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Short communication

Suppression of propanoic acid, acetic acid and 3-methylbutanoic acid production by other volatiles in a Swiss cheese curd slurry system

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ABSTRACT

The effect of the addition of 5–100 ppm 2,3-butanedione, butanoic acid, methyl mercaptan, ethyl butanoate, diethyl sulfide, 3-methylbutanoic acid, acetaldehyde and ethanol on production of propanoic acid, acetic acid and 3-methylbutanoic acid in a Swiss cheese slurry system was investigated. Enumeration of propionic acid bacteria (PAB) was done to monitor the microbial count in the systems. A Swiss cheese slurry system was used to simulate cheese manufacturing based on accelerated ripening for a 6-day incubation period. The headspace concentrations of propanoic acid, acetic acid and 3-methylbutanoic acid were significantly decreased by the addition of 2,3-butanedione, butanoic acid, and methyl mercaptan, but were generally unaffected by ethyl butanoate, diethyl sulfide, 3-methylbutanoic acid, acetaldehyde and ethanol. Microbial enumeration also showed that there was a significant reduction of PAB in slurry systems with 100 ppm 2,3-butanedione, butanoic acid, and methyl mercaptan.

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1. Introduction

Propanoic (propionic) acid, acetic acid, and 3-methylbutanoic acid are considered to be key volatiles and high impact flavor or flavor-active compounds, along with other compounds in Swiss cheese (Fröhlich-Wyder & Bachmann, 2004; Preininger, Warmke, & Grosch, 1996; Taylor, Wick, Castada, Kent, & Harper, 2013; Thierry & Maillard, 2002). Propanoic acid imparts the characteristic sweet and nutty flavor of Swiss cheese (Fröhlich-Wyder & Bachmann, 2004). Acetic acid imparts a pungent and vinegar aroma note, whereas 3-methybutanoic acid imparts the cheesy, rancid and sweaty aroma note in cheese (Fröhlich-Wyder & Bachmann, 2004; Thierry & Maillard, 2002; Thierry, Maillard, Richoux, Kerjean, & Lortal, 2005).

Propionic acid bacteria (PAB) of the species *Propionibacteria freudenreichii* produce the metabolites of propanoic acid and acetic acid from the propanoic acid fermentation pathway, and 3-methylbutanoic acid from the degradation of leucine (McSweeney, 2004; Thierry & Maillard, 2002; Thierry, Richoux, & Kerjean, 2004a). Acetic acid is also produced via the oxidation of lactate by lactic acid bacteria (LAB) and through citrate metabolism by non-starter LAB in cheese (Palles, Beresford, Condon, & Cogan, 1998). If the growth or metabolism of PAB is affected by the other volatiles present, this would inhibit the levels of the desirable volatiles propanoic acid, acetic acid and 3-methylbutanoic acid in the slurry.

A decline in desirable volatiles may be due to suppression of biochemical pathways by production of other compounds in competing pathways, or the inhibition of microorganisms initiating the production of these compounds. The goal of this study was to determine the effect of a number of volatiles produced during fermentation on the production of some of the key volatile organic compounds in relationship to the promotion or inhibition in microbial growth of PAB in a Swiss cheese curd slurry system.

2. Materials and methods

Fresh Swiss cheese curds were obtained, just before pressing, from an Ohio-based Swiss cheese manufacturing plant. Three batches of Swiss cheese curds with different manufacturing dates were shipped to the laboratory and immediately processed upon receipt. The Swiss cheese curd slurry system was developed by Singh and Kristoffersen (1971). Briefly, 500 g of fresh curd was combined with 250 mL of 3% sterile brine solution to give 0.9–1% final salt concentration in the slurry, and was mixed using a high-speed mixer to a smooth paste. The pH of the mixture was

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adjusted to pH 5.2 ± 0.1 by the gradual addition of 85% lactic acid and 4 M KOH solutions. Reduced glutathione (rGSH, 100 ppm) was then added to the mixture and the resulting slurry served as a control for the succeeding spiking studies. Standard solutions of 2,3-butanedione, butanoic acid, methyl mercaptan, ethyl butanoate, diethyl sulfide, 3-methylbutanoic acid, acetaldehyde and ethanol, at concentrations of 5, 50 and 100 ppm in the slurry, were independently added and homogenized in the curd slurry system using a high-speed mixer, and transferred aseptically to an airtight/re-sealable and sterile container and incubated in the dark at 30 °C for 6 d. For every sample, three replicates were produced.

Each of the slurry cheese samples (5 g) was weighed into individual 500 mL Schott bottles capped with a septum-lined screw cap. All bottles were incubated in a 40 ± 1 °C water bath for 1 h to allow for headspace equilibration prior to selected ion flow tube-mass spectrometry (SIFT-MS) scanning. The headspace sampling was done by inserting 3.5 cm of the SIFT-MS passivated sampling needle (3.8 cm) through the septa. An empty bottle was scanned between samples to act as a blank, and to zero the instrument before running the next sample.

A Voice 200^{TM} SIFT-MS (Syft Technologies, Christchurch, New Zealand) was used to detect and quantify the volatiles in the headspace of aqueous samples at ppb levels. The V200 was operated using the more sensitive selected ion mode (SIM) to quantify specific compounds of interest. SIM scans limit the number of masses to be counted per scan, thus allowing longer detection time per mass and delivers higher precision of quantification. A method containing compounds considered to be of high impact in Swiss cheese (Smit, Smit, & Engels, 2005; Taylor et al., 2013) was developed using the SIM scan mode and method development software of the V200. The scan duration was 2 min 10 s.

The method used in the enumeration of propionibacteria was patterned after Thierry and Madec (1995). Swiss cheese curd slurries (2 g each; control, 100 ppm butanoic acid, 100 ppm 2,3-butanedione and 100 ppm methyl mercaptan) was mixed with 20 mL 2% sodium citrate and mixed with a stomacher for 2 min. A 1 mL aliquot of the cheese curd mixture was mixed with 9 mL 0.1% peptone and serial dilutions were carried out using the mixture. A 0.1 mL of each diluted mixture was plated onto a lithium glycerol agar plate and incubated in an anaerobic gas chamber at 30 °C for 6 d.

Data fitting and least square means analyses were carried out using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC, USA). A P value < 0.05 was considered significant.

3. Results and discussion

3.1. Propanoic acid

Propanoic acid concentration in the headspace of the control slurry system was negligible for the first three days, then increased linearly from the 3rd to 6th day of incubation (Fig. 1). The third day of incubation corresponds to the end of the pre-cool step, two weeks into production, before the cheese goes into the warm room for curing (Castada, Harper, & Barringer, 2015). Microbial growth generally increases once the cheese enters the warm room. This increase is due to the metabolism of PAB in the slurry as they ferment lactate to propanoic acid, acetic acid and carbon dioxide gas (Fröhlich-Wyder & Bachmann, 2004). The propanoic acid content in ripe Swiss-type cheese (such as Emmentaler, approximately 5 months) was reported to reach an average concentration of 837 mmol kg^{-1} (Steffen, Eberhard, Bosset, & Rüegg, 1993).

The production of propanoic acid was completely inhibited by the addition of 5, 50 or 100 ppm of 2,3-butanedione or methyl mercaptan and 50 or 100 ppm butanoic acid to the slurry system (Fig. 1). It was previously reported that the presence of an "appreciable amount" of 2,3-butanedione has a lethal effect on propionibacteria species (Fröhlich-Wyder, Bachmann, & Casey, 2002). Our results suggest that the presence of as little as 5 ppm 2,3-butanedione, as well as 5 ppm methyl mercaptan or 50 ppm butanoic acid, interrupted either growth or metabolism of the PAB and completely inhibited propanoic acid production. The addition of ethyl butanoate, diethyl sulfide, 3-methylbutanoic acid, acetaldehyde, and ethanol had no significant effect on propanoic acid production (data not shown).

3.2. Acetic acid

The increase in the concentration of acetic acid in the control slurry system was linear throughout the entire incubation (Fig. 1), unlike for propanoic acid. Acetic acid is produced in the propanoic acid fermentation pathway, but is also produced via the oxidation of lactate by LAB and through citrate metabolism by non-starter LAB in cheese (Palles et al., 1998). It has been reported that the average concentration of acetic acid in ripe Swiss-type cheese (i.e., Emmentaler, approximately 5 months) is about 410 mmol kg^{-1} (Steffen et al., 1993). During the first three days, the increase in acetic acid was probably due to oxidation of lactate and citrate metabolism, and none of the additives inhibited acetic acid production during this time. After three days, several of the additives significantly inhibited acetic acid production, including 2,3-butanedione, methyl mercaptan and butanoic acid. The inhibition started on the same day as for propanoic acid, and was again likely due to inhibition of the propanoic acid fermentation pathway.

3.3. 3-Methylbutanoic acid

3-Methylbutanoic acid in the control slurry system increased during the first three days of incubation, and then remained constant (Fig. 1). Again, the concentrations of 3-methylbutanoic acid were significantly lower with the addition of 2,3-butanedione and methyl mercaptan by the 3rd day of incubation, compared with the control slurry systems (Fig. 1). The addition of 2,3-butanedione and methyl mercaptan appear to be inhibiting the growth of the PAB in these slurry systems such that the production of 3-methylbutanoic acid was significantly lower than in the control. *Propionibacterium freudenreichii* is the main contributor to the production of methylbutanoic acids in Swiss cheese (Thierry et al., 2004a; Thierry, Richoux, Kerjean, & Lortal, 2004b).

Furthermore, it was shown in these studies that the concentration of methylbutanoic acids in manufactured Swiss cheeses without PAB was 3–10 times lower than in the Swiss cheese samples with PAB. These findings are consistent with the results in this study, where the Swiss cheese slurries with inhibited PAB growth were observed to have lower concentrations of 3-methylbutanoic acid. The slurry systems with added butanoic acid and 3-methylbutanoic acid are not reported because addition of these compounds gave misleadingly high results for the measured headspace concentration of 3-methylbutanoic acid.

3.4. Enumeration of propionibacterium

Microbial enumeration of the PAB species in the control slurry sample and slurry samples containing 100 ppm butanoic acid, 100 ppm 2,3-butanedione or 100 ppm methyl mercaptan showed significantly reduced microorganism count with the added volatiles as incubation progressed (Fig. 2). The initial count of PAB was 2×10^3 cfu g^{-1}. After the 6th day of incubation, the PAB count in the