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Therese Jansson, Sidsel Jensen, Nina Eggers, Morten Clausen, Lotte Larsen, et al.. Volatile component profiles of conventional and lactose-hydrolyzed UHT milk-a dynamic headspace gas chromatographymass spectrometry study. Dairy Science & Technology, 2014, 94 (4), pp.311-325. 10.1007/s13594-014-0164-7. hal-01234865

# HAL Id: hal-01234865 https://hal.science/hal-01234865

Submitted on 27 Nov 2015

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### ORIGINAL PAPER

# Volatile component profiles of conventional and lactose-hydrolyzed UHT milk—a dynamic headspace gas chromatography-mass spectrometry study

Therese Jansson • Sidsel Jensen • Nina Eggers • Morten R. Clausen • Lotte B. Larsen • Colin Ray • Anja Sundgren • Henrik J. Andersen • Hanne Christine Bertram

Received: 16 January 2014 / Revised: 11 February 2014 / Accepted: 11 February 2014 / Published online: 14 March 2014 © INRA and Springer-Verlag France 2014

Abstract Lactose-hydrolyzed milk gains still increasing market share, and understanding the chemical characteristics of lactose-hydrolyzed milk products is important for the dairy industry. The aim of the present study was to identify and compare volatile compounds of commercial lactose-hydrolyzed and conventional ultra-high temperature (UHT) milk. For this purpose, the volatile compounds of lactose-hydrolyzed (<1% lactose), conventional (100% lactose), and filtered (60% lactose) UHT-treated milk were extracted using dynamic headspace sampling and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 24 volatile compounds were identified including ketones, aldehydes, and sulfides. Overall, principal component analysis (PCA) showed grouping of the different milk types, with loadings indicating a higher concentration of ketones in conventional versus lactose-hydrolyzed UHT milk, but PCA also indicated a marked batch-to-batch variation. Elucidation of individual volatile compounds detected also revealed that the content of ketones in general was higher in conventional UHT milk than in lactose-hydrolyzed milk; however, no significant differences in the volatile compound profiles could be identified between the various milk types as a result of the batch-to-batch variation. The present study

T. Jansson · S. Jensen · N. Eggers · M. R. Clausen · H. C. Bertram (🖂)

Department of Food Science, Research Center Aarslev, Aarhus University, Kirstinebjergvej 10, 5792 Aarslev, Aarhus, Denmark e-mail: hannec.bertram@agrsci.dk

L. B. Larsen Department of Food Science, Research Centre Foulum, Aarhus University, Blichers Allé 20, 8830 Tjele, Aarhus, Denmark

C. Ray · A. Sundgren Arla Foods Strategic Innovation Centre, Lindhagensgatan 126, 10546 Stockholm, Sweden

H. J. Andersen Roerdrumvej 2, 8220 Brabrand, Aarhus, Denmark



highlights a useful analytical method based on dynamic headspace sampling and GC-MS to profile volatiles important for the flavor characteristics of lactose-hydrolyzed and conventional UHT milk. In addition, the present study reveals that a considerable batch-to-batch variation exists in industrially produced batches of lactose-hydrolyzed UHT milk, which must be considered an important challenge for the dairy industry.

Keywords Lactose-hydrolyzed milk  $\cdot$  UHT  $\cdot$  Milk batch variations  $\cdot$  Dynamic headspace sampling  $\cdot$  GC-MS

# Abbreviations

DHS	Dynamic headspace sampling
DMDS	Dimethyl disulfide
DMS	Dimethyl sulfide
DMSO	Dimethyl sulfoxide
DMTS	Dimethyl trisulfide
FWER	Family-wise error rate
GC	Gas chromatograph
IS	Internal standard
LME	Linear mixed effect
LOD	Limit of detection
LOQ	Limit of quantification
MR	Maillard reaction
MS	Mass spectrometry
nd	Not detected
nq	Not quantified
PC	Principal component
PCA	Principal component analysis
REML	Restricted maximum likelihood
TIC	Total ion current
UHT	Ultra-high temperature
UV	Unit variance

# **1** Introduction

More than 70% of the world's population is unable to digest lactose as a consequence of a reduced or nonexistent  $\beta$ -galactosidase activity (Messia et al. 2007; Sahi 1994). This is referred to as "lactose malabsorption" or "lactose intolerance" depending on the degree of reduction in  $\beta$ -galactosidase activity. These conditions give rise to bacterial fermentation of lactose in the colon, resulting in the production of copious amounts of gas (a mixture of hydrogen, carbon dioxide, and methane) that causes abdominal symptoms such as bloating, cramps, and nausea (Zhong et al. 2004). During the past decade, several lactose-hydrolyzed milk products have been introduced to the commercial market to meet the needs of consumers with lactose intolerance.

High-quality lactose-hydrolyzed milk may be produced using filtration to remove approximately 40% of the lactose. The remaining lactose is enzymatically hydrolyzed by the addition of  $\beta$ -galactosidase, producing the monosaccharides glucose and



galactose. The decomposition of lactose to glucose and galactose in milk may influence the type and extent of chemical reactions in the milk taking place during processing and storage. For instance, Kato et al. (1986, 1988) reported that browning reactions and protein polymerization, both of which are characteristic for the advanced stages of the Maillard reaction (Brands et al. 2002), proceed more extensively in protein-galactose and protein-glucose models than in an equivalent protein-lactose model during heating (Brands et al. 2000). Consequently, lactose-hydrolyzed milk is expected to be more susceptible to Maillard reaction and Strecker degradation during storage than nonhydrolyzed ultra-high temperature (UHT) milk (Messia et al. 2007).

Various forms of thermal processing are used to prevent enzymatic and microbial spoilage and thereby extend the shelf life of milk. One commonly used heat treatment in the dairy industry is UHT processing (135–150 °C, 2–20 s; Kessler 2002). UHT-treated milk has a long shelf life and may be stored at room temperature for up to 8 months. However, it is well established that severe heat treatment and storage at ambient temperature lead to the development of off-flavors in milk (Jeon et al. 1978; Rerkrai et al. 1987; Valero et al. 2001).

Extensive investigations have been carried out to characterize the chemical changes giving rise to unwanted off-flavors in heat-treated milk (Rerkrai et al. 1987; Valero et al. 2001; Coppa et al. 2011; Vazquez-Landaverde et al. 2005; Toso et al. 2002; Contarini and Povolo 2002; Contarini et al. 1997). The severity of the heat treatment strongly affects the resulting volatile profile of milk. In particular, UHT milk is characterized by an increase in the amount of ketones (Vazquez-Landaverde et al. 2005), aldehydes (Vazquez-Landaverde et al. 2005), and sulfides (Vazquez-Landaverde et al. 2006).

In general, the amount of volatile compounds in milk is low and a concentration step is required to perform effective analyses. Dynamic headspace sampling (DHS) in combination with chromatographic techniques is a useful method for concentrating and analyzing volatiles with high reproducibility and sensitivity. Optimally, the volatiles should be collected under mild conditions to minimize sample manipulation and the potential for artifact formation (Dirinck et al. 1984). DHS in combination with gas chromatography-mass spectrometry (GC-MS) has been frequently used for characterizing the volatiles in milk (Valero et al. 2001; Toso et al. 2002; Vallejo-Cordoba et al. 1993) and is also used in the characterization of many other food matrices (Jensen et al. 2011; Bach et al. 2012). However, to our knowledge, no characterization of the volatile profile of heat-treated lactose-hydrolyzed milk obtained using the combination of DHS and GC-MS has been reported.

The aim of the present study was to identify and compare the volatile fractions of different types of commercially produced enzymatically hydrolyzed UHT milk (direct versus indirect) and conventional UHT milk. To ensure a representative pool of samples, several batches of milk were produced in a full-scale commercial dairy plant, which made it possible also to elucidate batch variations in industrially produced milks.

## 2 Materials and methods

#### 2.1 Chemical references

Decane (99%), tetradecane (99%), dodecane (99%), 2-methylbutanal (95%), hexanal (98%), octanal (99%), nonanal (95%), decanal (98%), 2-butanone (99%), 2-pentanone



(99%), 2-hexanone (99.5%), 2-heptanone (98%), 2-octanone (98%), 2-nonanone (99%), 6-methyl-5-heptene-2-one (99%), 6,10-dimethyl-5,4-undecadien-2-one (97%), dimethyl disulfide (98%), 4-methyl-1-pentanol (97%), toluene (99.5%), styrene (99%), and 2-pentyl furan (97%) were obtained from Sigma-Aldrich Inc. (Steinheim, Germany). Dimethyl sulfoxide (99%) was purchased from Serva electrophoresis GmbH (Heidelberg, Germany).

# 2.2 Milk samples

Milk was produced at a Danish commercial dairy plant (Arla Foods, Esbjerg, Denmark). A total of four types of milk (CONVI, LHD, LHI, and FILTI) were produced (Table 1) and stored at 22 °C until the day of analysis. CONVI, LHD, and LHI were produced in three batches in September and October 2012 and February 2013. For technical reasons, FILTI was included to separate the impact of filtration and enzyme treatment on the formation of volatiles. FILTI was only produced in two batches in September 2012. The milk was UHT-treated using either direct heat treatment (LHD) (steam is injected into the milk to heat it to 140–145 °C for a few seconds, followed by flash cooling; Tetra Pak 2003) or indirect heat treatment (CONVI, LHI, FILTI) (a tubular heat exchanger is used to heat the milk to 140-145 °C for a few seconds, followed by slow cooling (Tetra Pak 2003)) and packaged aseptically. Lactose in the lactose-hydrolyzed and the filtered milk (LHD, LHI, FILTI) was removed using ultrafiltration, to approximately 60% of the original lactose level. The remaining lactose was enzymatically hydrolyzed (LHD, LHI) by the addition of β-galactosidase (Jelen and Tossavainen 2003). A total of three replicates of each milk type and each batch were analyzed 17-21 days after production.

# 2.3 Dynamic headspace sampling

A 100-mL milk sample was placed in a conical flask with a glass cap insert and 200  $\mu$ L of a 10 ppm 4-methyl-1-pentanol solution was added to the milk as an internal standard (IS). Adsorbent traps (Tenax TA, 200 g Tenax TA, 35/60 mesh; Markes International Limited, Llantrisant, UK) were attached to the flask, which was subsequently placed in

Milk		No. of batches	Enzymatic hydrolysis	Heat treatment	Filtration	Level of lactose (% of original level)	Amount of lactose (% w/w)
CONVI	Conventional UHT	3	_	Indirect	_	100	5
LHD	Lactose-hydrolyzed direct UHT	3	+	Direct	+	<1	0.01
LHI	Lactose-hydrolyzed indirect UHT	3	+	Indirect	+	<1	0.01
FILTI	Filtered UHT	2	_	Indirect	+	60	3

**Table 1** Overview of milk types included in the study, heat treatments, and lactose levels. All milks contained 1.5% fat (w/w) and 3.5% protein (w/w)



a thermostatically controlled cabinet at 20  $^{\circ}$ C. The milk was sparged with nitrogen (40 mL.min) for 45 min while being stirred.

# 2.4 GC-MS

The adsorbent traps were desorbed (250 °C, 15 min) into a cold trap (U-TIIGCP, Markes International Limited, UK) using a thermal desorption system (ultra-UNITY<sup>TM</sup>, Markes International Limited, Llantrisant, UK). Helium was used as carrier gas at a constant pressure of  $6.985 \times 10^3$  Pa. The desorbed volatiles were focused at 1 °C for 5 min and then injected onto a gas chromatography column through a transfer line (140 °C) by raising the temperature of the trap to 300 °C under splitless conditions. The gas chromatograph (Finnigan trace GC ultra, Thermo, Waltham, MA) was equipped with a CP-Wax 52CB (Varian, Palo Alto, CA, USA) column (50 m×0.25 mm, film thickness 0.25 µm). The gradient program included an isothermal step at 32 °C for 1 min, a ramp to 35 °C at 0.2 °C.min, a ramp to 40 °C at 0.5 °C.min, a ramp to 60 °C at 2.5 °C.min, and a ramp to 220 °C at 10 °C.min, followed by a 15-min isothermal step.

The GC was connected to a single quadrupole mass spectrometer (Finnigan Trace dual-stage quadrupole (DSQ), Thermo, Waltham, MA, USA). The MS transfer line temperature was 250 °C and the ion source temperature was 200 °C. The mass spectrometer was scanned over a mass to charge (m/z) range of 45–650 (4.46 scans/s) and the spectra were obtained using a fragmentation voltage of 70 eV.

# 2.5 Method optimization

To optimize the sampling parameters, the effect of flow, sample volume, and headspace sampling time were initially studied. Final sampling parameter values were set to 40 mL.min, 100 mL, and 45 min, respectively. The sampling temperature was fixed at 20 °C. In addition, several trap and cold trap materials were tested, and the injection mode and GC temperature gradient were adapted to obtain optimal separation of the volatile compounds. The final trap material used was Tenax TA and the coldtrap material was U-T11GPC. The optimum parameter values were selected on the basis of peak shape, separation, intensity, and sensitivity.

### 2.6 Identification and quantitative analysis

A MS database (NIST MS search version 2.0, 2011) was used to identify 24 volatile compounds. In addition, all of the compounds except 3,3-dimethyl hexane, 2-undecanone, and dimethyl trisulfide were verified by comparison of mass spectral data and retention times with authentic reference compounds.

The identified volatile compounds were quantified using external standard curves prepared in duplicates. The four external calibrants, which were eluted in different regions of the chromatogram, were 2-pentanone (eluting at 10.7 min), hexanal (19.2 min), 2-heptanone (31.9 min), and decanal (eluting at 44.5 min). Analytes eluting in the same retention time window were assumed to have comparable response coefficient and equal linear range as the calibrant (Jensen et al. 2011). However, the ketone responses reminded more of the nearest ketone external calibrant and were quantified ( $\mu$ g.kg milk) according to the appropriate calibrant regression equation



(Table 2). The regression equation, limits of detection (LOD), and limits of quantification (LOQ) were calculated from the standard error of the *y*-intercept and the slope of the linear regression, for each standard curve (Table 2) (Dolan 2009a, b).

# 2.7 Multivariate data and statistical analysis

Multivariate data analysis in the form of principal component analysis (PCA) was applied including all quantified compounds to investigate grouping of the samples using the Simca software (version 13.0, Umetrics AB, Umeå, Sweden). Data were normalized against the area of the added IS and unit-variance (UV) scaled before PCA analysis.

To identify significant differences between the milk samples, univariate statistical analysis including linear mixed model and Tukey's honest significant difference ( $\alpha$ = 0.05) test was performed using the software R (version 2.15.2, R Foundation for Statistical Computing, Vienna, Austria). The variance-covariance was estimated using restricted maximum likelihood (REML) to avoid underestimations of random variations in the data. The statistical model (linear mixed effect, LME) included fixed factors for the four milk types (CONVI, LHD, LHI, FILTI) and the 11 batches and took into account the correlation between the three replicates from the same batch. The variance within milk types was estimated using a linear model and Tukey's honest significant difference ( $\alpha$ =0.05), and Bonferroni's method was used to maintain the family-wise error rate (FWER). Compounds below the LOD and LOQ were not included in the statistical analysis.

### **3 Results**

GC-MS analyses were conducted on the 11 different industrially produced milk batches included in the study. Representative total ion current (TIC) chromatograms obtained from conventional, lactose-hydrolyzed direct and indirect treated UHT milk, and filtered UHT milk are shown in Fig. 1. A total of 24 volatile compounds including aldehydes, ketones, hydrocarbons, and sulfides were identified and quantified in the milk samples (Table 3). A comparison of the chromatograms indicates that the conventional UHT milk contains a greater amount of the ketones 2-butanone (7.61 min), 2-pentanone (10.6 min), 2-heptanone (31.91 min), 2-nonanone (42.04 min), and 2-

**Table 2** Regression equations, limit of detection (LOD), limit of quantification (LOQ), and coefficient of determination ( $R^2$ ) for calibration curves in filtered UHT milk containing 1.5% fat (w/w)

Compound	Calibration concentrations (ug.kg)	Limit of detection (LOD) (ug.kg)	Limit of quantification (LOQ) (ug.kg)	Regression equation	R <sup>2</sup>
2-Pentanone	4.0–79.4	3.8	11.6	<i>y</i> =0.198 <i>x</i>	0.997
Hexanal	4.0-31.8	2.0	6.2	y=0.2295x+0.1999	0.994
2-Heptanone Decanal	6.4–64.3 16.3–244.1	9.4 13.9	28.4 42.1	y=0.2762x y=0.0108x+0.0711	0.985 0.994





Fig. 1 TIC-GC chromatograms of a conventional indirect treated UHT milk; b lactose-hydrolyzed direct treated UHT milk; c lactose-hydrolyzed direct treated UHT milk; d lactose-hydrolyzed indirect treated UHT milk; f filtered indirect treated UHT milk;

undecanone (46.2 min) compared to lactose-hydrolyzed milks (Fig. 1a–e). In addition, a batch-to-batch variation was observed for the ketones when two batches of direct treated lactose-hydrolyzed (Fig. 1b, c) and indirect treated UHT milk (Fig. 1d, e) were compared.

PCA was carried out on the 24 volatile compounds identified, and Fig. 2 shows the PCA score scatter plot (a) and the corresponding loading plot (b) of the volatile compounds identified in the milk types. In the score plot, principal components (PC) 1 and 2 describe 38% and 23.6% of the variation, respectively. The score plot also indicates a separation of the lactose-hydrolyzed samples along PC1 and filtered samples along PC2. However, the separation of the lactose-hydrolyzed milk away from the lactose-containing milk is batch dependent, and one batch each of LHD and LHI was more similar to the CONVI milk. Our data therefore exhibit a significant batch variation which tends to be more severe for lactose-hydrolyzed milk than for conventional milk. The loading scatter plot (Fig. 2b) reveals that the separation of the milk types along PC1 may be ascribed to differences in the concentration of ketones and the aldehydes octanal, nonanal, and decanal, while PC2 reveals differences in the concentration of the sulfides, dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and dimethyl sulfoxide (DMSO).

It was observed that CONVI tended to contain a greater amount of ketones than LHD or LHI milks, which in contrast were characterized by a higher content of the aldehydes nonanal and decanal, if the two outlier milk batches LHD (batch 1) and LHI (batch 3) were excluded. In general, no clear difference was seen between direct and



Rt [min]	Volatile compound	Conventic UHT (CO ← Batch	onal indirect NVI) no. →		Lactose-hr UHT (LH ← Batch 1	ydrolyzed di D) 10. →	rect	Lactose-h) UHT (LH) ← Batch r	/drolyzed ind [] 10. →	lirect	Filtered U (FILTI) ←Batch 1	IHT 10. ↓
		1	2	3	1	2	3	-	2	3	1	2
	Alkanes											
5.06	3,3-Dimethylhexane	$15.59^{a}$	$8.86^{\#}$	20.89 <sup>b</sup>	19.01 <sup>a</sup>	14.38 <sup>b</sup>	21.47 <sup>c</sup>	38.52 <sup>a</sup>	$30.16^{\mathrm{b}}$	23.58 <sup>c</sup>	31.70	2.51*
12.23	Decane	0.51*	0.55*	3.50*	1.27*	1.21*	0.72*	0.96*	2.19*	1.65*	0.27*	0.77*
34.50	Dodecane	$0.01^{*}$	pu	0.88*	<0.01*	$0.12^{*}$	0.71*	$0.52^{*}$	0.73*	1.60*	0.98*	2.45*
42.43	Tetradecane	pu	pu	pu	pu	pu	pu	pu	pu	pu	$10.51^{#}$	42.73
	Total content of alkanes	16.11	9.41	25.27	20.29	15.71	22.90	40.00	33.08	26.83	43.46	48.46
	Aldehydes											
8.85	2-Methylbutanal	0.06*	0.27*	0.48*	0.05*	pu	pu	pu	pu	0.18*	pu	pu
19.23	Hexanal	$0.02^{*}$	pu	2.59#	pu	pu	pu	pu	pu	1.67*	0.45*	1.13*
39.01	Octanal	pu	pu	pu	pu	pu	pu	pu	pu	pu	0.97*	4.03*
42.17	Nonanal	$1.04^{*}$	1.35*	nd	0.73*	5.71*	pu	5.29*	9.83*	pu	$13.50^{\#}$	6.49*
44.49	Decanal	pu	pu	pu	pu	4.20*	pu	0.4*	3.30*	pu	pu	3.75*
	Total content of aldehydes	1.12	1.62	3.07	0.78	9.91	pu	5.69	13.13	1.85	14.92	15.40
	Ketones											
7.61	2-Butanone	$91.43^{a}$	$73.90^{b}$	$105.10^{\circ}$	49.13	7.06#	$9.62^{#}$	$6.35^{#}$	$6.20^{#}$	99.11	$57.60^{a}$	50.50 <sup>b</sup>
10.73	2-Pentanone	$21.67^{a}$	34.51 <sup>b</sup>	24.25°	15.47	$5.36^{\#}$	$5.68^{#}$	$4.61^{#}$	$4.58^{#}$	20.82	$17.79^{a}$	$17.39^{a}$
18.95	2-Hexanone	<0.01*	0.13*	0.73*	pu	pu	pu	pu	pu	0.25*	pu	0.01*
31.91	2-Heptanone	$56.03^{a}$	$93.98^{b}$	76.09°	$49.20^{a}$	$15.60^{#}$	23.47 <sup>b</sup>	$14.73^{#}$	$14.05^{#}$	90.64	$45.96^{a}$	$45.30^{a}$
38.93	2-Octanone	0.17*	0.38*	0.47*	0.23*	$0.04^{*}$	0.01*	$0.04^{*}$	0.05*	0.19*	$0.26^{*}$	0.39*
40.65	6-Methyl-5-hepten-2-one	$0.34^{*}$	pu	pu	pu	0.33*	pu	$0.26^{*}$	0.44*	pu	0.23*	0.74*



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Rt [min]	Volatile compound	Conventi UHT (CC ← Batch	onal indirect DNVI) no. →		Lactose-h UHT (LH ← Batch	ydrolyzed d. ID) no. →	irect	Lactose-h UHT (LH ← Batch	ydrolyzed ind I) no. →	irect	Filtered U (FILTI) ←Batch r	HT 0. ↓
		-	2	я	_	2	c,	_	2	з	_	2
42.04	2-Nonanone	$10.68^{a}$	17.12 <sup>b</sup>	11.68°	9.55	2.86*	3.57*	3.02*	2.93*	14.35	11.09 <sup>a</sup>	11.46 <sup>a</sup>
46.19	2-Undecanone	$0.92^{*}$	$1.94^{*}$	$1.10^{*}$	0.90*	0.11#	0.09*	0.19*	0.22*	1.33*	$1.16^{*}$	1.32*
49.71	6,10-Dimethyl-5,9- undecadien-2-one (E)	*60.0	pu	pu	pu	pu	pu	pu	pu	0.10*	pu	pu
	Total content of ketones Sulfides	181.34	221.96	219.42	124.48	31.36	42.44	29.2	28.47	226.79	134.09	127.11
17.82	Dimethyl disulfide	$8.70^{a}$	$5.48^{\#}$	13.41 <sup>b</sup>	$4.92^{#}$	$6.37^{a}$	7.99 <sup>b</sup>	6.72	$4.83^{#}$	$5.70^{#}$	$3.94^{\#}$	$4.11^{#}$
41.67	Dimethyl trisulfide	$73.06^{a}$	51.74 <sup>b</sup>	136.74°	$24.91^{#}$	73.17 <sup>a</sup>	$77.76^{a}$	$56.42^{a}$	54.91 <sup>a</sup>	33.70#	$28.94^{#}$	32.17#
46.07	Dimethyl sulfoxide	7.24*	$20.44^{#}$	$13.99^{\#}$	5.30*	pu	pu	nd	pu	1.35*	12.22*	$41.63^{\#}$
	Total content of sulfides	89.00	77.66	161.14	35.13	79.54	85.75	63.14	59.74	40.75	45.10	77.91
	Others											
14.78	Toluene	$4.91^{#}$	$4.71^{#}$	8.14	12.44	1.91*	1.74*	$2.44^{#}$	$2.55^{#}$	$5.92^{#}$	$12.30^{a}$	12.53 <sup>a</sup>
36.33	2-Pentylfuran	0.32*	0.59*	0.79*	0.44*	0.14*	<0.01*	$0.14^{*}$	$0.21^{*}$	0.17*	0.53*	0.93*
37.34	Styrene	1.69*	1.25*	$1.80^{*}$	1.40*	0.48*	0.07*	0.63*	0.59*	$0.46^{*}$	*66.0	0.43*

ā nd not detected, asterisk concentration <LOD, number sign concentration <LOQ 5 17 V (4 ź į

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**Fig. 2** a PCA score plot with principal components 1 and 2, describing 38 % and 23.6 % of the total variation in the volatile compound profiles, respectively. *Circle A*: Conventional indirect UHT milk, *square B*: lactosehydrolyzed direct UHT milk, *triangle C*: lactose-hydrolyzed indirect UHT milk, *inverted triangle D*: Filtered indirect UHT milk, *numbers 1–3* indicate batch number. **b** Corresponding loading plot.  $R^2$ =0.739 and  $Q^2$ = 0.492, with three components

indirect lactose-hydrolyzed UHT (milk types LHD and LHI). The score plot in Fig. 2a partly places lactose-reduced, filtered UHT milk (FILTI) between the CONVI milk type and the LHD and LHI milk types, which may be ascribed to differences in ketones and the aldehydes nonanal and decanal (Fig. 2b). In contrast, FILTI is separated from the other milk types due to increased concentrations of tetradecane, DMSO, and 2-pentyl furan.

The volatile compounds were quantified based on the four calibration curves (Table 2). The concentrations of the 24 volatile compounds in the milk types are summarized in Table 3. However, some of the compounds were below the LOQ, and therefore, the given concentrations are only tentatively quantified. The volatile compounds varied widely in concentration within batches of similar milk types, which was visually seen in the PCA score scatter plot in Fig. 2a. Generally, ketones represented the chemical class with the highest number of detected compounds. In total, nine ketones were identified, and among these, 2-butanone, 2-heptanone, and 2-nonanone were present in the highest concentrations. These three methyl ketones were also the compounds that differed most in concentration between CONVI, LHD, and LHI according to the TIC in Fig. 1. In total, five aldehydes were identified, and of these, nonanal was the compound found in the highest concentration in all of the milk types analyzed. Of the three sulfides identified (DMDS, DMTS, DMSO), DMTS was found



to be present in the highest concentration in all milk types. Overall, no significant differences could be identified between the milk types (CONVI, LHD, LHI, or FILTI) for any of the detected volatile compounds. However, significant differences within milk types were observed due to high batch-to-batch variation in the industrially produced samples (Table 3, Fig. 2a).

### **4** Discussion

Reports on the volatile profile of lactose-hydrolyzed milk are relatively sparse (Jelen and Tossavainen 2003; Kallioinen and Tossavainen 2008; Messia et al. 2007; Tossavainen and Kallioinen 2007). DHS and thermal desorption combined with GC-MS is a useful and efficient method for collecting and analyzing volatiles (Naudé et al. 2009). In the present study, this method combined with multivariate data analyses was applied to obtain volatile profiles of lactose-hydrolyzed, filtered, and conventional UHT-treated milk. To our knowledge, no elucidation of the volatile profile of heat-treated lactose-hydrolyzed milk obtained using the combination of DHS and GC-MS has previously been reported.

A total of 24 volatile compounds were identified and quantified (Table 3). The number of ketones and aldehydes identified is in line with the results obtained by Vazquez-Landaverde et al. (2005), who identified eight ketones and nine aldehydes when investigating volatile compounds in UHT milk using headspace solid-phase microextraction GC. The 24 quantified volatile compounds (Table 3) ranged in concentration from <0.01 to 105.10  $\mu$ g.kg milk. The concentrations of the high-concentration compounds in the present study were somewhat higher, and the concentrations of the low-concentration compounds were lower than previously reported (Valero et al. 2001; Vazquez-Landaverde et al. 2005; Toso et al. 2002; Contarini et al. 1997; Vazquez-Landaverde et al. 2006; Al-Attabi 2009). This divergence in results is most probably due to differences in analytical and quantification procedures but also in the processing of the milk (e.g., heat treatment and packaging).

It is known that aldehydes and ketones contribute to the "stale" note found in UHT milk (Rerkrai et al. 1987; Vazquez-Landaverde et al. 2005) and this becomes more evident when the "cooked" flavor begins to decline (Thomas et al. 1975). PCA indicated that the secondary lipid peroxidation products octanal and nonanal were important for the differentiation of LHI and FILTI milks from the other milks (Fig. 2). However, the levels of octanal and nonanal were below LOD (Table 3), and further studies are therefore needed to substantiate this finding. The presence of the aldehydes is an indicator of lipid oxidation in the milk, and Vazquez-Landaverde et al. (2005) reported that although the aldehydes were less affected by heat treatment in comparison with ketones, an increase in the total concentration of aldehydes increased with the severity of heat treatment.

Ketones were the most abundant volatile compounds in all of the milk types in accordance with previous findings in conventional UHT milk (Vazquez-Landaverde et al. 2005; Contarini and Povolo 2002; Contarini et al. 1997). In general, the present study indicates that conventional UHT milk contained higher concentrations of ketones than lactose-hydrolyzed or filtered milk (Fig. 2a, Table 3).

The methyl ketones 2-butanone, 2-heptanone, and 2-nonanone were among the ketones present in the highest concentrations in both conventional and lactose-



hydrolyzed milk. This is consistent with the results of Vazquez-Landaverde et al. (2005), who concluded that 2-heptanone and 2-nonanone had a major impact on the flavor of heat-treated milk. In heat-treated milk, ketones are mainly products of the heat-initiated decarboxylation of  $\beta$ -oxidized saturated fatty acids or decarboxylation of  $\beta$ -ketoacids (Vazquez-Landaverde et al. 2005; Belitz et al. 2004). In general, ketone formation requires a low activation energy (Schwartz et al. 1966), and therefore, the production of these compounds progresses throughout the storage period following initiation of heat-induced oxidation (Kochhar 1996). As a result of a marked batch-tobatch variation in the milks, no significant differences in the content of ketones between the different milk types were evident. Intriguingly, 2-methylbutanal, a characteristic Maillard reaction product, was detected in all three batches of conventional UHT milk but only sporadically seen in the lactose-hydrolyzed milk. 2-Methylbutanal is a Strecker degradation product of isoleucine and is an important indicator for this reaction having taken place in severely heat-treated milk (Belitz et al. 2004). Adamiec et al. (2001) suggested that Strecker degradation of amino acids includes radical reactions, and more radicals may have been produced in the conventional UHT milk than in the lactose-hydrolyzed UHT milk based on the higher concentration of 2-methylbutanal in the former. The fact that Maillard reaction products formed in the final stage are well-known antioxidants (Yilmaz and Toledo 2005) and that the Maillard reaction proceeds more extensively in glucose- and galactose-protein model systems (Kato et al. 1986, 1988) may explain a more limited heat-activated lipid oxidation (lower ketone concentration). Furthermore, the presence of antioxidants may have affected the formation of 2-methylbutanal in lactose-hydrolyzed UHT milk, considering that this compound may be formed through a radical process as outlined above.

Three sulfide compounds were identified in the present study. Sampling and analysis of sulfides is difficult because of their high volatility and reactivity, which also explains the large variation in the concentration of these compounds (Table 3). The concentration of sulfides increases with the severity of heat treatment, primarily due to denaturation of  $\beta$ -lactoglobulin (Hutton and Patton 1952), which exposes the sulfur-containing amino acids cysteine and methionine to oxidation or Strecker degradation (Hutton and Patton 1952; Datta et al. 2002). DMDS and DMTS are products of Strecker degradation product dimethyl sulfide (DMS) is oxidized (Al-Attabi et al. 2008). The presence of sulfides in milk is an indication of Maillard reaction, and the compounds are characteristic for the flavor formation of UHT milk (Al-Attabi 2009). Surprisingly, DMS, which previously was found to be the most abundant sulfide in milk (Vazquez-Landaverde et al. 2006), was not identified in the present study, probably due to the high volatility of the compound, the mild collection conditions, and inadequate sensitivity of the method for sulfide compounds.

Four alkanes were identified in the milk (Table 3), and higher concentrations of 3,3-dimethylhexane and tetradecane were observed in LHI and FILTI compared with UHT and LHD. The origin of alkanes in milk is far from well-understood (Coppa et al. 2011); however, the compounds may be formed from fatty acid oxidation and thermal degradation of amino acids (Lien and Nawar 1973; Lien and Nawar 1974; Macku and Takayuki 1991; Whitfield 1992). There are no obvious explanations why a difference in alkane concentration was observed between the



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different milk types in the present study. However, these compounds were considered to have a minor impact on the volatile profile of the different milk types.

Previous studies have reported that direct UHT treatment represents a less severe process than indirect UHT treatment (Badings et al. 1981) due to the shorter heating and cooling times. Perkins et al. (2005) found that the amount of ketones was approximately two times higher in 1-week-old indirect UHT-treated milk than in direct UHT-treated milk. Badings et al. (1981) found that the compounds primarily responsible for the differences between the two heat-treatment procedures were not only 2-heptanone and 2-nonanone, but also DMDS, 2-hexanone, 2-octanone, and 2-undecanone. In the present study, there were no clear differences in the volatile compound profiles between the two lactose-hydrolyzed milk types LHD and LHI, suggesting that the direct and indirect heat treatment do not have the same effect on lactose-hydrolyzed UHT milk as previously observed for conventional UHT milk. However, the volatile profile of the milk types can be expected to develop differently during storage (Perkins et al. 2005), an aspect which was not investigated in the present study.

Lactose-hydrolyzed milk is an alternative to conventional milk, and therefore, the dairy industry strives to produce lactose-hydrolyzed milk with characteristics resembling conventional milk. However, the present study reveals that considerable product differences between milk batches exist, which may impair the consumers' recognition of the product. Lactose-hydrolyzed milk produced through partial physical removal of lactose and subsequent enzymatic hydrolysis of the remaining lactose is a rather new product, and a full understanding of its quality characteristics is important to ensure consumer acceptance. Consequently, the grouping of conventional, filtered, and lactose-hydrolyzed UHT milk in Fig. 2 is highly relevant in relation to optimizing the flavor profile of lactose-hydrolyzed milk.

Moreover, significant batch variations were present in all types of milk which is deemed to be a challenge in the dairy industry. Consumers are relatively sensitive to variations in flavor (Thomas 1981). The batch variation in two out of six batches of the lactose-hydrolyzed milk was due to differences in the ketone concentrations. This may be ascribed to a number of factors, including seasonal variations in the raw milk (Auldist et al. 1998; Ostersen et al. 1997) and differences in processing. The latter seems most feasible as industrial-scale thermal processing can subject certain batches of milk to a more severe heat load. This would explain the difference in ketone concentration, as a higher ketone concentration may result from more severe heat treatment (Vazquez-Landaverde et al. 2005; Contarini and Povolo 2002; Contarini et al. 1997). Similar variations in ketone concentration between batches and brands have been noted in previous studies by Perkins et al. (2005) and Vazquez-Landaverde et al. (2005).

## **5** Conclusion

The present study focused on the analysis of short-term stored lactose-hydrolyzed and conventional UHT milk, being analyzed 2–3 weeks after production at the dairy plant. GC-MS analysis indicated that the content of ketones in general was higher in conventional UHT milk than in lactose-hydrolyzed UHT milk; however, no significant differences in the volatile compound profiles could be observed between the milk types



as a result of a batch-to-batch variation. However, it is hypothesized that the volatile profile of conventional and lactose-hydrolyzed UHT milk will change during storage and that differences in the volatile profiles of the milk will increase during extended storage. Further studies are necessary to obtain a comprehensive understanding of how the volatile profile of lactose-hydrolyzed milk will change during storage, and how these changes affect the quality of the milk.

Acknowledgments The authors gratefully acknowledge the excellent technical assistance of Birgitte Foged. The present study is part of the Ph.D. work of Therese Jansson and was financially supported by Arla Foods Amba, Food Future Innovation (FFI), Danish Dairy Research Foundation, and Aarhus University.

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