Formation of biogenic amines by *Lactobacillus wasatchensis* in experimental Swiss-type cheeses and related opening defects

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Introduction

Lactobacillus wasatchensis (DSM 29958T) is an obligate heterofermentative, nonstarter lactic acid bacterium that has recently been discovered and isolated from downgraded Cheddar cheese showing late gas formation ("gassy defect"). Even though this textural defect may not create a specific sensory defect, the formation of slits or cracks severely restricts the cheese's suitability to be cut and sliced. Apart from product losses, the downgrading of such cheeses entails substantial financial loss.

The abilities of *Lb. wasatchensis* to utilize both ribose and galactose, to grow under elevated salt concentrations, acidic conditions and low temperatures (4 to 5% s/m, ~pH 5.2, 6 to 12°C) have been shown to be important characteristics that contribute to gas formation in Cheddar cheese. However, little is known about its occurrence in other cheese varieties, its thermal resistance, and metabolic characteristics that may contribute to gas formation such as citrate utilization and decarboxylation of selected amino acids.

Material & Methods

In the present study, eight experimental Swiss-type cheeses were made from pasteurized milk inoculated with *Lb. wasatchensis* (DSM 29958T) at a level of about 10^4 cfu ml^{-1} using scalding temperatures in the range of 35-58°C. During cheese ripening (3 months), eye formation was monitored using 2D X-ray, computer tomography and cross-sectional views. Moreover, population density of *Lb. wasatchensis* was measured by a species-specific qPCR and ripening was monitored by determination of volatile carboxylic acids, biogenic amines, OPA-value and pH.

Results

The qPCR results (**Table 1**) indicate that *Lb. wasatchensis* (DSM 29958T) was gradually inactivated at scalding temperatures ≥ 50°C. As a result, no gas formation was detectable in the cheeses no. 5 to 8 after a ripening time of 29 days (**Fig. 1**). However, during the subsequent ripening time, CO_2 formation by the *Propionibacterium freudenreichii* culture resulted in cheese 1 (control) and cheeses 5 to 8 in eye formation typical of Swiss cheese (**Fig. 2**). In in cheese no. 2, growth and CO_2 formation by propionibacteria was limited due to the high water content and the resulting lower pH.

The inoculated *Lb. wasatchensis* type strain produced high amounts of cadaverine and putrescine with maximal concentrations of 2200 mg kg^{-1} and 660 mg kg^{-1}, respectively (**Table 1**). Through the decarboxylation of lysine and ornithine to the corresponding biogenic amines, about 600 mL CO_2 per kg cheese were formed. This resulted in the formation of cracks and slits in the cheese scalded at 35°C, whereas the cheeses 3 (40°C) and 4 (45°C) showed a too intensive and improper eye formation with many interconnected or irregularly shaped eyes (**Fig. 2**). On the other hand, the formation of basic degradation products favored the pH increase, which, as can be seen from the OPA values, promoted proteolysis and thus made the cheese body susceptible to slit and crack formation. Formation of biogenic amines seems to be a key factor in explaining opening defects caused by *Lb. wasatchensis*.

The use of citrate as a carbon source can be considered as a strain-specific feature that supports the growth of different species of homo- and heterofermentative lactic acid bacteria. The results of the present study indicate that *Lb. wasatchensis* is not capable of metabolizing citrate. This finding was also confirmed in in vitro tests (data not shown).

Table 1. Chemical characterization of experimental Swiss-type cheeses made from pasteurized milk inoculated with *Lactobacillus wasatchensis* at a dose of 10^4 cfu ml^{-1} (no. 1 = control without *Lb. wasatchensis*). Different scalding temperatures in the range of 35-58°C were applied.

Cheese number		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Scalding temperature		53°C	35°C	40°C	45°C	50°C	53°C	56°C	58°C
Lb. wasatchensis	GE g^{-1}	Not detected	8.4E+07	3.6E+07	2.8E+07	1.3E+06	2.1E04	7.9E+02	1.2E+04
Cadaverine	mg kg^{-1}	0	1900	2200	1940	12.3	0	0	0
Histamine	mg kg^{-1}	0	47.3	6.7	0	0	0	0	0
Putrescine	mg kg^{-1}	0	660	622	484	0	0	0	0
Tyramine	mg kg^{-1}	0	46	0	0	0	0	0	0
Total biogenic amines	**mg kg^{-1}**	**0**	**2650**	**2820**	**2420**	**12.3**	**0**	**0**	**0**
pH 24 h	-	5.29	5.08	5.24	5.27	5.31	5.31	5.28	5.25
pH 90 d	-	5.64	5.72	5.79	5.82	5.63	5.65	5.75	5.72
Moisture	g kg^{-1}	381	436	379	375	378	373	370	375
Fat	g kg^{-1}	306	278	307	309	304	306	318	307
OPA	mmol kg^{-1}	161	237	254	269	151	174	206	208
Acetic acid	mmol kg^{-1}	40.08	33.08	42.63	41.85	45.32	45.05	41.67	43.33
Propionic acid	mmol kg^{-1}	77.7	65.1	82.7	82.3	87.4	88.7	75.5	78.8
Total carboxylic acids	**mmol kg^{-1}**	**118.6**	**99.7**	**126.2**	**125.0**	**133.5**	**134.8**	**118.0**	**123.0**
Citric acid	mmol kg^{-1}	6.0	5.3	5.3	6.4	6.0	6.1	6.2	6.4

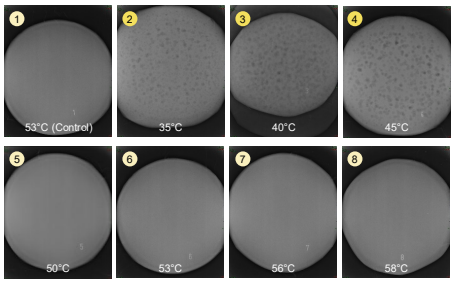

Figure 1. 2D X-ray images of 29 days aged experimental Swiss-type cheeses produced with different scalding temperatures containing *Lb. wasatchensis* (DSM 29958T).

Figure 2. Cross-sectional views of 90 days aged experimental Swiss-type cheeses produced with different scalding temperatures containing *Lb. wasatchensis* (DSM 29958T).

Summary: *Lactobacillus wasatchensis* (DSM 29958T) has been identified a potent gas producing species that promotes the "gassy defect" in Cheddar cheese. The present study shows that gas formation by this species is largely due to the formation of biogenic amines. Due to the limited heat resistance, this new species is not relevant for the production of hard cooked cheeses such as Emmentaler PDO and Gruyère PDO cheese.

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