

Influence of the contamination level of *Lactobacillus parabuchneri* in vat milk and of the cheese-making conditions on histamine formation during ripening.

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Introduction

In recent years, *L. parabuchneri* has been identified as one of the most potent histamine producing species in cheese, which can cause histamine levels of >1000 mg kg⁻¹ in ripened cheeses. The consumption of cheeses contaminated with elevated histamine content provokes a persistent burning sensation in the mouth and may cause skin redness, diarrhea and other digestive disorders. People with histamine intolerance are particularly affected and may show even more severe symptoms. *L. parabuchneri* is a Gram-positive, facultative anaerobic, and catalase negative rod that grows at 15°C, but not at 45°C. Histidine decarboxylase (HDC, EC 4.1.1.22) activity is a strain-specific characteristic of *L. parabuchneri*. The HDC gene cluster is located on a genomic island and was introduced to the genome by horizontal gene transfer. In addition to histidine decarboxylase activity, the obligate hetero-fermentative species can convert arginine into ornithine, CO₂, and ammonia using the arginine deiminase (ADI, EC 3.5.3.6) pathway and is capable of converting lactic acid to acetic acid, CO₂, and propane-1,2-diol under anaerobic conditions. As a result of these metabolic properties *L. parabuchneri* has some selective growth advantages in cheese. However, little is known about the damage threshold of this species in cheese milk and its survival in cheese production.

Material & Methods

In the present study four cheese isolates of *L. parabuchneri* (FAM 21731, FAM 21823, FAM 21836, and FAM 23097) were inoculated into pasteurized milk at four different levels (10¹, 10², 10³ and 10⁴ cfu g⁻¹) in order to study strain-specific differences regarding thermal resistance and histamine formation in experimental cheeses manufactured at four different scalding temperatures (44, 48, 52 and 56°C). Gene equivalents (GE) of *L. parabuchneri* during cheese ripening was monitored after 1, 15, 45, 90 and 180 days using a species-specific qPCR assay. Formation of histamine was measured after 45, 90 and 180 days using UPLC.

Results

The results show that a very low population density of about 10⁰–10¹ cfu g⁻¹ is sufficient to induce a strong formation of histamine. Independently of the inoculation level up to 992 mg histamine kg⁻¹ was accumulated in the cheeses scalded at 44°C within a ripening period of 180 days.

Moreover, *L. parabuchneri* was found to be rather heat tolerant. Three of four tested strains survived scalding at 52°C for 20 minutes; a histamine level of 481 mg kg⁻¹ could be detected after 180 days, which is nearly half the amount measured in the cheeses scalded at 44°C. However, scalding at 56°C for 20 min proved to be a suitable measure for the inactivation of *L. parabuchneri* and thus for the prevention of histamine

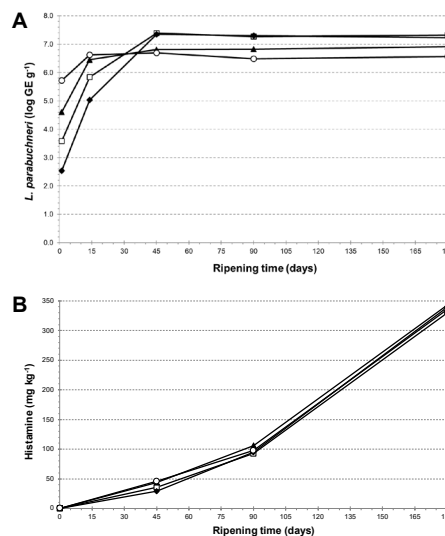


Figure 1: Gene equivalents (GE) of *L. parabuchneri* (A) and histamine formation (B) in experimental cheeses made from vat milks inoculated with *L. parabuchneri* strains at a level of 10⁰–10¹ cfu mL⁻¹ (◆), 10¹–10² cfu mL⁻¹ (□), 10²–10³ cfu mL⁻¹ (▲), and 10³–10⁴ cfu mL⁻¹ (○). The results represent mean values of four cheeses each produced from milk individually contaminated with one of the four *L. parabuchneri* strains FAM 21731, FAM 21823, FAM 21836 and FAM 23097.

formation. Practical experience confirms that hard and extra-hard raw milk cheeses such as Gruy ere PDO, Parmigiano Reggiano PDO or Sbrinz PDO only contain low histamine contents. For all other raw milk cheeses produced under milder thermal conditions, contamination with *L. parabuchneri* poses a serious risk to food safety and product quality. To prevent the survival of *L. parabuchneri* in biofilms, which may be a persistent source of contamination, a minimum temperature of 60°C is recommended for the cleaning of milking or processing equipment.

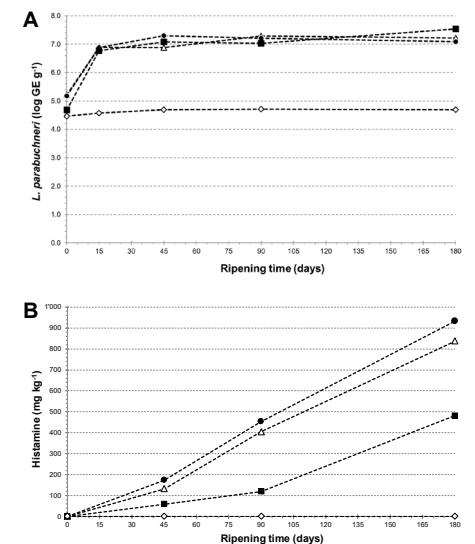


Figure 2: Gene equivalents (GE) of *L. parabuchneri* (A) and histamine formation (B) in experimental cheeses made from vat milks inoculated with *L. parabuchneri* strains at a level of 10²–10³ cfu mL⁻¹ and scalded at maximal temperatures of 44°C (●, n = 4), 48°C (△, n = 4), 52°C (■, n = 3), and 56°C (◇, n = 4). With one exception, the values represent mean values of four cheeses each produced from milk individually contaminated with the four *L. parabuchneri* strains FAM 21731, FAM 21823, FAM 21836 and FAM 23097. The strain FAM 21836 was already inactivated at a maximum scalding temperature of 52°C and was therefore not included at this temperature.

Summary: *L. parabuchneri* has been identified as an important histamine producing species that survives the scalding conditions of most cheeses varieties. Even slight contamination in the processed milk may cause strong histamine formation during cheese ripening. Therefore, the prevention of contaminations in raw milk and exclusion of contaminated milk from processing are important measures to ensure the safety and quality of raw milk cheeses.

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