Food Packaging and Shelf Life xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Relationship between total microbial numbers, volatile organic compound composition, and the sensory characteristics of whole fresh chilled pasteurized milk

Mohammad Alothman, Karen A. Lusk, Patrick J. Silcock*, Phil J. Bremer

Department of Food Science, University of Otago, 276 Leith Walk, 9054 Dunedin, New Zealand

ARTICLE INFO

Keywords:
Total microbial numbers
Descriptive sensory analysis
Post-pasteurization contamination
PTR-MS
Shelf life

ABSTRACT

Total microbial numbers (TMN*) is a widely used, yet broad indicator of the quality of fresh chilled pasteurized milk (FCPM). Changes in the sensory characteristics (trained panel, $n = 9$), volatile organic compounds (VOC) composition (proton transfer reaction mass spectrometry, PTR-MS), and TMN were assessed for FCPM stored at 4.5 °C ± 0.5 for up to 26 days (12 days past the stated shelf life). Significant differences ($p \leq 0.05$) in its VOC composition (m/z 41, 43, 45, and 47) and the sensory characteristics (10 out of 17 attributes) occurred at various TMN levels over this time. However, owing to differences in the spoilage potential of the microorganisms present there was only a weak relationship between TMN, VOC composition, and the sensory characteristics, which indicates that both the nature of the microorganisms present as well as the numbers present play a critical role in determining the shelf-life of FCPM.

1. Introduction

Milk and other dairy products are recognized as important constituents of the human diet. As a commodity, the definition of 'quality' milk varies among farmers, producers, and consumers. While it must be of a physiological composition that meets the standards, free from added water, antibiotics, and mastitis indicators for producers, consumers define quality milk as that which meets their flavour, taste, colour, and texture expectations.

Fresh cow's milk has been described as having a subtle sweet taste, clean flavour, and no aftertaste (Bendall, 2001; Molina, Amigo, & Quirós, 2008). The quality of milk degrades with time due to the growth and metabolic activity of microorganisms. Spoilage microorganisms such as Gram-negative rods (e.g., *Pseudomonas* sp.,) which frequently re-contaminate pasteurized milk, or Gram-negative, spore-forming bacteria that survive pasteurization (e.g., *Bacillus* sp.) have the ability to alter the sensory profile of fresh chilled pasteurized milk (FCPM) by utilizing milk constituents as substrates in biochemical reactions that produce volatile organic compounds (VOC) (aromas) that maybe unpleasant (Chandler & McMeekin, 1989; Fromm & Boor, 2004).

Although FCPM producers rely on total microbial number (TMN) as an indicator of the end of shelf-life, consumers usually rely on sensory assessment to determine the acceptability of FCPM (Silcock et al., 2014). Although it has been established analytically that the concentration of VOCs only start to change when TMN exceeds 7 $\log_{10}$ CFU mL^{-1} (Silcock et al., 2014), it is important to determine if the VOCs would be detectable by consumers and perceived as spoilage.

Sensory descriptive analysis (DA) has been used to study chilled pasteurized milk (Lawless & Claassen, 1993; Watson & McEwan, 1995). DA can be used to determine the sensory profile of a product and/or to measure the intensity of its characteristic attributes using a trained panel (Drake, 2007; Lawless & Heymann, 2010). It can also be used to characterize changes that occur in a product during storage where a descriptive test is applied to characterize and/or quantify changes that may have occurred compared to a control (IFT, 1981). The deviation from reference method can be implemented in DA to evaluate a number of samples against a chosen reference and is useful especially when evaluating samples with subtle differences (Stoer & Lawless, 1993). The method is performed by placing the reference sample in the middle of an unstructured line, or category type scale for each attribute that is anchored with labels which relate to the intensity of the reference sample (Chiralertpong, Acree, Barnard, & Siebert, 2008; Deterre,

Abbreviations: (W)FCPM, (Whole) fresh chilled pasteurized milk; VOCs, Volatile organic compounds; DA, sensory descriptive analysis; PTR-MS, Proton transfer reaction – mass spectrometry; HDPE, High-density polyethylene; PPM, Part per million; TMN, Total microbial numbers; CFU, Colony forming unit; sccm, Standard cubic centimetre per minute; n-cps, Normalized counts per second; GLM, General linear model; MFA, Multiple factor analysis; PPC, Post-pasteurization contamination; m/z, Mass-to-charge ratio

* Corresponding author.
E-mail addresses: mohammad.alothman@otago.ac.nz (M. Alothman), karen.lusk@otago.ac.nz (K.A. Lusk), pat.silcock@otago.ac.nz (P.J. Silcock), phil.bremer@otago.ac.nz (P.J. Bremer).

https://doi.org/10.1016/j.fpsl.2017.11.005
Received 24 July 2017; Accepted 13 November 2017

Table 1
Sensory attributes, definitions, and reference standards for the descriptive sensory analysis of whole fresh chilled pasteurized milk (WFCPM).

	Sensory Attribute	Definition and related terms	Reference standard
ODOUR	Cheesy	Sharp, similar to the odour of cheddar cheese, parmesan, feet, dirty socks, and fake cheese.	50 ppm butyric acid in trim milk
	Creamy	Similar to the odour of 100% cream	100% cream (Meadow Fresh cream, Goodman Fielder, Auckland, New Zealand).
	Malty/biscuity/ grainy	Similar to the odour of grain, cereal, musty, oat.	A 50% (w/v) mixture of cornflakes (Kellogg's Cornflakes, Newmarket, Auckland, New Zealand) and trim milk (Meadow Fresh Brand, Goodman Fielder, Auckland, New Zealand) soaked for 30 min and strained.
	Cultured	Similar to the sour aroma and the odour of sour cream, yoghurt.	A 75% (w/v) mixture of sour cream (Country of Goodness Original sour cream, Anchor, Fonterra Foodservices, Manurewa, New Zealand) and trim milk.
	Barny/cowy	Similar to the odour of dairy shed, manure, decaying, wood, cowpat, dairy barn, farm.	30 ppb *p*-cresol in trim milk
	Fruity	Similar to the fruity odour, perfume, tropical fruit, floral.	10 ppm γ-Decalactone in trim milk
FLAVOUR	Sweet taste	As in the sweetness associated with sucrose (not vanilla, malt, nor biscuit)	No physical reference was provided
	Creamy	As in the flavour of 100% cream	100% cream (Meadow Fresh cream, Goodman Fielder, Auckland, New Zealand).
	Cheesy	As in the flavour of parmesan, cheddar cheese	No physical reference was provided
	Cultured	As in the flavour of sour cream, yoghurt	A 75% (w/v) mixture of sour cream and trim milk.
	Malty/biscuity/ cereal	As in the flavour of malt (but not the sweetness)	A (50% w/v) mixture of cornflakes and trim milk soaked for 30 min and strained.
	Barny/cowy	As in the flavour associated with barny/cowy odour	No physical reference was provided
	Fruity	As in the fruity, tropical fruit, perfumy flavour.	No physical reference was provided
MOUTH FEEL	Thickness	As in the mouth feel caused by the viscosity of the matrix (i.e., milk)	Cream/Trim milk.
AFTER SWALLOW	Mouth coating	Referring to the coating film formed on the tongue/mouth after swallowing milk.	No physical reference was provided
	Sweet aftertaste	As in the sweetness of sucrose (not vanilla, malt, nor biscuit)	No physical reference was provided
	Creamy	As in the after swallow flavour associated with cream.	100% cream

Delarue, Innocent, & Giampaoli, 2012; Larson-Powers & Pangborn, 1978; Olabi & Lawless, 2008).

Although sensory evaluation is considered to be the ultimate approach to measure the quality of food products, many researchers investigated the use of analytical methods to evaluate the role of VOCs and/or non-volatile components in evoking a variety of sensory perceptions (Aparicio, Morales, & Alonso, 1996; Morales, Alonso, Rios, & Aparicio, 1995). Proton transfer reaction – mass spectrometry (PTR-MS) is a technique that allows for online-measurement of VOCs at concentrations as low as parts per trillion by volume (pptv) (Lindinger, Hansel, & Jordan, 1998). PTR-MS has been used to characterize the headspace fraction of dairy products such as milk, cheese, butter, and fruit yoghurt (Biasioli et al., 2006; Mei, Reineccius, Knighton, & Grimsrud, 2004; Ruth et al., 2008; Silcock et al., 2014). The objective of the current study was to determine the relationship between changes in the sensory properties, total microbial numbers, and the VOC composition of WFCPM.

2. Materials and methods

2.1. Milk samples

WFCPM in 1 L high-density polyethylene (HDPE) translucent bottles were purchased from a local supermarket at various times and stored in the dark at 4.5 °C ± 0.5 to be evaluated during 5 separate sessions (replications), which were carried out over 5 consecutive days (n = 5). All the purchased bottles were collected directly from the supermarket cold room not the chiller display cabinet to ensure that the WFCPM was not exposed to light but so that it was transferred and handled under commercial conditions. The collection of WFCPM continued until 5 separate testing sets were obtained, which each consisted of 9 WFCPM bottles that were stored for 3, 8, 12, 16, 20, 22, 23, 24, or 26 days by each of the sensory evaluation sessions (best before date was 14 days post production). The three-day old WFCPM was used as the fresh WFCPM reference (control) against which all the rest of the aged WFCPM samples were evaluated. On each sensory evaluation session, aliquots of 300, 10, and 100 mL from each of the reference and the aged WFCPM bottles were withdrawn for sensory, microbial, and headspace analyses, respectively. The remainder WFCPM from each 1 L bottle was discarded. WFCPM aliquots taken for VOCs headspace analysis were frozen at −18 °C and evaluated after the completion of the sensory study.

2.2. Determination of the total microbial number (TMN) in WFCPM

Total microbial numbers in the WFCPM samples were determined each evaluation day using standard microbiological methods whereby dilutions of the WFCPM (0.1% peptone) were spread plated in triplicates on plate count agar plates (Difco Laboratories Inc., Becton, Dickinson and Company, New Jersey, USA) (Johnson & Wichern, 2007). Plates were incubated at 25 °C for 72 h before the numbers of colonies were counted. Results were expressed as $\log_{10}$ colony forming unit per millilitre of WFCPM ($\log_{10}$ CFU mL^{-1} milk).

2.3. Descriptive sensory analysis

An existing DA panel (up to 10 years of experience) consisting of nine members (8 females and one male) who had been selected as per ISO-8586-1 (1993) was used. Panellists were trained following international standards (ISO-8586-2, 1994) in the descriptive sensory analysis of WFCPM over a total of 9 training sessions (18 h). During the first five 2 h training sessions, panellists developed and reached a consensus on the sensory attributes to describe the changes in WFCPM during storage. This was achieved by the comparison of WFCPM (3-day) with a series of aged WFCPM samples and the provision of possible physical reference standards with sensory qualities potentially found in WFCPM to help them identify the odour, flavour, mouth feel, and aftertaste attributes of fresh and aged WFCPM. The final sensory attributes and corresponding reference standards used for product evaluation are listed in Table 1. The remainder of the training sessions focused