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BIOGENIC AMINE CONTENT IN CHEESE PRODUCED WITH DIFFERENT SELECTED LACTIC ACID BACTERIAL STRAINS

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ABSTRACT. The aim of the work was to study the effect of selected Lactobacillus strains having different decarboxylase activities on the formation of biogenic amines during manufacturing and storage of semi-hard cheeses produced under laboratory conditions. Results were compared with cheese made with a starter culture generally used in the dairy industry. The present experiment proved that cheeses fermented with selected Lactobacillus strains contained lower amounts of biogenic amines, especially tyramine and histamine, than the sample fermented by mixed lactic acid bacterial culture.

Keywords: *biogenic amines, cheese, starter cultures*

INTRODUCTION

Biogenic amines (BAs) are aliphatic, alicyclic or heterocyclic low molecular weight bases. They can be found in all kind of food products at low level.

In fermented foods BAs are mainly formed due to the microbial decarboxylation of free amino acids [1]. Among BA producers are starter and non-starter bacteria [2]. The microorganisms involved in fermentation process can cause high concentrations of BAs.

The consumption of high amounts of BAs can cause several problems to the consumer. The determination of the toxicity threshold is difficult, since it is dependent on the individual response and on the presence of co-factors [3]. In the European Union a limit is established for histamine

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in fish (200 mg/kg) [4]. Some countries have regulated the maximum amounts of histamine in different foods at a national level. There are recommendations for histamine (100 mg/kg), tyramine (100-800 mg/kg) and phenylethylamine (30 mg/kg) [5].

Cheeses represent an ideal condition for amine production, i.e. the presence of free amino acids and the presence of bacteria which is able to decarboxylase amino acids [6]. Several efforts have been made in food science and in the food industry to reduce or to prevent formation of biogenic amines in food. Using selected starter culture is one of the most encouraging methods for the reduction of biogenic amine content in food [7,8,9].

The aim of this work was to study the influence of three selected *Lactobacillus* strains (*Lactobacillus paracasei subsp. paracasei 2750*, *Lactobacillus fermentum DT41*, *Lactobacillus curvatus 2770*) on the formation of BAs during manufacturing and storage in cheese.

RESULTS AND DISCUSSION

Microbiological analysis

Figure 1 illustrates the development of the total microflora over a period of 6 weeks. The total viable counts varied between 8-9 log CFU/g depending on the used starter culture. At the end of the first week in the cheese made with the industrial starter culture the total aerobic plate count was slightly lower than in cheeses made with *Lactobacillus* strains. During the 6 weeks of storage this difference reduced, and at the end of the 6 weeks, the plate counts were around 8 log CFU/g in all types of cheeses.

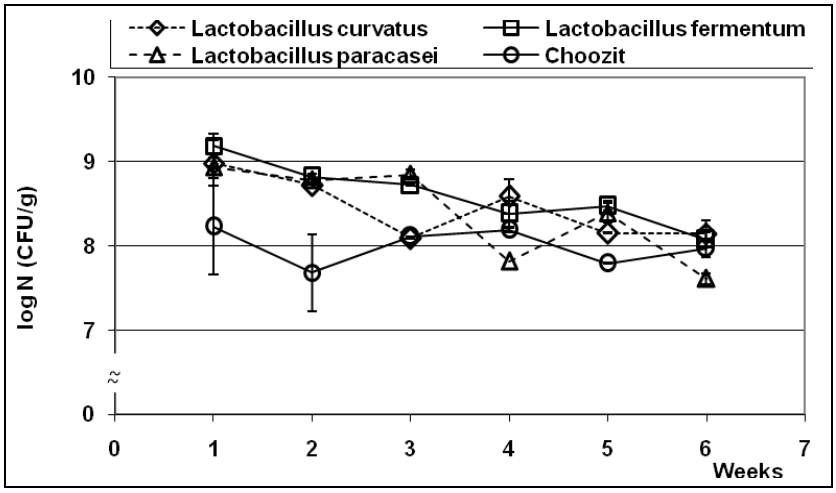

Figure 1. Aerobic plate counts of cheese samples during 6 weeks of storage at 13 °C

Biogenic amine analysis

The changes of total BA content of cheeses are summarized in Figure 2. The amount of total BAs was significantly lower in cheeses made with *Lactobacillus* strains than in cheese made with industrial starter culture (Choozit). The total BA content ranged between 25-42 µg/g, 25-85 µg/g, 14-112 µg/g and 3-207 µg/g in samples inoculated with *Lactobacillus curvatus*, *Lactobacillus fermentum*, *Lactobacillus paracasei* and Choozit Cheese Culture, respectively.

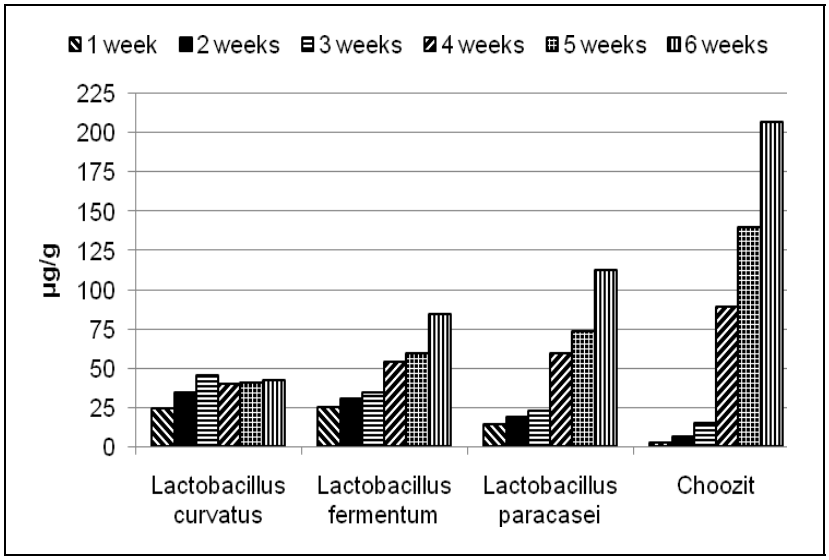

Figure 2. Changes of biogenic amines in cheese samples inoculated with different starter cultures during 6 weeks of storage

The biogenic amines determined were cadaverine (Cad), putrescine (Put), histamine (Him) and tyramine (Tym). Cad and Put were the major amines in cheeses with selected *Lactobacillus* strains (Fig. 3,4,5), while Tym and Him were the predominant BAs in cheese manufactured with Choozit Cheese Culture (Fig. 6).

The biogenic amine content was significantly lower in samples inoculated with selected *Lactobacillus* strains than in samples manufactured with the cheese culture used in the dairy industry. The BA composition was much better (no Tym and lower Him content) in cheeses made with selected starter cultures, than in cheeses made with the industrial starter culture “Choozit Cheese Culture”.

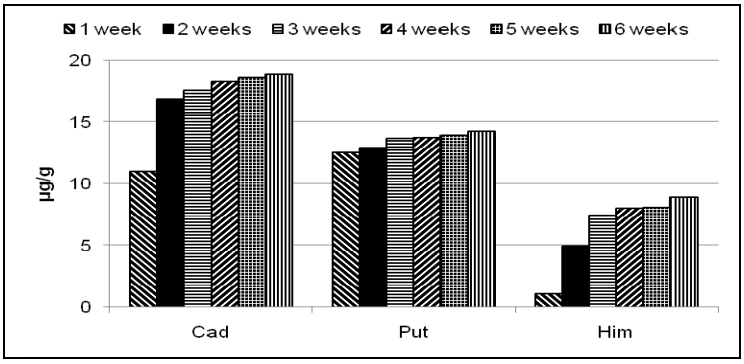

Figure 3. Changes of biogenic amines in cheese samples inoculated with *Lactobacillus curvatus 2770* during 6 weeks of storage

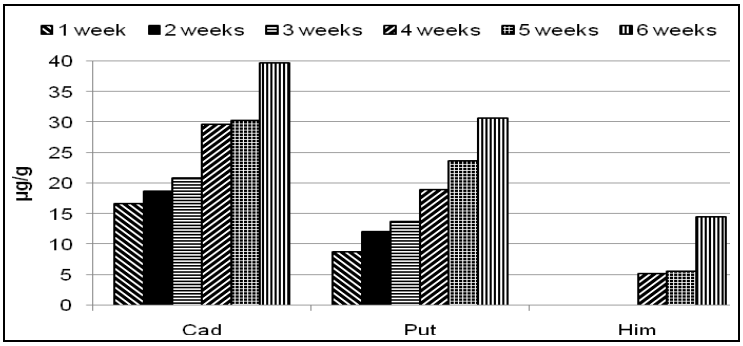

Figure 4. Changes of biogenic amines in cheese samples inoculated with *Lactobacillus fermentum DT 41* during 6 weeks of storage

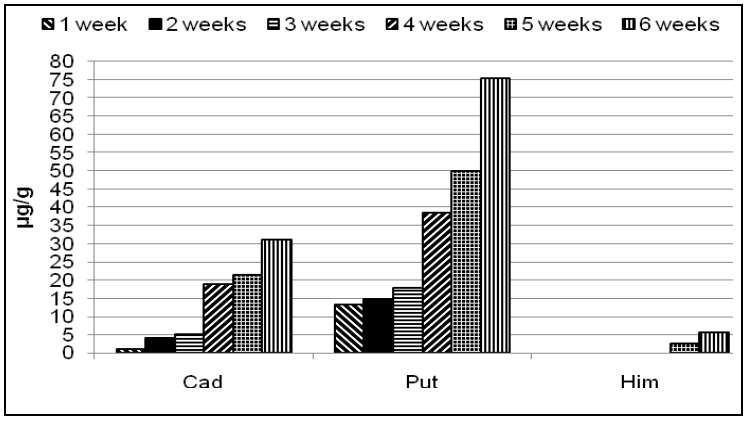

Figure 5. Changes of biogenic amines in cheese samples inoculated with *Lactobacillus paracasei subsp. paracasei 2750* during 6 weeks of storage

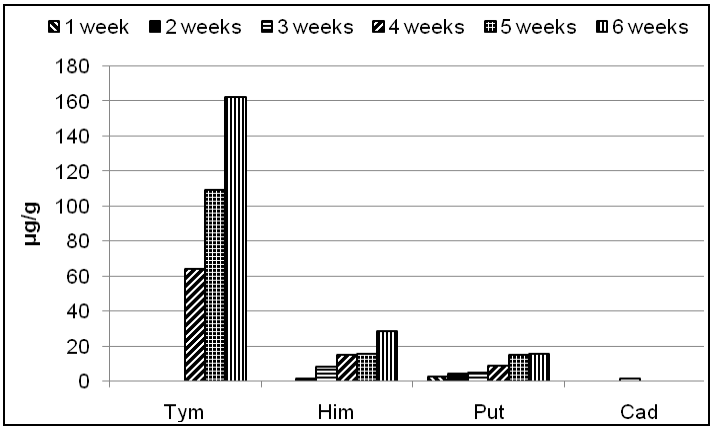

Figure 6. Changes of biogenic amines in cheese samples inoculated with Choozit during 6 weeks of storage

CONCLUSIONS

Our results indicates that it is recommended to use selected starter cultures to obtain a healthier product regarding the biogenic amine content and composition of cheeses.

EXPERIMENTAL SECTION

Cheese manufacture

10 litres of pasteurized, non-homogenized bovine milk with 2,8 % fat content (Dabastej Ltd., Dabas, Hungary) was heated to 30°C during continuous agitation (15 traverses per minute) in a laboratory-scale stainless steel jacketed cheese vat (FT20 Cheese Vat, Armfield Ltd., UK). Then 2,5 g of $CaCl_2$ were added. Four various starter cultures with different decarboxylase activity were used in the experiments: *Lactobacillus fermentum DT41*, *Lb*. *curvatus 2770*, *Lb*. *paracasei subsp*. *paracasei 2750* and Choozit Cheese Culture used in the dairy industry (Danisco A/S, Denmark). Milk was inoculated by the starter culture and 5 cm^3 of rennet were added (Chy-Max Special, Ch. Hansen, Denmark). Milk was allowed to coagulate. After coagulation the curd was cut. The curd-whey mixture was gradually heated to 39°C (2°C/12 min) under continuous agitation. When 39°C was reached the curd-whey mixture was stirred for 1 more hour. Then whey was drawn and curds were put into a hoop and put into a cheese press. Pressure was increased continuously up to 5 kg/cheese kg. Cheese was pressed overnight at room temperature then removed from the press and put into brine (20% salt concentration) for 24 hours. Then cheese was dried for 1 day at room temperature and vacuum packaged in Cryovac BB4L foil bags (Sealed Air Corporation, USA) (oxygen permeability 30 cm^3/m^2/24 h at 23°C, 0% RH and 1 bar). The four various

cheeses were stored at 13±2°C in a cooling cabinet (J 600-2, Thermotechnika Ker. Ltd., Hungary). Samples were taken every week until the cheese samples were used up (6 weeks). After sampling cheeses were vacuum packaged again and put back to storage.

Microbiological analysis

Aerobic plate counts were determined as follows: 10 g of sample was placed in a stomacher bag (with nylon mesh bag, pore size 1.0 mm) with 40 cm^3 of diluents (1 g peptone; 8.5 g NaCl in 1000 cm^3 distilled water). Five-time dilution cheese homogenates were stomached for 2 minutes, and 10-fold dilution series were prepared. These were routinely cultivated on Plate Count Agar (PCA, MERCK). PCA plates were incubated at 30°C for 2-3 days. Samples were taken regularly for aerobic plate count determination during 6 weeks of storage.

Biogenic amine analysis

Samples (2 g) were extracted with 10 cm^3 10% trichloroacetic acid for 1h at room temperature, at 100 rpm using a Laboshake (Gerhardt, Germany). Samples were filtered through a 0.25 µm membrane filter (Nalgene, USA). Biogenic amine analysis was performed with an AA 400 Amino Acid Analyser (Ingos, Czech Republic) [10].

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