THE INFLUENCE OF A STARTER CULTURE ON THE AMINE FORMATION CAPABILITY OF AN AMINE-POSITIVE LACTOBACILLUS STRAIN

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Keywords: biogenic amines, starter culture, amine-positive lactic acid bacterium, meat

Background

Biogenic amines are basic nitrogenous compounds occuring in foods mainly due to the amino acids decarboxylation activity of some microbes. The presence of histamine, tyramine, tryptamine and phenylethylamine especially in conjuction with potentiating factors such as amine oxidase-inhibiting drugs, alcohol and other food amines (e.g. spermine, spermidine, putrescine, cadaverine) may cause food poisoning (Askar and Treptow, 1986).

The fermentation process of sausage manufacturing offers both microorganisms and protein-free amino acids required to amine formation together with environmental factors (pH, temperature) favoring bacterial growth and decarboxylase activity. An important parameter suggested for preventing amine accumulation is the controlling of natural fermentation by addition of amine-negative starter culture (Eitenmiller et al., 1978, Taylor et al., 1978).

Objectives

The purpose of this work was to study the effect of a lactic acid bacterial starter culture on the amine formation capability of an amine-positive contaminant lactic acid bacterial strain (G 106) isolated from dry sausage (Maijala and Eerola, 1993). The work was done in a *in vitro*-scale using minced meat as a model of dry sausage.

Methods

Before the trial the amine formation capability of G 106 and commercial starter culture were tested in MRS-broths containing 0.5% tyrosine and histidine. Broths were incubated at 20 °C for 72 hours in a shaker and amines were determinated as described in the study of Maijala and Eerola (1993). Pure cultures of G 106 and starter culture were inoculated separately into MRS-broths (containing 0.1% tyrosine and histidine) and incubated at 30 °C for 24 h, anaerobically. Bacterial suspensions were added to minced meat. Inoculated amounts of bacteria/g meat mass were enumerated by cultivating the broths on MRS-S agars.

The raw materials, beef and pork meat, were obtained from local slaughterhouse. The surface of a 500 g meat pieces (pork and beef) were first burned with bunsen flame and then removed with a sterile knife. Meat was cut into smaller pieces and divided to 5 samples, all containing pork and beef (1:1). Meat was minced in a Moulinex mixer (chamber and blade washed with 70% ethanol), NaCl (3%) and D-glucose(0.6%) were added. The sample of raw material was taken before the inoculation of strains.

The raw material was divided into five portions (A-E), of which A was a control without any inoculation of strains, B was manufactured with starter culture (6.82 $\log_{10}$(CFU/g)), C with G106 (7.07 $\log_{10}$(CFU/g)), D with starter culture and G106 (6.82 + 7.07 $\log_{10}$(CFU/g)) and E with starter culture and G106 (6.82 + 2.82 $\log_{10}$(CFU/g)).

A 10 g sample was serially diluted with a diluent containing 0.1% peptone and 0.85% NaCl in sterile deionized water. Aerobic mesophyllic microorganisms (PCA, at 30 °C 48 h), lactic acid bacteria (LAB) (MRS-S, at 20 °C 5 d anaerobically), enterobacteria (VRBG, at 37 °C 24-48h), enterococci (Slanetz-Bartley, at 44.5 °C 48h) and micrococci (blood agar, at 37 °C 24 h) were enumerated from raw materials (two plates from one dilution / sample). From 1st, 4th and 7th days samples only LABs were enumerated (one plate from three dilution series / sample).

Biogenic amines were analysed from 2 g of sample with 0.4 M perchloric acid and detected as their dansyl derivatives by high performance liquid chromatography (Eerola et al., 1993). pH values were measured directly from samples using a PHM Laboratory pH meter (Radiometer Oriola Prolab), equipped with a combination electrode (GK2401C).

Results and discussion

The microbial quality of raw material was good. There were only some colonies detected in PCA, MRS-S and blood agar plates and no enterobacteria and enterococci were found. The evolution of LAB was similar in B, C, D and E, increasing to 7.8-8.4 $\log_{10}$

(CFU/g) on the 1st day. Sample A contained LAB below 4 $\log_{10}$ (CFU/g) in 1st and 4th days samples but it reached the level of 8.28 $\log_{10}$ (CFU/g) at the 7th day. Two different types of LAB colonies were observed on MRS-S plates (sample B: starter culture, sample C: G 106, sample D: both). In D sample G 106 was the predominant LAB on the 4th and 7th days samples.

The pH value of raw material was 5.4 and it decreased in samples B, D and E in a similar way to 5.0-5.1. pH increased from 5.1 to 5.3 in C-samples from the 4th to 7th day. In control samples pH increased from 5.4 to 5.9 during the trial.

The levels of histamine, tyramine and phenylethylamine formed are presented in Figure 1. The amounts of other biogenic amines determined did not increased during the trial (tryptamine, putrescine, cadaverine and spermidine were <10 mg/kg and spermine 29-42 mg/kg).

Tyramine was formed in a control samples, indicating existence of an amine-positive contaminant bacteria in the raw material. Sample B (manufactured with starter culture only) contained less tyramine than other samples thus proving that starter culture had effect on this contaminant bacteria derived from the raw material. However, starter culture could not inhibit the formation of histamine and tyramine in both inoculation levels of G106. The lower inoculation level only delayed the formation process. Phenylethylamine was formed only when G106 was inoculated at higher amount. The starter culture had effect on contaminant G106 only at the lower inoculation level.

As a conclusion the effect of the starter culture was dependent on the kind of contaminant microorganism and also on the different biogenic amine.

Literature

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Figure 1. The evolution of histamine, tyramine and phenylethylamine during the trial.