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AROMA FORMATION IN A CHEESE MODEL SYSTEM BY DIFFERENT LACTOBACILLUS HELVETICUS STRAINS

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Abstract

A model cheese system consisting of mild, young, normal-fat, semi-hard cheese, homogenised with water to a dry matter content of 40% was inoculated with six different *Lactobacillus helveticus* strains and ripened for four weeks. After ripening, considerable increases were seen in content of free amino acids. Furthermore, the strains effected formation of different aroma patterns, and it is concluded that the model system is sensitive and that the six *Lactobacillus helveticus* strains resulted in patterns of volatiles that are likely to be associated with different sensory characteristics.

Introduction

Traditionally, *Lactobacillus helveticus* was not used in semi-hard cheese production, but research has shown that these bacteria may be used to markedly increase amino acid release and to improve the sensory profile of low-fat semi-hard cheese (1). Their impact on aroma production in cheese is suspected to be strain dependent, and in this study six different strains of *Lactobacillus helveticus* were compared and evaluated using a cheese model system.

Experimental

From a screening using molecularbiological methods of a large number of *Lactobacillus helveticus* isolates from cheese, cultures and bacteria banks, strains with potentially different properties were selected (See Table 1). The strains were cultivated with 1% inoculation level in 8x20 ml MRS for 19 hours at 37°C. In the beginning of stationary phase the bacteria were harvested by centrifugation for 10 min at 4500 rpm (708 xg), 4°C (Sigma 3-10). The supernatant was removed and the pellet was diluted in 1 ml sterile water before use. The total number of bacteria was determined on MRS plates. A cheese model was made from mild, young (one week) normal-fat, semi-hard cheese, cut into pieces and homogenised with water to a dry matter content of approx. 40%. The cheese paste obtained was heated to 80°C to inactivate enzymes and unwanted bacteria and nisin was added to improve lysis of the *Lactobacilllus* cells and as a preservative. The mixture was poured into sterile beakers with 100 g in each and the lid was closed immediately. After cooling to 40°C, *Lactobacillus helveticus* culture was added by stirring (each beaker was stirred app. 60 times) with sterile metal spoons in an aseptic bench, and the samples (100 g) were sprayed with natamysin to avoid growth of fungi. Finally the samples were

ripened for four weeks at 20°C. Reference samples of the model with no added *Lactobacillus* were incubated in parallel to assure absence of activities of unwanted microorganisms.

Table 1. *Strains of Lb. helveticus used in the study.*

Strain
Lb. helveticus CNRZ 32
Lb. helveticus CNRZ 303
Lb. helveticus ATCC 15009 (type strain)
Lb. helveticus LHC2
Lb. helveticus MI 2275 (Isolate from cheese culture)
Lb. helveticus MI 2359 (Isolate from cheese)

After 2 and 4 weeks of ripening, the samples were analysed for volatiles by dynamic headspace sampling and GC-MS (method modified after (2)). For determination of total amount of amino acids and of 12 specific amino acids, cheese samples were extracted with 6.0% 5-sulfosalicyl acid, derivatised with 6-aminoquinolyl-N hydroxysuccinimidyl carbamate (AQC), and analysed using RP-HPLC with scanning fluorescence detection (AccQTag).

Results

All the examined strains of *Lactobacillus helveticus* effected considerable increases in total concentrations of free amino acids (Table 2). Mainly the same amino acids increased in all the cheese models (data not shown), and for half of the 12 analysed amino acids (Leu, Ile Val, Phe, Pro and Lys) the increase was highest for cheese models with *Lactobacillus helveticus* ATCC 15009 and MI 2275, indicating higher lysis The presence of the same aminopeptidase activities in all the examined strains was revealed. Amino acids are important flavours, but since they also are intermediates in aroma production, differences in overall flavour of the cheese models were still expected.

Table 2. *Total content of amino acids.*

Lactobacillus helveticus strain	mmol/kg
Control	4.8
CNRZ 32	17.9
LHC2	18.3
MI 2359 (from cheese)	18.8
CNRZ 303	19.3
ATCC 15009 (type strain)	19.8
MI 2275 (from cheese culture)	25.9

This was confirmed by (Figure 1). When type of strain was related to aroma pattern using PLS regression, a clear separation of most of the cheese models was obtained, thus demonstrating considerable impact of *Lactobacillus helveticus* on volatiles normally considered potentially important aroma compounds in cheese (3). This was obtained after 2 and 4 weeks of ripening in the model system used.

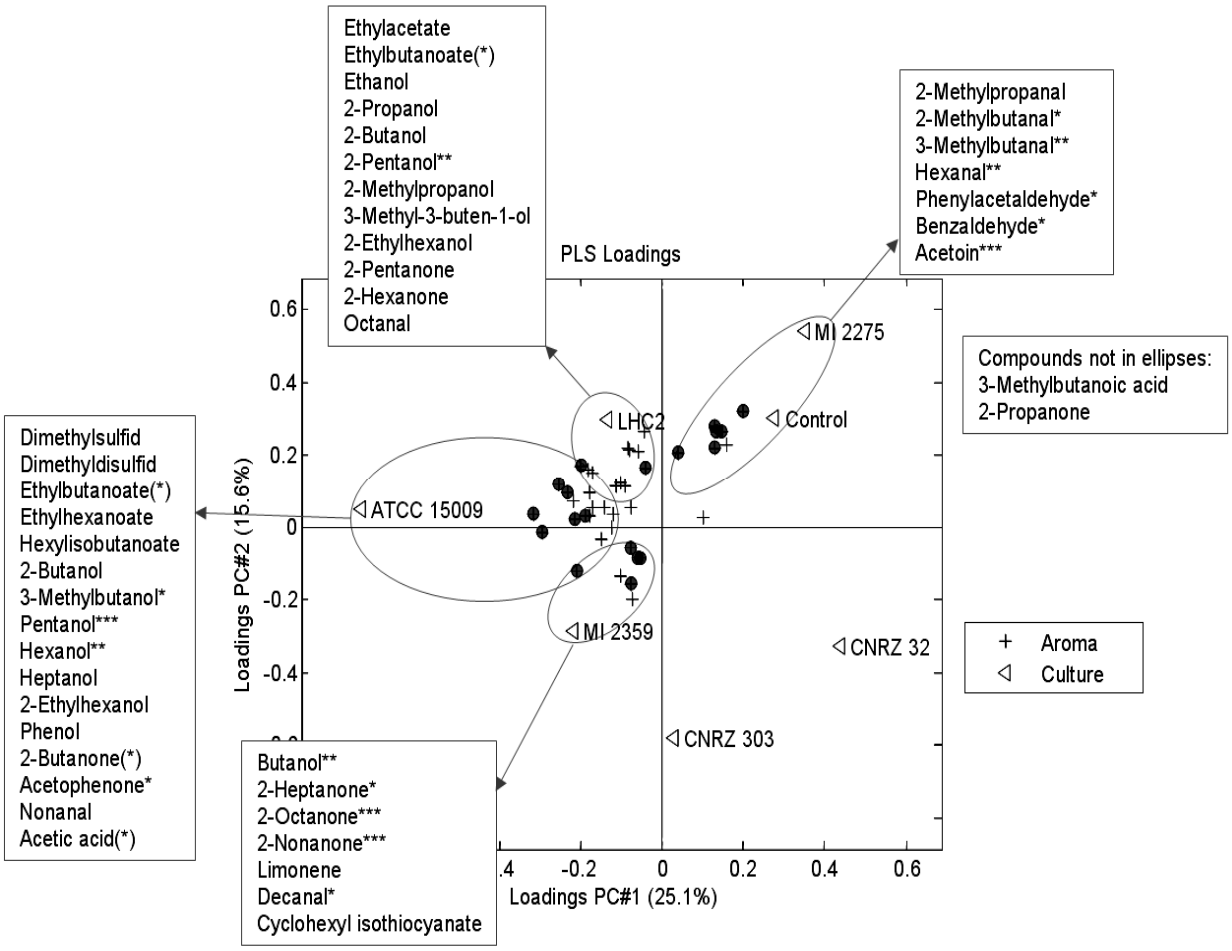

Figure 1. *Loadings plot from a dummy PLS relating aroma data to strain. A red circle indicates a volatile compound where the concentration is significantly influenced by the culture (ANOVA). The boxes list compounds having higher concentration in the model and asterisks indicate significant influence of culture (ANOVA, *: p<0.05; **: p<0.01; *** p<0.001).*

Most compounds increased in concentration as compared to the control (however, phenylacetaldehyde, hexanal and 3-methylbutanal were lower than the control for all *Lactobacillus helveticus* strains except MI 2275 (data not shown). The results indicate that MI 2275 has high capacity of amino acid catabolism since all the volatiles found in significantly higher amounts than in the control were possibly amino acid derived (Table 2) (3). The other strains had increased levels of mainly alcohols and also ketones derived from lipids (except 3-methylbutanol which is amino acid derived), indicating significant lipase/esterase activities. It should be noted that there was a characteristic difference for MI 2359, being the only strain with increased levels of several methyl ketones. Free fatty acids are the principal methyl ketone precursors through β-oxidation to β-keto acids (4) and the increased formation in the MI 2359 model could be a result of increased lipase activity.

It is concluded that the model system has a potential in screening experiments by achieving useful results about processes going on during cheese ripening within the short time of only 4 weeks. Furthermore, the six *Lactobacillus helveticus* strains tested had a clear effect on release of free amino acids and of volatiles that are likely

to be associated with different sensory characteristics. Cheese making experiments are needed to confirm the interesting effects in different cheese varieties.

Table 2. *Volatiles present in significantly higher amounts in models with Lb. helveticus strains than in controls (ANOVA, p<0.05). The strain CNRZ32 did not significantly increase the amount of any volatile component.*

MI 2275	LHC2	ATCC	CNRZ 303	MI 2359
3-Methylbutanol		3-Methylbutanol		
2-Methylbutanal		Butanol	Butanol	Butanol
3-Methylbutanal	Pentanol	Pentanol		Pentanol
	Hexanol	Hexanol		Hexanol
		2-Butanone		Decanal
Benzaldehyde				2-Heptanone
Acetoin				2-Octanone
				2-Nonanone

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