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Measurement of CO_2 from metabolic activity of adjunct cultures during cheese ripening

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Introduction

Eye formation is an important characteristic trait for many semi-hard cheese varieties. Deviations from the defined eye characteristics result in disadvantages for sale and therefore lower prices. Control of the eye formation during cheese ripening is important but still challenging. Besides conditions during moulding and pressing, the amount of CO_2 formed by starters and NSLAB in different metabolic pathways and the amount of eye nuclei present in the cheese body influence the shape, number and size of eyes. In Swiss semi-hard cheeses, small round eyes are desired only.

Material and Methods

A new system for the dynamic measurement of gas formation on the basis of highly sensitive pressure sensors was developed and used to study the influence of *Lactobacillus parabuchneri* adjunct cultures on the formation of CO_2 in experimental Tilsit cheeses (fig. 1).
Four different cheeses were produced out of microfiltered raw skim milk, added pasteurised cream and eye nuclei with one replicate on day 1 (7.5±0.14 kg) and day 2 (6.7±0.26 kg) respectively: Standard; with histamine producing adjunct *L. parabuchneri* FAM21731 (hist+); with histamine negative strain *L. parabuchneri* FAM21835 (hist-); and with both strains. The whole wheels ripened each in a hermetically sealed container. Gas volumes diffused into eyes and out of the cheeses were measured over the length of the ripening period. O_2 was removed (<0.1%) by addition of argon before measuring. No smear developed due to the anaerobic conditions. Volatile carboxylic acids, propane-1,2-diol, biogenic amines and free amino acids were determined in order to calculate the contributions of different metabolic activities to CO_2 formation.

Fig. 1: Dynamic gas volume measuring system developed in collaboration with abiotec Ltd., Rheinfelden, Switzerland

Results and discussion

The gas volume monitor showed significantly higher gas diffusion for all cheeses with *L. parabuchneri* adjuncts ($p < 0.01$ for hist+ and hist-; $p < 0.001$ for combination; fig. 2). At day 142 it was 220 mL/kg for the standard cheese and 395 mL/kg with both strains together. Approximately half of the total gas production in the cheeses with *L. parabuchneri* was due to the arginine deiminase (ADI) pathway forming ornithine (fig. 3). The hist+ and hist- strains formed about the same amount of CO_2 with the ADI-pathway. Contrarily, the formation of gamma amino butyric acid (GABA) by the decarboxylation of glutamic acid was the most abundant source of CO_2 in the standard cheese and about double as high as with the adjuncts. Other important sources of CO_2 were the lactate fermentation into propane-1,2-diol by all *L.parabuchneri* and the formation of high amounts of 480-590 mg/kg histamine by decarboxylation of histidine for the cheeses with the hist+ strain. Formation of propane-1,2-diol is characterstic for *L. parabuchneri*. As the cheeses were ripened without smear, the pH of the cheeses remained about 0.17 pH-units lower than with normal smear-ripening and this increased the formation of propane-1,2-diole and combined CO_2. The higher CO_2-formation in the cheeses with adjuncts increased the eye number (not significant; fig. 2). With 650 mL/kg CO_2 dissolved in the cheese body (Jakobsen et al. 2009), the totally measured and dissolved CO_2-formation was 1.5 to 2.0 times as high as calculated based on the metabolic products (fig. 2 and 3). There must be other metabolic pathways forming CO_2 or other gas, e.g. heterofermentative lactic acid fermentation, propionic acid - and butyric acid fermentation, and amino acid - or citrate catabolism.

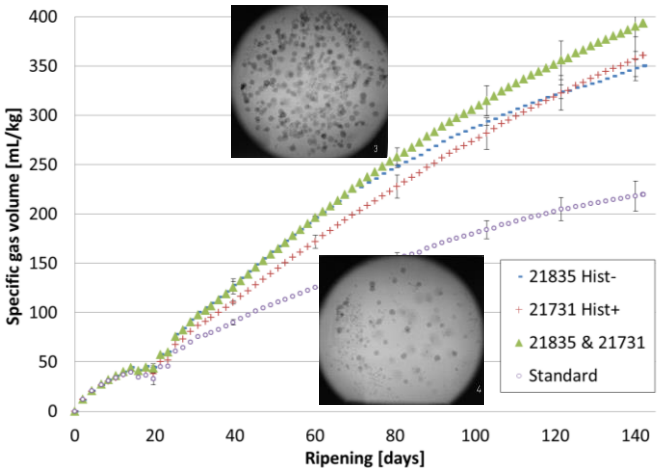

Fig. 2: Specific gas volumes measured during ripening of the three cheeses with *L. parabuchneri* and a standard cheese (n=2).

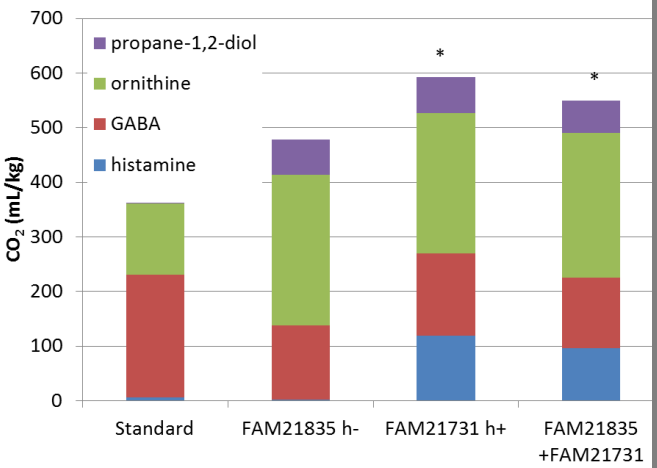

Fig. 3: CO_2 volumes calculated on the basis of the metabolic products of the four cheeses after 142 days of ripening (n=2).

Abstract

A newly developed gas volume monitor based on a sensitive pressure sensor served to measure CO_2 formed in cheeses with adjunct *L. parabuchneri* strains, either histamine + or histamine - or in combination. For all cheeses with *L. parabuchneri*, higher gas formation was measured. The arginine deiminase pathway was the main contributor, in contrast to the standard cheese where decarboxylation of glutamic acid to GABA produced the most CO_2. Measured gas volumes were 1.5 – 2.0 times as high as calculated. The gas volume monitor is a very useful tool to investigate the formation of CO_2 and other gases in cheeses over the whole ripening period in real-time.

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