# ORIGINAL PAPER

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# Influence of defined milk products on the flavour of white coffee beverages using static headspace gas chromatography-mass spectrometry/olfactometry and sensory analysis

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Abstract The influence of milk processing and ingredients on the sensory properties of white coffee beverages has, as yet, not been thoroughly investigated, so in this study analysed milk products processed in a defined manner were added to a standardized, fresh coffee beverage; the resulting odour, taste and retronasal odour perception were measured by intensity tests, and selected volatiles were analysed by static headspace gas chromatography/mass spectrometry. After adding pasteurized consumers' milk, the effects of a different fat content (3.5 and 1.5%) and fat dispersion were studied. The milk with the lower fat content and with smaller fat globules, resulting from double homogenization (each 250/50 bar), induced a more intense coffee-related retronasal odour perception, whereas the milk-related impression was nearly the same. The addition of casein increased the creamy and milky retronasal odour perception and reduced the coffee-related taste and retronasal odour. These correlations may result in a custom-made development of milky coffee beverages controlled in their nasal and retronasal odour and taste by the defined processing of the milk component. With instrumental analysis it was observable that the effect of the addition of twice-homogenized, twice-pasteurized low-fat milk and of the addition of whole milk was similar. In both cases more volatiles were released from the beverage than with the addition of low-fat milk that was homogenized once. This is an unexpected result, because the whole milk has a higher fat

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K. Borcherding · W. Hoffmann Institute of Dairy Chemistry and Technology, Federal Research Centre for Nutrition and Food, Hermann-Weigmann-Str. 1, 24103 Kiel, Germany content than the low-fat milk and therefore a greater retention of the flavour compounds was expected. Is it possible that the flavour compounds are not dissolved in fat and that interactions happen only with fat globule membrane constituents?

**Keywords** Static headspace · Milk treatment · Fat globule membrane · Aroma release · Low-fat milk · Whole milk · White coffee

# Introduction

The popularity of coffee beverages is based on their energizing effect and the evaporated milk served for the rounding of coffee beverages; in the 1980s and 1990s coffee cream became more important [1]. In the last few years different consumer milk sorts were used for coffee specialities such as cappuccino or café au lait. Particularly younger consumers, who reject traditional coffee beverages, prefer products with relatively high amounts of milk because it softens the bitter coffee taste. To date, the influences of the milk treatment and of the milk constituents on odour, taste and retronasal odour perception of white coffee beverages are relatively unknown, although numerous investigations have been performed on pure roasted coffee [2-11]. Bücking and Steinhart as well as Griess and Büttner used milk products for their investigations, but these were limited to commercial products [12-14].

The aim of this project was the determination of the relevant influencing factors for the flavour of white coffee beverages. For this, the share of the ingredients (milk proteins and milk fat) and those parameters that are of importance for industrial production and manufacturing of coffee and milk products were varied. Changes in the fat content of a food matrix are known to affect aroma release. The influence of fat is expressed by the hydrophilic and/or hydrophobic interdependency [15]. The lipophilic flavour compounds are bound to the fat mole-

**Table 1** Overview of the re-<br/>combined-milk samples

Recombined milk	Fat content (%)	Casein content (%)	Volume-related diameter $d$ [4.3] ( $\mu$ m)
А	3.5	2.8	0.61
В	3.5	4.2	0.54
С	3.5	5.6	0.47
D	7.0	2.8	0.63

cules by reversible van der Waals forces [16]. In the presence of proteins, the flavour release is reduced because of the ability of the proteins to form covalent and reversible hydrogen bonds with the flavour substances [17]. Kinsella [18] indicated that proteins form different bonds with aldehydes and ketones owing to their different size, structure and surface.

Defined manufactured and physicochemically analyzed milk products were added to a standardized, fresh coffee beverage. The effects on the nasal and retronasal odour and taste were monitored sensorially (i.e. by intensity tests) and analytically (i.e. by external static headspace sampling, SHS, gas chromatography, GC/mass spectrometry, MS) by the changes in the intensity of defined descriptors and volatiles, respectively.

# **Materials and methods**

## Coffee

Arabica coffee beans from Kenya were dark-roasted (roasting degree 70 scale division; roasting temperature 266 °C; supplied by Tchibo, Hamburg, Germany). The beans were stored at -18 °C in screw-cap vessels under a nitrogen atmosphere. The coffee brew was prepared in a household drip coffee maker (Tchibo mat, Tchibo) with 42 g coffee powder and 800 ml tap water.

#### Milk products

#### Production of recombined milk

In preliminary investigations the effects of the individual main constituents of milk on the coffee beverage were analyzed. For this purpose, powders with high casein and/or whey protein content (in each case 82% or greater) were manufactured from fresh raw milk (of the experimental farm of the Federal Research Centre for Nutrition and Food, Kiel) by membrane filtration (microfiltration with 0.1- $\mu$ m pore size, ultrafiltration with a 10-kDa cutoff value, following diafiltrations) and subsequent freeze-drying [19]. Emulsified milk fat was obtained by repeated washing of the cream with ultrafiltration permeate and supplemented with 0.02% carrageen (Petco, Ganderkesee, Germany) for the reduction of creaming. Lactose was purchased from Biolac (Variolac of 99 edible grade, Biolac, Harbarnsen, Germany). These four isolated main milk constituents were added to a synthetic milk salt solution by Jenness and Koops [20]. The resulting 'recombined milk' (A) should correspond to an average consumer's whole milk (3.5% fat, 2.8% casein, 0.7% whey protein, 4.7% lactose). This was valid not only for the composition but also for the thermal treatment (short-time pasteurization) as well as for the distribution of the fat globules by appropriate homogenization. Figure 1 shows the procedure of the process. Thermal treatment and homogenization were performed in pilot plants (maximum flow 300 l/h, APV Invensys, Unna, Germany). Further recombined-milk samples with a deviating composition were also produced. Table 1 represents an overview of the different recombined-milk samples.

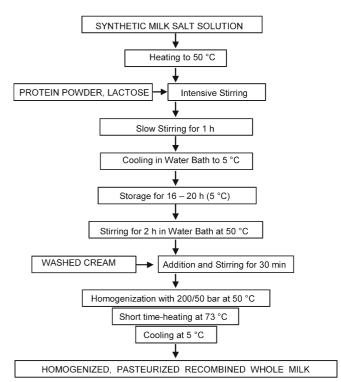


Fig. 1 Procedure for the production of recombined whole milk

#### Double homogenization of low-fat milk

Low-fat milk (1.5% fat) and whole milk (3.5% fat) were made of fresh raw milk (in pilot plants). The whole milk was homogenized once by a pressure at 200/50 bar. The low-fat milk samples were homogenized twice by 250/50 bar each at 60 °C. Both milk variants were heated for 20 s at 73 °C.

#### Chemicals

Compounds 1–5 (Table 3) were obtained from Merck (Darmstadt, Germany), compound 6 was purchased from ACROS (Gelnhausen, Germany) and compound 7 was obtained from Aldrich (Steinheim, Germany).

Measurement of the particle size distribution in milk samples

The determination of the particle size distribution was made using a laser diffraction spectrophotometer (Coulter LS 230, Beckman Coulter, Krefeld, Germany). For the calculation of the fat globule diameters, the refraction indices of Hinrichs were selected [21].

#### Sensory evaluation

A sensory panel of assessors (consisting of 15 students and coworkers of the Institute of Biochemistry and Food Chemistry,

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**Table 2** Overview of the d[4.3]and d[3.2] values and of thetotal fat globule surface ofonce- and twice-homogenizedmilk samples

Homogenized, pasteurized milk	<i>d</i> [4.3] (µm)	$d[3.2] \ (\mu m)$	Fat globule surface (m <sup>2</sup> /kg)
3.5% fat, 1×200/50 bar	0.47	0.30	~750
1.5% fat, 1×200/50 bar	0.35	0.19	$\sim 510$
1.5% fat, 2×250/50 bar	0.23	0.14	$\sim 690$
Only values $> 0.195$			
1.5% fat, 1×200/50 bar		$\sim 0.40$	$\sim 240$
1.5% fat, 2×250/50 bar		$\sim 0.32$	$\sim 300$

#### Table 3 Selected potent odorants in white coffee beverages

Comp	pound	Retention in	ndex	Aroma quality	
		OV-1701	Literature data <sup>a</sup>	-	
1	2,3-Butanedione	677	680	Buttery	
2	2-Methylbutanal	735	734	Fruity/malty	
3	Dimethyl disulfide	815	814	Cabbagelike	
4	2,5-Dimethylpyrazine	995	991	Roasted/cacaolike	
5	Phenylacetaldehyde	1,153	1,157	Sweet/honeylike	
6	2-Ethyl-3,5-dimethylpyrazine	1,191	1,192	Roasted/earthy	
7	2-Methoxyphenol	1,237	1,219	Burnt/phenolic	

<sup>a</sup> Final report (16 SV 1078/9) SPAN (2003)

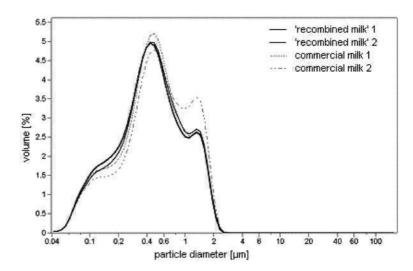
 Table 4 Sensory analysis—standard deviations (Figs. 4, 5, 7, 8)

	Descriptor	Black coffee	Recombined milk				Whole	Low-fat milk	Low-fat milk
			A	В	С	D	milk	(homogenized twice)	(4.2% casein)
Odour	Buttery	0.6	1.2	1.0	1.0	1.0	0.9	0.9	0.7
	Milky	0.4	1.5	0.8	1.0	0.7	1.3	1.4	1.6
	Creamy	0.5	1.5	1.5	1.5	1.1	1.0	0.8	1.1
	Sour	1.1	1.0	0.9	0.8	1.0	1.0	1.0	0.7
	Caramel	1.0	1.2	1.2	1.2	1.4	1.3	1.1	1.4
	Aromatic	0.9	0.9	0.8	1.1	1.3	0.6	0.8	0.6
	Roasted	0.9	1.1	0.8	0.8	1.3	1.0	1.3	1.1
	Coffee	0.9	1.3	1.0	1.1	1.0	1.0	1.0	1.0
Taste/retronasal	Coffee	1.0	0.9	1.0	1.0	0.9	1.1	1.2	0.9
odour	Roasted	1.0	0.9	0.6	1.0	1.1	1.2	1.1	1.1
	Bitter	1.1	1.1	0.9	1.0	0.7	1.2	1.1	0.8
	Burnt	1.4	1.1	0.9	1.0	0.8	1.2	1.0	0.7
	Sour	1.3	0.9	0.7	0.7	0.8	1.1	1.2	0.9
	Creamy	0.3	1.6	1.4	1.2	1.2	1.3	1.1	1.1
	Milky	0.2	1.4	0.8	0.7	1.1	1.2	1.1	1.1
	Buttery	0.5	1.3	1.1	1.3	0.9	0.9	0.8	1.1

Department of Food Chemistry, University of Hamburg) trained according to DIN 10950 performed predominantly intensity tests according to DIN 10966 and triangular tests (ISO standard 4120). The sensory panel was designed to reflect the untrained consumer; therefore, students were selected and trained by several repeated tastings of the coffee in order to sensitize the examiners to the specific characteristics of the coffee. The panel compiled characteristic descriptors for the odour and taste of the black and the white coffee beverages. These descriptors were determined in preliminary group sessions of the panel, and the intensities of these attributes were evaluated using a six-point scale from 0 (no smell/taste) up to 5 (dominant, very strong smell or taste). For the examinations, the freshly brewed coffee beverages were supplemented with the same amount (1:1, w/w) of the milk products. In each case approximately 60 g of these white coffee beverages was put into paper cups with covers (Market Grounds, Hamburg, Germany). The duration of the sensory analysis did not exceed 10 min. The tasting temperature of the white coffee beverage was 30-35 °C and the pH value was between 6.2 and 6.3. The pH value of the black coffee with the same amount of water was at 5.1. The project aimed at milky coffee beverages with a very high degree of milk (1:1, w/w) in the beverage. In the mixture, the usual drinking temperature is above 30 °C. In order to be able to compare results of experiments with a lower degree of milk, this temperature was adhered to throughout the entire study. The statistical data analysis was calculated with the statistics program SPSS. Paired *t* tests were performed to compare the means of the individual descriptors with different additives in the coffee beverage (Table 4).

## SHS analysis

For the external SHS the parameters and the modified device according to Bücking and Steinhart [14] were used. A freshly brewed coffee beverage (132.5 g) and additive (132.5 g) were filled into the lower of the two glass vessels of the device. After an equilibrium time of 15 min at room temperature, the lower vessel was replaced by an empty one. The volatiles were swept from the vessel with a nitrogen flow of 100 ml/min for 30 min and the volatiles were trapped on Tenax TA tubes (6 mm×16 cm with 100 mg Tenax TA 60/80, Supelco, Bellefonte, USA). **Fig. 2** Volume-related particle size distribution of recombinedmilk samples and commercial whole milk samples (from Nordmilch and Hansano)



#### GC/MS and GC/olfactometry

The trapped flavour compounds were desorbed from the Tenax TA tubes by use of a thermal desorber autosampler system TDS A (Gerstel, Mühlheim a. d. R., Germany) and injected into a CIS 3 cold injection system (Gerstel, Mühlheim a. d. R., Germany) with liquid nitrogen cooling (-150 °C). The volatiles studied were identified by their mass spectra, odour impression and retention time in comparison with standard substances. GC analysis was performed with a Hewlett-Packard model series II gas chromatograph (HP 5890) coupled with an HP 5971A mass selective detector (MSD) and a sniffing port for GC/olfactometry analysis.

The MSD was run in the electron impact mode at 70 eV. A BGB-1701 column (BGB-Analytik, Adliswil, Switzerland; 14% cyanopropylphenylpolysiloxane, 60-m×0.25-mm inner diameter, i.d., 0.5- $\mu$ m film thickness) was used with the following temperature programme: isotherm at 40 °C for 3 min, raised at 5 °C/min to 75 °C, raised at 3 °C/min to 200 °C, then raised at 10 °C/min to 285 °C, and held for 5 min. The capillary column was connected to a splitter (Graphpack 3D/2, Gerstel, Mühlheim a. d. R., Germany), and the effluent was split 1:1 into two uncoated, deactivated restriction capillaries (95-cm×0.1-mm i.d. and 300-cm×0.18-mm i.d., respectively) leading to the MSD (280 °C) and to the sniffing port (makeup gas nitrogen).

## **Results and discussion**

Coffee beverages with recombined milk

After the determination of the effects of the enriched milk constituents on the odour and taste of the coffee beverage had been completed, the recombined-milk samples were manufactured. The concentrates were reunited for the examination of the cumulative effect in the coffee beverage. Compared with commercial pasteurized and homogenized whole milk samples, the recombined milk (A) exhibited the typical color in use of a yellowish lactose powder (edible grade) and showed a relatively flat, nearly neutral retronasal flavour perception. The particle size distributions measured with the laser diffraction spectrophotometer also agreed well (Fig. 2). The volume-related mean diameter d[4.3] was within the range of 0.6  $\mu$ m and represents largely only the fat globule size. An increasing casein amount of 4.2 or 5.6% (B, C) resulted in bimodal

distribution curves with a second maximum (above 0.1  $\mu$ m), probably near the mean casein micelle diameter (Fig. 3). The *d*[4.3] was accordingly reduced.

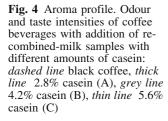
If the commercial whole milk samples and the recombined milk were added to the hot coffee beverage, the sensory intensity tests between both variants resulted in very small differences (milky odour). It is obvious that the addition of recombined milk could represent a whole milk additive. The results of the intensity test for the recombined milk with 2.8% casein (A) and for the modified recombined milks with 4.2 and 5.6% casein (B, C) are shown in Fig. 4. The intensities of the coffee descriptors clearly decreased on addition of the recombined-milk samples, while the milk descriptors were noticed accordingly. With increasing amounts of casein, the odour descriptors sour and roasted declined significantly in intensity. This is also valid for the descriptors burnt and sour, whereas the milky odour (nasal and retronasal) increased. The influence of casein for the descriptor sour for the odour and taste was already noticeable in the sensory analysis of the single milk constituents. With an increase in the amount of casein to 4.2 and 5.6%, the intensity of some descriptors changed but the differences were not significant.

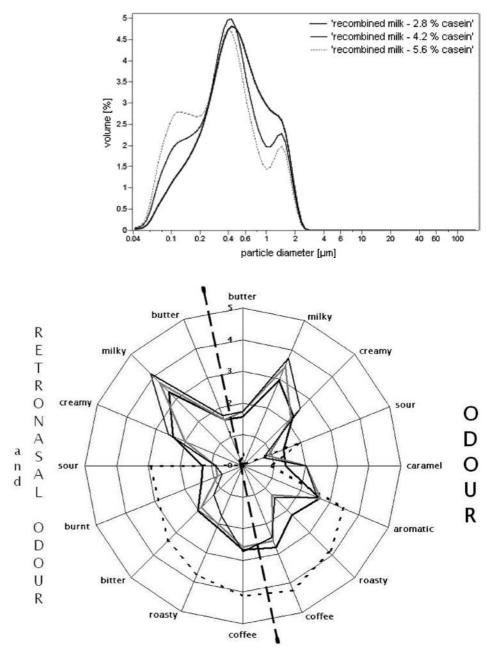
An increase in the amount of fat in the recombinedmilk samples of 3.5% (A) to 7.0% (D) led to a further decrease in the intensity of the coffee descriptors (Fig. 5). In the case of the milk descriptors no significant differences between the two recombined-milk samples were detectable.

Coffee beverages with twice-homogenized low-fat milk

The low-fat milk with 1.5% fat should always undergo a more intense homogenization, in order to get a fat globule surface and a full body comparable to those of whole milk. A short-time pasteurized whole milk (73 °C, 20 s), homogenized at 200/50 bar, served as the control. The resulting volume-related mean fat globule diameter d[4.3] was 0.47  $\mu$ m (Fig. 6). If the average value was related to

Fig. 3 Volume-related particle size distribution of recombinedmilk samples with different amounts of casein





the fat globule surface, d[3.2] values of approximately 0.30  $\mu$ m resulted. The low-fat milk was short-time pasteurized after the first and after the second homogenization. The original lipoprotein lipase was thermally inactivated by the first heating. Without this, the active lipase could have led to an 'off flavour' because free fatty acids could have been released from triglycerides [22]. The double homogenization of the low-fat milk (250/50-bar homogenizing pressure) resulted in a d[4.3] value of approximately 0.23  $\mu$ m and a d[3.2] value of approximately 0.14  $\mu$ m.

Т

S

Т

F

From the d[3.2] values (in microns) the specific volume-related surface ( $S_V$ ) can be calculated in metres squared per millilitre of fat [23]:

$$S_{\rm V} = \frac{6}{d[3.2]}.$$

The fat globule surface per kilogram of milk could be determined because the fat content of the milk samples is known. For the whole milk (d[3.2]=0.30  $\mu$ m) the fat globule surface was approximately 750 m<sup>2</sup>/kg milk (Table 2). For the twice-homogenized and twice-pasteurized low-fat milk only a slightly smaller surface of 690 m<sup>2</sup>/kg milk was calculated. However, it has to be considered that with the decrease of the fat content and the fat globule diameter casein micelles also increasingly enter into the calculation. The bimodal distribution curve shows that the demarcation of casein and fat particles could only be estimated. If a border is set at 0.195  $\mu$ m, which seems to

**Fig. 5** Aroma profile. Odour and taste intensities of coffee beverages with addition of recombined-milk samples with different amounts of fat: *dashed line* black coffee, *thick line* 3.5% fat (A), *grey line* 7.0% fat (D)

Fig. 6 Volume-related particle

size distribution of differently

treated milk samples (p-pas-

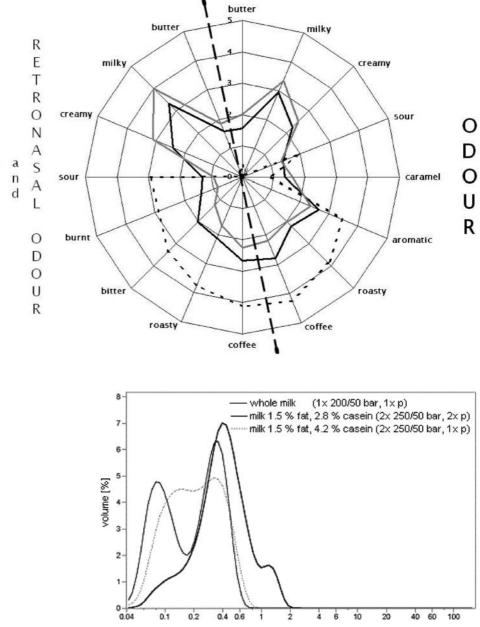
teurized)

Т

A

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E



## particle diameter [µm]

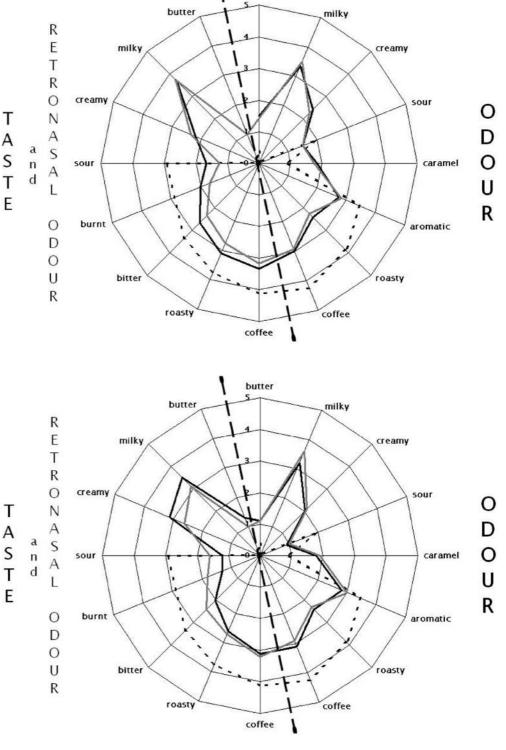
be not completely unrealistic for the process from the distribution curves, the calculated d[3.2] value increases from approximately 0.14 to 0.32  $\mu$ m. Accordingly, the fat globule surface is reduced to approximately 300 m<sup>2</sup>/kg milk. According to a rough estimation of Walstra [24], the fat globule surface is covered by approximately 10 mg protein/m<sup>2</sup> fat. This means that whole milk needs about 0.7–0.8% protein and low-fat milk only about 0.3% for the emulsifying of the fat globules. Walstra assumed that with a decreasing fat globule diameter the protein loading of the fat globules rises, because the adsorption of the casein micelles by equivalently dimensioned or only slightly larger fat globules is favoured.

With triangular tests it was examined if the effects of the addition of twice-homogenized low-fat milk samples and whole milk to the coffee beverage differed. This was confirmed. Hence an intensity test was performed in order to identify the descriptors responsible. Twice-homogenized and pasteurized milk with 1.5% fat did not differ practically from the whole milk for the entire odour and the milk-typical descriptors for the retronasal odour (Fig. 7). The coffee-typical descriptors for taste and retronasal odour were generally perceived more with the addition of the low-fat milk. With the twice-homogenized low-fat milk with a raised casein content of 4.2%, the creamy and milky retronasal odours were noticed more intensively in comparison with the whole milk, whereas Fig. 7 Aroma profile. Odour and taste intensities of coffee beverages with addition of differently treated milk samples: *dashed line* black coffee, *thick line* whole milk, *grey line* lowfat milk (homogenized twice)

Fig. 8 Aroma profile. Odour and taste intensities of coffee beverages with addition of differently treated milk samples: *dashed line* black coffee, *thick line* whole milk, *grey line* low-fat milk with 4.2% casein (homogenized twice)

the sour taste descriptor was now noticed less. In previous investigations it had already been noticed that the addition of casein reduced the sour taste. The addition of casein increased the viscosity from 2.09 to 2.50 mPa s and thus the perception of the creamy characteristic is understandable, while this cannot affect the odour to the same degree (Fig. 8).

Several characteristic and coffee specific-flavour compounds were investigated by SHS and subsequent GC/MS analysis. In Fig. 9 the results of just a few of those volatiles are presented. The selection of these characteristic compounds is based on the aroma quality, and the chemical group. Table 5 shows the standard deviation of the headspace data presented in Fig. 9. It can be observed



butter

Fig. 9 Static headspace—gas chromatography/mass selective detector peak areas (average of three measurements) of selected flavour compounds shown as a percentage related to a white coffee beverage (once homogenized low-fat milk as an additive; corresponds to 0%). WM whole milk; LFM low-fat milk

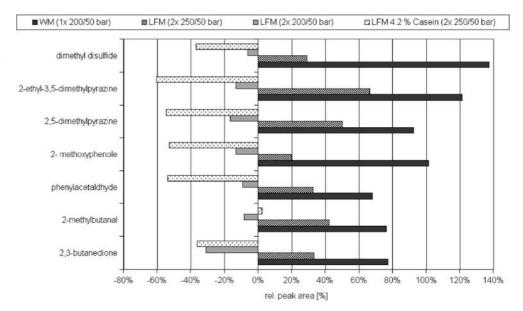


 Table 5 Static headspace sampling—standard deviations (Fig. 9)

	Low-fat milk (2×250/50 bar)	Low-fat milk (2×200/50 bar)	Low-fat milk (4.2% casein, 2×250/50 bar)	Whole milk
2,3-Butanedione	13	19	24	7
2-Methylbutanal	3	33	2	1
Dimethyl disulfide	35	26	10	19
2,5-Dimethylpyrazine	17	19	18	42
2-Ethyl-3,5-dimethylpyrazine	35	20	21	23
Phenylacetaldehyde	33	29	3	54
2-Methoxyphenol	29	27	18	70

that the effects of the addition of twice-homogenized (250/50 bar), twice pasteurized low-fat milk and the addition of whole milk were similar. In both cases, more volatiles were released from the beverage than with the addition of low-fat milk that had been homogenized only once. This is an unexpected result because the whole milk has a higher fat content than the low-fat milk and therefore a greater retention of the flavour compounds was expected. It is known that fat acts as a solvent for lipophilic aroma compounds and reduces the volatility of these compounds [15]. Some studies reported that increases in the solid fat content cause an increase in the release of volatiles [25, 26]. In our case the white coffee beverage had a temperature of approximately 30 °C, and thus only 2-8% of the milk fat is present in solid form. We assumed that no distribution of the flavour compounds in the fat phase is possible, because the fat globule membrane prevents the transfer. In this case the retardation or release characteristics of the volatiles are only based on reciprocal effects of membrane compounds, probably mainly with proteins. The treatment of the milk influences the composition of the fat globule membrane. During homogenization the total surface of the fat globules is increased and the material of the original fat globule membrane is not sufficient to cover the new fat globules. Because of the hydrophilic and hydrophobic regions of the casein micelles they can immediately cover the exposed new fat globules. If the milk homogenization takes place below 65 °C, only casein adsorbs on the fat globules. The whey proteins begin to denature above temperatures of 65 or 70 °C. Only in a denatured state can an accumulation take place to the fat globules. The new secondary membranes have locally very different thicknesses, which depend on the coincidental adsorption of the new membrane components [27]. If the thermal treatment takes place after the homogenization, an association of the denatured whey proteins with the residue of the original membrane or with the case in is possible [28]. In particular, the  $\beta$ -lactoglobulin associates with the hydrophilic components of the case in ( $\kappa$ -case in). If the thermal treatment takes place before the homogenization, then 'whey protein/casein complexes' formed in the milk serum before the formation of the secondary membrane starts. Therefore, it can be concluded that the secondary membrane is not identical in both cases. Whether the ratio of casein and whey protein at the membrane differs is controversial [29, 30]. These complex coherences could be a reason for the unexpected results of the investigations.

## Conclusions

The present results show that pasteurized consumer milk with a reduced fat content of 3.5 to 1.5% and more finely distributed fat globules affects mainly the coffee taste and retronasal odour descriptors of a white coffee beverage. The descriptors for the milky odour (nasal and retronasal) were almost invariably noticed. The creamy and milky retronasal odour perception of such low-fat milk in a coffee beverage could be strengthened by the addition of casein. This causes a decrease in the intensity of the coffee taste and retronasal odour at the same time. Thus, milk processing offers product development possibilities for controlling the sensory characteristics of white coffee beverages. Further examinations of flavour characteristics and the influence of casein contained in the secondary fat globule membrane or in the milk serum would be interesting.

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