Model Studies on the Influence of Coffee Melanoidins on Flavor Volatiles of Coffee Beverages

Thomas Hofmann,[†] Michael Czerny,[†] Sonia Calligaris,[‡] and Peter Schieberle*[,†]

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-85748 Garching, Germany, and Dipartimento di Scienze degli Alimenti, Universitá degli Studi di Udine, Via Marangoni 97, 33100 Udine, Italy

Addition of the total melanoidin fraction isolated by water extraction from medium-roasted coffee powder to a model solution containing a set of 25 aroma compounds mimicking the aroma of a coffee brew reduced, in particular, the intensity of the roasty, sulfury aroma quality. Model studies performed by static headspace analysis revealed that especially three well-known coffee odorants, that is, 2-furfurylthiol (FFT), 3-methyl-2-butene-1-thiol, and 3-mercapto-3-methylbutyl formate, were significantly reduced in the headspace above an aqueous model solution when melanoidins were added. In particular, the low molecular weight melanoidins (1500−3000 Da) led to the most significant decrease in FFT. In contrast, for example, aldehydes remained unaffected by melanoidin addition.

Keywords: *Melanoidins; flavor binding; 2-furfurylthiol; coffee flavor*

INTRODUCTION

At a given concentration, the absolute amount of a certain aroma molecule in the headspace above a food matrix mainly depends on two factors: (i) its volatility and (ii) its reactivity versus the matrix compounds. The volatility, as a physical parameter, is influenced by the overall composition of the food matrix; for example, higher amounts of fats and oils generally lower the volatility of hydrophobic odorants such as long-chain aldehydes. The reactivity of the odorant as a chemical parameter is also influenced by the matrix, for example, the presence of certain reactive groups. For instance, the reaction of carbonyls with amino groups or thiols in proteins may lead to the formation of covalent bonds. This phenomenon is named flavor binding. From a flavor standpoint, however, changing odorant concentrations selectively will consequently influence the overall aroma of the respective food.

Studies on various odorants in model solutions containing β-lactoglobulin recently revealed reversible hydrophobic interactions between the biopolymer and the volatiles under investigation (*1*, *2*). It was shown that the strength of hydrophobic interaction of methyl ketones was strongly dependent on the chain length of the odorants, with the affinity constants increasing significantly from 2-heptanone to 2-nonanone (*2*).

In addition, evidence was found also for an irreversible binding of odorants to macromolecules. Carbonyl compounds such as vanillin or benzaldehyde were reported to bind to proteins, most likely via a Schiff base formed with the ϵ-amino group of lysine side chains (*3*). Recently Mottram et al. (*4*) reported that heating of odor-active disulfides, namely, bis(2-furanylmethyl) disulfide and bis(2-methyl-3-furanyl) disulfide, in an aqueous solution containing egg albumin resulted in a >100-fold decrease in disulfide concentrations with a large proportion of the disulfides being reduced into the corresponding thiols. The authors suggested that this effect was caused by an interchange of thiol and disulfide groups with sulfhydryl and disulfide groups of the protein. This was further proven in model experiments with pure water or maltodextrin solutions, for which no loss of disulfide was observable (*4*).

Besides proteins, other polymers also present in foods might be involved in flavor binding, such as the brown macromolecules that are formed during thermal processing of foods. These so-called melanoidins are a chemically undefined class of high molecular weight compounds formed when carbohydrates and amino compounds are reacted at higher temperatures, for example, during roasting of coffee beans.

Due to the presence of a variety of functional groups, it can be assumed that melanoidins may have the potential to covalently bind odorants. The purpose of the present study was, therefore, to gain first insight into such properties of melanoidins from model experiments with selected key coffee odorants and isolated coffee melanoidins.

MATERIALS AND METHODS

Chemicals. Acetaldehyde, methylpropanal, 3-methylbutanal, 2,3-butanedione, 2,3-pentanedione, and 2-furfurylthiol (FFT) were from Aldrich (Steinheim, Germany). 3-Mercapto-3-methylbutyl formate (*5*) and 3-methyl-2-butene-1-thiol (*6*) were synthesized following procedures reported recently. The following isotopically labeled internal standards were synthesized as described in the literature: [$^2H_7$]methylpropanal (*7*); [$^2H_2$]-3-methylbutanal (*8*); [$^{13}C_4$]-2,3-butanedione (*9*), and [$^{13}C_2$]-pentanedione (*10*). [$^{13}C_2$]Acetaldehyde was purchased from Cambridge Isotope Laboratories (Andover, MA).

Isolation of Volatiles from Coffee Brew. Coffee powder (*Coffea arabica* var. Caturra; medium-roasted) was freshly prepared by grinding coffee beans in liquid nitrogen. A coffee

* Author to whom correspondence should be addressed (telephone +49-89-289-13265; fax +49-89-289-14183; e-mail Peter.Schieberle@Lrz.tum.de).

[†] Deutsche Forschungsanstalt für Lebensmittelchemie.

[‡] Universitá degli Studi di Udine.

10.1021/jf0012042 CCC: $20.00
Published on Web 04/20/2001

Table 1. Concentrations of Coffee Odorants Used in the Reconstitution of a Coffee Brew Aroma[a] (Model A)

odorant	concn[b] (μg/100 mL)
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	720
acetaldehyde	470
2,3-butanedione	210
2,3-pentanedione	160
2-methylbutanal	87
2(5)-ethyl-4-hydroxy-5(2)-methyl-3(2*H*)-furanone	80
methylpropanal	76
4-ethenyl-2-methoxyphenol	74
3-methylbutanal	57
vanillin	21
methane thiol	17
2-methoxyphenol	12
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	8
4-ethyl-2-methoxyphenol	4.8
2-ethyl-3,5-dimethylpyrazine	1.7
2-furfurylthiol	1.7
methional	1.0
3-mercapto-3-methylbutyl formate	0.57
2,3-diethyl-5-methylpyrazine	0.36
(*E*)-β-damascenone	0.16
3-isobutyl-2-methoxypyrazine	0.15
2-methyl-3-furanthiol	0.11
2-ethenyl-3,5-dimethylpyrazine	0.10
3-methyl-2-butene-1-thiol	0.06
2-ethenyl-3-ethyl-5-methylpyrazine	0.02

[a] The aroma recombinate was prepared by adding small volumes of stock solutions of each aroma compound (in ethanol; total volume of 50 μL/100 mL) to phosphate buffer (0.1 mol/L; pH 5.4) (*12*). [b] Concentrations were taken from ref *12*.

brew was prepared by percolation of coffee powder (2 min) with boiling water (50 g of powder/L of water). The brew (100 mL) was subjected to solvent-assisted flavor evaporation [SAFE; (*11*)] to carefully isolate an aqueous distillate of the coffee volatiles.

Preparation of Coffee Brew Models. *Model A*. Twenty-five odorants previously identified as key odorants in a coffee brew (*12*) were dissolved in phosphate buffer (100 mL; cf. Table 1) at the same concentrations as they occur in a fresh coffee brew.

Model B. Acetaldehyde (470 μg), methylpropanal (76 μg), 3-methylbutanal (57 μg), 2,3-butanedione (210 μg), and 2,3-pentanedione (160 μg) were dissolved in phosphate buffer (100 mL; 0.1 mol/L; pH 5.4).

Model C. 3-Methyl-2-butene-1-thiol (400 μg), 2-furfurylthiol (FFT) (600 μg), and 3-mercapto-3-methylbutyl formate (500 μg) were dissolved in phosphate buffer (20 mL; 0.1 mol/L; pH 5.4).

Isolation of Coffee Melanoidins. Coffee powder (50 g) was extracted with hot tap water (80−90 °C) until no colored material could be extracted (~1 L). The aqueous solution was extracted with dichloromethane to remove lipids and, finally, concentrated by freeze-drying (yield = 12.5 g from 50 g of powder = 25%); 1.25 g of this material (equals 5 g of coffee powder and 100 mL of water) was dissolved in distilled water (20 mL) and put onto the top of a glass column (75 × 5 cm i.d.) filled with Sephadex G-25 fine in water (Pharmacia, Uppsala, Sweden). Elution was done with distilled water at a flow rate of 4 mL/min. Four fractions of different molecular weights were collected: fraction I (258 mg; 13000−60000 Da); fraction II (221 mg; 8000−12000 D); fraction III (570 mg; 3000−6000 Da); and fraction IV (141 mg; 1500−3000 Da). The effluent was monitored at 405 nm using a Gilson photometer (Abimed, Düsseldorf, Germany). The molecular weight ranges were approximated by means of polystyrene sulfonates as the calibration standards.

Static Headspace Analysis. Either solutions of models B and C or the SAFE distillate (10 mL each) were pipetted into a septum-sealed vessel (240 mL total volume) and equilibrated for 30 min at 30 °C. Aliquots of the headspace were withdrawn with a gastight syringe (1−10 mL) and injected onto an HRGC column (60 m × 0.32 mm fused silica capillary; RTX-5: methyl polysiloxane−5% phenyl, film thickness = 3 μm) installed in a gas chromatograph type CP 9001 (Chrompack, Frankfurt, Germany). Separation was done by increasing the oven temperature from 30 to 230 °C at a rate of 6 °C/min.

Table 2. Influence of the Addition of Coffee Melanoidins on the Overall Aroma of a Coffee Recombinate

	intensity		
aroma quality	coffee brew	model A without melanoidins[a]	model A with melanoidins added[b]
sweet-caramel	1.6	2.1	1.9
earthy	1.9	1.7	1.9
sulfury-roasty	2.3	2.1	1.2
smoky	2.0	1.4	1.6

[a] Aroma recombinates (10 mL) were prepared as given in Table 1. The odor profiles were determined as recently reported ref *5*. [b] As given in footnote *a* but addition of melanoidins (125 mg).

Quantitation of Odorants. Solutions of (i) model B, (ii) model B plus melanoidins (125 mg), or (iii) of only melanoidins (125 mg) in phosphate buffer (10 mL; 0.1 mol/L, pH 5.4), respectively, were pipetted into a septum-sealed vessel (240 mL total volume) and equilibrated at 30 °C. Because the addition of the isotopically labeled standards directly to the aqueous solutions would not indicate the losses caused by, for example, melanoidin addition (due to binding of the labeled standard), the following approach was used: An aliquot (5 mL) of the headspace above the sample and, in addition, a headspace volume (5 mL) above phosphate buffer (10 mL; 0.1 mol/L; pH 5.4; 30 °C) containing only the internal standards [$^{13}C_2$]acetaldehyde (5.37 μg), [$^{2}H_7$]methylpropanal (2.60 μg), [$^{2}H_2$]methylbutanal (2.74 μg), [$^{13}C_4$]-2,3-butanedione (2.61 μg), and [$^{13}C_2$]-2,3-pentanedione (3.03 μg) were consecutively withdrawn into a syringe and then injected into the MS system. From preliminary experiments using two separate aqueous solutions of either the five unlabeled aroma compounds or the labeled internal standards, the response factors were determined.

For model C, only a comparison of the amounts of thiols present in the headspace before and after melanoidin addition was performed.

Mass Spectrometry (MS). MS was performed using a CP 9001 gas chromatograph (Chrompack, Frankfurt, Germany) equipped with the fused silica capillary CP-Wax 52 CB (25 m × 0.32 mm, 1.2 μm film thickness, Chrompack) and coupled with the mass spectrometer Incos XL (Finnigan, Bremen, Germany) running in the chemical ionization mode (MS/CI). MS spectra were recorded for the analytes and the internal standards as reported recently (*12*).

RESULTS AND DISCUSSION

In a first experiment, an aqueous aroma recombinate was prepared in analogy to ref *12* using 25 coffee aroma compounds in the same concentrations as determined in an original coffee brew (Table 1). The solution was divided into two equal portions, and one aliquot was sensorially evaluated in comparison to an original coffee brew without any further additions (Table 2). The second aliquot was spiked with melanoidins that had been isolated from coffee powder prior to the sensory experiments. The spike (125 mg/10 mL) was equivalent to the amounts extracted during preparation of a coffee brew. Coffee melanoidins were isolated prior to the experiments from freshly ground coffee powder by extraction with water.

As reported very recently (*12*), the aroma qualities sweet-caramel, earthy, and sulfury-roasty were nearly identical in their intensities in a freshly prepared coffee brew and in the aroma recombinate (model A without addition). However, addition of melanoidins reduced, in

Table 3. Odorant Concentrations (Micrograms per Liter of Air) in the Headspace above an Aqueous Model Solution of Five Carbonyl Compounds As Influenced by the Addition of Melanoidins[a]

	control		melanoidins added	
odorant	1 h	3 h	1 h	3 h
acetaldehyde	34.6	25.9	35.8	27.4
methylpropanal	13.0	17.2	13.5	15.7
3-methylbutanal	13.8	12.9	13.4	12.6
2,3-butanedione	3.8	5.4	3.8	5.7
2,3-pentanedione	3.7	5.1	3.7	5.5

[a] The solution of the volatiles in phosphate buffer (model B) was equilibrated at 30 °C.

particular, the intensity of the sulfury-roasty odor quality (cf. Table 2) after an equilibration time of 30 min at 40 °C.

To gain a first insight into the flavor binding properties of coffee melanoidins, their influence on the headspace concentrations of some highly volatile aroma compounds, that is, acetaldehyde, methylpropanal, 3-methylbutanal, 2,3-butanedione, and 2,3-pentanedione, known as contributors to coffee flavor (*5*) was investigated. The five odorants were dissolved in phosphate buffer (model B), and the melanoidins (125 mg/10 mL) were added. Quantitation was then performed by means of stable isotope dilution assays. Because the isotopically labeled standard might interact with the matrix as the flavor compound itself, addition of the standards directly to the solution would not make sense. Therefore, a "reference solution" of labeled standards was prepared in the same buffer at 30 °C and the same amount of headspace gas was withdrawn as from the sample. After 1 h of equilibration, the amounts of the five odorants present in the headspace above the model solution without melanoidins added (control) did not differ significantly from those of the model mixture with melanoidins added (Table 3). Extending the storage time to 3 h resulted in small shifts in odorant concentrations, but again no clear influence of the melanoidins on the concentrations of these odorants in the headspace was detectable. In another control sample containing only the melanoidins in phosphate buffer, only small amounts of acetaldehyde (2 μg) were measured, indicating that no contamination of the melanoidins with coffee odorants had occurred during isolation.

An aqueous distillate of a fresh coffee brew was isolated by SAFE distillation (*11*). An aliquot (10 mL) was spiked with coffee melanoidins (125 mg) corresponding to the "natural" concentrations. Both solutions were equilibrated for 30 min at 40 °C in a closed vessel, and then gas chromatograms obtained by headspace isolation were compared. Surprisingly, the chromatograms looked nearly identical (data not shown), thus indicating that also the predominant coffee volatiles were not much influenced by the presence of the melanoidins. This gave us the idea that obviously some trace compounds, which were not detected by the flame ionization detector, may be responsible for the decrease in aroma intensity (cf. Table 2).

Because the sensory experiments had revealed that, especially, the sulfury-roasty odor quality of the coffee recombinate was much reduced by adding the coffee melanoidins, the investigations were now focused on FFT, 3-mercapto-3-methylbutyl formate (MMBF), and 3-methyl-2-butene-1-thiol (MBT), known as the key sulfur-containing aroma compounds in coffee (*6*).

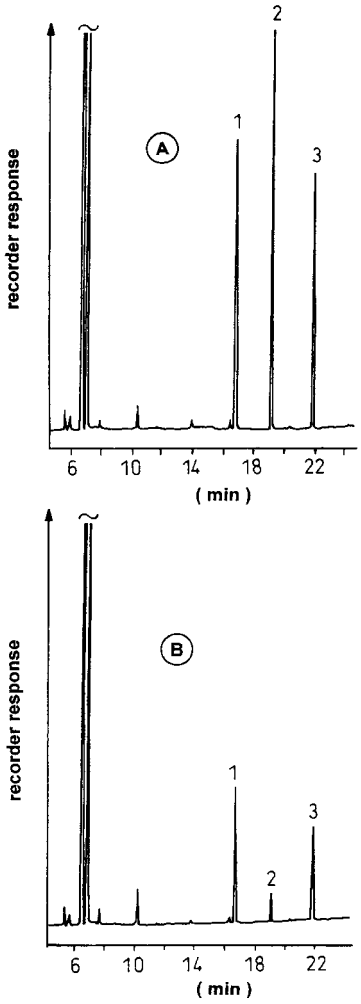

Figure 1. HRGC of static headspace samples of model C (10 mL) before (A) and after (B) addition of coffee melanoidins (125 mg); equilibration time was 30 min. Peaks: 1, 3-methyl-3-butene-1-thiol; 2, 2-furfurylthiol; 3, 3-mercapto-3-methylbutyl formate.

A mixture of the three thiols (model C; 10 mL) was prepared in phosphate buffer either without or with addition of coffee melanoidins. The decrease in concentration was determined by headspace/HRGC by comparing the control (without melanoidins added) to the sample with added melanoidins. The results revealed that the amounts of each of the three thiols were significantly reduced in the presence of melanoidins (Figure 1). Further studies showed that the decrease in concentration proceeds very rapidly (Figure 2). In particular, 50% of FFT was "lost" after 20 min and, after 30 min, the FFT was nearly absent in the headspace. Similar behaviors were observed for MBT and MMBF (Figure 2).

To get some information on the molecular weight of the "active" melanoidins, these were separated by gel chromatography (Figure 3). Four fractions (I−IV) of decreasing molecular weight were pooled and used in the experiments. The data based on model C containing the three thiols showed that FFT (2 in Figure 4) was most effectively reduced by each fraction, with fraction IV being the most effective. MBT (1 in Figure 4) was less affected than FFT, but even fraction II, the least effective one, was able to decrease the flavor compound by ~75%. The melanoidin fractions showed a similar behavior toward MMBF (3 in Figure 4).

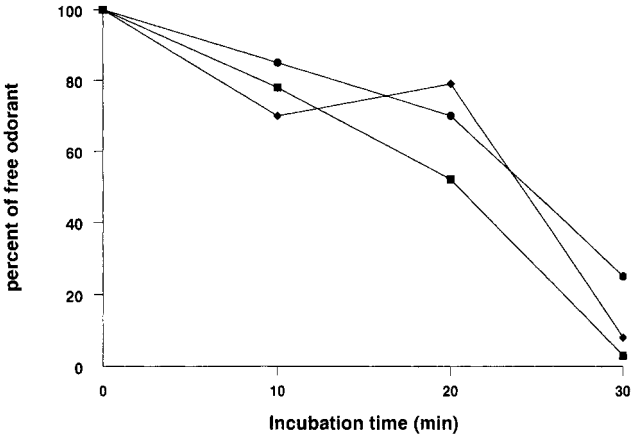

Figure 2. Influence of equilibration time on the headspace concentrations of FFT (■), 3-methyl-2-butene-1-thiol (◆), and 3-mercapto-3-methylbutyl formate (●).

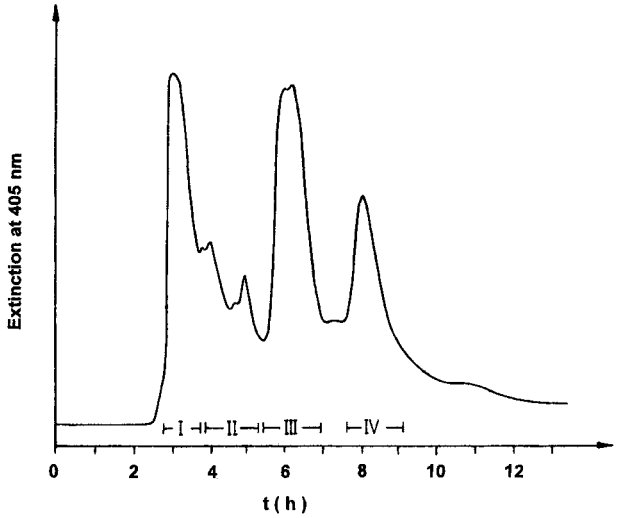

Figure 3. Fractionation of coffee melanoidins by gel chromatography (fraction I, 13000−60000 Da; fraction II, 8000−12000 Da; fraction III, 3000−6000 Da; fraction IV, 1500−3000 Da).

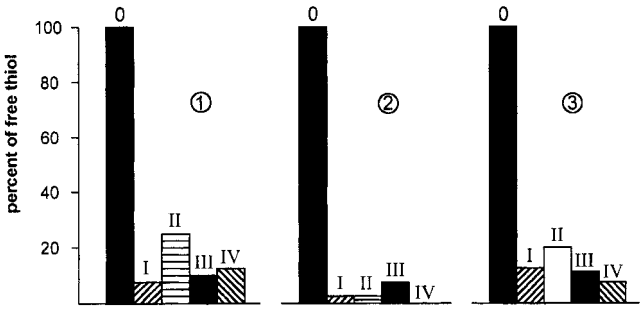

Figure 4. Amount of "free" thiols present in the headspace of model C after the addition of melanoidin fractions I−IV and equilibration (30 min, 30 °C): (1) 3-methyl-2-butene-2-thiol; (2) FFT; (3) 3-methyl-3-mercaptobutyl formate. "0" indicates control without melanoidins added.

Assuming that the thiols may be bound to thiol groups in the melanoidins, for example, to cysteine, the addition of an excess of another thiol should result in a regeneration of free thiols. However, when dithioerythritol (DTE) was added to model C containing the three thiols and the coffee melanoidins (fraction 4) after a reaction time of 30 min, only a certain portion of FFT (2 in Figure 5) was regenerated. MBT and MMBF (1 and 3 in Figure 3) were liberated to a much lesser extent.

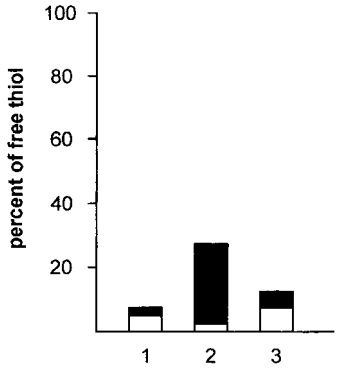

Figure 5. Influence of the addition of dithioerythritol (DTE) on the liberation of 3-methylbutene-1-thiol (1), FFT (2), and 3-mercapto-3-methylbutyl formate (MMBT; 3) bound to melanoidins. The model solution containing the three odorants and melanoidin fraction IV was stored for 30 min to induce "binding" and, after the addition of DTE, for another 90 min to liberate the odorants. Amounts released are given as black bars.

In general, the results allow the conclusion that melanoidins have a potential to bind flavor-active thiols. This process may be involved in the staling of coffee brews observed up on storage. Because treatment with a reducing agent did not much regenerate the thiols, it can be speculated that they preferably either add to double bonds present in the melanoidins, forming thioethers or, alternatively, generate thioacetals by a reaction with carbonyl groups.

In general, it can be assumed that this type of binding may also affect the flavors of other food flavors containing thiols and "brown colors" such as meat, bread crust, or roasted sesame seeds. Studies to structurally define the binding sites are underway.

LITERATURE CITED

(1) Sostmann, K.; Guichard, E. Immobilized β-lactoglobuline on a HPLC-column: a rapid way to determine protein-flavour interactions. *Food Chem.* **1998**, *62*, 509−513.

(2) Androit, I.; Harrison, M.; Fournier, N.; Guichard, E. Interactions between methyl ketones and β-lactoglobulin: sensory analysis, headspace analysis, and mathematical modeling. *J. Agric. Food Chem.* **2000**, *48*, 4246−4251.

(3) Hansen, A. P.; Heinis, J. J. Decrease of vanillin flavor perception in the presence of casein and whey proteins. *J. Dairy Sci.* **1991**, *74*, 2936−2940.

(4) Mottram, D. S.; Szauman-Szumski, C.; Dodson, A. Interactions of thiol and disulfide flavor compounds with food components. *J. Agric. Food Chem.* **1996**, *44*, 2349−2351.

(5) Czerny, M.; Mayer, F.; Grosch, W. Sensory study on the character impact odorants of roasted arabica coffee. *J. Agric. Food Chem.* **1999**, *47*, 695−699.

(6) Semmelroch, P.; Grosch, W. Studies on character impact odorants of coffee brews. *J. Agric. Food Chem.* **1996**, *44*, 537−543.

(7) Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem.* **1996**, *44*, 2366−2371.

(8) Schieberle, P.; Grosch, W. Changes in the concentrations of potent crust odorants during storage of white bread. *Flavour Fragrance J.* **1992**, *7*, 213−218.

(9) Schieberle, P.; Hofmann, T. Evalution of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *J. Agric. Food Chem.* **1997**, *45*, 227−232.

(10) Mayer, F.; Czerny, M.; Grosch, W. Influence of provenance and roast degree on the composition of potent odorants in Arabica coffees. *Eur. Food Res. Technol.* **1999**, *209*, 242−250.

(11) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavor evaporation. A new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237−241.

(12) Mayer, F.; Czerny, M.; Grosch, W. Sensory study on the character impact aroma compound of coffee beverage. *Eur. Food Res. Technol.* **2000**, in press.

Received for review October 4, 2000. Revised manuscript received February 20, 2001. Accepted February 26, 2001.

JF0012042