, 2004a; Thage et al., 2004b). Because different kinds of flavours are produced from the degradation of different amino acids, the AT profiles of the LAB present in cheese are thus likely to have an impact on flavour development. The AT activity depends on the availability of an amino group acceptor, commonly α-ketoglutaric acid, which may be a limiting factor for AT activity in cheese, and it has been suggested that LAB strains with the ability to produce and use glutamate dehydrogenase (GDH) to regenerate Glu back to α-ketoglutaric acid could be used to increase flavour development in cheese (Yvon, Berthelot, & Gripon, 1998; Banks et al., 2001; Tanous, Kieronczyk, Helinck, Chambellon, & Yvon, 2002). This activity, however, is in turn likely to be limited in the interior of cheese, which has a low redox potential and is anaerobic. Oxidised $NAD(P)^+$ cannot be regenerated easily in an oxygen-free environment and, consequently, the availability of reducible substances, as well as the enzymatic pathways, may be limiting factors.

Recently, we demonstrated that three *Lb. paracasei* subsp. *paracasei* strains (CHCC 2115, 4256 and 5583) had different expression of AT activities against BcAA, ArAA and Asp (Thage et al., 2004a). CHCC 2115 had 5–10 times higher AT activity against all three BcAAs when compared with the AT activity of the other strains, and BcAT activity was 20 times higher than ArAT activity and two times higher than AspAT activity; CHCC 4256 had Asp-, Leu- and PheAT activity at a similar level, whereas Ile- and ValAT activities were 50% lower, and the Tyr- and TrpAT activities were 20–50% lower than the LeuAT activity; for strain CHCC 5583, the only BcAA that was transaminated was Leu, and the LeuAT activity was 50% lower than the Asp- and ArAT activities. In this current investigation, the impact of these three *Lb. paracasei* strains on aroma production in reduced-fat semi-hard cheese was investigated.

2. Materials and methods

2.1. Cultivation of Lactobacillus cells

Three strains of *Lb. paracasei* subsp. *paracasei* (CHCC 2115, 4256 and 5583) were obtained from the culture collection of Chr. Hansen A/S (Hørsholm, Denmark). The organisms were precultured by inoculating a single colony into 10 mL De Man Rogosa Sharpe (MRS) broth (De Man, Rogosa, & Sharpe, 1960) that was incubated anaerobically at 30 °C for 22 h. Two millilitre of this cell suspension was added into 200 mL of MRS broth containing 2% glucose (pH 6.0), and incubated anaerobically at 30 °C for 22 h (referred to as stock culture). A sample (50 mL) of this culture was then inoculated into 5 L of the same media, and incubated under the same conditions. This 5 L of cell suspension was harvested by centrifuging at 4 °C for 15 min at 13,500 *g*, and the cell material was resuspended in autoclaved reconstituted skimmed milk to a final volume of 1000 mL. Portions (100 mL) of suspension were filled into tubes and frozen immediately at −50 °C; these inoculation tubes contained approximately 5×10^9 cfu mL^{-1} of each adjunct culture, in order to obtain a viable cell concentration of 5×10^6 cfu mL^{-1} in the cheese milk during cheese trials.

2.2. Cheese-making experiments

Reduced-fat (30% fat in dry matter, FDM and 60% moisture in non-fat substance, MNFS, semi-hard, round-eyed Samsø 30+) cheese was made from pasteurised (72 °C for 15 s) bovine milk using calf rennet (Standard premium 225, Chr. Hansen A/S) and undefined DL-starter (CH-N11, Chr. Hansen A/S). KNO_3 (0.01%) was added to the cheese milk in order to prevent clostridia from growing. On three different days, four cheese vats of 170 kg milk were manufactured into Samsø 30+ cheese of approximately 8 kg each; two cheeses were made from each vat. Each day, three vats were inoculated with starter and adjunct cultures of *Lb. paracasei* subsp. *paracasei* (5×10^6 cfu mL^{-1}), and a