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Interactions between coffee melanoidins and flavour compounds: impact of freeze-drying (method and time) and roasting degree of coffee on melanoidins retention capacity

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Abstract

The objective of this work was to study the putative interactions between flavour compounds and coffee melanoidins. After extraction, melanoidins were freeze-dried and several flavour compounds from different chemical classes were tested in aqueous solution. The retention of flavour compounds by melanoidins was found to be different in function of the method or time of freeze-drying. Thus, for the same freeze-drying method, the retention capacity of melanoidins increased when the aliphatic chain length of a homologous series of flavour compounds increased. This observation seems to favour the hydrophobic nature of the interactions between melanoidins and flavour molecules. Moreover, for the same aroma compound, the retention capacity of coffee melanoidins was found to vary in function of the freeze-drying method used. Freeze-drying could therefore be involved in the modification of the surface properties of melanoidins or in their denaturation, modifying their retention ability towards volatile flavour compounds. At last, retention by coffee melanoidins decreased with the roasting degree of coffee.

Keywords: Aroma; Melanoidins; Interaction; Headspace; Freeze-drying; Roasting

1. Introduction

The perception of flavour compounds in a food matrix depends on the composition of this matrix. It has been shown that the macromolecules, such as proteins, are involved in the retention of flavour compounds (Franzen & Kinsella, 1974; Guichard, 2002). Many studies realised on milk proteins have shown that hydrophobic interactions exist with the most hydrophobic flavour compounds and that these interactions have some influence on flavour perception (Guichard & Langourieux, 2000). Similar mechanisms were observed with other proteins such as caseins or soy proteins (Lubbers, Landy, & Voilley, 1998). Phenolic compounds are also known to interact with flavour compounds (King & Solms, 1982). Retention by carbohydrates increases with the molecular weight of flavour compounds, within a same chemical class and decreases with their polarity (Goubet, Le Quéré, & Voilley, 1998). As carbohydrates are mostly used as thickener agents, their effect on flavour retention and release is a combination of binding and increased viscosity (Roberts, Elmore, Langley, & Bakker, 1996; Yven, Guichard, Giboreau, & Roberts, 1998).

In roasted coffee, a large proportion of macromolecules is composed of unidentified materials, including melanoidins. Melanoidins are macromolecular and brown: they result of the reactions between reducing sugars and compounds possessing a free amino group, such as free amino acids and the amino groups of peptides. So melanoidins are considered as the final products of Maillard reaction (Ames, Caemmerer, Velisek, Cejpek, Obretenov, & Cioroi, 1999). With the assumption that coffee melanoidins might interact with flavour compounds, thus modifying aroma perception in coffee, Mestdagh and Collin (1999), in their review, reported that interactions between flavour compounds and melanoidins have not yet been studied. In fact, only the effect of ions on melanoidins has been reported in the literature: interactions

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of melanoidins with metallic ions (Gomyo & Horikoshi, 1976), flocculation of melanoidins induced by inorganic ions (Migo, Del Rosario, & Matsumura, 1997), and the effect of pH and calcium ions on the destabilisation of melanoidins (Migo, Matsumura, Del Rosario, & Kataoka, 1993).

More recently, Hofmann, Czerny, Calligaris, and Schieberle (2001; Hofmann & Schieberle, 2002) studied the interactions between coffee melanoidins and several aroma compounds, such as odour active thiols. They showed that these thiols were covalently bound to melanoidins. However no study deals with interactions between coffee melanoidins and other flavour compounds such as ketones or esters. The aim of the present work is to study the interaction between melanoidins extracted from coffee and flavour compounds from different chemical classes that may represent the variability which is encountered in coffee volatiles; moreover, these compounds are currently used in flavour mixtures. This study is also a part of an EU COST Action 919, Melanoidins in food and health (Ames, 2002).

2. Materials and methods

2.1. Materials

Two types of coffee were tested: the first one was a commercial coffee (100% Arabica), and the other one, a mix of 50% Arabica (Brazil), 30% Arabica (Colombia) and 20% Robusta (the Ivory Coast), was kindly supplied by Nestec (Orbe, Switzerland), as sample for the COST Action 919, Melanoidins in food and health. The Nestec coffee was provided in three different roasting degrees (roasting colour, roasting loss): light (CTn 109, 14.1%), medium (CTn 86, 15.8%), dark (CTn 59, 18.8%). Flavour compounds were purchased from Sigma Aldrich (Saint Quentin Fallavier, France). Their purity was evaluated by GC–MS analysis (>90%).

2.2. Isolation of coffee melanoidins

According to Hofmann et al. (2001), melanoidins were isolated from coffee beverages. Coffee beans were grinded and coffee powder (50 g) was extracted with hot pure water (80–90 °C) until no coloured material could be extracted (~1.2 l). The aqueous phase was extracted twice with dichloromethane (200 ml) to eliminate lipids and then freeze-dried. The yield of obtained melanoidins was 18% relative to coffee powder.

Several extractions of melanoidins were realised. With the freeze-drier (FTS Systems Inc., New York), two methods can be used. Using the first method (method 1), the samples were frozen in glass-bottles using a thin layer of sample, and then the bottles were connected to the vacuum manifold of the freeze-drier. The vacuum was established in each bottle, and the freeze-drying was realised at room temperature. Using the second method (method 2), the sample deposited in small aluminium containers was frozen for one night at −80 °C, and then the containers were placed on a tray in the freeze-drier chamber, the vacuum was established, and the freeze-drying was realised at a controlled temperature (−20 °C during 1 h, 0 °C during 1 h and the rest of the time at 20 °C).

2.3. Headspace analysis

Aqueous NaCl solution (50 mM, solution 1) was adjusted with HCl (1 N) to pH 5.4 corresponding to the pH of coffee. The freeze-dried melanoidins were dispersed (solution 2) in this NaCl (50 mM, pH 5.4) solution at a concentration of 6 g l^{-1}, and if necessary the pH was adjusted with HCl (1 N). Solutions of flavour compounds (100 mg l^{-1}, solution 3) were prepared daily in the same NaCl solution. The samples, placed in amber flasks (40 ml) closed with mininert valves (Supelco, France), were composed of 2.5 ml of the aroma solution (solution 3) and 2.5 ml of either the NaCl solution (solution 1) or the melanoidins solution (solution 2). The final aroma compound concentration was therefore 50 mg l^{-1} and that of melanoidins was 3 g l^{-1}. Solutions to be analysed, with or without melanoidins, were stirred and equilibrated at 30 °C for 90 min. A vapour phase aliquot (1 ml) was taken with a gastight syringe (1 ml, SGE) and injected on a Carlo Erba 8000 gas chromatograph equipped with a DB-Wax column (J&W Science, i.d. 0.32 mm, 30 m, film thickness 0.5 μm). For the parts 4 and 5 of this publication, headspace analyses were automated using an MPS2 multipurpose sampler (Gerstel AnaPlications, Brielle, The Netherlands). The GC analyses were realised at an oven temperature of 110 °C, for most of the compounds, except for diacetyl (40 °C), and for linalool, ethyl nonanoate and benzaldehyde (130 °C). Temperatures of the injector and detector were respectively 250 and 260 °C. The H_2 carrier gas velocity was 37 cm s^{-1} at 143 °C.

The FID signal was sampled every 50 ms using a PC-driver four-channel plug-in acquisition board developed in the laboratory (Almanza & Mielle, 1990). After analysis, the data were processed using a software developed in the laboratory (Almanza, Couton, Mielle, & Nicolardot, 1989). The experiments were done in triplicate. The area means, corresponding to samples, with or without melanoidins, were calculated. The percentage of aroma compounds retention by coffee melanoidins were also calculated with the following equation:

$$\text{retention } (\%) = \frac{\text{area without melanoidins} - \text{area with melanoidins}}{\text{area without melanoidins}}$$

The percentages of retention, corresponding to different samples, were compared by the Newman–Keuls test.

3. Results and discussion

3.1. Retention of aroma compounds by melanoidins

Table 1 shows that: (1) The melanoidins potential retention level was rather low (generally lower than 10%) for all the aroma compounds tested. For comparison, the retention of flavour compounds by β-lactoglobulin is much higher: thus the percentage of retention for 2-heptanone by β-lactoglobulin (20%) (Andriot, Harrison, Fournier & Guichard, 2000) is significantly higher than the one observed here (3%). (2) The percentage of retention for some aroma compounds in the presence of melanoidins from a commercial coffee varies according to chemical classes. For the esters or ketones, the percentage of retention increased with the aliphatic chain length. These observations led to conclude that for these compounds hydrophobic interactions occur. The same phenomenon was observed with β-lactoglobulin, a milk lactoserum protein (Andriot et al., 2000; Pelletier, Sostmann, & Guichard, 1998). These results are opposite to those obtained by Hofmann et al. (2001; Hofmann & Schieberle, 2002), who showed that aldehydes and di-ketones did not interact with coffee melanoidins, whereas odour-active thiols were covalently bound to coffee melanoidins. Moreover, a recent study, while confirming thiols are highly interactive compounds, showed that 1% of coffee brew melanoidins induced a significant retention for di-ketones and pyrrole-derivatives (Charles-Bernard, Kraehenbuehl, & Roberts, 2003). In the present study, the results obtained with another coffee sample (Nestec, medium roasting) were opposite (Table 2). In fact, linalool was retained by melanoidins, but a salting-out effect was observed for esters and ketones, i.e. a flavour release effect was noticed. These behaviour differences could probably be explained by the nature and roasting of coffee, or by the freeze-drying time: the commercial coffee melanoidins were freeze-dried during 12 h whereas the other coffee melanoidins were freeze-dried during 24 h. Interestingly, Obretenov, Demyttenaere, Abbaspour Tehrani, Adams, Keršiene, and De Kimpe (2002) showed that the retention of isoamyl acetate by model melanoidins varied according to the way of melanoidins synthesis. Moreover, Demyttenaere, Escudero Alonso, Abbaspour Tehrani, Keršiene, Roberts, and De Kimpe (2003) showed that the release of three flavour compounds decreased with increasing model melanoidins concentration. For example, a salting-out effect was observed for linalool at low concentration of melanoidins (100 ppm) and no significant effect was observed at higher concentration of melanoidins (1000 ppm). Further experiments were realised in order to precise the effect of freeze-drying time and method and the influence of roasting degree.

3.2. Effect of freeze-drying time on melanoidins retention capacity

The effect of the freeze-drying time on retention of aroma by melanoidins was studied for two compounds of different chemical classes (2-nonanone and ethyl octanoate). With the same sample of coffee extract (Nestec, medium roasting), three different freeze-drying times (14, 19 and 24 h) were tested. Fig. 1 shows that the percentage of retention decreases significantly with the increase of freeze-drying time. For a freeze-drying time of 24 h, a salting-out effect was observed for both compounds. Therefore, freeze-drying may modify the structure of melanoidins, and so their capacities to retain flavour compounds, as was already suggested for another macromolecule, β-lactoglobulin, by Jouenne (1997). It is noteworthy that using melanoidins extracted from this coffee sample (Nestec, medium roasting), 27% of 2-nonanone is retained after 14 h of freeze-drying using method 1, whereas only 14% of 2-nonanone was retained with the commercial coffee after 12 h of freeze-drying (Table 1). These observed differences are supposedly related to the origin of the coffee or its roasting degree (see below).

Table 1
Results of flavour compounds retention by melanoidins extracted from commercial coffee [freeze-drying time: 12 h in glass-bottles (method 1)] [Number of replicates (N) = 3]

Flavour compounds	Retention of flavour compounds (%)
Benzaldehyde	9 ± 1
1-Octen-3-ol	−1 ± 2
Diacetyl	6 ± 2
2-Heptanone	3 ± 2
2-Octanone	6 ± 3
2-Nonanone	14 ± 2
Isoamyl acetate	7 ± 14
Ethyl hexanoate	19 ± 2

Table 2
Results of flavour compounds retention by melanoidins extracted from Nestec coffee [medium roasting; freeze-drying time: 24 h in glass-bottles (method 1)] (N = 3)

Flavour compounds	Retention of flavour compounds (%)
Linalool	8 ± 1
2-Octanone	−16 ± 3
2-Nonanone	−34 ± 11
Ethyl hexanoate	−45 ± 10
Ethyl octanoate	−20 ± 5
Ethyl nonanoate	−14 ± 6

3.3. Effect of freeze-drying method

Two methods of freeze-drying were tested to confirm that freeze-drying plays a fundamental role in the retention behaviour of coffee melanoidins. The retention of melanoidins extracted from Nestec coffee (medium roasting) obtained by these methods [melanoidins samples in glass-bottles (method 1) or in containers on a tray (method 2)] were tested on the same two compounds (2-nonanone and ethyl octanoate). Fig. 2 shows that the retention capacity of melanoidins depends on the freeze-drying method used. Thus, after 19 h of freeze-drying, melanoidins contained in small containers deposited on the freeze-drier tray, displayed a retention capacity towards flavour compounds (up to 47% for ethyl octanoate). The capacity of retention depends not only on the method, but also on the freeze-drying time. In fact, for both compounds, the retention by melanoidins decreased with the increase of freeze-drying time, and this retention is always lower with the method of freeze-drying in glass-bottles (Figs. 1 and 2).

Moreover, the melanoidin's visual aspects were different with the two methods of freeze-drying. The melanoidins obtained with the glass-bottle method (method 1) seemed caramelised (glassy surface), whereas, the melanoidins obtained with the other method (method 2) had always the same powder aspect. With an infrared analytical balance, the quantity of water in the samples was measured. The humidity percentage was 9% in the melanoidins obtained with the tray method (method 2), whereas for those obtained with the glass-bottles method (method 1), the moisture was evaluated at 5%. The freeze-drying in glass bottles seems to be more efficient than the freeze-drying in tray. With the tray method even after 36 h of freeze-drying, no salting out effect was observed, suggesting that this method led to melanoidins whose surface and/or denaturation states did not prevent their capacity of retention towards aroma compounds. As freeze-drying method and time were found to be very important, we decided to perform complementary experiments for melanoidins retention behaviour, using the same freeze-drying method and different samples of melanoidins.

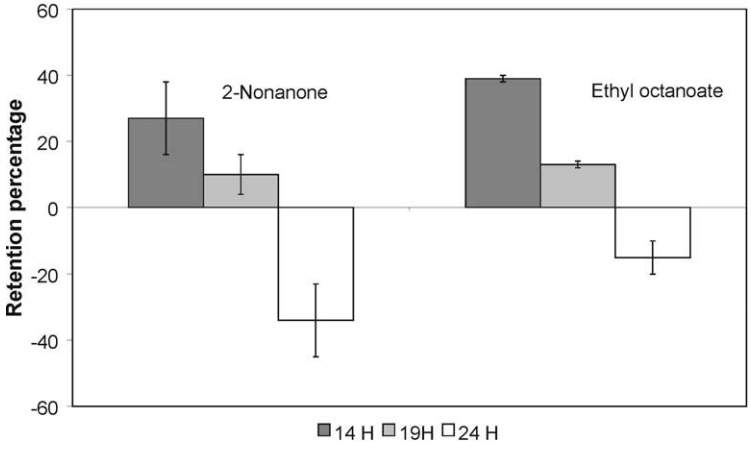

Fig. 1. Effect of freeze-drying time on melanoidins (extracted from Nestec, medium roasting) retention coffee [freeze-drying realised in glass-bottles (method 1)].

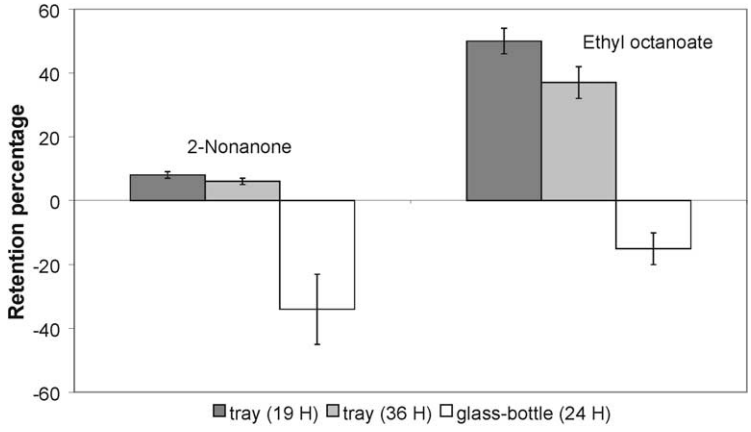

Fig. 2. Effect of freeze-drying method [melanoidins samples in glass-bottles (method 1) or in containers on a tray (method 2); melanoidins extracted from Nestec, medium roasting].

Table 3
Results of flavour compounds retention by melanoidins extracted from commercial and Nestec (medium roasting) coffees [freeze-drying in the same conditions and simultaneously, 24 h in containers on a tray (method 2)] ($N = 3$)

Flavour compounds	Retention of flavour compounds		Level of significativity (P value)
	Commercial	Nestec (medium roasting)	
Benzaldehyde	12±2	11±1	0.607
Oct-1-en-3-ol	7±2	6±1	0.566
Linalool	3±3	1±3	0.393
Diacetyl	5±2	9±1	0.055
2-Heptanone	4±2	4±2	0.615
2-Octanone	14±0	13±1	0.372
2-Nonanone	21±2	25±1	0.006
Isoamyl acetate	7±3	5±3	0.139
Ethyl hexanoate	6±1	7±1	0.131
Ethyl octanoate	37±2	46±2	0.001
Ethyl nonanoate	68±1	75±1	0.002

3.4. Effect of coffee melanoidins samples on retention of flavour compounds

The freeze-drying for two coffee samples (commercial and Nestec, medium roasting) was realised in the same conditions, i.e. 24 h in containers on a tray (method 2), and simultaneously. Three freeze-drying steps were necessary to obtain a sufficient quantity of melanoidins for the experiments; so the different batches were pooled and mixed to eliminate the impact of freeze-drying. Interactions between melanoidins and all flavour compounds are reported in Table 3. Some retention between flavour compounds and melanoidins occurred, whatever the coffee (commercial or Nestec). For benzaldehyde, oct-1-en-3-ol, linalool and diacetyl, interactions were weak: the percentage of retention of flavour compounds was less than 20%. On the other hand, for esters or ketones, the retention by melanoidins increased with the aliphatic chain (up to 75% for ethyl nonanoate with Nestec sample). Except for 2-nonanone, ethyl octanoate and ethyl nonanoate which are the most hydrophobic compounds of the series under study, the retention capacities were not significantly different ($P > 0.05$) for the melanoidins extracted from the two different coffee samples. Moreover, a measurement of the colour of the two coffee (powder and brew) samples did not show any difference between the two coffees: we can suppose that the roasting process was similar. The same applied for the humidity percentage: the amount of water in the two samples of melanoidins was evaluated at 3.8%.

3.5. Effect of roasting degree on retention of flavour compounds by melanoidins

We also studied the roasting degree of Nestec coffee (Table 4). For four compounds, the retention by coffee melanoidins decreased when the degree of roasting increased. Ottinger and Hofmann (2000) have studied the influence of roasting on the melanoidin spectrum by gel permeation chromatography. They showed that the melanoidin spectrum was significantly influenced by the roasting degree: the amounts of high-molecular weight melanoidins increased drastically with increasing roasting loss of the coffee bean, i.e. final roasting temperature. In the dark roasted coffee, the high-molecular weight melanoidins were the predominant type of melanoidins. Moreover, Chockchaisawasdee and Ames (2001) showed that the ability of the low molecular weight components to bind saccharin was greater than that of the high molecular weight fraction. Thus we could suppose that high-molecular weight melanoidins may retain the flavour compounds to a lower extend. On the other hand,

Table 4
Effect of roasting degree (light, medium, dark) on flavour retention compounds by melanoidins extracted from Nestec coffees [freeze-drying in the same conditions and simultaneously, 24 h in containers on a tray (method 2)] ($N = 3$)

Flavour compounds	Retention of flavour compounds			Level of significativity (P value)
	Light roasting	Medium roasting	Dark roasting	
2-Nonanone	13±2	13±3	12±2	0.651
Ethyl octanoate	27±2 (a)	26±2 (a)	22±1 (b)	0.027
Ethyl nonanoate	45±6 (a)	55±2 (a)	26±8 (b)	0.003
Benzaldehyde	10±2	8±2	8±1	0.333

Samples with the same letters (a,b) are not significantly different at the 5% level.

the differences observed could not be due to a difference of water activity: the percentage of humidity was measured, and was respectively 3.3% for light roasting, 3.9% for medium roasting and 4% for dark roasting.

4. Conclusion

Melanoidins are the preponderant part of coffee brew non volatiles that may interact with volatile flavour compounds (Charles-Bernard et al., 2003). The interactions between coffee melanoidins and selected flavour compounds have been found to depend on the nature of coffee, the chemical class of flavour compounds and also on the method and time of freeze-drying used to obtain the melanoidins. For methyl ketones and esters, when the melanoidins state allows interactions to occur, these interactions seem to involve hydrophobic mechanisms. When the freeze-drying time increased, the retention capacity of coffee melanoidins towards flavour compounds decreased, probably due to a denaturation of the melanoidins or to differences in water activity. Moreover, increasing the roasting degree of coffee affected negatively the retention capacity of coffee melanoidins.

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