Flavour retention and haze formation by chocolate polyphenols

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ABSTRACT

Interactions of flavours and proteins with chocolate polyphenols were investigated. Flavour retention was measured by dynamic headspace gas chromatography. Relative recovery factors were calculated from standard addition experiments performed on polyphenol-containing model media. Aroma retention was found to depend on the (+)-catechin level, the aroma structure, and the temperature applied. Chocolate oligomeric procyanidins proved more active than monomers; catechin showed no significant interaction with proteins. Chocolate oligomers induced high colloidal instability at high temperature, especially with gliadin.

INTRODUCTION

Polyphenols, widely distributed in plants, are found in many common foods (fruits, vegetables…) and beverages (wine, tea, beer…). Polyphenols are known to interact with a large range of molecules, including proteins and aromas.
Polyphenols and proteins form soluble complexes that can grow to colloidal size (light scattering) or even form sediments[1].
Protein-polyphenol associations are usually weak, non-covalent, and reversible (hydrogen bonds or hydrophobic interactions)[2].
Interaction seems to depend on various parameters, such as polyphenol and/or protein structure and concentration, the temperature applied, the solvent used, the ionic force, and the pH[3]. Santos Buelga and Scalbert[2] report that the haze-forming activity of a protein increases with its proline content (e.g. it is greater for salivary proteins than for gelatine) and its degree of glycosylation. The haze-forming activity of polyphenols depends on their structure, their flexibility[2], their molecular weight, and their rate of galloylation[4,5]. De Freitas and Mateus[3] report that (+)-catechin has a higher affinity than (-)-epicatechin for salivary proteins rich in proline (PRPs). According to Siebert *et al.*[1], the haze-forming activity of procyanidins increases with the degree of polymerisation.
Procyanidin dimers with C4-C8 bonds bind PRPs more effectively than dimers with C4-C6 bonds (subunit: (+)-catechin). According to Mc Manus *et al.*[5], haze also increases with the number of galloyl groups. De Freitas and Mateus[3] report that epicatechin-3-O-gallate and the B2-3-O-gallate dimer complex PRPs more effectively than the corresponding non-galloyl polyphenols.
The polyphenol content can also influence flavour release by generating hydrogen bonds and/or hydrophobic bonds[6]. By dynamic headspace analysis, Perpète *et al.*[7] showed that the availability of linear aldehydes is not affected by 100 ppm (+)-catechin, whilst branched aldehydes such as 2- and 3-methylbutanal are strongly retained (21-23% retention at 20°C). In hydroalcoholic solutions (10%), Dufour *et al.*[8] found isoamylacetate, ethylhexanoate, and benzaldehyde to be retained more readily than limonene by 5000 ppm catechin. On the other hand, condensed wine tannins caused benzaldehyde volatility to decrease only slightly, while a salting-out effect was even observed with limonene. No interaction was found to occur with isoamylacetate or ethylhexanoate[8]. According to Okuda[9], gallotannins bind aldehydes very efficiently. In malt spiked with gallotannins, significant extraction-yield decreases were observed for phenylacetaldehyde, furfural, and benzaldehyde[10]. According to Dufour *et al.*[11], acyl-substituted guaiacyl substances interact more readily than alkyl-substituted analogues with malvidin-3,5-O-diglucoside (anthocyanin). King and Solms[12] found naringin, a lemon flavan-4-one, to decrease the volatility of ethyl benzoate and 2,3-diethylpyrazine, whilst no retention occurred with limonene. Gallic acid has been found to interact more readily than naringin with vanillin, 2-methylpyrazine, and ethylbenzoate[6,13].

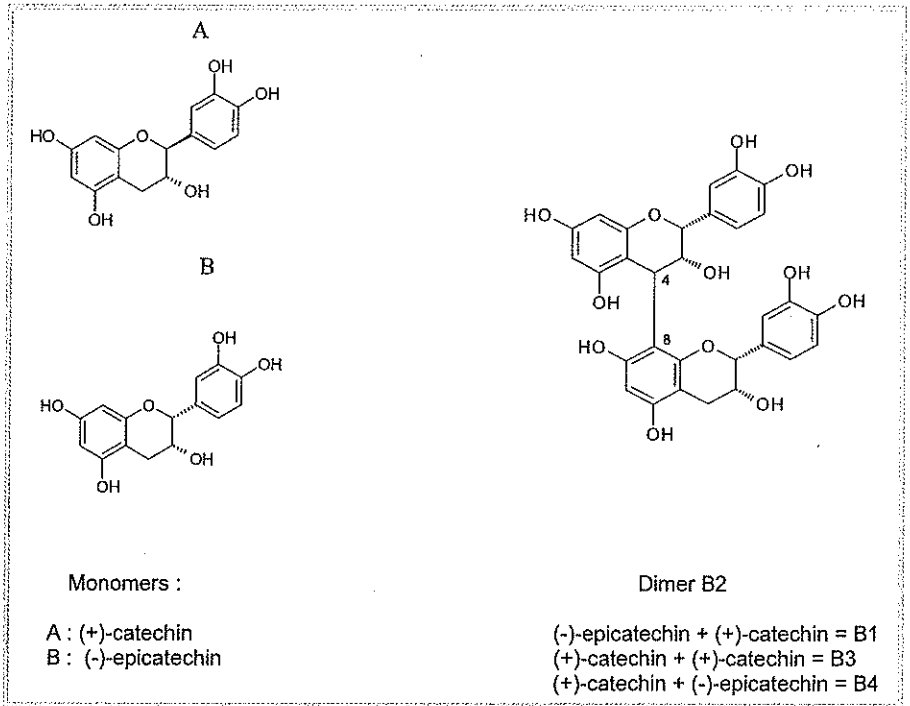

Figure 1. Monomers and procyanidin dimers found in cocoa products.

Chocolate is an exceptional source of polyphenols, mainly (-)-epicatechin, (+)-catechin, and procyanidins[14] (Figure 1).
Attention has recently focused on chocolate polyphenols because of their very high antioxidant activity[15-18] and other potential physiological effects (antimutagenic, immunomodulatory activities…[19-22]). The aim of the present work was to investigate how chocolate polyphenols interact with aroma and proteins in aqueous media (such as functional beverages).

MATERIAL AND METHODS

Materials. For colloidal instability experiments, chocolate procyanidins were extracted by a method recently developed in our laboratory[23]. This extract contains 4% monomers, 6% dimeric to hexameric oligomers, higher weight molecules, and 2.25% of caffeine and theobromine. Chocolate and cocoa liquors were supplied by Belcolade (Puratos Group, Erembodegem, Belgium).
Chemicals. Proteins (gelatin and gliadin), caffeine, theobromine, (+)-catechin and acetic acid were purchased from Sigma-Aldrich (Bornem, Belgium). Aqueous solutions were made with Milli-Q (Millipore, Bedford, MA) double-distilled water (resistance = 18 mΩ). All the aromatic compounds were from Sigma-Aldrich (Bornem, Belgium).

Flavour analysis - Dynamic headspace gas chromatography analysis

A Hewlett Packard Model 5890 gas chromatograph equipped with a Chrompack Purge and Trap injector, a flame ionisation detector and a Shimadzu CR3A integrator were used. Samples were injected into the chromatograph in three steps as follow:

(1) pre-cooling of the trap: the trap was cooled at – 95°C for 2 min by a stream of liquid nitrogen;
(2) purging of the sample: the temperature of the purge vessel was set at 10°C, 25°C, 40°C or 50°C. The sample (9 ml) was purged with helium (12 mL / min) for 15 min. The gas stream was passed through a condenser kept at –15°C by means of a cryostat (Colora WK 15) to remove water vapour and then through an oven at 200°C. The volatiles were finally concentrated in the cold trap maintained at – 95°C (liquid nitrogen);
(3) Desorption of the volatiles: cooling was stopped, and the surrounding metal capillary was immediately heated to 220°C for 5 min. The carrier gas swept the trapped compounds into the analytical column.

Analysis was carried out on a 50 m x 0.32 mm, wall-coated, open tubular (WCOT) CP-Sil5 CB (Chrompack, Antwerpen, Belgium) capillary column (film thickness, 1.2 μm). Oven temperature, initially kept at 36°C for 15 min, was programmed to rise from 36°C to 120°C at 2°C / min then to 200°C at 15°C / min, remaining at the maximum for 9 min thereafter. Helium carrier gas was used at a flow rate of 1 mL / min. Injection and detection temperatures were 200 and 220°C, respectively. The assessment of the technique reproducibility has been previously described[24] (coefficients of variation under 10% for five analyses of the same standard mixture).
Chocolate aroma recoveries were measured by dynamic headspace analysis at 10°C, 20°C, 30°C, 40°C and 50°C. Increase amounts of aromas were added to Milli-Q double-distilled water (Millipore, Bedford, MA; resistance = 18 mΩ) with 0, 100 or 500 ppm of (+)-catechin or with 0, 100, 1000 mg of dark chocolate. Aromas concentrations (0-500 ppb) were chosen close to those found in dark and milk chocolates[25;26]. By comparing the calibration slopes obtained by dynamic headspace analysis, it was possible to assess the relative recovery of aromas in the presence of flavanoids.

Protein-polyphenol interactions – Haze analysis

Protocol has been adapted from Siebert *et al.* (1996). Proteins (gliadin or gelatin : 400 ppm except for the chocolate procyanidins assay : 200 ppm) and polyphenols ((+)-catechin : 400 ppm, chocolate procyanidin extract : 200 ppm) or alkaloids (caffeine or theobromine : 400 ppm) were combined in 0.02 M acetate buffer (pH 4.2). The mixture was held in ice (0°C) or in a water bath at 100°C for 30 min. After attemperation at 25°C, haze was measured with a Hach ratio turbidimeter 2000D (Loveland, CO, USA) using 30 mL cuvettes. All analyses were done in duplicate.

RESULTS

Polyphenol-aroma interactions

Flavour retention was measured by dynamic headspace gas chromatography. Relative aroma recovery factors were calculated from standard addition experiments in model media containing polyphenols (i.e. (+)-catechin, dark chocolate, or cocoa liquor). As depicted in Figures 2 and 3, flavour retention was found to depend on the (+)-catechin concentration, the aroma structure, and the temperature. (+)-Catechin at 500 ppm was able to bind 20-40% of the branched Strecker aldehydes (isobutanal, 2- and 3-methylbutanal) at 10 and 20°C (the binding rate was 10-20% at 30-50°C). Surprisingly, retention of limonene was much higher (90% retention whatever the temperature). In the case of benzaldehyde, the retention rate rose to 60% when the temperature was increased to 50°C, probably because the temperature rise caused hydrophobic bonds to form between phenyl moieties (entropic linkages favoured at higher

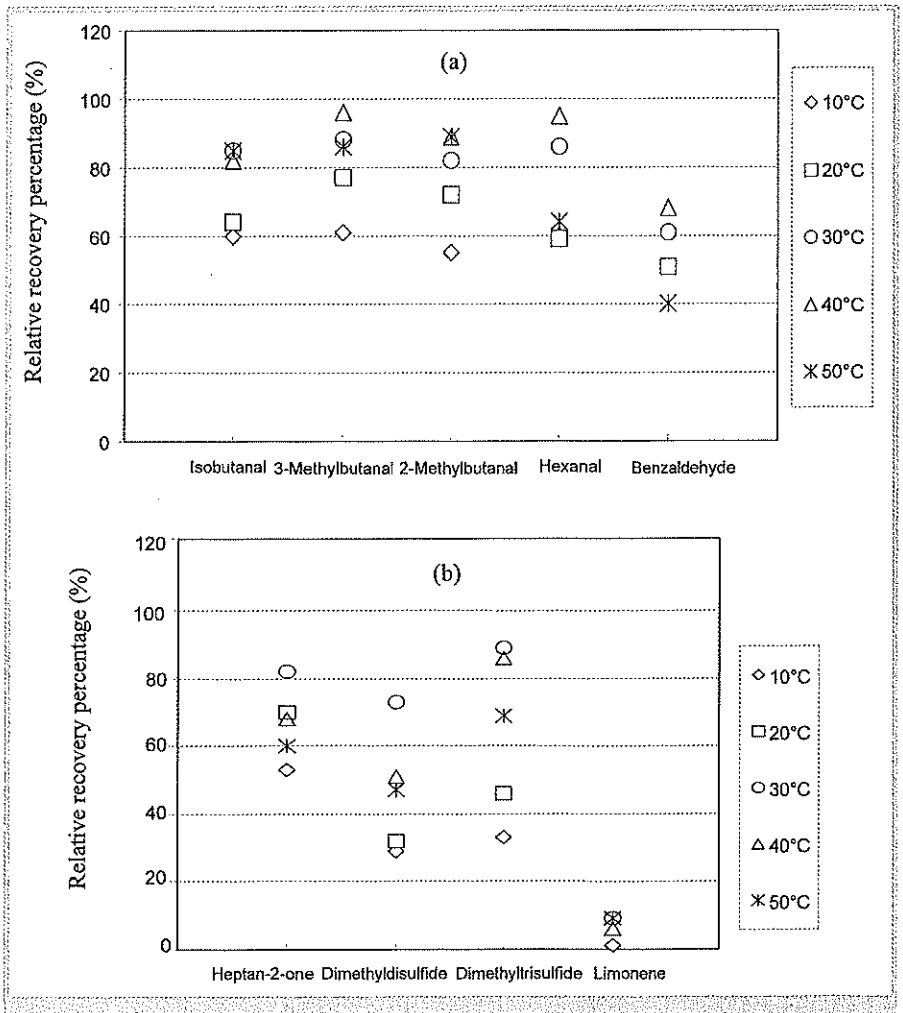

Figure 2. Relative recovery percentages (%) for (a) aldehydes and (b) other aromas in a dynamic headspace system containing 500 ppm (+)-catechin.

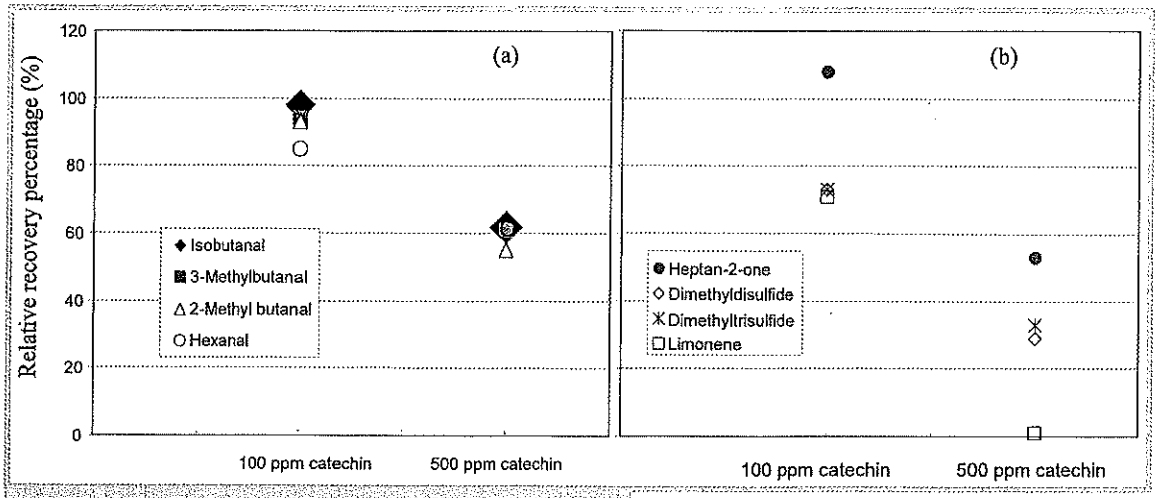

Figure 3. Relative recovery percentages (%) at 10°C for (a) aldehydes and (b) other aromas in a dynamic headspace system containing 100 or 500 ppm (+)-catechin (percentages not determined for benzaldehyde).

temperature). On the other hand, sulfur compounds (dimethyldisulfide and dimethyltrisulfide) were more abundantly retained (50-70%) at lower temperature, indicating efficient enthalpic interactions.

Similar experiments were conducted at 10°C after spiking the medium with either 0.1-1 g dark chocolate or 1g cocoa liquor (Table 1). Again, the aroma structure and the concentration of the added cocoa products emerged as key parameters. The availability of aldehydes was affected in all cases by addition of dark chocolate or cocoa liquor. Limonene was almost completely retained in the polyphenol-enriched media. Intermediate retention rates were measured for polysulfides. As expected, flavour retention was higher with cocoa liquor (richer in polyphenols) than with dark chocolate (aldehydes: ~20% more; sulfur compounds: 10-40% more). Dark chocolate, which contains cocoa liquor, cocoa butter, sugar, and lecithin, can be viewed as diluted cocoa liquor. Lipids are probably rejected from the aqueous phase, although some interactions cannot be excluded.

Polyphenol-protein interactions

We also studied haze formation between polyphenols and proteins rich in proline (gelatine, gliadin). In order to determine the type of chemical interactions involved, we performed experiments at either 0 or 100°C. Haze was quantified at the optimal pH 4.2[1]. Results obtained with chocolate procyanidins, (+)-catechin, theobromine, and caffeine are depicted in Tables 2 and 3.

(+)-Catechin did not significantly interact with proteins, whilst chocolate procyanidins caused strong haze formation (30 to 100 times more than the monomer, despite the lower concentration added). These results confirm that haze formation requires oligomeric sructures. At 100°C, colloidal instability increased even further (hydrophobic interactions), especially in the case of

Table 1: Relative recovery percentages at 10°C in the dynamic headspace system in presence of dark chocolate or cocoa liquor.

Added in 9 ml aqueous solution :	0.1 g Chocolate	1g Chocolate	1g Cocoa liquor
Aldehydes	(%)	(%)	(%)
Isobutanal	77	74	57
3-Methylbutanal	75	66	46
2-Methylbutanal	74	71	53
Hexanal	63	69	56
Ketones			
Heptan-2-one	114	115	85
Sulfur compounds			
Dimethyldisulfide	40	40	1
Dimethyltrisulfide	15	10	2
Terpenes			
Limonene	2	1	8

Table 2: Haze formation in presence of 400 ppm1 or 200 ppm2 gliadin (NTU : nephelos turbidity units) after cold (0°C) or warm (100°C) treatments.

	Haze formation after 30 min at 0 °C		Haze formation after 30 min at 0 °C followed by attemperation at 25 °C		Haze formation after 30 min at 100 °C followed by attemperation at 25 °C	
		Mean		Mean		Mean
Caffeine (400 ppm)[1]	2.41	2.50	2.59	2.44	1.90	1.91
	2.58		2.29		1.92	
Theobromine (400 ppm)[1]	2.6	2.94	2.30	2.82	2.15	2.40
	3.27		3.33		2.64	
Catechin (400 ppm)[1]	12.60	11.98	5.87	5.87	7.22	7.20
	11.65		5.80		7.18	
Chocolate extract (200 ppm)[2]	318	322	312	320	826	824
	325		328		821	

Values below 20 NTU were measured on the range of 0-20 NTU; values up to 200 were measured on the range 0-2000 NTU.

Table 3. Haze formation in presence of 400 ppm1 or 200 ppm2 gelatin (NTU : nephelos turbidity units) after cold (0°C) or warm (100°C) treatments.

	Haze formation after 30 min at 0 °C		Haze formation after 30 min at 0 °C followed by attemperation at 25 °C		Haze formation after 30 min at 100 °C followed by attemperation at 25 °C	
		Mean		Mean		Mean
Caffeine (400 ppm)[1]	1.02	1.03	1.01	1.00	0.91	0.90
	1.05		0.99		0.89	
Theobromine (400 ppm)[1]	1.46	1.15	1.38	1.09	1.21	0.96
	0.83		0.80		0.70	
Catechin (400 ppm)[1]	1.19	1.17	1.12	1.09	2.20	2.05
	1.15		1.06		1.89	
Chocolate extract (200 ppm)[2]	208	205	238	232	357	354
	201		225		350	

Values below 20 NTU were measured on the range of 0-20 NTU; values up to 200 were measured on the range 0-2000 NTU.

gliadin (no hydroxyproline as opposed to 12-14% in gelatine). Caffeine and theobromine, two main alkaloids present in the chocolate polyphenol extract[23], did not interact at all with proteins.

CONCLUSIONS

Binding of volatile compounds and proteins to chocolate polyphenols could have a significant effect on flavour, astringency, and colloidal stability of chocolate-based aqueous products. Flavour retention depends on the polyphenol concentration, aroma structure, and temperature. Chocolate oligomers displayed a greater retention capacity than catechin in such interactions. Likewise, oligomerisation strongly increases protein-polyphenol linkages, especially at high temperature. According to several authors[4;27;28], haze formation with proteins could decrease the bioavailability of polyphenols. Hence, although much higher antioxidant activity is associated with oligomers[23;29], clinical studies should be undertaken to assess the impact of oligomerisation on human health.

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