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Use of inverse gas chromatography with humidity control of the carrier gas to characterise aroma–starch interactions

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Abstract

The interactions between aroma compounds and native corn starch were studied by inverse gas chromatography (IGC). A system of humidification of the carrier gas has been set up and generated a fixed and stable relative humidity of 56±3%. The IGC system worked under pressure (2.1 bars), using starch as stationary phase without any support. This technique allowed to maintain the starch matrix with a constant water content of 10%. The specific retention volumes of volatiles (1-hexanol, 2-hexanol, octanal, ethyl hexanoate and *d*-limonene) were measured under dry and humid conditions. Retention was higher under humid conditions, especially for 1-hexanol. Retention indices of volatiles with various functions and carbon chain lengths were determined on starch and compared to RI on Carbowax. RI on starch increased with the carbon chain length, like on Carbowax. Retention on starch and Carbowax followed the same general order, relative to the functional group.

1. Introduction

Interactions between aroma compounds and food components play an important role in the perception of flavour. Knowledge of these interactions is useful in improving the process and the flavouring of food products (Dubois, Lubbers & Voilley, 1995). Carbohydrates, particularly starch, are present in many low-water content foods, like cereal-based products. They are also widely used as solid support for aroma compounds (Goubet, Le Quere & Voilley, 1998), and as fat-replacers (Godshall, 1997).

Binding of volatiles to starch has been studied by different techniques. Inclusion complexes of starch with different aroma compounds have been characterised by amperometric iodine titration (Nuessli, Conde-Petit, Trommsdorff & Escher, 1995; Rutschmann & Solms, 1990), X-ray diffraction and differential scanning calorimetry (Nuessli, Sigg, Conde-Petit & Escher, 1997). Interaction parameters have generally been measured using starch solutions, by static headspace (Le Thanh, Thibeaudeau, Thibault & Voilley, 1992) or by extraction methods (Rutschmann, Heiniger, Pliska & Solms, 1989). Concerning starch systems with low water contents, only a few studies exist. Hau, Gray and Taylor (1996) demonstrated the existence of interactions between native starch and aroma compounds. Furthermore, Hau et al. (1998) suggested that the physical state of extruded starch as well as water contents have an effect on volatile binding.

The technique used here was inverse gas chromatography (IGC). IGC has already been used to study interactions between polymers and monomers (Kontominas & Giacin, 1993), between aroma compounds and food packaging polymers (Gavara, Catala & Hernandez-Munoz, 1997; Aucejo, Pozo & Gavara, 1998) and between aroma compounds and carbohydrates (Delarue & Giampaoli, 2000). Water and ethanol sorption on polysaccharides (starch, pectins) was widely tested by this technique (Demertzis, Riganakos, Giannakakos & Kontominas, 1991; Greenway, 1986; Paik & Gilbert, 1986; Valeri, Demertzis & Akrida-Demertzi, 1998).

In most IGC studies, the tested matrix is mixed with materials such as glass wool or Chromosorb (Demertzis et al., 1991; Paik & Gilbert, 1986), or coated on glass beads (Kontominas & Giacin, 1993), in order to reduce the pressure drop in the column. However, the second

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medium mixed with the starch may influence the retention parameters. In this IGC study, native corn starch was used as the stationary phase, without any support.

To maintain the initial water content of native starch during the experiment, we set up a system to humidify the carrier gas. To evaluate the influence of water on aroma-starch interactions, we measured the retention of aroma compounds on corn starch under dry and under humid conditions.

2. Materials and methods

2.1. Materials

2.1.1. Volatile compounds

1-Hexanol, 2-hexanol, hexanal, heptanal, octanal, 2*(E)*-hexenal, 2*(E)*-heptenal, 2*(E)*-octenal, 2-hexanone, cyclohexanone, ethyl butanoate, ethyl hexanoate, octane, undecane and dodecane were obtained from Sigma Aldrich Chimie (Saint Quentin Fallavier, France). Nonane, decane, butanol and pentanol were obtained from Merck (Nogent sur Marne, France), benzaldehyde and *d*-limonene were obtained from René Laurent (Le Cannet, France). The purity of the aroma compounds ranged from 95 to 99%.

2.1.2. Starch

Corn starch with an amylose content of 70 to 80% and less than 1% extractable lipids was provided by Roquette Frères (Lestrem, France). The starch was sieved to obtain particle sizes between 40 and 63 µm.

2.2. Methods

2.2.1. Inverse gas chromatography

Glass columns (11.5 cm×4.5 mm i.d.) were deactivated with dimethyldichlorosilane (DMCS) before being packed with starch. Column packing was facilitated with the combination of an electric vibrator and a vacuum system. The mass of the stationary phase was 1.15±0.01 g.

The starch column was placed in the oven of a Carlo Erba 6000 Vega II gas chromatograph and connected to the injector and detector with deactivated fused silica tubing (0.32 mm i.d.). The gas chromatograph, equipped with a flame ionisation detector (FID), was operated under isothermal conditions at a temperature of 40°C. The injector and detector temperatures were maintained at 250°C. The nitrogen flow-rate was regulated at 20 ml min^{-1} with a precision Nupro needle valve. This flow rate provided a pressure of 2.1 bars at the head of the column, measured with a precision manometer.

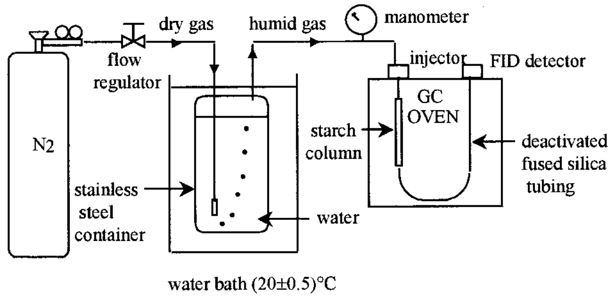

Fig. 1. IGC set-up with controlled humidification of the carrier gas.

For experiments performed under humid conditions, carrier gas bubbled in a 19 l stainless steel pressure resistant container (Paar, USA) filled with water (16 l, 40 cm high) (Fig. 1). The container was maintained at 20±0.5°C by a temperature-regulated water bath. A final nitrogen stream of 56±3% relative humidity (RH) under 2.1 bars of pressure was obtained by this scheme.

The RH of the carrier gas was determined by weighing the water trapped on a column containing copper sulphate granules, over a 5-h period. To measure the RH under 2.1 bars, the copper sulphate column was placed between the humidifier and the starch column (Fig. 2). RH of nitrogen under atmospheric pressure has been measured without the starch column.

Pure volatile compounds (0.05 µl) were injected in a split less mode with a 0.5 µl Hamilton syringe (Supelco, Bellefonte, USA). Retention times were determined by a Spectra Physics SP 4290 integrator. Specific retention volumes V_g were calculated using Eq. (1), where t (min) is the retention time of the solute, t_0 (min) is the retention time of a non-retained compound (methane), F (ml min^{-1}) is the carrier gas flow rate, m (g) is the mass of stationary phase, T (K) is the temperature of the column. The James and Martin compressibility factor j is calculated using Eq. (2), where P_i and P_0 are the inlet and outlet pressures, respectively.

$$V_g = j\frac{(t - t_0)\cdot \mathrm{F}}{m}\cdot\frac{273}{T} \quad (1)$$

$$j = \frac{3}{2}\cdot\frac{(P_i/P_0)^2 - 1}{(P_i/P_0)^3 - 1} \quad (2)$$

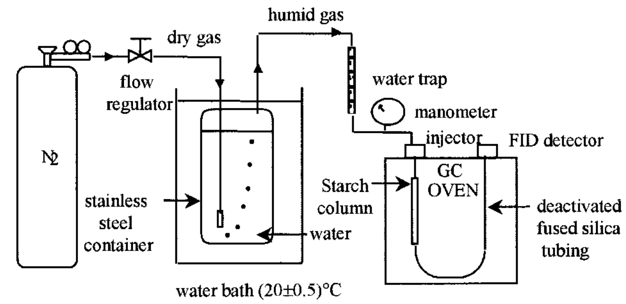

Fig. 2. IGC set-up to measure humidification of the carrier gas under 2.1 bars.

Retention indices on starch were measured using series of C8–C12 alkanes. These RI were compared to published RI on Carbowax (Kondjoyan & Berdagué, 1996).

3. Results and discussion

3.1. Optimisation of the IGC technique

The initial water content in native corn starch was 12.5% (w/w). When we used IGC under dry conditions (dry nitrogen), a considerable dehydration of the starch matrix occurred. The loss of weight was about 8% at 40°C after a volume of 10 l nitrogen passed through the column (corresponding to 1 day of work). This led to variations of the flow rate and of the retention times during the experiment. To solve these problems, we decided to reduce starch dehydration by humidifying the carrier gas. The RH of nitrogen depended on pressure and mainly on temperature, so that the humidification system had to be thermostated. The container temperature 20±0.5°C had to be lower than the room temperature (22–29°C) to avoid condensation of water in the connection tubes.

Assuming ideal gas behaviour, the vapour pressure of water (P_v) was then calculated from the total amount of water collected ($M_{H_2}O$), the total volume of carrier gas (V_{N_2}) that passed through the column during the 5 h period, the gas pressure (P) and temperature (T) [Eqs. (3)–(5)]. The RH was calculated by comparing this calculated vapour pressure with the tabulated saturated vapour pressure of water (P_{vsat}) (Wexler & Greenpan, 1971) at 20°C [Eq. (6)].

$$P_v = \frac{w \cdot P}{w + (M_{H_2O}/M_{N_2})} \tag{3}$$

$$w = \frac{m_{H_2O}}{m_{N_2}} \tag{4}$$

w is the mixing ratio: the mass of water versus the mass of dry nitrogen; m_{N_2} is the mass of dry nitrogen; M_{H_2O} and M_{N_2} are, respectively, the molecular mass of water and nitrogen.

$$m_{N_2} = \frac{P \cdot V_{N_2} \cdot M_{N_2}}{RT} \tag{5}$$

$$RH(\%) = \frac{P_v}{P_{vsat}} \times 100 \tag{6}$$

The measured RH of nitrogen under atmospheric pressure, without the starch column, was 96±3%, approaching the saturation value (100% RH). This demonstrated the efficiency of the humidification system under atmospheric pressure. When the starch column was connected, the provided pressure (2.1 bars) reduced the RH to 56±3%.

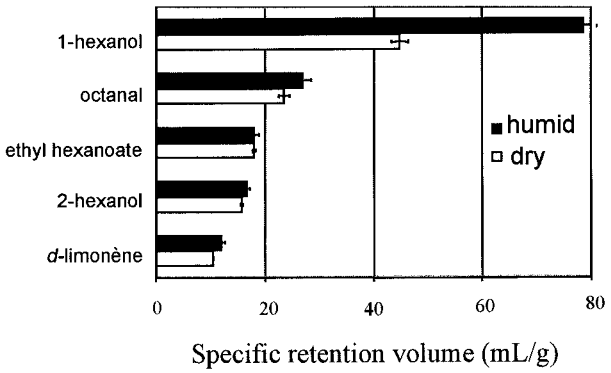

Fig. 3. Specific retention volume of aroma compounds on corn starch under dry and under humid conditions. Dry conditions: dry nitrogen, 4.5% water content in starch. Humid conditions: 56±3% RH Nitrogen, 10% water content in starch.

When IGC experiments were performed under these humid conditions, the dehydration of starch was reduced. The loss of weight was only about 2.5 instead of 8% under dry conditions. Starch moisture was then maintained to a constant value of 10% during all the experiment (8 h). In semi-dynamic conditions (cabinet with gentle air circulation over the sample), the RH of 56% would be sufficient to provide a water content of 14% in starch at 40°C, as shown by isotherm sorption curves of water on corn starch (Shotton & Harb, 1965). But in dynamic conditions of IGC, starch moisture was lower (10 instead of 14%). However, these conditions were satisfactory, because they enabled approximation of the 12.5% initial moisture of starch and gave repeatable retention times and a stable flow rate.

3.2. Aroma compounds retention on starch

3.2.1. Influence of water

Retention of aroma compounds on starch was compared under dry and under humid conditions after 8 hours of experiment (Fig. 3). Dry conditions (dry nitrogen) corresponded to 4.5% water content in starch. Humid conditions (nitrogen with 56% RH) corresponded to 10% water content in starch. All injections of aroma compounds were performed in triplicate. Less than 6% variation was observed in retention times for replicate injections. Three different columns were tested. Column to column variations were less than 6%.

In general, the specific retention volume is higher under humid conditions. The difference is particularly high for 1-hexanol, quite high for octanal, weak for the other compounds. Water has more influence on the more retained compounds. These compounds present a

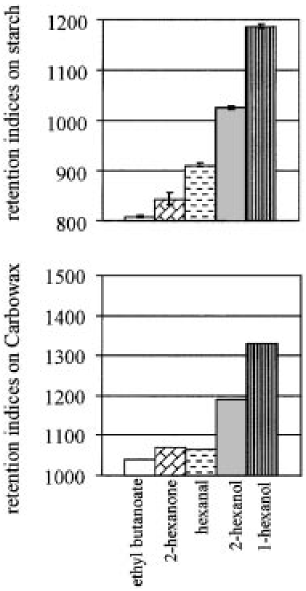

Fig. 4. Retention indices of C6 aroma compounds with different functional groups on native starch (10% water content) and Carbowax.

strong adsorption on starch through the formation of hydrogen bonds. Presence of water enhances the starch activity and so the possibilities of adsorption.

Presence of water has a great influence on aroma-carbohydrate interactions. Hau et al. (1998) found an increase of the diacetyl binding rate on extruded starch with increasing the water content of starch (19–43%).

Starch is a semicrystalline polymer, composed of highly branched amylopectine molecules (crystalline phase) and linear amylose molecules (amorphous phase), associated in granular entities. The native corn starch tested in this study contained around 75% of amylose. This starch was predominantly amorphous and existed in the glassy state at this water content. Water plays an important role in the physical properties of starch (Garcia, 1996). Water exerts a plasticising effect on the polymer by replacing hydrogen bonds between amylose chains by hydrogen bonds between water molecules and glucose residues. For a water content between 0 and 10%, water is strongly fixed by hydrogen bounds to the hydroxyl groups of starch macromolecules, and is organised as a monomolecular layer. For a water content between 10 and 65%, other water molecule layers are fixed successively with weaker hydrogen bounds. This freer water may behave as a solvent for other molecules, facilitating their diffusion through the starch matrix.

Under our dry conditions, starch contained only 4.5% water. Interactions between aroma compounds and starch probably resulted of an adsorption phenomenon.

Under IGC humid conditions, starch contained 10% of water. A continuous gas flow charged with water passed through the starch column. All the water molecules of the carrier gas were not fixed to the matrix. Hence, free water could partially dissolve hydrophilic molecules, which could then penetrate the matrix. The retention of these molecules also increases with the water content of starch; this phenomenon could involve the partition coefficient of the compounds between the gas phase and the water present in the starch matrix. The existence of several binding mechanisms could then be proposed; on the one hand adsorption of volatiles on the free OH-sites of starch, on the other hand diffusion of the molecules through the matrix, facilitated by free water. Moreover, water may separate amylose chains, by plasticising effect and thus liberate new fixation sites of amylose for molecules containing polar functions. In this case, the adsorption phenomenon is increased by the presence of water.

3.2.2. Influence of the molecule chemical structure

To evaluate the nature of starch–aroma interactions, we studied retention of volatiles with various functional groups and different chain lengths. Experiments were carried out under humid conditions. Retention indices (RI) were calculated on starch and compared with RI on Carbowax, i.e. a polar stationary phase.

All the RI on starch are lower than RI on Carbowax. This means that starch is a less polar stationary phase than Carbowax.

For series of molecules with six carbon atoms (Fig. 4), retention on starch and Carbowax follows the same general sequence, relative to the functional group. RI increases in the following sequence: ester < ketone < aldehyde < alcohol. Secondary alcohol is less retained

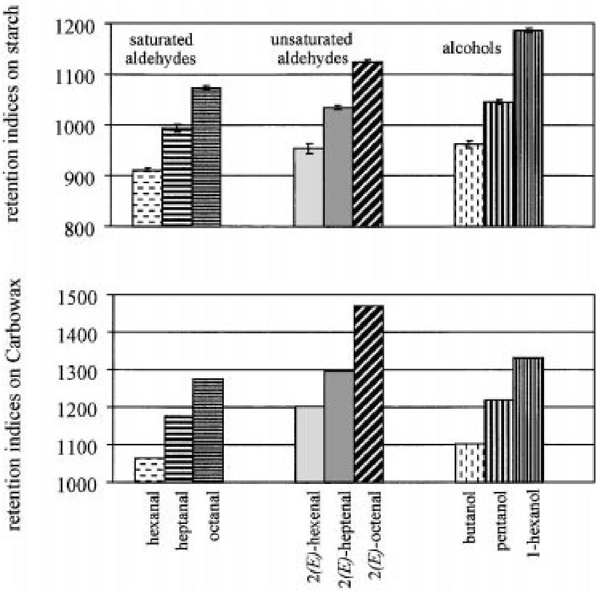

Fig. 5. Retention indices of three series of aroma compounds on native corn starch (10% water content) and Carbowax.

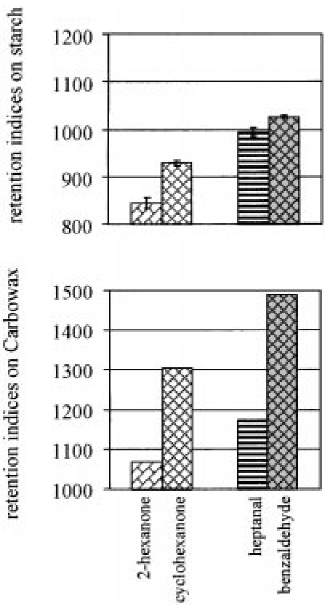

Fig. 6. Comparison of retention indices of cyclic and linear C6 and C7 aroma compounds on native corn starch and Carbowax.

than the corresponding primary alcohol, due to the lower accessibility of its hydroxyl group.

For aldehydes, unsaturated aldehydes and alcohol series (Fig. 5), retention on starch increases with the carbon chain length like on Carbowax. Retention increases with the boiling point of the molecule; this follows the general rule in gas chromatography. The presence of a double bound in each set of aldehydes (C6, C7, C8) greatly increases the RI on Carbowax, and has a weaker effect on starch. The presence of a cyclic group (Fig. 6), especially aromatic, has more influence on the retention in Carbowax than in starch. These differences are most likely related to the two techniques used: liquid-gas chromatography for Carbowax and solid-gas chromatography for starch. With Carbowax, the volatile compounds are partially dissolved in the stationary phase. Because of this solvatation effect, Carbowax creates stronger interactions than starch, especially with unsaturated compounds.

These results lead us to conclude to the existence of hydrogen bonds between aroma compounds and glucose residues of starch, as postulated by Maier (1972) for alcohols, aldehydes and ketones. However, binding volatiles to a food matrix appears to be the sum of different phenomena: surface binding (adsorption), diffusion from the surface into the interior of the matrix, various physical and chemical bindings effects (Hau et al., 1998). Our results agree with these hypotheses; we suppose a predominant adsorption phenomenon (involving hydrogen bonds) and probably a partial diffusion phenomenon in presence of water for hydrophilic compounds. This penetration was observed by BeMiller and Pratt (1981) for water and water-soluble alcohols, in an ungelatinised granule.

4. Conclusion

The control of the pressure and of the humidification of carrier gas in IGC enables maintaining of the starch matrix at a constant water contents between 4 and 10%.

Application of humidified IGC to native corn starch has revealed existence of selective interactions with aroma compounds. These interactions mainly result from an adsorption phenomenon involving hydrogen bonds. Furthermore, for the more hydrophilic compounds, solvatation by water could produce a partial diffusion into the matrix. The IGC approach gives us insight into the dynamics of volatile compounds for a solid matrix like starch.

In further investigations, sorption isotherms of volatiles on starch and solubility coefficients will be determined rapidly with this method. Modification of the physical structure of polymers after thermal treatment could also be investigated in relation to aroma bindings.

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