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Microbially induced changes in the volatile constituents of fresh chilled pasteurised milk during storage

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ABSTRACT

Off-odours caused by volatile organic compounds (VOCs) are often the first indicators consumers have of milk spoilage. In this study the VOCs associated with three types (trim, 0.25–0.40% fat; lite, 1.40–1.50% fat; and full-cream, 3.18–3.28% fat) of fresh chilled pasteurised milk (FCPM), held for up to 17 days at 4.5 ± 0.5 °C, were measured using proton-transfer-reaction mass spectrometry (PTR-MS). The chemical identification of VOCs in the headspace of the milk was supported by SPME–GC–MS analysis. Bacterial numbers (aerobic plate count at 25 °C) in the milk were also estimated. Replicate sets of milk types treated with sodium azide (NaN_3) to inhibit microbial activity were investigated. The relationship between microbial numbers and VOCs was not linear; rather the concentrations of VOCs only started to change after a threshold number of bacteria ranging from 10^6–10^8 CFU mL^{-1} was reached. This combined approach provided new insights on the effect of microbial growth on FCPM shelf-life.

1. Introduction

Fresh chilled pasteurised milk (FCPM) packaged in high-density polyethylene (HDPE) containers is generally given a shelf-life of 14 days if stored under appropriate temperature and light conditions. Milk producers frequently use total bacterial numbers to determine the end of shelf-life, while consumers usually rely on a sensory assessment, mainly odour, to determine if the milk is suitable to consume.

Regardless of the assessment method, it is generally accepted that the growth of micro-organisms limits the shelf-life of FCPM (Fromm & Boor, 2004; Sørhaug & Stepaniak, 1997). If post-pasteurisation contamination is low, shelf-life is limited by Gram-positive psychrotolerant, endospore-forming bacteria, such as *Paenibacillus* and *Bacillus* spp. that survive

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pasteurisation and have the ability to grow at typical refrigeration temperatures (Fromm & Boor, 2004; Huck, Hammond, Murphy, Woodcock, & Boor, 2007; Huck, Sonnen, & Boor, 2008). If post-pasteurisation contamination occurs due to the design, age or operations of the milk packaging plant, milk spoilage has been found to be associated with the growth of psychrotrophic Gram-negative bacteria such as *Pseudomonas* spp. (Craven & Macauley, 1992; Dogan & Boor, 2003; Sørhaug & Stepaniak, 1997). Despite efforts to reduce post-pasteurisation contamination, it is still believed to be a major cause of spoilage of FCPM (Martin et al., 2011).

Many bacterial species, including *Pseudomonads* spp., produce enzymes such as proteinases and lipases (Champagne et al., 1994; Sørhaug & Stepaniak, 1997) that break down proteins and fat in the milk, altering its physicochemical, functional and sensory properties and eventually resulting in consumers rejecting the milk. Such enzymes are only believed to occur at a significant concentration in milk when the bacterial counts reach around 10^6–10^7 colony forming units (CFU) per millilitre (Champagne et al., 1994). Bacterial numbers of 10^7 CFU mL^{-1} have also been defined as marking the end of shelf-life for milk (Griffiths & Phillips, 1986).

When consumers sniff milk to determine if it is suitable to drink they are actually assessing the odour-active volatile organic compounds (VOCs) present in the headspace of the milk container. These VOCs comprise compounds from the fresh milk and compounds arising due to a combination of chemical, microbial, enzymatic or light-induced reactions (Shipe et al., 1978).

For milk stored in light-exposed containers a relatively good correlation has been reported between sensory quality and the formation of VOCs, including light-induced and auto-oxidation compounds such as carbonyls (hexanal, pentanal, heptanal) and dimethyl disulphide. In contrast, in light-protected containers there was no correlation between VOCs produced and the milk's sensory attributes. Under all storage conditions there was a poor relationship between microbiological data and either VOC or sensory data (Karatapanis, Badeka, Riganakos, Savvaidis, & Kontominas, 2006). A poor correlation between the sensory properties and microbial numbers was also reported from studies on the shelf-life of both raw or pasteurised milk (Duyvesteyn, Shimoni, & Labuza, 2001). Gas chromatography–mass spectrometry (GC–MS) has been widely used in the analysis of milk for the early detection of microbial spoilage metabolites such as dimethyl sulphide, dimethyl disulphide, methanethiol, methional (3-(methylthio)propionaldehyde), skatole (3-methylindole), 3-methylbutanol, acetaldehyde, acetic acid, 1-octen-3-one, and 1,5-cis-octadien-3-one (Belitz, Grosch, & Schieberle, 2001; Cormier, Raymond, Champagne, & Morin, 1991; Marsili & Miller, 1998; Töpel, 2007; Toso, Procida, & Stefanon, 2002). Although GC–MS is the reference analytical method for the analysis of food VOCs (Snow & Slack, 2002), it is prone to artefacts and losses and it is a relatively laborious and costly test (Andersen, Hansen, Feilberg, 2012; López-Feria, Cárdenas, & Valcárcel, 2008). In general, GC–MS analysis of milk is made by prior extraction of the volatile fraction of milk using various methods like solid phase micro-extraction (SPME), vacuum distillation or simultaneous steam distillation and extraction (Contarini & Povolo, 2002). However, these methods are time-consuming and some require a large sampling volume, making them not entirely suitable for routine monitoring of a large number of samples (Contarini, Povolo, Leardi, & Toppino, 1997). Electronic nose instruments have been trialled as rapid methods to assess milk quality (Magan, Pavlou, & Chrysanthakis, 2001) and while they have had some success, they do not provide the detailed chemical information offered by GC–MS methods and there is concern that the technique lacks sensitivity (Marsili, 1999).

Proton-transfer-reaction mass spectrometry (PTR-MS) is a chemical ionisation-based technique for the gas-phase analysis of VOCs (Lindinger, Hansel, & Jordan, 1998). The technique allows rapid, direct, and accurate determination of the concentration of VOCs at trace levels (down to low part per trillion by volume, pptv, levels) (Jordan et al., 2009). PTR-MS has distinct advantages over GC–MS, as sample pre-treatment is not required, the technique is insensitive to inorganic air constituents and sample gas humidity variations, and it is a fast, sensitive and non-destructive method (Aprea et al., 2006). A drawback of PTR-MS is that the chemical identity of a measured VOC often cannot be determined unequivocally, especially in the complex gas matrix of food headspace, owing to the low mass resolution ($m/\Delta m$ of 1 Da) of the quadrupole mass spectrometer; hence multiple compounds, clusters and fragments from other VOCs can potentially contribute to the integral signal of a detected ion. Therefore, for confirmation of sample identification a secondary technique such as SPME–GC–MS should be used. PTR-MS has been successfully applied for the headspace analysis of several foods (Biasioli, Gasperi, Yeretzian, & Tilmann, 2011), including dairy products (Fabris et al., 2010; Soukoulis et al., 2010; van Ruth et al., 2008). Operational details of the PTR-MS technique can be found in the literature (Blake, Monks, & Ellis, 2009; de Gouw & Warneke, 2007).

The objectives of the present study were to investigate changes in VOCs in the headspace of refrigerated (4.5 ± 0.5 °C) bovine milk of different fat content (trim at 0.25–0.40% fat, lite at 1.40–1.50% fat, and full-cream at 3.18–3.28% fat) as a result of microbial activity over a storage period of up to 17 days. To control against changes arising from light-induced oxidation, the samples were kept in brown bottles in the dark throughout the storage period. In order to determine the relative impact of growth of the psychrotrophic Gram-negative bacteria such as *Pseudomonas* spp. and chemical or enzymatic processes on VOC production in the milk, a duplicate series of the analysed milk samples that were treated with sodium azide (NaN_3), a bacteriostatic agent that works by inhibiting respiration in Gram-negative bacteria (Lichstein & Soule, 1943), were also analysed. Bacterial numbers were determined using standard techniques used in the dairy industry. PTR-MS was used as the principal tool for monitoring the temporal development of the VOCs in the milk headspace over storage time. Off-line SPME–GC–MS analysis of the headspace of a subset of the frozen milk samples was made in order to aid chemical identification of the VOCs detected by PTR-MS.

2. Materials and methods

2.1. Milk samples

Fresh chilled pasteurised milk (FCPM) was purchased at a local supermarket (Dunedin, Otago, NZ) in 2 L high-density