ORIGINAL RESEARCH

Composition of volatile compounds in bovine milk heat treated by instant infusion pasteurisation and their correlation to sensory analysis

ANNI B HOUGAARD, JANNIE S VESTERGAARD, CAMILLA VARMING, WENDER L P BREDIE and RICHARD H IPSEN*

Department of Food Science, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

Volatile compounds in skim milk and nonstandardised milk subjected to instant infusion pasteurisation at 80°C, 100°C and 120°C were compared with raw milk, high temperature short time pasteurised milk and milk pasteurised at 85°C/30 s. The composition of volatile compounds differed between infusion pasteurisation treated samples and the reference pasteurisations. The sensory properties of skim milk subjected to instant infusion pasteurisation were described by negative attributes, such as cardboard sour and plastic flavours, which are not associated normally with fresh milk. Partial least squares modelling showed good correlation between the volatile compounds and the sensory properties, indicating the predictive and possible causal importance of the volatile compounds for the sensory characteristics.

Keywords Milk, Instant infusion pasteurisation, Volatile compounds, Descriptive sensory analysis.

INTRODUCTION

Heat treatment of milk is normally performed to provide microbiological inactivation, and thereby ensure consumer safety and prolong the shelf life. A standard treatment is high temperature short time (HTST) pasteurisation with the minimum requirements of holding the milk at 72°C for 15 s.

Heat treatment is known to cause changes in the flavour of milk and one of the factors contributing to this is changes occurring in the composition of volatile compounds. The degree of this change is related to the intensity of heat treatment (Calvo and de la Hoz 1992). The content of several ketones, aldehydes and sulphur compounds has been reported to increase when the intensity of heat treatment is increased, e.g. from pasteurisation to ultra high temperature (UHT) treatment or in-bottle sterilisation (Contarini *et al.* 1997; Contarini and Povolo 2002). Increasing the intensity of heat treatment by changing the time and/or temperature of a certain processing method may provide less pronounced changes in the composition of volatile compounds (Bassette and Jeon 1983; Valero *et al.* 2000).

Heat treatment by direct steam infusion is characterised by high heating and cooling rates and short holding times, and as applied in the Pure-Lac™ (Elopak, Spikkestad, Norway and SPX APV) process, it has been reported to provide improved shelf-life and increased freshness, sweetness and general acceptance in comparison with pasteurised milk (Fredsted *et al.* 1995). The flash cooling, applied in direct heat treatment technologies to remove the excess water added as steam during heating, has an additional deodourising effect, which may reduce the content of some volatile compounds in the products (Bassette and Jeon 1983; Fredsted *et al.* 1995; Contarini *et al.* 1997; Edmond 2001). Based on these findings, it is suggested that heat treatment of milk using instant infusion at temperatures in the range from 80°C to 120°C would provide a gentle pasteurisation where some of the flavour attributes of unprocessed raw milk is preserved also during storage, i.e. resulting in milk with a fresh taste compared with standard pasteurisation methods.

The aim of this study was thus to describe the differences in composition of volatile compounds induced by instant infusion pasteurisation (IIP) of milk in comparison with raw milk, HTST pasteurisation and a more intensive pasteurisation at 85°C/30 s. Furthermore, it was the aim to study the

*Author for correspondence. E-mail: ri@life.ku.dk

sensory characteristics of IIP milk, focusing on characteristics related to freshness after storage and compared with the two latter mentioned pasteurisation methods. In addition, the correlation between composition of volatile compounds and descriptive sensory analysis of skim milk samples subjected to IIP, HTST and the 85°C/30 s pasteurisation was investigated.

MATERIALS AND METHODS

Heat treatment of milk

Two batches of milk were obtained on two consecutive days from a Danish dairy factory (Rødkærsbro Mejeri, Arla Foods, Rødkærsbro, Denmark). Batch 1 constituted 3000 L of skim milk, which had not been heat treated, but had been microfiltrated (membrane pore size 1.4 μm) in connection with the skimming process. Batch 2 was 3000 L of raw, nonstandardised (NSTD) milk. Both skim milk and NSTD milk were heat treated in a pilot scale instant infusion plant (SPX APV, Silkeborg, Denmark) with a flow of 500 L/h, using a holding time of 0.2 s and treatment temperatures 80°C (IIP 80), 100°C (IIP 100) and 120°C (IIP 120). Milk samples were aseptically packed in plastic bags consisting of a nylon foil laminate with an inner layer of polyethylene (Rapak, Rugby, UK). Two reference treatments were applied; HTST pasteurisation at 72°C for 15 s, and a more intensive pasteurisation at 85°C for 30 s (85°C/30 s), both treatments performed in a pilot scale plate heat exchanger (SPX APV). The reference samples were drawn under laminar air flow into sterile polyethylene terephthalate bottles (Grathwol A/S, Karlslunde, Denmark). A sample of the raw skim milk and NSTD milk was collected in polyethylene terephthalate bottles as well. All samples were cooled and stored at 5°C until further handling.

All milk samples were transferred to 250 mL polyethylene bottles (Plastiques Gosselin, Hazebrouck Cedex, France) before freezing or further refrigerated storage. Samples of fresh milk were frozen at −45°C on day 2 after heat treatment for skim milk samples, and on day 1 after heat treatment for the fresh NSTD milk samples. To investigate the effect of storage, samples of both milk types were stored at 5°C until day 7 after heat treatment and subsequently at −45°C until further analysis.

Dynamic headspace sampling of volatile compounds

Milk samples were thawed at 5°C overnight. 150 g of milk were weighed into a 1 L purge bottle and

1 mL of internal standard was added (50 ppm 4-methyl-1-pentanol). A magnet was added before attachment of a purge head with a Tenax-TA trap containing 250 mg of Tenax-TA with mesh size of 60/80 and density 0.37 g/mL (Buchem bv, Apeldoorn, The Netherlands). The bottles were placed in a temperature controlled waterbath at 40°C on magnetic stirrers with a stirring speed of 200 rpm. Temperature equilibration was done for 10 min before turning on nitrogen flow. The samples were purged for 1 h by purging above the sample with a nitrogen flow of 100 mL/min. After purging, 10 min of dry purging was performed, blowing nitrogen backwards through the trap at 50 mL/min to remove excess water trapped due to the relatively high temperature used during sampling. The traps were stored at refrigeration temperature until further analysis.

Gas chromatography–mass spectrometry (GC–MS) analysis

The collected volatile compounds were thermally desorbed using an automated thermal desorber (ATD 400; Perkin Elmer, Norwalk, CT, USA). The trap was heated to 250°C, and desorption time to the cold trap (5°C, 30 mg Tenax-TA; Buchem bv, Apeldoorn, The Netherlands) was 15 min, with a helium flow of 60 mL/min. The volatile compounds were desorbed from the cold trap to the GC-column by flash heating from 5°C to 300°C, using a split ratio of 1:10. Separation and identification of aroma compounds were performed on a Hewlett-Packard (Palo Alto, CA, USA) G1800A GC–MS system equipped with a J&W Scientific DB-Wax (J&W Scientific, Inc., Folsom, CA, USA) capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness) using helium as carrier gas (1 mL/min). The column temperature was kept at 45°C for 10 min, increased with 6°C/min to 240°C, and kept isothermal for 10 min. The mass selective detector used the electron ionisation mode at 70 eV, and the mass/charge (*m/z*) range between 15 and 300 was scanned. The volatile compounds were identified by interpretation of their ms spectra and comparison with those in the Wiley275.L library (HP product G1035A; Hewlett-Packard, Palo Alto, CA, USA). The software GCD Plus ChemStation G1074B (Version A.01.00; Hewlett-Packard) was used for data analysis. All analyses were carried out in triplicate.

Sensory descriptive analysis

Sensory descriptive analysis (Lawless and Heymann 1999) was performed with ten trained

panelists on the skim milk samples IIP 80, IIP 120 (batch 1) and the two reference treatments HTST and 85°C/30 s. The samples were transferred to 1000 mL polyethylene bottles (unknown producer) immediately after processing, cooled and stored at 5°C until further analysis after 7 days of storage. Three training sessions of 2–2.5 h duration were conducted 1 week prior to the evaluation in order to familiarise the assessors with the descriptors and intensity scales. Consensus about descriptors and anchor points of the scales was obtained after thorough discussion of the descriptors, using different samples as examples and specific references prepared for each descriptor. The 17 descriptors developed comprised three odour, two appearance and 12 flavour terms as shown in evaluation order in Table 1. The intensity of the descriptive terms was scored on an unstructured 15 cm line scale anchored 'none' to the left and 'extreme' to the right. Samples (50 mL) were evaluated in odour-free jars with lid and tempered to 16°C prior to evaluation.

In each of two evaluation sessions the four heat treated samples and two commercial control samples (organic, nonhomogenised skim milk) were evaluated. The control samples representing a fresh sample (day 1 after milking) and a stored sample (purchased day 1 after milking and stored as the heat treated samples) were included as references on the intensity scale. The serving order of the samples was randomised. All samples were evaluated in triplicate in a randomised balanced block design.

Data analysis

The effect of treatment on the composition of volatile compounds was tested by one-way ANOVA and Duncan's multiple range *post hoc* test. The analysis was performed in SAS 9.1 (SAS Institute, Inc., Cary, NC, USA).

Sensory raw data were examined with regard to panel performance. For each session, *F*-values and MSE-values for each assessor and descriptor were calculated (results not shown). Analyses for panel performance were performed with the open source software Panel Check version 1.3.2 (SourceForge Inc., Mountain View, CA, USA). Overall effects of treatments were analyzed conducting a 3-way ANOVA (effects of and interactions between assessor, product and replicate) and least significant difference as *post hoc* test in SPPS version 17.0 (SPSS

Table 1 List of 17 sensory descriptors used for evaluation of milk presented in evaluation order

Descriptive term	*Definition*[a]	*Direction*[b]
Odour	Odour associated with	
O-Boiled milk[c]	Boiled milk	Pos
O-Maize	Brine from canned maize in milk	Pos
O-Cardboard/sour	Cardboard soaked in milk	Neg
Appearance	Sensation associated with the appearance	
A-Grey colour	Grey colour	Neutral
A-Yellow colour	Yellow colour	Neutral
Flavour/taste	Sensation associated with flavour/taste of	
F-Boiled milk	Boiled milk	Pos
F-Watery	Diluted milk	Neg
T-Sugar sweet	Sugar in milk	Pos
F-Toffee	Sweet toffee in milk	Pos
F-Maize sweet	Brine from canned maize in milk	Pos
F-Cardboard/sour	Cardboard soaked in milk	Neg
F-Metallic	$Fe_2(SO_4)_3$, $7 H_2O$ in milk	Neg
F-Plastic/chemical	Odourised plastic	Neg
T-Bitter	Quinine in milk	Neg
Aftertaste	Sensation associated with aftertaste/mouthfeel of	
AT-Astringent 0	Astringency 0 s after mouth emptying	Neutral
ATMF-Cardboard/sour	Cardboard soaked in milk	Neg
AT-Astringent 20	Astringency 20 s after mouth emptying	Neutral

[a]Definition of the sensory terms as derived during vocabulary development using reference samples.

[b]Direction of the descriptor; Pos: positive; Neg: negative.

[c]Suffix to sensory term indicates method of assessment by panelists; O, odour; A, appearance; F, flavour; T, taste; ATMF, aftertaste/mouthfeel.

Inc., Chicago, IL, USA). Principal components analysis (PCA) and partial least squares (PLS) modelling were performed in MATLAB R2008a (The Mathworks, Inc., Natick, MA, USA) with the PLS-Toolbox version 5.2.2 (Eigenvector Research, Inc., Wenatchee, WA, USA).

RESULTS AND DISCUSSION

Composition of volatile compounds

A total of 59 volatile compounds were identified in the milk samples. A total of 20 of these compounds were selected for further data analysis, primarily based on an expectation of contribution to milk flavour from reported low threshold values (Burdock 2005). The selected compounds are shown in Tables 2 and 3 for skim milk and NSTD milk, respectively. The remaining compounds mainly constituted of aliphatic and aromatic hydrocarbons and alcohols (data not shown). The majority of the volatile compounds detected in both skim milk and NSTD milk have been reported in one or more studies of milk subjected to different heat treatments, such as pasteurisation, ultrapasteurisation in a steam infusion plant, UHT treatment, or in-bottle sterilisation (Bassette and Jeon 1983; Contarini *et al.* 1997; Valero *et al.* 2001; Solano Lopez *et al.* 2005).

Comparing Tables 2 and 3 shows that 2-decanone and 2-undecanone were only detected in skim milk, and 2-heptanone, 2-octanone and 2-nonanone were detected in higher amounts in skim milk as compared with NSTD milk. These differences may, at least partially, be due a protective effect of the milk fat against release of volatile compounds from the NSTD milk during the dynamic headspace sampling (Wilkes *et al.* 2000). The content of 2-propanone and 2-butanone in both skim milk and NSTD milk was significantly higher in the reference samples (raw milk, HTST and 85°C/30 s pasteurised milk) than in the samples subjected to IIP (Tables 2 and 3). This could be due to stripping of volatile compounds during flash cooling, as suggested in other studies (Bassette and Jeon 1983; Contarini *et al.* 1997). This explanation is further corroborated by the tendency, though not significant, of decreasing amounts of 2-propanone and 2-butanone with increasing temperature of IIP where the flash effect is enhanced due to the concurrent increased drop in temperature. The alcohols 2-propanol and 2-butanol showed similar tendencies of low amounts or no detection in IIP treated samples, and it may be speculated that they are also stripped during the flash cooling step (data not shown).

The content of ethyl acetate exhibited an opposite effect, with higher content in the IIP treated samples than in the reference samples (Tables 2 and 3). Increased content of ethyl acetate in UHT milk as compared with raw and pasteurised milk was also reported by Vazquez Landaverde *et al.* (2005). An explanation for this observation has not been established, but it has been suggested that ethyl acetate could be formed in milk by a heat catalysed esterification of ethanol and acetic acid (Vazquez Landaverde *et al.* 2005). However, IIP may provide better catalysing effect than the reference treatments due to the direct contact between milk and steam. It could also be considered whether the difference in ethyl acetate level was related to the fact that the milk fat globules are partially disrupted in the IIP treatment, probably due to cavitation during flash cooling (Hougaard *et al.* 2009), leaving the milk fat more susceptible to enzymatic reactions.

Methyl ketones with an odd number of C atoms may be formed during heat treatment of milk by thermal decarboxylation of β-keto acids and have primarily been reported in UHT milk (Badings 1991; Calvo and de la Hoz 1992) or in higher amounts in UHT milk as compared with pasteurised and raw milk (Vazquez Landaverde *et al.* 2005). However, in the skim milk of this study, all methyl ketones were found in lower amounts in the IIP treated samples, with only one exception, 2-octanone, which does not contain an odd number of C atoms, and consequently may be formed by a different reaction. In the NSTD milk, only 2-propanone and 2-butanone were found in lower amounts in the IIP treated samples as compared with the references. A number of heptanones and octanones were detected in the IIP treated samples, but not in the reference samples.

Several aldehydes were detected both in the reference samples and in the milk subjected to IIP, and the levels of aliphatic aldehydes, except pentanal, were higher in raw and HTST treated samples than in IIP treated samples, but no clear relation between IIP treatment intensity and amount of aldehydes was observed (Tables 2 and 3).

The only sulphur compound detected was dimethyl disulphide, and this compound was primarily found in the IIP treated milk samples showing a tendency to increasing amounts with increasing treatment temperature (Tables 2 and 3). Dimethyl disulphide may be formed by oxidation of methanethiol from the amino acid methionine (Calvo and de la Hoz 1992), and has previously been detected in milk treated using direct UHT treatment, but not in pasteurised milk (Contarini *et al.* 1997).

Table 2 Volatile compounds detected in skim milk samples subjected to different heat treatments

Compound	Day*	*Treatment***					
		Raw	HTST	85°C/30 s	IIP 80	IIP 100	IIP 120
Ketones							
2-Propanone	2	763[a]	828[a]	740[a]	190[b]	100[b]	66.1[b]
	7	321[b]	793[a]	676[a]	166[b]	121[b]	93.8[b]
2-Butanone	2	973[a]	1043[a]	1015[a]	168[c]	91.4[c]	57.2[c]
	7	695[b]	968[a]	1046[a]	159[c]	97.1[c]	61.1[c]
3-Heptanone	2	ND***	ND	ND	11.3[b]	13.1[a]	9.17[c]
	7	ND	ND	ND	11.4[b]	12.3[a]	9.55[c]
2-Heptanone	2	32.4[c]	36.1[a]	33.2[bc]	24.0[e]	25.1[d]	19.4[f]
	7	32.9[bc]	34.6[ab]	34.8[ab]	24.7[e]	27.1[d]	21.0[f]
3-Methyl-2-Heptanone	2	ND	ND	ND	10.2[b]	11.2[a]	8.67[c]
	7	ND	ND	ND	9.66[b]	10.0[b]	8.74[c]
4-Octanone	2	ND	ND	ND	2.16[a]	2.27[a]	1.90[b]
	7	ND	ND	ND	1.83[b]	2.15[a]	1.66[c]
3-Octanone	2	ND	ND	ND	3.94[c]	5.16[a]	3.66[cd]
	7	ND	ND	ND	3.93[c]	4.37[b]	3.36[d]
2-Octanone	2	11.3[e]	11.9[e]	11.4[e]	55.9[bc]	62.8[a]	47.1[d]
	7	10.1[e]	11.2[e]	11.0[e]	54.4[c]	57.9[b]	48.6[d]
2-Nonanone	2	49.2[a]	49.7[a]	49.9[a]	13.1[c]	12.6[cd]	9.62[e]
	7	47.7[a]	44.4[b]	47.9[a]	14.0[c]	12.8[cd]	10.8[de]
2-Decanone	2	37.2[a]	37.6[a]	38.5[a]	9.29[d]	7.16[de]	4.40[e]
	7	31.2[c]	31.6[c]	34.3[b]	8.98[d]	6.64[de]	4.74[e]
2-Undecanone	2	5.14[c]	4.82[c]	6.35[b]	2.29[d]	2.48[d]	1.94[d]
	7	7.52[a]	4.92[c]	6.13[b]	2.94[d]	2.61[d]	2.20[d]
Aldehydes							
Pentanal	2	12.7[bc]	12.7[bc]	9.10[d]	10.3[cd]	18.3[a]	20.5[a]
	7	ND	8.4[d]	14.4[b]	7.36[d]	18.4[a]	21.6[a]
Hexanal	2	119[a]	119[a]	22.8[c]	22.7[c]	25.7[c]	18.4[c]
	7	2.56[d]	62.0[b]	57.6[b]	29.1[c]	28.2[c]	20.8[c]
Heptanal	2	8.30[a]	7.59[b]	4.07[e]	3.05[f]	3.82[e]	3.49[ef]
	7	0.80[g]	5.86[c]	5.01[d]	3.67[ef]	4.14[e]	3.59[ef]
Octanal	2	7.32[ab]	7.01[ab]	6.52[abc]	3.21[de]	4.30[cd]	5.18[bcd]
	7	1.48[e]	6.01[abc]	6.05[abc]	4.33[cd]	5.67[abc]	7.52[a]
Nonanal	2	20.6[a]	19.0[ab]	16.4[bcd]	10.0[f]	13.2[def]	11.5[ef]
	7	6.71[g]	15.7[cd]	18.4[abc]	14.0[de]	14.0[de]	10.8[ef]
Benzaldehyde	2	0.23[e]	0.76[bcde]	1.26[ab]	0.43[cde]	1.32[ab]	1.10[abcd]
	7	0.33[de]	1.42[ab]	1.15[abc]	ND[e]	1.81[a]	1.53[ab]
Ester							
Ethyl acetate	2	16.5[c]	18.0[c]	19.6[c]	484[a]	445[a]	367[b]
	7	36.9[c]	16.7[c]	18.3[c]	444[a]	458[a]	379[b]
Sulphur compound							
Dimethyl disulphide	2	ND[e]	0.41[de]	ND[e]	3.11[c]	4.05[b]	5.01[a]
	7	1.26[d]	0.36[de]	ND[e]	2.96[c]	4.23[ab]	4.93[ab]
Terpene							
Limonene	2	2.21[c]	4.16[b]	5.57[a]	1.97[c]	2.86[bc]	3.19[bc]
	7	2.17[c]	2.87[bc]	5.89[a]	3.23[bc]	2.89[bc]	2.72[c]

Values are determined as: Mean of [Area quantifier ion (compound)/area quantifier ion (internal standard)] × 1000, $n = 3$.
Values within one compound without common superscript are significantly different ($P < 0.05$).
*Day: day after processing; **Treatments: HTST, high temperature short time pasteurisation, IIP, instant infusion pasteurisation at 80°C, 100°C and 120°C; ***ND, Not detected.

Table 3 Volatile compound detected in nonstandardised milk samples subjected to different heat treatments

Compound	Day*	Treatment**					
		Raw	HTST	85°C/30 s	IIP 80	IIP 100	IIP 120
Ketones							
2-Propanone	1	795[a]	825[a]	926[a]	220[b]	86.5[b]	86.8[b]
	7	983[a]	818[a]	931[a]	270[b]	139[b]	166.7[b]
2-Butanone	1	492[a]	482[a]	518[a]	81.6[b]	55.0[b]	34.3[b]
	7	503[a]	479[a]	518[a]	88.0[b]	63.0[b]	45.5[b]
3-Heptanone	1	ND[c]***	ND[c]	ND[c]	5.79[a]	5.32[b]	5.33[b]
	7	ND[c]	ND[c]	ND[c]	5.29[b]	5.95[a]	5.80[a]
2-Heptanone	1	0.60[f]	1.61[f]	3.88[e]	11.8[bc]	11.3[c]	11.7[bc]
	7	4.33[e]	1.94[f]	6.43[d]	11.9[bc]	13.0[b]	15.4[a]
3-Methyl-2-heptanone	1	ND[c]	ND[c]	ND[c]	2.71[a]	2.75[a]	1.72[ab]
	7	ND[c]	ND[c]	ND[c]	0.76[bc]	1.54[abc]	1.67[ab]
4-Octanone	1	ND[c]	ND[c]	ND[c]	0.57[a]	0.30[abc]	0.59[a]
	7	ND[c]	ND[c]	ND[c]	0.20[bc]	0.34[abc]	0.39[ab]
3-Octanone	1	ND[a]	ND[a]	ND[a]	0.34[a]	0.60[a]	0.33[a]
	7	ND[a]	ND[a]	ND[a]	ND[a]	0.22[a]	0.44[a]
2-Octanone	1	ND[b]	ND[b]	ND[b]	12.8[a]	12.3[a]	12.2[a]
	7	ND[b]	ND[b]	ND[b]	11.8[a]	12.5[a]	13.5[a]
2-Nonanone	1	ND[d]	ND[d]	0.56[cd]	1.39[bc]	1.36[bc]	1.16[bc]
	7	0.52[cd]	ND[d]	1.39[bc]	1.54[b]	1.68[b]	2.54[a]
2-Decanone	1	ND	ND	ND	ND	ND	ND
	7	ND	ND	ND	ND	ND	1.20
2-Undecanone	1	ND	ND	ND	ND	ND	ND
	7	ND	ND	ND	ND	ND	ND
Aldehydes							
Pentanal	1	32.7[cd]	35.8[bc]	9.40[e]	19.5[de]	46.4[b]	49.3[b]
	7	21.1[de]	26.7[cd]	18.1[de]	30.5[cd]	65.1[a]	69.5[a]
Hexanal	1	129.7[a]	112[a]	10.3[d]	15.2[cd]	18.0[cd]	13.3[cd]
	7	94.2[b]	124[a]	22.0[cd]	31.9[c]	22.1[cd]	18.7[cd]
Heptanal	1	5.59[a]	4.61[b]	0.58[f]	1.74[cde]	1.77[cde]	1.41[def]
	7	5.01[ab]	5.63[a]	1.06[fe]	2.68[c]	2.18[cd]	2.46[c]
Octanal	1	2.48[ab]	2.01[b]	1.47[b]	1.85[b]	1.28[b]	1.52[b]
	7	3.10[ab]	2.51[ab]	1.19[b]	1.89[b]	1.75[b]	5.57[a]
Nonanal	1	4.58[ab]	3.30[b]	2.47[b]	3.48[b]	2.67[b]	2.70[b]
	7	5.69[a]	3.67[b]	2.80[b]	3.48[b]	2.85[b]	3.48[b]
Benzaldehyde	1	0.61[a]	0.39[a]	1.02[a]	0.52[a]	1.39[a]	ND[a]
	7	0.84[a]	1.07[a]	0.97[a]	0.90[a]	0.51[a]	1.28[a]
Ester							
Ethyl acetate	1	331[e]	335[e]	354[e]	608[ab]	616[ab]	469[d]
	7	343[e]	331[e]	343[e]	573[bc]	666[a]	538[c]
Sulphur compound							
Dimethyl disulphide	1	ND[d]	ND[d]	ND[d]	ND[d]	4.88[b]	9.20[a]
	7	ND[d]	ND[d]	ND[d]	1.76[cd]	3.60[bc]	8.16[a]
Terpene							
Limonene	1	1.07[ab]	1.20[ab]	1.82[ab]	0.99[ab]	1.18[ab]	0.62[ab]
	7	2.17[a]	1.63[ab]	1.63[ab]	0.92[ab]	1.33[ab]	1.10[ab]

Values are determined as: Mean of [Area quantifier ion (compound)/area quantifier ion (internal standard)] × 1000, $n = 3$.
Values within one compound without common superscript are significantly different ($P < 0.05$).

*Day: day after processing; **Treatments: HTST, high temperature short time pasteurisation, IIP, instant infusion pasteurisation at 80°C, 100°C and 120°C; ***ND, Not detected.

Storage of the milk generally had minor impact on the volatiles within the different heat treatments, except from the levels of aliphatic aldehydes in raw skim milk that decreased considerably during storage. Changes in the composition of volatile compounds in ultrapasteurised and UHT treated milk have been investigated by several authors, and generally the content of aldehydes and methyl ketones are found to increase during storage (Rerkrai *et al.* 1987; Valero *et al.* 2001; Solano Lopez *et al.* 2005). However, storage at refrigeration temperature leads to markedly slower changes (Bassette and Jeon 1983; Rerkrai *et al.* 1987), which may explain the minor effect observed after refrigerated storage for 7 days.

A PCA was performed, including all samples of both skim milk and NSTD milk, and the levels of the 20 volatile compounds. A biplot of the scores and loadings obtained for principal component (PC)1 and PC2, explaining 75% of total variance, is shown in Figure 1, and this plot reveals four distinct groups of samples, separated by milk type (skim milk vs NSTD milk) and applied processing method (IIP vs references) Roughly, it can be said that PC1 separated the IIP treatments from the reference treatments, and PC2 separated the skim milk from the NSTD milk. No obvious separation of the different heat treatment temperatures was observed, and no effect of refrigerated storage for 7 days could be inferred from the PCA model (sample labels not shown in Figure 1). The observed grouping show that the composition of volatile compounds in IIP treated milk was different from the composition of volatile compounds in the reference samples. However, the distance was shorter between the two groups of NSTD milk than between the skim milk groups, which may be due to difficulties in releasing the volatile compounds from the milk as discussed above, but it may also indicate that the differences in composition of volatile compounds resulting from the different applied heat treatments were in fact less pronounced for NSTD milk than for skim milk.

Sensory properties of IIP treated skim milk

The results of the sensory descriptive analysis showed, that 12 of the 17 descriptors were found significant in describing the differences between the four heat treatments (Table 4). The results also showed, that two out of three of the significant
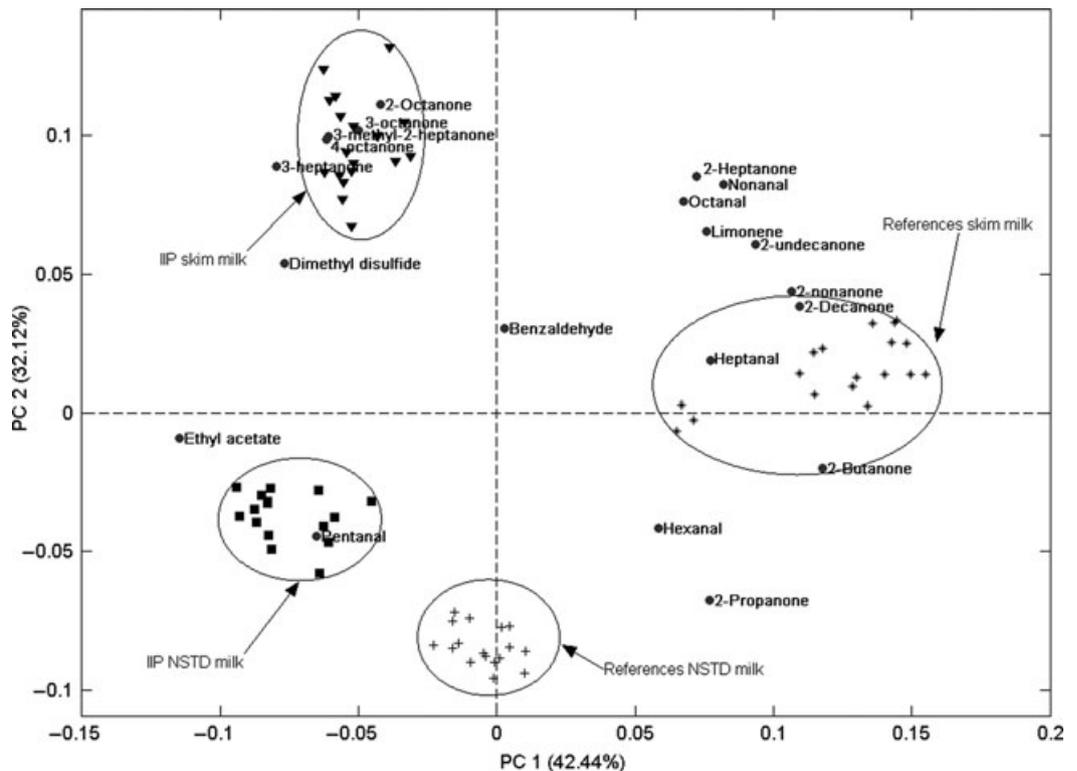

Figure 1 Biplot showing PC1 vs PC2 from PCA of the composition of 20 volatile compounds in milk samples subjected to IIP at 80°C, 100°C or 120°C, or reference treatments (raw milk, HTST pasteurisation, 85°C/30 s pasteurisation). IIP, instant infusion pasteurisation; HTST, high temperature short time; NSTD, nonstandardised; PC, principal component; PCA, principal components analysis.

descriptors were describing differences between the heat treatment technologies; the odour of maize ($P \leq 0.01$) and the odour and flavour of boiled milk ($P \leq 0.01$ and $P \leq 0.001$) were all perceived lower for IIP samples than for reference pasteurised samples whereas the watery ($P \leq 0.001$), cardboard sour ($P \leq 0.001$) and plastic and chemical like ($P \leq 0.001$) flavours, as well as the cardboard

Table 4 Mean values (over assessor and replicates) of 17 sensory descriptors for each of the four heat treatments

	Treatment [A]			
Sensory descriptor	*Sign[B]*	*85°C/30 s*	*HTST*	*IIP 80*	*IIP 120*
O-Boiled milk[C]	**	4.04[b]	3.85[b]	2.26[a]	2.05[a]
O-Maize	**	4.49[b]	4.10[b]	2.61[a]	2.40[a]
O-Cardboard/sour	**	3.53[a]	3.62[a]	6.74[b]	4.61[a]
A-Grey colour	n.s.	4.89	4.57	5.11	4.99
A-Yellow colour	n.s.	3.67	4.09	4.08	3.52
F-Boiled milk	***	4.63[b]	4.10[b]	2.84[a]	2.53[a]
F-Watery	***	6.33[a]	5.48[a]	7.97[b]	8.23[b]
T-Sugar sweet	*	4.34[b]	4.57[b]	3.74[ab]	3.37[a]
F-Toffee	**	2.40[b]	2.65[b]	1.77[ab]	1.08[a]
F-Maize sweet	**	3.39[ab]	4.05[b]	3.10[ab]	2.34[a]
F-Cardboard/sour	***	3.13[a]	2.91[a]	5.83[b]	4.72[b]
F-Metallic	n.s.	3.37	2.66	4.17	4.25
F-Plastic/chemical	***	2.71[a]	2.31[a]	4.50[b]	4.67[b]
T-Bitter	*	1.81[ab]	1.42[a]	2.33[b]	2.51[b]
AT-Astringent 0	n.s.	4.66	4.95	5.51	5.75
ATMF-Cardboard/sour	***	2.32[a]	2.01[a]	4.39[b]	4.06[b]
AT-Astringent 20	n.s.	7.60	7.95	8.47	7.83

[A]Treatments: HTST, high temperature short time pasteurisation, IIP, instant infusion pasteurisation at 80°C and 120°C.
[B]Significance levels: n.s., not significant; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.
[C]Means within a row with the same superscript letter are not significantly different (least significant difference test, $P \leq 0.05$).
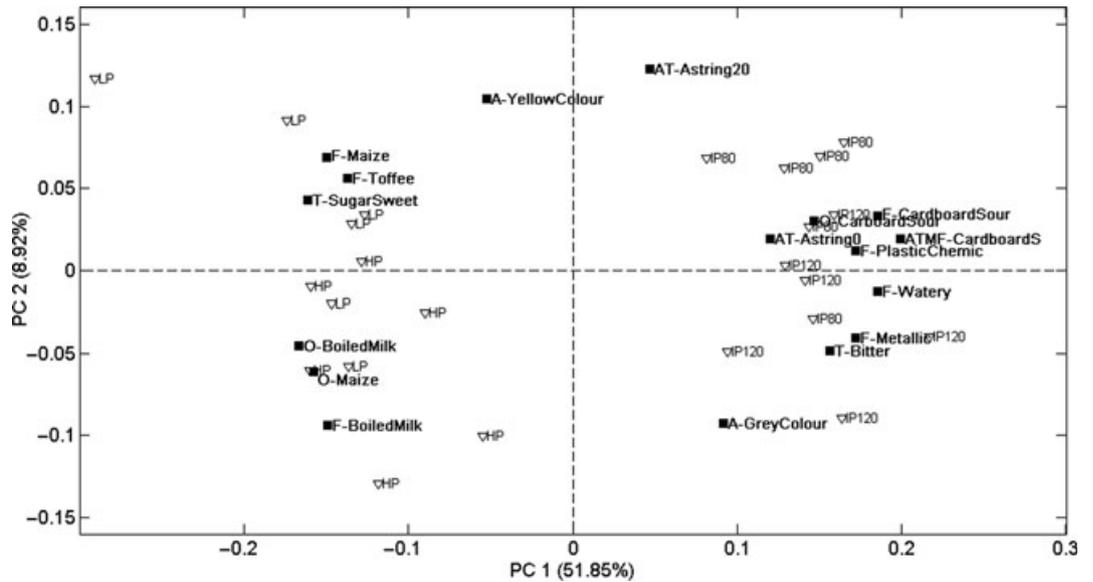

Figure 2 Biplot of PC1 vs PC2 from PCA on sensory data from evaluation of skim milk samples subjected to IIP at 80°C or 120°C (IP 80 and IP 120, respectively) or the reference treatments: HTST pasteurisation (LP) and 85°C/30 s pasteurisation (HP). IIP, instant infusion pasteurisation; HTST, high temperature short time; PC, principal component; PCA, principal components analysis.

sour aftertaste ($P \leq 0.001$) were perceived higher for IIP samples than for reference pasteurised samples. Bitterness ($P \leq 0.05$) was perceived higher for IIP samples than for the HTST sample, and a pronounced cardboard sour odour ($P \leq 0.01$) was found to be perceived higher for the IIP 80 sample than for the other samples. Sweetness related descriptors, i.e. sugar sweet taste ($P \leq 0.05$) and toffee flavour ($P \leq 0.01$), were found to be perceived lower for the IIP 120 sample than for reference pasteurised samples. Maize sweet flavour ($P \leq 0.01$) was perceived lower for IIP 120 sample than for the HTST sample.

An overview of the sensory data analyzed by PCA revealed that more than 50% of the total variance in the data was primarily related to the heat treatment technology, separating the reference treatments (HTST and 85°C/30 s) from the IIP treatments in PC1 (Figure 2). The sensory descriptors watery flavour, cardboard sour odour, flavour and aftertaste as well as plastic and chemical like

flavour were found to be associated positively with the IIP treatments. The reference treatments resulted in skim milk associating positively with the odours and flavours of boiled milk and maize as well as sugar sweet taste and toffee flavour. In PC2, explaining 9% of total variance, a tendency to separation of the two temperatures of IIP is found, indicating a more pronounced astringent aftertaste (not significant) in samples treated at 80°C (Figure 2).

As seen from Table 1, the most pronounced sensory characteristics of the IIP treated skim milk are all related to negative descriptors, whereas the sensory properties of the reference heat treatments are related to positive descriptors. It can thus be concluded, that in comparison with the two reference heat treatments the findings in this study did not support the suggestion based on earlier reports by Fredsted *et al.* (1995) of IIP treatment preserving sensory characteristics associated to freshness of milk, as freshness is generally regarded as a positive milk property.

Correlation between composition of volatile compounds and sensory properties
A PLS model may be used to evaluate the ability of the composition of volatile compounds to describe the sensory properties of the milk samples. A cross validated PLS2 model with three latent variables was calculated, explaining a total of 92.4% of the variance in the volatile compounds and 79.7% of the variance in the sensory data. Regression coefficients of the cross validated predictions vs measured sensory attributes are presented in Table 5. The regression coefficients were generally high with 9 out of 17 sensory descriptors showing r^2 values above 0.7, clearly indicating that the composition of volatile compounds in the heat treated milk samples can be used for prediction of the sensory descriptors. The PLS regression vectors for the sensory terms with the highest correlations between predictions and measured values are plotted in Figure 3. The regression vectors for boiled milk odour and flavour and for toffee flavour, which were positively correlated to the reference samples in the PCA (Figure 2), are seen to differ substantially in shape from the vectors for the rest of the descriptors (Figure 3), which were positively correlated to the IIP treated samples (Figure 2). For example, for the volatile compounds 2-propanone, ethyl acetate, 2-butanone and 3-heptanone the regression vectors of the sensory terms positively associated with the IIP treated samples points in the opposite direction of the regression vectors of the reference treatments. These results emphasise
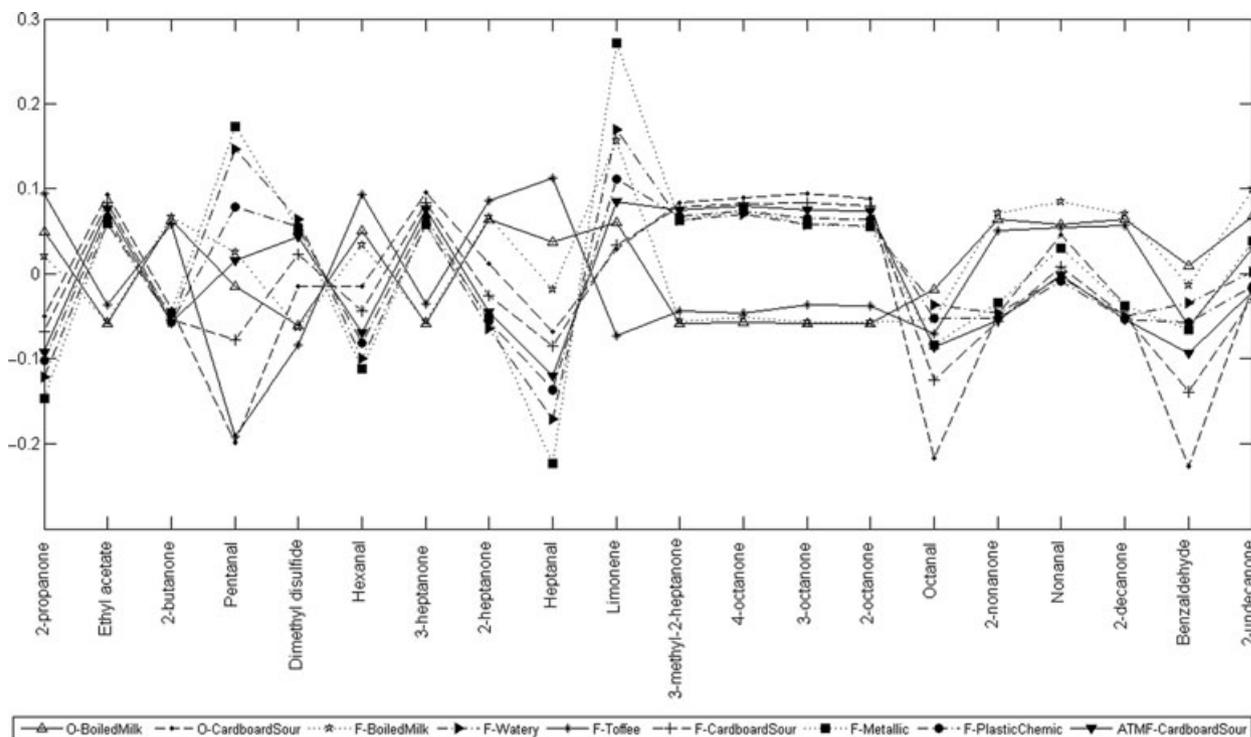
Table 5 Regression coefficients (r^2) of cross validated prediction vs measured sensory descriptor and RMSECV[a] for a partial least squares 2 model with three latent variables on skim milk samples subjected to instant infusion pasteurisation at 80°C and 120°C, high temperature short time pasteurisation and 85°C/30 s pasteurisation using the content of 20 volatile compounds for prediction

Sensory descriptor[b]	r^2	RMSECV
O[c]-Boiled milk	0.702	0.528
O-Maize	0.572	0.667
O-Cardboard/sour	0.896	0.441
A-Grey colour	0.610	0.143
A-Yellow colour	0.044	0.384
F-Boiled milk	0.769	0.439
F-Watery	0.943	0.280
T-Sugar sweet	0.669	0.307
F-Toffee	0.797	0.287
F-Maize	0.641	0.412
F-Cardboard/sour	0.782	0.591
F-Metallic	0.846	0.268
F-Plastic/chemical	0.879	0.386
T-Bitter	0.618	0.308
AT-Astringent 0[c]	0.489	0.350
ATMF-Cardboard/sour	0.971	0.184
AT-Astringent 20[c]	0.003	0.546

[a]RMSECV, Root mean square error of cross validation.
[b]Sensory descriptors as defined in Table 1.
[c]Suffix to sensory term indicates method of assessment by panelists; O, odour; A, appearance; F, flavour; T, taste; ATMF, aftertaste/mouthfeel.

Figure 3 Regression vectors for selected sensory attributes from partial least squares 2 analysis (three latent variables) on composition of volatile compounds and sensory properties.

that the composition of volatile compounds was important for the sensory properties, and that the volatile compounds detected in milk subjected to IIP contributed to sensory descriptors that are not associated with freshness of milk.

CONCLUSIONS

The composition of volatile compounds in skim milk or NSTD milk subjected to IIP at different temperatures was found to differ from the composition of volatile compounds in raw milk, HTST pasteurised milk, and milk pasteurised at 85°C/30 s. Separation of milk samples according to milk type (skim or NSTD) and heat treatment technology [References (including raw) and IIP] was obtained when applying principal component analysis on volatile compounds. Based on the current results, it seems highly unlikely that IIP, as previously suggested, can provide pasteurisation of milk with preservation of the fresh taste of raw milk. This was confirmed for the skim milk by descriptive sensory analysis, where the IIP treated samples were positively correlated to negative descriptive terms, which can not be associated with freshness. Furthermore, good predictions of the sensory

properties of skim milk were obtained from the composition of volatile compounds in partial least squares analysis.

REFERENCES

Badings H T (1991) Milk. In *Volatile Compounds in Foods and Beverages*, pp 91–106. Maarse H, ed. New York, USA: Marcel Dekker, Inc.

Bassette R and Jeon I J (1983) Effect of process-times and storage-times and temperatures on concentrations of volatile materials in ultra-high-temperature steam infusion processed milk. *Journal of Food Protection* **46** 950–953.

Burdock G M (2005) *Fenaroli's handbook of flavour ingredients*, 5th edn. Boca Raton, USA: CRC Press.

Calvo M M and de la Hoz L (1992) Flavour of heated milks. A review. *International Dairy Journal* **2** 69–81.

Contarini G and Povolo M (2002) Volatile fraction of milk: comparison between purge and trap and solid phase microextraction techniques. *Journal of Agricultural and Food Chemistry* **50** 7350–7355.

Contarini G, Povolo M, Leardi R and Toppino P M (1997) Influence of heat treatment on the volatile compounds of milk. *Journal of Agricultural and Food Chemistry* **45** 3171–3177.

Edmond S P (2001) Continuous heat processing. In *Thermal technologies in food processing*, pp 29–48. Richardson p, ed. Cambridge: Woodhead Publishing Ltd.

Fredsted L B., Rysstad G and Eie T (1995) "Pure-Lac™: the new milk with protected freshness and extended shelf life". In *Heat Treatments & Alternative Methods*, pp. 104–125. Brussels: International Dairy Federation.

Hougaard A B, Hammershøj M, Vestergaard J S, Poulsen O and Ipsen R H (2009) Instant Infusion paseurization of bovine milk I Effects on bacterial inactivation and physical-chemical properties. *International Journal of Dairy Technology* **62** 484–492.

Lawless H T and Heymann H (1999) *Sensory Evaluation of Food. Principles and Practices*. New York, USA: Aspen Publishers, Inc.

Rerkrai S, Jeon I J and Bassette R (1987) Effect of various direct ultra-high temperature heat treatments on flavour of commercially prepared milks. *Journal of Dairy Science* **70** 2046–2054.

Solano Lopez C E, Taehyun J and Alvarez V B (2005) Volatile compounds and chemical changes in ultrapasteurized milk packaged in polyethylene terephthalate containers. *Journal of Food Science* **70** C407–C412.

Valero E, Villamiel M, Sanz J and Martinez Castro I (2000) Chemical and sensorial changes in milk pasteurised by microwave and conventional systems during cold storage. *Food Chemistry* **70** 77–81.

Valero E, Villamiel M, Miralles B, Sanz J and Martinez Castro I (2001) Changes in flavour and volatile components during storage of whole and skimmed UHT milk. *Food Chemistry* **72** 51–58.

Vazquez Landaverde P A, Velazquez G, Torres J A and Qian M C (2005) Quantitative determination of thermally derived off-flavour compounds in milk using solid-phase microextraction and gas chromatography. *Journal of Dairy Science* **88** 3764–3772.

Wilkes J G, Conte E D, Kim Y, Holcomb M, Sutherland J B and Miller D W (2000) Sample preparation for the analysis of flavours and off-flavours in foods. *Journal of Chromatography A* **880** 3–33.