Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 98 (2006) 201–208
Food Chemistry
www.elsevier.com/locate/foodchem

Investigation of the retronasal flavour release during the consumption of coffee with additions of milk constituents by 'Oral Breath Sampling'

M. Denker [a], M. Parat-Wilhelms [a], G. Drichelt [a], J. Paucke [a], A. Luger [a], K. Borcherding [b], W. Hoffmann [b], H. Steinhart [a,*]

[a] *Institute of Biochemistry and Food Chemistry, Department of Food Chemistry, University of Hamburg, Grindelallee 117, D-20146 Hamburg, Germany*
[b] *Institute of Dairy Chemistry and Technology, Federal Research Centre for Nutrition and Food, Hermann-Weigmann-Str. 1, D-24103 Kiel, Germany*

Received 22 October 2004; received in revised form 3 March 2005; accepted 17 March 2005

Abstract

The aim of this study was the investigation of the influence of milk proteins (casein and whey proteins) and milk fat on the release of flavour compounds from white coffee beverages in the oral cavity. For this reason a retronasal headspace technique for measurement of the after-flavour was adapted. A 'Gas Sampler' equipped with a mouthpiece was used as an 'Oral Breath Sampler (OBS)'. Analyses were performed by gas chromatography with mass spectrometric detection. It was noticed that the sampling at different hours resulted in different standard deviations. The flavour release is more constant in the morning (Variation coefficient from 3% to 28%; median: 10%) than in the afternoon (7–52%; median: 23%). The relationships between flavour release and some salivary parameters like salivation rate, buffer capability and protein content were also studied. The 'Oral Breath Sampling' was considered to be a valuable sampling method for the analysis of the retronasal aroma release from coffee beverages.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Oral Breath Sampling; Retronasal; Casein; Whey proteins; Milk fat

1. Introduction

Flavour is an important factor influencing the consumer's choice of foods. Hence, many methods for the analysis of volatile flavour compounds have been developed to give information on the total volatile composition of the food or the volatiles in the air above the food (orthonasal perception). Especially, the flavour of coffee is well-investigated (Czerny, Mayer, & Grosch, 1999; Grosch, 1996, 1998; Mayer, Czerny, & Grosch, 2000; Semmelroch & Grosch, 1996; Shibamoto, 1991).

Recently, studies were performed with the aim to investigate the flavour perception during the consumption (retronasal perception). For this reason many mouth model systems enabling the investigation of the effects of saliva, temperature and mastication on flavour release were developed (Deibler, Lavin, Linforth, Taylor, & Acree, 2001; Rabe, Krings, & Berger, 2004; Roberts & Acree, 1995; van Ruth, Roozen, & Cozijnsen, 1994). They simulate in a more or less simple way the eating and drinking process, respectively. Recently, the complex swallowing process was visualised by video fluoroscopy and real-time magnetic resonance imaging (MRI) providing information about the transfer of aroma compounds to the odour receptors in the nasopharynx (Büttner, Beer, Hanning, & Settles, 2001).

* Corresponding author. Tel.: +49 40 42838 4357; fax: +49 40 42838 4342.
E-mail address: hans.steinhart@chemie.uni-hamburg.de (H. Steinhart).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.03.034

Beside the mouth model systems, in vivo methods examining the gas area in the nose or mouth were developed. The group of Graus accomplished breath-by-breath analysis of nose-space while drinking coffee (Graus, Yeretzian, Jordan, & Lindinger, 2001). But this gas area contains a smaller concentration of odourants than the gas area of the oral cavity. It should be noted that the breath in the nose-space are more likely to represent the profile experienced during drinking. For the investigation of the retronasal flavour a method, which makes it possible to examine the flavour release in the oral cavity directly after the consumption of foods, was developed by Roozen and Legger-Huysman (1995). This Oral Breath Sampling (OBS) was considered to be a valuable sampling method for the analysis of the release of volatile compounds from coffee beverages by human volunteers. Contrary to the model systems, in vivo measurements have the advantage that the results reflect the reality better. On the other hand the results may be highly variable, because the conditions cannot be controlled as in in vitro systems.

In the presence of proteins, the flavour release is reduced because of the proteins' capability to form covalent and reversible hydrogen bonds with the flavour substances (Leland, 1997). Kinsella (1990) indicated that proteins form different bonds with aldehydes and ketones due to their different size, structure and surface. Fat acts as a solvent for lipophilic aroma compounds and reduces the volatility of these compounds. The influence of fat is expressed by the hydrophilic and/or hydrophobic interdependency (de Roos, 1997). The lipophilic flavour compounds are bound to the fat molecules by reversible van der Waals forces (Plug & Haring, 1993). Changes in the fat content of a food matrix are known to affect aroma release. Bücking, Roozen, and Steinhart (1998) reported that the amount of volatiles decreases with an increasing fat content of a milk additive to a coffee beverage.

The OBS technique was adapted. The aim of this project was the determination of the relevant influencing factors for the flavour of milky coffee beverages. For this, the components and those parameters that are of importance in the industrial production and manufacturing, respectively, of coffee and the milk products were to be varied. The effects of those variations were sensorically (intensity tests) and analytically (external static headspace (SHS) and OBS followed by GC/MSD) monitored by the changes in the intensity of defined descriptors. In this paper, only the OBS results are represented. As Milk products, different kinds of milk, 'recombined milk' and isolated components such as milk proteins (casein and whey protein) and milk fat were used.

2. Material and methods

2.1. Coffee

Arabica coffee beans from Kenya were dark roasted (roast 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 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.

2.2. Milk products

For the method development commercial milk (3.5% fat, 'milram pur', Nordmilch, Bremen, Germany) was used. The addition of this milk to the coffee amounted to 100% (1:1, w:w). After this, enriched milk proteins (casein and whey proteins) and milk fat were added to the coffee beverage (Table 1). Different milk protein or milk fat quantities were always suspended in 30 g water to avoid dilution effects. The milk protein powders and the enriched milk fat (emulsion in water with 36.5% fat stabilized with 0.02% carrageen) were supplied by the Federal Research Centre for Nutrition and Food, Location Kiel, Institute for Dairy Chemistry and Technology. The specified amounts of the proteins were always suspended in 30 g water and immediately added to 150 g coffee beverage, so the addition amounted to 20% (1:5, w:w).

Table 1
Characteristics and amounts of the used milk constituents

Milk constituent	Characteristics of the used products			Added amounts (g/30 g water)
	Content (%)	Dry matter (102 °C) (%)	Total protein (%)	
Casein	84.0	98.4	87.3	2.7[a], 4.0[a]
Whey protein	82.0	99.2	83.9	0.8[a], 1.5[a]
Milk fat[c]	36.5	–	0.3	4.0[b], 6.0[b]

[a] Enriched powders.
[b] Absolute fat content.
[c] Stabilized with 0.02% carrageen.

2.3. White coffee beverages

Fresh prepared coffee brew (∼80 °C) was mixed with the milk products (∼20 °C) and stirred by the glass stirrer.

2.4. Chemicals

Compounds 1–6 (Table 2) were obtained from Merck (Darmstadt, Germany), compound 7 was purchased from ACROS (Gelnhausen, Germany) and compound 8 was obtained from Aldrich (Steinheim, Germany). Flavour compounds 1–7 were dissolved in diethylether (∼1%) and 2-methoxyphenol (8) was dissolved in methanol (∼2%). This compounds were used as references for the GC analysis to compare the spectra and retention indices.

2.5. Oral Breath Sampling

A 'Gas Sampler' (Gerstel, Germany) equipped with a mouthpiece was used as OBS. Assessors took a quantity of 20° mL fresh brewed coffee for a defined period (10 s) into the mouth area and rinsed the oral cavity with it. After the swallowing of the beverage, the lips enclosed the mouthpiece. While the assessor was breathing through the nose, the gas area of the oral cavity was removed by the OBS.

The number of assessors was three and the number of replicates was between two and four. With a flow of 0.3 L/min and a volume of 1.0° L the duration amounted to approx. 3.3 min; i.e., with three assessors the duration of the sampling was approx. 10 min. In this period, the temperature of the coffee beverage decreased from 55 °C to 45 °C. The volatiles were collected on Tenax TA (tubes 6 mm × 16 cm with 100 mg Tenax® TA 60/80, Supelco, Bellefonte, USA) and then examined via thermal desorption, cryofocusing and GC–MS analysis.

2.6. Gas chromatography/mass spectrometry

The trapped flavour compounds were desorbed from the Tenax TA™ tubes by use of a thermal desorber auto sampler system TDS A (Gerstel, Mühlheim a. d. R., Germany) and injected into a cold injection system CIS 3 (Gerstel, Mühlheim a. d. R., Germany) with liquid nitrogen cooling (−150 °C). The desorption temperature was 300 °C. Gas chromatography (GC)/mass spectrometry (MS) analysis was performed with a Hewlett–Packard model series II gas chromatograph (HP 5890 GC series II) coupled with HP 5971A mass selective detector (MSD). The MSD was run in the electron impact mode at 70 eV. A BGB-1701 column (BGB-Analytik, Adliswil, Switzerland; 14% cyanopropyl phenylpolysiloxane, 60 m × 0.25 mm i.d., 0.5 μm film thickness) was used with the following temperature program: 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.

Paired *t* tests were performed to compare the area means of individual tracer compounds with different additives in the coffee beverage. The statistical data analysis was calculated with the statistic program SPSS.

2.7. Saliva characterisation of the assessors

Salivation rate. The released quantity of the saliva was determined twice by the same persons as in the OBS-experiments, by the complete spit-outs of saliva over a period of 5 min. The salivation was stimulated by chewing paraffin tablets (test set "CRT Dentobuffer", Ivoclar Vivadent AG, Schaan, Liechtenstein). Two hours before the collection of the saliva the assessors were not allowed any eating, drinking or smoking.

Buffer capacity. The buffer capacity was determined with indicator strips of the CRT Dentobuffer test set. A drop of the saliva was transferred with a pipette on a test stick, after 5 min the developed colour was compared with a colour scale. This colour scale differentiates between high (blue), middle (green) and low buffer capacity (yellow).

Protein content. The determination of the protein content of the saliva took place according to the photometric method of Bradford (1976).

Table 2
Selected potent odorants in white coffee beverages

Compound	Retention index		Aroma quality
	OV-1701	Literature data[a]	
2,3-Butanedione	677	680	Buttery
3-Methylbutanal	729	730	Malty
2-Methylbutanal	735	734	Fruity/malty
2,3-Pentanedione	780	788	Buttery
Dimethyldisulfide	815	814	Cabbage-like
Phenylacetaldehyde	1153	1157	Sweet/honey-like
2-Ethyl-3,5-dimethylpyrazine	1191	1192	Roasty/earthy
2-Methoxyphenol	1237	1219	Burnt/phenolic

[a] Final report (16 SV 1078/9) SPAN (2003).

3. Results and discussion

3.1. Method adaptation

The adaptation was carried out with a black coffee beverage. The Gas Sampler permitted to vary the volume, the flow and the duration of the sampling. In the context of this method development, the flow and the volume were varied. The duration of the sampling is defined by these two parameters. Apart from these instrumental settings, the drinking process was standardized. The assessors were instructed to rinse the mouth with the coffee and perform defined mouth movements to mix the coffee before swallowing in order to reach a maximum of volatiles in the oral cavity.

The flow was varied within the range of 0.2–0.4 L/min and the volume from 0.5 to 1.2 L. A volume of 1 L and a flow of 0.3 L/min were selected as optimal. During the evaluation the selected volume showed a sufficient yield for eight selected volatiles (data not shown). The flow of 0.3 L/min was not unpleasant for the assessors. The duration of the sampling is also crucial because a longer sampling period is unpleasant for the assessors and the investigation material cools down. For these reasons the number of assessors was limited to three.

3.1.1. Variation between the assessors

The following investigations were performed with the assistance of two female and one male co-workers of the institute for biochemistry and food chemistry. First of all the salivation rate, the buffer capacity and the protein content of the saliva were measured to determine physiological differences between the assessors. The results of the saliva measurements are shown in Table 3. The saliva composition is individual to a human being and may influence the release of volatiles. During the consumption of coffee a proportion of the flavour-enriched liquid remains as a thin film in the oral cavity, so that the salivation influences the flavour release and hence the aftertaste (Harrison, 1998). When volatiles are released from food into the saliva phase, interactions may occur either between the volatile compounds and solutes of small molecular weight (e.g., salts) and/or between proteins (e.g., mucin) and volatiles in the liquid phase (Friel & Taylor, 2001). The inorganic salts in the saliva can cause an increased flavour release by a 'salting out effect'. Linforth reported that some volatiles (linalool and dimethyl pyrazine) do not interact with salivary components (Linforth, Martin, Carey, Davidson, & Taylor, 2002). On the other hand the headspace concentration of other compounds such as aldehydes (hexanal, decanal and phenylacetaldehyde) decreases in the presence of mucin. Büttner showed that this decrease in the concentrations may be due to a chemical reduction of the aldehydes to the appropriate alcohols (Büttner, 2002a, 2002b).

The saliva investigations showed that differences exist between the characteristics of the saliva of the assessors. There is a possibility that these physiological differences contribute to an increased variation range in the measurements with the OBS. But differences between individuals occur always, so that with in vivo measurements relatively wide ranges must be accepted.

Table 3
Saliva characteristics of the three assessors

Assessor	Salivation rate (mL/min)	Buffer capacity	Protein content (mg/100 g)
P I	1.6 ± 0.2	Medium	102
P II	1.2 ± 0.2	High	107
P III	1.0 ± 0.2	Medium	52

3.1.2. Variation of the sampling hour

During the first investigation, it was noticed that the sampling at different hours results in different standard deviations. For verification purposes, all measurements were performed during the morning and the afternoon. These investigations took place with three assessors consuming a coffee beverage with milk (1:1 ratio) to consume. The assessments were done in 4 days with two sessions per day. Fig. 1(a) shows the areas of the volatile compounds in the morning and Fig. 1(b) those in the afternoon. The result of this experiment was that individuals showed different amounts of release from the same amount of sample. But the flavour release over all assessors is more homogenous in the morning than in the afternoon. In particular, it was observed that the variance in peak areas of highly volatile compounds varied to a larger extent than those of the less volatile compounds. This can be due to a different respiration rate. In 1988, Soeting and Heidema already assumed from breath-by-breath analyses that differences in aroma release among assessors was related to their frequency of swallowing and the associated airflow through nose and mouth (Soeting & Heidema, 1988). During the OBS-experiment saliva was accumulated and swallowed by the assessors, but at different time, and so affected the results. The observed differences in the range were confirmed by these experiments. For this reason, all following investigations with the OBS were only performed in the morning.

3.2. Influence of milk proteins and milk fat on the retronasal aroma release from coffee beverages

With the addition of whey protein, an obvious decrease in the release of all selected volatile compounds to partly 50% (2-methoxyphenol) with increasing whey protein addition was observed (Fig. 2). The retardation

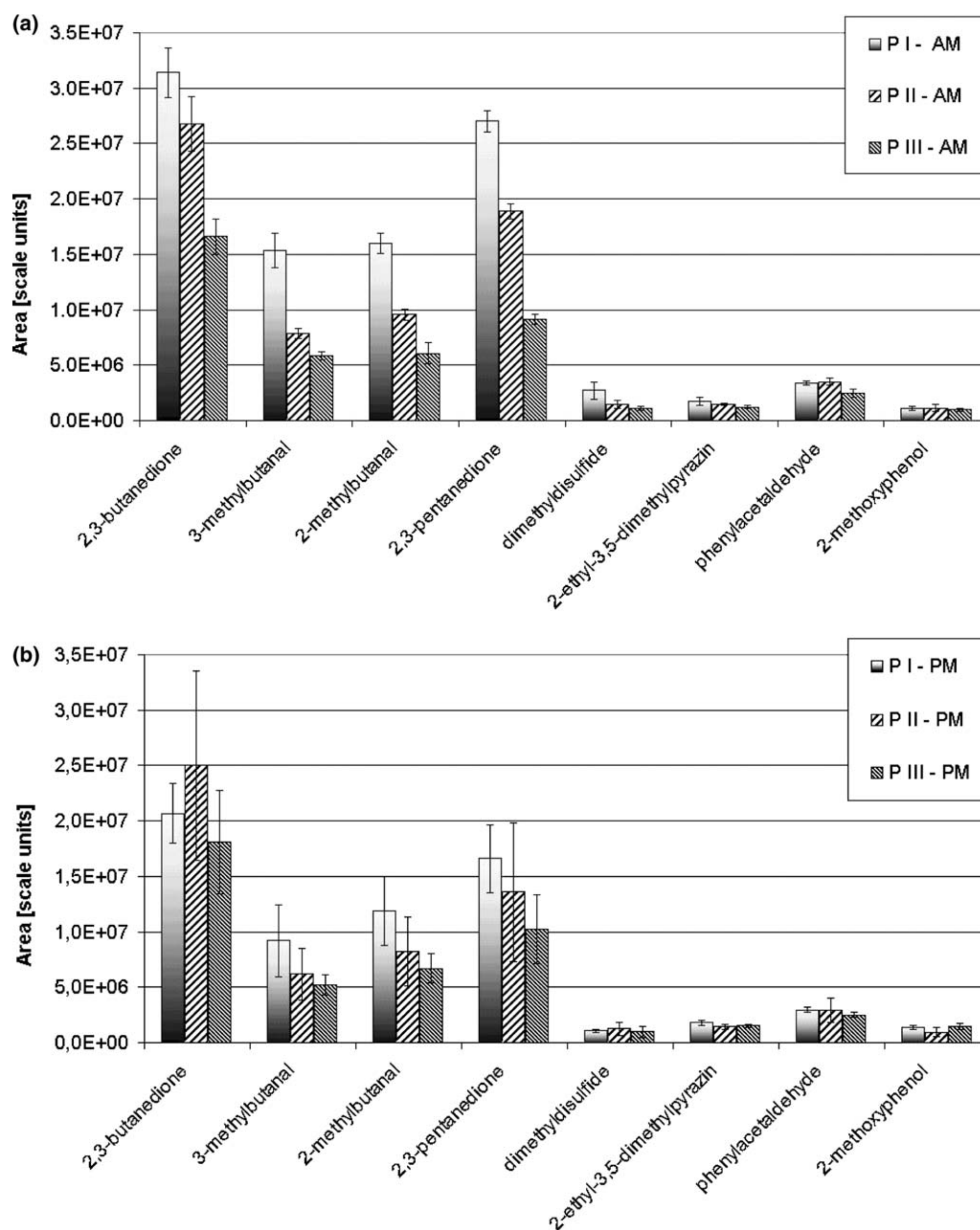

Fig. 1. (a) Oral Breath Sampling – sampling in the morning (AM): GC/MSD peak areas of selected aroma compounds (average of four replicates for each assessor). (b) Oral Breath Sampling – sampling in the afternoon (PM): GC/MSD peak areas of selected aroma compounds (average of four replicates for each assessor).

of 2,3-pentanedione, phenylacetaldehyde, 2-ethyl-3,5-dimethylpyrazine and 2-methoxyphenol (guajacol) could not be increased by adding more whey protein to the coffee beverage. Though a similar effect was observable with the addition of casein, the retardation was weaker than with the whey protein. For phenylacetaldehyde and 2-ethyl-3,5-dimethylpyrazine even a slight increase in the flavour release was recognizable before it decreased with higher amounts of the casein additive. In the case of the retronasal perception, only a small reduction of the release was observable. A possible explanation may be that the retarding properties of the added proteins compete with the releasing properties of the saliva which results in a lower effect upon the flavour release. Statistic computations (SPSS: paired *t*-test) show only significant ($\alpha = 0.05$) differences between beverages with milk protein additives and black coffee beverages (only water as additive) for the less volatile compounds (phenylacetaldehyde, 2-ethyl-3,5-dimethylpyrazine, 2-methoxyphenol). Between the different amounts of casein was a significant difference found for phenylacetaldehyde and 2-ethyl-3,5-dimethyl pyrazine.

For the OBS measurements during the consumption of the coffee beverages with additives of milk

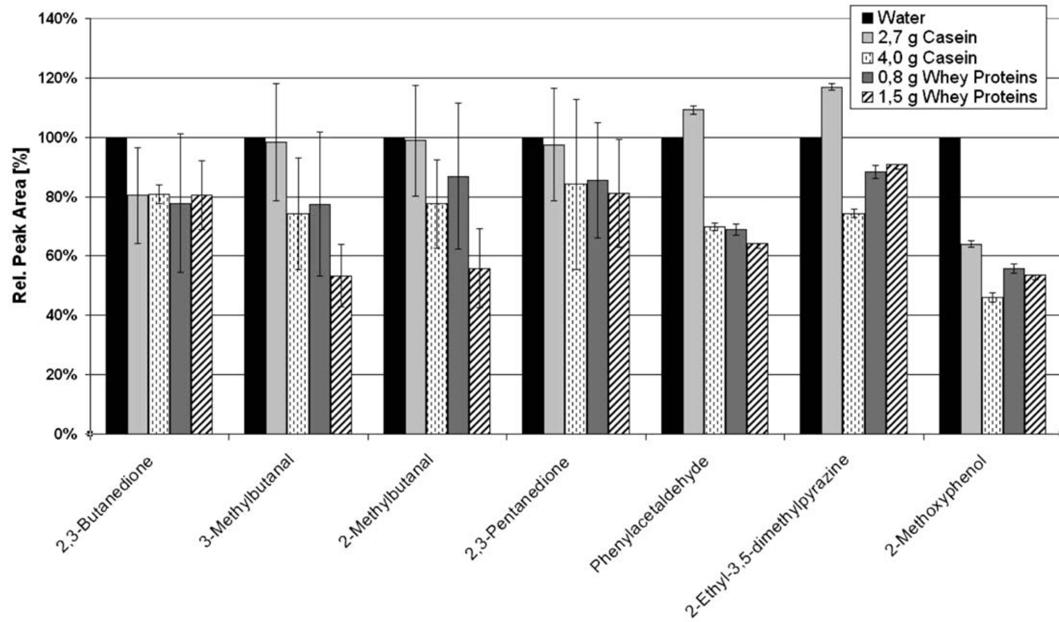

Fig. 2. Oral Breath Sampling – effect of milk proteins on flavour perception: GC/MSD peak areas (average of three assessors) of selected flavour compounds shown as percentage related to black coffee (only water as additive).

protein-solutions it has to be considered that the flavour compounds are not only affected by the milk proteins but also by the saliva composition. The protein components of saliva and the mucosa membranes must be regarded as potential binding sites for volatiles that are released in the mouth (Taylor, 1996). In the same manner interactions of the added proteins and the saliva are conceivable, so that the volatiles in this case react differently as in the headspace analysis.

An obvious decrease in the release of all flavour compounds is to be recognized with the addition of milk fat (Fig. 3). Practically no difference in the retention of 3-methylbutanal, 2,3-pentandione and phenylacetaldehyde is to be observed with increasing amounts of milk

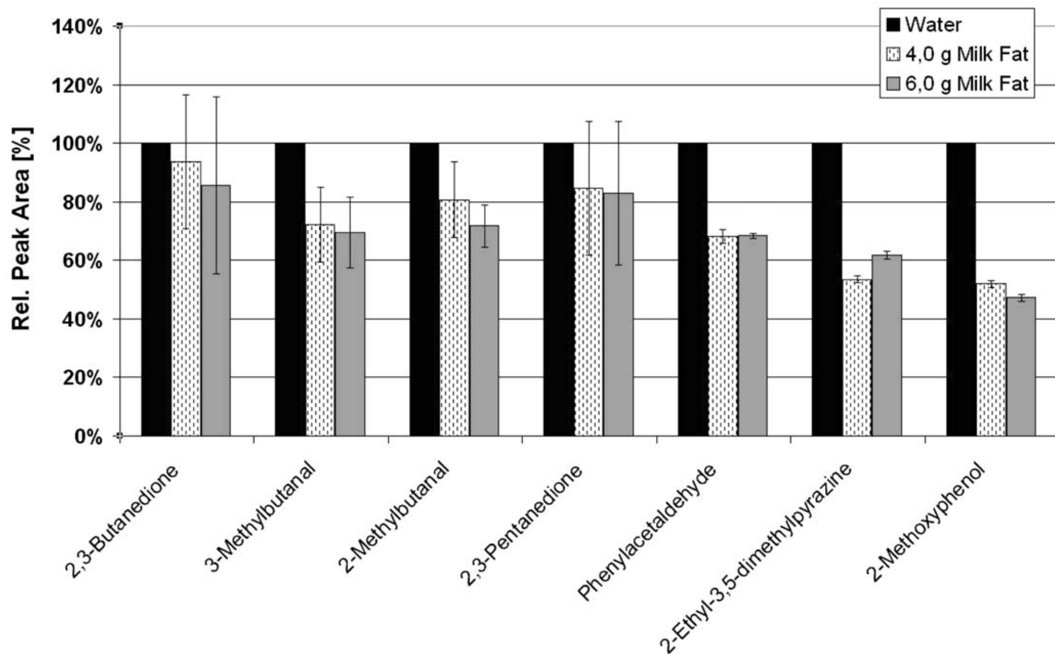

Fig. 3. Oral Breath Sampling – effect of milk fat on flavour perception: GC/MSD peak areas (average of three assessors) of selected flavour compounds shown as percentage related to black coffee (only water as additive).

fat. Only the retention of 2-methoxyphenol showed a significant difference between the different amounts of milk fat in the coffee beverage. Many of the important flavour compounds are hydrophobic and dissolve only very poorly in the water-rich coffee beverage. Thus their vapour pressure is high and the equilibrium distribution between the phases shifts in favour of the vapour phase. With the addition of milk fat or fatty milk products it is possible for the lipophilic flavour compounds to distribute not only between the water and the air phases but also into the hydrophobic fat phase. The results presented here are in line with these theories. However, the group of Jo demonstrated in a model system that various ketones and aldehydes were only very little affected in the release by the addition of high lipid concentrations (Jo & Ahn, 1999).

4. Conclusions

Our investigations confirmed that the physiological characteristics of the individual assessors lead to differences in flavour release, and a time of day dependence was determined with in vivo measurements. These differences cause large ranges in the results of the OBS measurements. For these reasons, the measurements with the OBS should be only performed in the morning, so that the deviations are minimized.

The amount of volatiles in the mouth decreased with an addition of milk proteins, with whey proteins showing a larger influence than the casein. With the selected additions of casein, a dependence on quantity was observed during the flavour release in the oral cavity. Milk fat also influenced the release of the examined flavour substances during the OBS sampling.

Oral Breath Sampling is a useful method for determination of the interaction between volatiles and other ingredients in coffee beverages.

Acknowledgements

Thanks to our OBS-panel, and to the Tchibo-Frisch-Röst-Kaffee GmbH for supplying the coffee beans.

This research project was supported by the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn), the AiF and the Ministry of Economics and Labour. AiF-Project No. 12970 N.

References

Bradford, M. M. (1976). Rapid sensitive method for the quantification of microgram quantitive of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Bücking, M., Roozen, J., & Steinhart, H. (1998). Effects of milk additives on the coffee flavour release in the oral cavity. In Guichard, E. (Ed.), *Cost action 96*, Athen.

Büttner, A. (2002a). Influence of human saliva on odorant concentrations. 2. Aldehydes, alcohols, 3-alkyl-2-methoxypyrazines, methoxyphenols, and 3-hydroxy-4,5-dimethyl-2(5H)-furanone. *Journal of Agricultural and Food Chemistry, 50*, 7105–7110.

Büttner, A. (2002b). Influence of human salivary enzymes on odorant concentration changes occurring in vivo. 1. Esters and thiols. *Journal of Agricultural and Food Chemistry, 50*, 3283–3289.

Büttner, A., Beer, A., Hanning, C., & Settles, M. (2001). Observation of the swallowing process by application of videofluoroscopy and real-time magnetic resonance imaging – consequences for retronasal aroma stimulation. *Chemical Senses, 26*, 1211–1219.

Czerny, M., Mayer, F., & Grosch, W. (1999). Sensory study on the character impact odorants of roasted arabica coffee. *Journal of Agricultural and Food Chemistry, 47*, 695–699.

de Roos, K. (1997). How lipids influence food flavor. *Food Technology, 51*, 60–62.

Deibler, K. D., Lavin, E. H., Linforth, R. S. T., Taylor, A., & Acree, T. E. (2001). Verification of a mouth simulator by in vivo measurements. *Journal of Agricultural and Food Chemistry, 49*, 1388–1393.

Friel, E. N., & Taylor, A. J. (2001). Effect of salivary components on volatile partitioning from solutions. *Journal of Agricultural and Food Chemistry, 49*, 3898–3905.

Graus, M., Yeretzian, C., Jordan, A., & Lindinger, W. (2001). Mouth coffee aroma: breath-by-breath analysis of nose-space while drinking coffee. In *ASIC, Proceedings of the 19th International scientific colloquium on coffee*, Trieste.

Grosch, W. (1996). Warum riecht Kaffee so gut? *Lebensmittelchemie, 30*, 126–134.

Grosch, W. (1998). Flavour of coffee. A review. *Nahrung, 42*, 344–350.

Harrison, M. (1998). Effect of breathing and saliva flow on flavor release form liquid foods. *Journal of Agricultural and Food Chemistry, 46*, 2727–2735.

Jo, C., & Ahn, D. U. (1999). Fat reduces volatiles production in oil emulsion system analyzed by purge-and-trap dynamic headspace/gas chromatography. *Journal of Food Science, 64*, 641–643.

Kinsella, J. E. (1990). Flavor perception and binding. *Inform, 1*, 215–226.

Leland, J. V. (1997). Flavor interactions: the greater whole. *Food Technology, 51*, 75–80.

Linforth, R., Martin, F., Carey, M., Davidson, J., & Taylor, A. J. (2002). Retronasal transport of aroma compounds. *Journal of Agricultural and Food Chemistry, 50*, 1111–1117.

Mayer, F., Czerny, M., & Grosch, W. (2000). Sensory study of the character impact aroma compounds of a coffee beverage. *European Journal of Food Research and Technology, 211*, 272–276.

Plug, H., & Haring, P. (1993). The role of ingredient–flavour interactions in the development of fat-free foods. *Trends in Food Science and Technology, 4*, 150–153.

Rabe, S., Krings, U., & Berger, R. G. (2004). In vitro study of the influence of physiological parameters on dynamic in-mouth flavour release from liquids. *Chemical Senses, 29*, 153–162.

Roberts, D. D., & Acree, T. E. (1995). Simulation of retronasal aroma using a modified headspace technique: investigating the effects of saliva, temperature, shearing, and oil on flavor release. *Journal of Agricultural and Food Chemistry, 43*, 2179–2186.

Roozen, J. P., & Legger-Huysman, A. (1995). Sensory analysis and oral vapour gas chromatography of chocolate flakes. In H. P. Kruse (Ed.), *Proceedings of the 4th Wartburg aroma symposium, aroma perception, formation and evaluation*, Wartburg.

Semmelroch, P., & Grosch, W. (1996). Studies on character impact odorants of coffee brews. *Journal of Agricultural and Food Chemistry, 44*, 537–543.

Shibamoto, T. (1991). An overview of coffee aroma and flavor chemistry. In *ASIC, Proceedings of the 14th international scientific colloquium on coffee*, San Francisco.

Soeting, W. J., & Heidema, J. (1988). A mass spectrometric method for measuring flavor concentration/time profiles in human breath. *Chemical Senses, 13*, 607.

Taylor, A. J. (1996). Volatile flavor release from foods during eating. *Critical Reviews in Food Science and Nutrition, 36*, 765–784.

van Ruth, S. M., Roozen, J. P., & Cozijnsen, J. L. (1994). Comparsion of dynamic headspace mouth model systems for flavour release from rehydrated bell pepper cuttings. *Trends in Flavour Research*. Elsevier Science, pp. 59–64.