Food Chemistry 43 (1992) 129–135

Interactions between volatile and non-volatile compounds in the presence of water

M. Le Thanh, P. Thibeaudeau, M. A. Thibaut & A. Voilley

Laboratoire de Biologie Physico-Chimique, ENS.BANA, Campus Universitaire 21000 Dijon, France

(Received 19 November 1990; revised version received and accepted 4 April 1991)

The interactions between volatile aromas and non-volatile compounds were studied by two techniques coupled with gas chromatography. These two techniques (head-space analysis and sorption) show that there exist interactions which depend on the nature of the volatile and non-volatile compounds and on the water content: for instance, the activity coefficient (measured by head-space analysis) of acetone and ethyl acetate in solution at infinite dilution increases in the presence of glucose. The sorption of a mixture of six aroma substances (acetone, ethyl acetate, diacetyl, 2-propanol, *n*-hexanol and benzaldehyde) on carbohydrates (glucose, maltodextrin, starch and β-cyclodextrin) and on caseinate at pH 3, 7 and 9 varies with the water content. The quantity of pure acetone sorbed on the carbohydrate substrates is greater than the total quantity of the mixture of six volatile compounds sorbed. The carbohydrate substrates sorb a lower quantity of volatile compounds than caseinate.

It can be supposed that hydrogen bonds exist between carbohydrates, water and aroma substances, and also that hydrophobic interactions occur in the presence of casein.

INTRODUCTION

Interactions play an important role in the physico-chemical behaviour of food. They may occur between all components and can be very different in nature. This work deals more particularly with the interactions between volatile and non-volatile components. The fixation of volatile compounds by different substrates was investigated in solution at infinite dilution and at high concentration. The effect of equilibrium relative humidity (ERH) on the retention of volatile compounds was also assessed.

Interactions between volatile aroma substances and non-volatile compounds are of two types: attractive (fixation of volatile compounds on non-volatile substrates) or repulsive (release of the volatile compounds). The nature of these interactions depends on the physico-chemical properties of the compounds and, in particular, on the binding that may occur between them. According to Maier (1970), Solms *et al.* (1973), Voilley (1988) and Voilley *et al.* (1990), the fixation of aroma substances in food results from several binding processes:

- Covalent bonds: these bonds are irreversible. Examples are aldehyde or ketone fixation by amino groups of proteins.
- Hydrogen bonds between substrates and polar volatile substances such as alcohols.
- Hydrophobic bonds between apolar volatile compounds and proteins.
- Formation of inclusion complexes with volatile components.

Le Maguer (1972) and Castenada (1975) observed that the fixation of volatile compounds by starch occurred only in the presence of water. Voilley and Rifai (1982) studied the fixation of six aroma substances by food substituents as a function of equilibrium relative humidity. They showed that, for all the substrates, the retention of volatile components was very high in the ERH range 0–30%. Loss of these compounds, was, however, almost complete for ERH greater than 75%. The principal factors influencing the fixation of aroma substances are the nature of the substrate and of the volatile compounds.

Two techniques were used to determine the presence or absence of interactions between volatile compounds, water and non-volatile substrates: head-space analysis and sorption coupled with gas chromatography. This

Food Chemistry 0308-8146/91/$03.50

led the authors to study the activity coefficient of aroma substances at infinite dilution and to assess the quantity fixed by different non-volatile compounds. Quantitative analysis of the vapour phase in equilibrium with the liquid phase in gas chromatography was used to determine the composition of the aroma substances of different foodstuffs. This technique may also be applied to determine the activity coefficients of volatile compounds in solution. At equilibrium and for a given temperature, the concentration of a volatile compound in the head-space depends on its nature, on its concentration in the liquid solution or in the solid substrates and on the nature of interactions between this compound and the other components.

The sorption isotherms allow the determination of the number of active sites or of the effective surface for a given compound; the partial vapour pressure of this compound can also be related to the quantity fixed by non-volatile substrates. The authors' work involved well defined model systems of volatile compounds and substrates such as carbohydrates (glucose, maltodextrins, starch and β-cyclodextrin) and caseinate at different pH in the presence of water.

MATERIALS AND METHODS

Materials

Volatile compounds

Acetone, ethyl acetate, 2-propanol, *n*-hexanol, diacetyl and benzaldehyde were obtained from Merck (Darmstadt, D-6100). They represent some major chemical groups found in food aromas—aldehyde, ketone, ester, alcohol and phenol—and they have various degrees of water solubility.

Substrates

The substrates used were glucose and casein (from Merck, Darmstadt, D-6100), maltodextrins of various degree of hydrolysis (DE 61·5, 31, 20), starch and β-cyclodextrin (from Roquette Frères, Lestrem, F-62136). (DE = Dextrose equivalent (reducing power expressed as g glucose/100 g dry matter).)

Methods

Head-space analysis

This method measures the equilibrium concentration of the aroma between the liquid phase and the vapour phase at a given temperature. An inert gas (nitrogen) passes through the liquid phase (100 g solution containing 10 μl pure volatile compound) at a constant flow-rate and carries the volatile compounds into the head-space. A sample of the vapour phase is automatically injected into the gas chromatograph at regular intervals. The equilibrium liquid–vapour is considered to be reached when the concentration of aroma compounds in the gas phase remains constant. The initial concentration of volatile substances is determined by injection of the liquid into the chromatograph. In the non-ideal solution, the partial pressure of a component i (P_i) in the vapour phase is proportional not only to the mole fraction of compound i in the solution (X_i) but also to the activity coefficient in solution at infinite dilution (γ_i^∞):

$$(P_i) = (P_i^s X_i \gamma_i^\infty)$$

where P_i^s is the vapour pressure of pure compound i.

According to Dalton's law, $Y_i = P_i/P$ where P is total pressure at the same temperature, and concentration of volatiles in the gas phase could be expressed as:

$$Y_i = \frac{P_i^s X_i \gamma_i^\infty}{P}$$

where Y_i is the mole fraction of compound i in the gas phase, and γ_i^∞ represents the tendency for intermolecular forces to develop between the component i and the major constituents of the liquid, in comparison with the strength of intermolecular forces among the major components of the liquid themselves. In the case where activity coefficient decreases, this demonstrates the occurrences of molecular forces between solute and volatile compound. If it increases, this result can be explained by the interactions between substrate and solvent (in the authors' case, the solvent is water).

Technique of preparation of the samples for sorption

The casein was prepared as follows: frozen bovine caseinate was thawed at 4°C, adjusted to pH 7 with NaOH and filtered. The casein was precipitated by adjusting to pH 4·6 with HCl. The precipitate was washed twice and redissolved in water by adjusting to pH 7 with NaOH. This solution was filtered and the casein again precipitated by adjusting to pH 4·6 with HCl. The precipitate was filtered and washed twice with the water. The purified casein was used to make three substrates by dissolving in water with HCl to pH 3, with NaOH to pH 7 or pH 9. These solutions were then freeze-dried.

The samples were prepared as follows: 2 ml of solution containing 15% glucose, maltodextrins or starch, 10% caseinate or 5% β-cyclodextrin were poured into small vials and freeze-dried.

Sorption

The freeze-dried samples of the different substrates were placed in desiccators containing saturated salt solutions that maintain a fixed water activity (Table 1). The temperature of the desiccators was maintained at 25 ± 0·5°C, until equilibrium was reached (as shown by the constant weight), then 10 ml of pure acetone or 10 ml of each pure component was put in a desiccator beside the freeze-dried substrates. During the sorption process, the samples were weighed every 48 h; when the equilibrium was reached, these substrates were rehydrated with pure

Table 1. Saturated salt solutions

Salt	Equilibrium relative humidity (%)
Lithium chloride (LiCl)	11
Potassium acetate ($KC_2H_3O_2$)	22
Magnesium chloride ($Mg(Cl)_2$)	32
Magnesium nitrate ($Mg(NO_3)_2$)	52
Sodium chloride (NaCl)	75
Barium chloride ($BaCl_2$)	90

water, homogenized and injected directly into the gas chromatographic column. The quantity of volatile sorbed by substrates was calculated by the amount of volatile in the sample measured by gas chromatography.

Gas chromatographic conditions: a Hewlett Packard model 5710A gas chromatograph with a flame ionization detector (FID) and a Hewlett Packard 3380A integrator was used. The stainless steel column (3 m × 3·15 mm i.d.) was packed with 100/120 mesh Carbowax 20M-10%. The injector and detector temperatures were maintained at 200°C. The column was operated at 75–160°C, with 2°C/min increase. Nitrogen flow-rate was 20 ml/min, hydrogen flow-rate was 25 ml/min and air flow-rate was 250 ml/min.

RESULTS

The authors verified the influence of the substrates on the activity coefficient of acetone and ethyl acetate compared to data reported in other studies by using an intermediate concentration in the liquid phase.

Activity coefficient of acetone and ethyl acetate in water and in the presence of glucose or sodium caseinate at pH 7

The activity coefficients of the volatile compounds are related to their saturated vapour pressures (Table 2). In general, the higher the saturated vapour pressure, the lower the activity coefficient in dilute solution. This trend was not valid for ethyl acetate which has a low solubility in water.

The activity coefficients of acetone and of ethyl acetate in water, in 32% glucose solution and in 10% sodium caseinate at pH 7 were determined by head-space analysis. In the presence of glucose, their activity coefficients were significantly higher than those observed in pure water. Results are presented in Table 3. The authors' results confirmed those obtained by Sorrentino *et al.* (1986).

Land and Reynolds (1981) found no difference for diacetyl with different substrates in the presence of 0–5% glucose, but observed a decrease in the presence of bovine serum albumin, even at a concentration of 0·5%. Marinos-Kouris and Saravacos (1975) reported that, with the addition of sucrose (from 15 to 60%), the activity coefficients of volatile compounds (alcohols, ketones and esters) increased. Many authors showed that the addition of protein to solutions containing flavour compounds decreased the concentration of the latter in the head-space (Franzen & Kinsella, 1974; Gremli, 1974). In the present authors' case, in the presence of 10% sodium caseinate at pH 7, there is no significant change of activity coefficient. The authors' divergent results for sodium caseinate are attributed to the residual NaCl present from the preparation of caseinate, because salts increase the concentration of volatile compounds in the vapour phase (Jenning, 1965; Nawar, 1966; Land & Reynolds, 1981).

Water sorption at different ERH

In the sorption method, results obtained were the average of three measurements and the variation coefficient was less than 5%.

The water sorption isotherms of glucose, maltodextrin, starch, β-cyclodextrin and caseinate at pH 3, 7 and 9 are shown in Fig. 1. The amount of water sorbed

Table 2. Physico-chemical properties of volatile compounds

Parameters (25°C)	Volatile compounds					
	Acetone	Ethyl acetate	Diacetyl	2-Propanol	*n*-Hexanol	Benzaldehyde
P_i^s (mm Hg)[a]	239·19	104·20	27·56	42·75	0·99	0·75
Solubility in water (g/100ml)	Very soluble	8·6	25	Very soluble	0·59	0·33
Activity coefficient in water (γ^∞)	7·8[b] 7·1[c]	60[b] 64·4[c]	14[c]	7·1[c]	609·5[c]	1485·8[c]

[a] P_i^s value calculated by Antoine equation.
[b] Present authors' value.
[c] Voilley *et al.* (1988).

Table 3. Effect of glucose and sodium caseinate at pH 7 on the activity coefficient of acetone and ethyl acetate

Volatile compound	In water	In glucose solution (%, w/w)			In sodium caseinate solution (%, w/w) at pH 7		
		15	32	50	2·5	5·0	10
Acetone	7·8 (2·7)[c]	9·9[a]	13·1 (3·4)	14·7[a]	6·8[b]	7·7[b]	8·6 (4·7)
Ethyl acetate	60 (3·2)	108[a]	130 (3·5)	180[a]	65·4[b]	64·8[b]	72 (3·8)

[a] Sorrentino *et al.*, (1986).
[b] Fares (1987).
[c] Variation coefficient for four analyses (%).

increased from 0 to 40% with increasing ERH. For carbohydrates, the amount of water sorbed decreased in the following order:

β-cyclodextrin > starch > MD DE 20 > MD DE 31 > MD DE 61·5 > glucose

The caseinate sorbed more water than the carbohydrates and the sodium caseinate sorbed more than the hydrochloride caseinate.

Sorption of volatile compounds on carbohydrates and casein

Sorption of volatile compounds on carbohydrate

The sorption of pure acetone and a mixture of six volatile compounds at ERH 11% and 32% is shown in Table 4.

The quantity of pure acetone sorbed was variable and ranged from 0·05 g/100 g dry matter for maltodextrin to 4·4 g/100 g dry matter for starch. The fixation of volatile compounds on maltodextrins decreased slightly with the degree of hydrolysis. The amount of pure acetone sorbed by glucose and maltodextrin increased with ERH whereas, with starch and β-cyclodextrin, it decreased. The same phenomenon was found for the mixture of six volatile compounds. The total of the mixture of volatile compounds that was sorbed at the same ERH was lower than the amount of pure acetone. The starch sorbed more acetone than the β-cyclodextrin but less of the mixture of six volatiles than the β-cyclodextrin.

Table 5 shows the percentage of each volatile in the mixture of six volatiles that was sorbed in each substrate. The amount sorbed depends both on the nature of the volatile compounds and of the substrates. Sorption decreased in the following order. For glucose and maltodextrin:

benzaldehyde > acetone > 2-propanol > diacetyl > *n*-hexanol > ethyl acetate

For starch and β-cyclodextrin:

2-propanol > acetone > diacetyl > benzaldehyde > *n*-hexanol > ethyl acetate

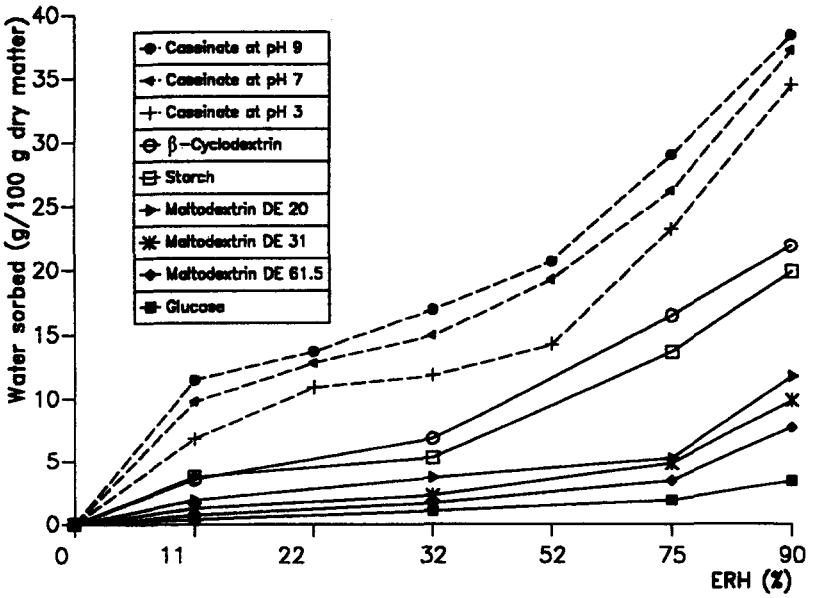

Fig 1. Sorption isotherms of water.

Table 4. Amount of volatile compounds sorbed by carbohydrate substrates as a function of equilibrium relative humidity (g/100 g dry matter)

Substrate	ERH(%)			
	11		32	
	Acetone	Mixture of 6 volatile compounds	Acetone	Mixture of 6 volatile compounds
Glucose	0·1	0·04	0·1	0·08
Maltodextrins	—	—	—	—
DE 61·5	0·10	0·06	0·16	0·14
DE31	0·08	0·05	0·10	0·09
DE20	0·05	0·04	0·10	0·07
Starch	4·4	1·94	3·6	1·1
β-cyclodextrin	4·0	2·7	3·6	2·4

ERH, Equilibrium relative humidity.
DE, Dextrose equivalent (g glucose/100 g dry matter).

Sorption on caseinate

The quantity of volatile compounds in the mixture sorbed on caseinate ranged between 4·9 and 93%. The acidic casein sorbed much less than the neutral or alkaline casein (see Table 6). This trend is the same even when the ERH is high. Table 5 shows the percentage of each volatile in the mixture of six volatiles that was sorbed on caseinate. This percentage is constant whatever the type of casein and the ERH. The amount sorbed depends only on the nature of the volatile compounds. Sorption decreased in the following order:

acetone > ethyl acetate > 2-propanol > benzaldehyde > diacetyl > *n*-hexanol

DISCUSSION

At infinite dilution, the activity coefficient of a volatile compound depends on the saturated vapour pressure and on its solubility in water. Compounds with a high activity coefficient and an apparently low volatility are more concentrated in the gaseous phase. The

Table 5. Percentage of each volatile compound sorbed by different substrates (%)

Substrates	Volatile compounds					
	Acetone	Ethyl acetate	Diacetyl	2-Propanol	n-Hexanol	Benzaldehyde
Glucose Maltodextrin	15 ± 2	5 ± 1	7 ± 1	12 ± 2	9 ± 1	52 ± 4
Starch β-Cyclodextrin	23 ± 1	5 ± 1	7 ± 1	37 ± 1	12 ± 1	16 ± 1
Caseinate	43 ± 2	20 ± 2	7 ± 1	16 ± 1	4 ± 1	10 ± 1

Table 6. Amount of the mixture of six volatile compounds sorbed on casein as a function of equilibrium relative humidity (g/100 g dry matter)

Substrates	Equilibrium relative humidity (%)					
	11	22	32	52	75	90
Sodium caseinate:						
at pH 7	9·2	10	14	66	71	93
at pH 9	9·0	10·1	11·5	56	68	75
Casein hydrochloride						
at pH 3	4·9	8·9	10·8	22	35	49·5

considerable increase of activity coefficient of acetone and ethyl acetate in the presence of glucose can be explained by the water–glucose interactions.

Water sorption varies with the nature of the substrates for the same ERH. Water is sorbed in a greater amount by casein than by carbohydrates. Ruegg and Blanc (1976) showed that the difference in the sorption between sodium caseinate and casein hydrochloride can be explained by the difference in hydration between Na^+ and Cl^- because Cl^- ions are much less hydrated than Na^+ ions.

The importance of the nature of the volatile compounds as shown in our work confirms other studies. Menting and Hoogstad (1967) reported that, at the same water content, alcohols were more strongly fixed by a maltodextrin than other volatile compounds (ethanol > acetone > ethyl acetate > benzene). The quantity sorbed of substances of the same homologous series increases with decreasing molecular weight. Arai *et al.* (1970) and Maier (1975) showed that alcohols, aldehydes and ketones were more strongly bound by carbohydrates and proteins and that, at the same water content, acetone was more strongly bound than ethyl acetate. The present authors' work confirms these results. Acetone was also bound to a greater extent than *n*-hexanol. The volatility of the volatile compounds affects the sorption processes. The more volatile the compounds, the greater the amount sorbed. Polarity, molecular masses and solubility of the volatile compounds also affect the sorption. The quantity of pure acetone sorbed on carbohydrate substrates is greater than the total amount of volatile compounds sorbed from a mixture. In the authors' case, intermolecular hydrogen bonds between acetone and 2-propanol or *n*-hexanol may have formed in the mixture of pure volatile compounds.

The nature of the substrate plays an important role. Carbohydrate substrates sorb a lower amount of volatile compounds than casein. 2-Propanol, *n*-hexanol and benzaldehyde are more strongly sorbed on carbohydrate substrates than ethyl acetate. Hydrogen bonds may occur between them. Acetone and ethyl acetate are strongly sorbed by casein. This may be due to the presence of hydrophobic bonds between them. The caseinate substrates became orange-red in colour when they sorbed the volatile compounds. This phenomenon was observed after 48 h of sorption at ERH of 90 and 75% and occurred later for the other ERH. The coloration intensity increased in the order: caseinate pH 3, caseinate pH 7 and caseinate pH 9. The appearance of such a coloration may be due to the reaction of non-enzymatic browning.

Rutschmann *et al.* (1989) confirmed the formation of starch inclusion complexes with different organic compounds such as decanal, 1-naphthol, mono-stearate, limonene. The nature of starch inclusion complexes and degree of complex formation depends on the nature of the organic compounds.

By comparing the behaviour of volatile compounds by two different methods, the authors were able to see that the volatile components were sorbed by the casein whatever the amount of water in the foodstuffs (solid state in the sorption and solution in the head-space method). With glucose, in solution acetone and ethyl acetate are released but, in the solid state, these compounds are retained. These results confirm our hypothesis that the glucose interacts essentially by hydrogen bonding, and casein by hydrophobic interactions.

CONCLUSION

The study of the volatility of aroma compounds at infinite dilution and of their sorption by substrates at high concentration shows the importance of water content in the sorption of volatile compounds. Sorption of the six flavour substances in a mixture on non-volatile freeze-dried carbohydrate compounds and on freeze-dried caseinate depends on ERH. The amount of pure acetone sorbed on carbohydrates is greater than the total quantity of six volatile compounds sorbed from the mixture. Carbohydrate substrates sorb less volatile compounds than caseinate. Hydrogen bonds may exist between carbohydrate, water and flavour substances. In addition, hydrophobic interactions may occur in the presence of casein.

ACKNOWLEDGEMENTS

The authors thank Dr M. C. Bourne, professor of Food Science & Technology, Cornell University for his helpful discussion. They are also grateful to the Society Roquette Frères for providing the maltodextrin samples.

REFERENCES

Arai, S., Noguchi, M., Yamashita, M., Kato, H. & Fujimaki, M. (1970). Studies on flavour components in soybean—Part VI—Some evidence of protein flavour binding. *Agric. Biol. Chem.*, **34**, 1569–73.

Castenada, J. A. (1975). Contribution a l'étude de la fixation de modèle de substances volatiles sur l'amidon. These de Docteur Ingenieur, Université Paris VI, Paris.

Fares, K. (1987). Comportement de substances d'arôme dans des solutions de caseines. These 3ieme cycle, ENSBANA, Dijon, France.

Franzen, K. L. & Kinsella, J. E. (1974). Parameters affecting the binding of volatile flavor compounds in model food systems. *J. Agr. Food Chem.*, **22**, 675–8.

Gremli, H. A. (1974). Interaction of flavor compounds with soy protein. *J. Am. Oil Chemists' Soc.*, **5**, 95–7A.

Jenning, W. G. (1965). Influence of temperature and salt addends on vapor equilibration of headspace. *J. Food Sci.*, **30**, 445–9.

Land, D. G. & Reynolds, J. (1981). The influence of food

components on the volatility of diacetyl. In *Flavour '81*. Ed. P. Schreier, Walter de Gruyter, Berlin, pp. 701–5.

Le Maguer, M. (1972). Rétention des matières volatiles sur des substrats à humidité variable. Thèse de Docteur Ingénieur, Université Paris VI, Paris.

Maier, H. G. (1970). Volatile flavoring substances in foodstuffs. *Angew. Chem.*, **9**, 917–88.

Maier, H. G. (1975). Binding of volatile aroma substances to nutrients and foodstuffs. Paper presented at Proc. Int. Symp. Aroma Research, Zeist, Pudoc, Wageningen, pp. 143–57.

Marinos-Kouris, D. & Saravacos, G. (1975). Volatility of organic compounds. Paper presented at 5th Int. Congress Chem. Eng. (CHISA 75), Prague, pp. 24–7.

Menting, L. C. & Hoogstad, B. (1967). Volatile retention during the drying of aqueous carbohydrate solution. *J. Food Sci.*, **32**, 87–90.

Nawar, W. W. (1966). Some considerations in interpretation of direct headspace gas chromatographic analyses of food volatiles. *Food Technol.*, **20**, 115–17.

Ruegg, M. & Blanc, B. (1976). Effect of pH on water vapor sorption by caseins. *J. Dairy Sci.*, **59**, 1019–24.

Rutschmann, M. A. & Solms, J. (1990). Formation of inclusion complexe of starch with different organic compounds —Part II, III, IV, V. *Lebensm.-Wiss. u-Technol.*, **23**, 70–92.

Rutschmann, M. A., Heiniger, J., Pliska, V. & Solms, J. (1989). Formation of inclusion complexe of starch with different organic compounds—Part 1. *Lebensm.-Wiss. u-Technol.*, **22**, 240–4.

Solms, J., Osman-Ismail, F. & Beyeler, M. (1973). The interaction of volatiles with food components. *Can. Inst. Food Sci. Technol. J.*, **6**, A10–16.

Sorrentino, F., Voilley, A. & Richon, D. (1986). Activity coefficients of aroma compounds in model food systems. *A.I.Ch.E.*, **32**, 1988–93.

Voilley, A. (1988). Interactions macromolécules-petites molécules non ionisées. In *Cahier de l'ENSBANA—No. 6, Dijon*, ed. D. Lorient, B. Colas & M. Le Meste. Tec et Doc, Librairie Lavoisier, Paris, pp. 49–63.

Voilley, A. & Rifai, M. (1982). Fixation de substances d'arôme sur des supports alimentaires et activité de l'eau. *Sci. Aliments*, 2 (no. hors serie II) 53–60.

Voilley, A., Fares, K., Lorient, D. & Simatos, D. (1988). Interactions between proteins and aroma compounds. In *Functional Properties of Food Proteins*, ed. R. Lasztity & M. Ember-Karpati. Proceedings of International Seminar, Budapest, 7–9 September.

Voilley, A., Lamer, C., Dubois, P. & Feuillat, M. (1990). Influence of macromolecules and treatments on the behavior of aroma compounds in a model wine. *J. Agr. Food. Chem.*, **38**, 248–51.