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Novel methodology for the in situ assessment of CO_2 production rate and its application to anaerobic ripened cheese

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ABSTRACT

CO_2 is produced by many microorganisms present in cheese and it can affect cheese quality both during processing and storage. The knowledge of the extent of CO_2 production by cheese microorganism (CO_2 production rate coefficients) may be used to predict gas exchange in cheese/packaging systems, helping dairy industries in the right choice of the packaging (higher/lower gas permeability) and mastering of cheese ripening. However very few coefficients for CO_2 production rate have been published and the ones assessed in vitro (not inside real food) may not well describe the activity of the microorganisms in situ. We have therefore developed a methodology for the in situ assessment of CO_2 production rate and applied it to cheese with propionic acid fermentation. The proposed methodology is based on infra-red measurement of CO_2 and it allows measuring its accumulation up to 1% with 0.001% resolution, while monitoring the level of oxygen. The method showed a good repeatability, with a low coefficient of variation within samples (6.6%) and between samples (8.4%) compared to 10–30% between samples found in literature. The method was compared with a gas chromatography based method, which is also described.

1. Introduction

CO_2 is one of the most important gases produced by microorganisms in fermented food products, from the yeast fermentation of *Saccharomyces cerevisiae* in traditional bread, beer and wine, to the numerous metabolic pathways yielding carbon dioxide as end product in dairy foods. Within the dairy industry, the presence of different levels of gas (e.g. N_2, CO_2, H_2, NH_3) is common in most natural cheeses as it can frequently be observed by the presence of regular or irregular openings in the casein matrix (Martley & Crow, 1996). CO_2 can be produced in cheese by several different microorganisms such as types of moulds, yeasts and bacteria, which can use different substrates. These may be originally present in milk (e.g. lactose, citrate) or found in cheese as end product of the metabolic pathways of microorganisms (e.g. lactate, urea and amino acids amongst others) (Beresford, Fitzsimons, Brennan, & Cogan, 2001). CO_2 production can be seen as a positive or negative phenomenon in cheeses: CO_2 is the main gas responsible for desired eye growth in Emmental and Swiss–Dutch (Maasdam) types, while its production is considered as a defect in other cheese varieties such as Cheddar and Italian extra hard cheeses (Parmigiano Reggiano, Grana Padano) where the presence of holes in the cheese paste is not desired (Polychroniadou, 2001). The understanding of the factors which affect CO_2 production in cheese and the quantification of the effects may help cheese industries in the selection of desired microorganisms or in the optimisation of the ripening conditions. Notwithstanding the importance of CO_2 during cheese ripening, we found only few studies in scientific literature which deals with the in situ assessment of CO_2 produced in cheese. Some of them used non-CO_2 specific approaches where the volume or pressure increase in the sample chamber was converted into moles of CO_2, using the ideal gas law (Fedio, Ozimek, & Wolfe, 1994; Huc et al., 2014). Fedio et al. (1994) assumed that the volume increase of packed blocks of Swiss cheese (Emmental type) was solely related to CO_2 production of propionic acid bacteria. Huc et al. (2014) assumed that increase of total pressure inside the hermetic chamber containing the semi-hard cheese samples was solely related to the accumulation of CO_2 released by the cheese. Other researchers used approaches more specific to CO_2 chemical–physical properties such as infra-red absorption, thermal conductivity properties, and reactions with a chemical scavenger. Vivier, Compan, Moulin, and Galzy (1996) quantified the CO_2 released from blocks and slices of Feta cheese by using infra-red (IR) CO_2 specific analyser. Roger, Desobry, and Hardy (1998) and Rodriguez-Aguilera, Oliveira, Montanez, and Mahajan (2009) simultaneously measured O_2 consumption and CO_2 production in mould ripened cheese (Camembert-type) by using a gas chromatography technique (thermal conductivity detector) and a dedicated gas analyser (IR and chemometrical based gas analyser for CO_2 and O_2 respectively). Blanc, Bosset, and Pauchard (1980) measured the CO_2 release from loafs of Gruyère cheese by scavenging the gaseous CO_2 released by the cheese with a soda solution of known molarity and

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titrating it with acidic solution of known volume and molarity. All cited authors assessed CO_2 production by using an accumulation method, i.e. by measuring the CO_2 released from the cheese and accumulated in a hermetic chamber of known volume or by directly measuring the volume increase of deformable packs (Fedio et al., 1994). The maximum level of CO_2 partial pressure in the sample chamber usually differs for different samples assessed with the cited accumulation methods. Different levels of CO_2 partial pressure may differently affect the activity of some microorganisms (Dixon & Kell, 1989), leading to higher variance in the assessed CO_2 production rate coefficient. Furthermore, the CO_2 partial pressure accumulated at the end of different experiments considered as repetitions may differ and it may thus differently affect microorganisms inside the cheese (Dixon & Kell, 1989). We therefore propose a novel methodology for assessing diffusion-free CO_2 production rate in anaerobic ripened cheese, which overcome the disadvantages of the above cited methods. The methodology includes the use of a customized version of a closed loop respirometry system, similar to the ones previously used for assessing respiration activity in small animals (Gifford, Clay, & Peterman, 2013; Molero et al., 2004). We compared the results of the novel methodology with the ones of an accumulation method applied on cheese samples originated from the same cheese batch.

2. Materials and methods

2.1. Model cheese product

Semi-hard cheese blocks of 1 kg were kindly supplied by a cheese company. The cheeses were not salted to avoid salt gradient during ripening, thus allowing a constant homogeneous chemical composition within the cheese during ripening: 42% moisture, 26.5% fat, pH 5.5–5.7. Common characteristics of the cheese used concern the presence of intentionally added CO_2 producing bacteria (propionic acid bacteria, PAB) at the level of 10^6 cfu/ml milk and the absence of hetero-fermentative lactic acid bacteria (LAB). Thus the production of CO_2 in the model cheese could be attributed mainly to PAB fermentation. All cheeses were ripened under foil at 13 °C until the 14th day from renneting. Afterwards, cheeses were sampled (Section 2.4) and the samples were foil packed and conditioned at the analysis temperature. The foil used for ripening and conditioning the samples was chosen as high barrier to gas and vapour transfer, thus the chemical composition, in terms of macronutrients, of the cheese blocks and packed cheese samples, remained unchanged during the relatively short ripening time. Furthermore, the barrier foil ensured anaerobiosis both during ripening and sample conditioning. Anaerobic condition is considered fundamental for obtaining high quality (texture, flavour) cheese with intense PAB fermentation (Benjelloun, Rochex, Lecouturier, Dechemi, & Lebeault, 2005; Fröhlich-Wijder & Bachmann, 2004).

2.2. Sampling position

The chemical composition of each cheese was measured within the same two sampling regions used for the assessment of CO_2 production rate: core position and rind position. The core position was situated 3 cm away from all rinds, resulting in a sample weighing 60 ± 5 g. The rind position included the cheese rind, with a thickness of 0.5 cm (Fig. 1).

2.3. Chemical analyses

The chemical composition (dry matter, pH, fat, organic acids) of three cheeses per production was measured in order to verify that the cheese production was on target (fat: 26.5% *w*/*w*, moisture (100—dry matter): 42% *w*/*w*, pH at 2 weeks from renneting: 5.50–5.70). Analytical methods used are reported: dry matter (NF EN ISO 5534: 2004, constant weight in fan oven at 102 °C), pH (FD V04-035 July 2009, pH evaluation of a prepared homogenised curd slurry with penetration electrode), fat (NF V04-287 Feb 2002, Heiss acido-butyrometric method), and organic acids (internal method based on high performance liquid chromatography, including an ionic exclusion column and conductivity detector, coupled to anionic suppressor). Possible variations in the sample conditions during the experiments where monitored by measuring weight and pH of the samples both before and after the experiment.

2.4. Microbiological analyses

Propionic acid bacteria were enumerated with a method based on the count of the diluted colonies grown on agar plates (15 g agar per litre) enriched mainly with sodium lactate and yeast extract (YELA medium) after 1 week of anaerobic incubation at 30 °C (Petran, Grieme, & Foong-Cunningham, 2013). The results are expressed in colony-forming unit per gramme of cheese (cfu/g). The count was performed in the sampling region described in Section 2.2 and on cheeses produced within the same batch of the ones used for CO_2 production rate assessment.

2.5. Assessment of CO_2 production rate

The CO_2 production rate was assessed both directly measuring the release of gaseous CO_2 from the cheese during time (Section 2.5.2; Section 2.5.3) and by assaying the organic acids formed as a result of the propionic acid fermentation (Section 2.5.4).

2.5.1. Sample preparation

Samples for the assessment of CO_2 production rate and chemical composition assays were taken within the same location of cheeses produced within the same batch (Section 2.2). Two cheeses were used for obtaining a sample mass of about 120 g, which was pre-cut with

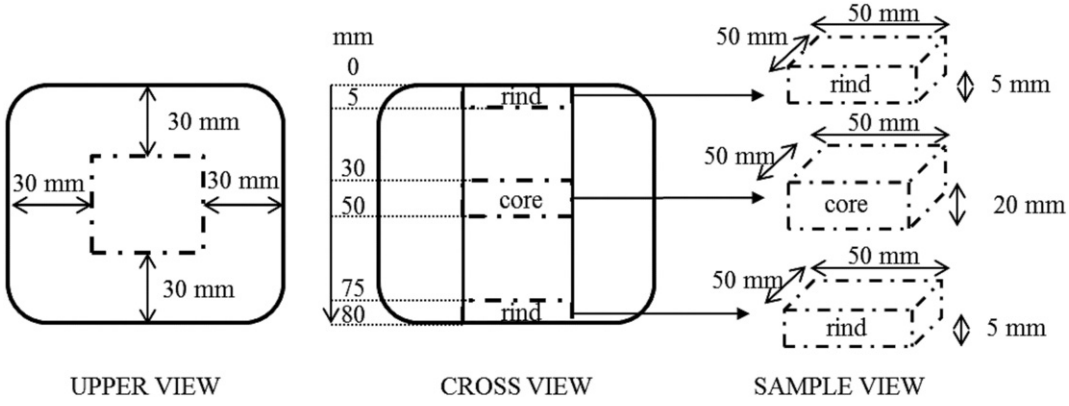

Fig. 1. Illustration of rind and core sampling positions inside model cheese.