

Study of Interaction Phenomena between Aroma Compounds and Carbohydrate Matrixes by Inverse Gas Chromatography

Julien Delarue and Pierre Giampaoli*

Laboratoire de Chimie des Substances Naturelles, Département Science de l'Aliment, Ecole Nationale Supérieure des Industries Alimentaires, 1 Avenue des Olympiades, 91744 Massy Cedex, France

Besides the conventional techniques used for the investigation of food–flavor interactions, inverse gas chromatography (IGC) may provide a useful tool for the direct assessment of the retention of aroma compounds in a matrix. IGC consists of single reference compound injections at a fixed temperature onto a column which stationary phase is the material of interest. We have applied this technique to high amylose corn starch, wheat starch, and β -cyclodextrin matrixes, using aroma compounds presenting various functional groups. Low energy interactions were measured which may result from polar or hydrophobic binding. Starch behaved under these conditions as a polar chromatographic phase. We also focused on the influence of a physical modification of the starch on its retention properties toward 3,7-dimethyl-2,6-octadien-1-al. Retention was found to be greater after a specific thermal treatment. As checked by DSC (differential scanning calorimetry) and DMTA (dynamic mechanical and thermal analysis), the observed changes are believed to be related to the glass transition of the starch. For the time being, the dehydration of the matrix associated with the lack of control of the relative humidity within the column is the main restriction to this technique.

Keywords: IGC; carbohydrate; aroma; heat treatment

INTRODUCTION

The understanding of the behavior of volatile flavor compounds in the food matrix is one of the most studied and most challenging issue in food science nowadays as attested by the numerous reviews on the topic in the past few years (Solms and Guggenbuehl, 1990; Plug and Haring, 1994; Bakker, 1995; Leland, 1997).

Many efforts have been made to model and gather data about the partitioning of aroma compounds (Taylor, 1998), the binding properties of food materials (Godshall, 1997), and ultimately the flavor release behavior during the eating process (Overbosch et al., 1991; Linforth and Taylor, 1993). Thus, interaction phenomena are particularly relevant for product development and for technological purposes such as the optimal setting of the operating parameters for food drying (King, 1995), and cooking and baking processes (de Roos and Graf, 1995). One strategy for flavor release control is the proper choice of carrier materials for flavor encapsulation.

Carbohydrates that are present in most food products must be closely considered. They are especially relevant for food products with low water contents. Our knowledge of their interactions with volatile flavor compounds has been recently reviewed by Goubet et al. (1998). It is striking that most studies in the field investigated sorption and interaction behaviors using only more or less sophisticated headspace analysis techniques (Voilley and Bosset, 1986; Elmore and Langley, 1996). Besides, models were developed to predict partition and diffusion of volatile compounds during drying of food products (Etzel, 1993).

However, the behavior of aroma compounds in food may not solely depend on the chemical properties of the components into consideration (Bakker and Mela, 1996;

Roberts et al., 1996). As pointed out by the investigations about flavor encapsulation, it may also depend on the physical properties of the food matrix (Hau et al., 1996; Whorton and Reineccius, 1995). Clearly and despite the many studies led on the topic, there is a need for a holistic approach to food–flavor interactions (Leland, 1997).

Here we aimed to develop a tool for the study of food–aroma interactions in low water content systems. Our approach consisted in the study of the stationary phase as the material of interest in gas chromatography. This technique known as inverse gas chromatography (IGC) or “molecular probe” is derived from synthetic polymer chemistry and was initially developed by Smisrød and Guillet (1969) to study the glass transition of polymers. Few authors used similar methods for characterizing food aroma interactions (Maier, 1969; Castaneda-Bravo, 1975; Smith et al., 1981), mainly to measure sorption isotherms on carbohydrates. However, this approach is likely to provide dynamic information about flavor retention, as related to the physical state of the food matrix.

MATERIALS

We used β -cyclodextrin and two kinds of starch as the carbohydrate stationary phases for the IGC: high amylose corn starch (particle median diameter: 10.2 μm) with an amylose content of 70–80% and less than 1% of extractable lipids; wheat starch (particle median diameter: 20.0 μm) with 25% of amylose and less than 0.1% of extractable lipids. All of them were kindly provided by Roquette Frères (Lestrem, France). We chose starches with different amylose contents because amylose was suspected to be relevant either for aroma retention (Rutschmann and Solms, 1990) and for the physical behavior of the starch. β -Cyclodextrin was chosen as a reference carrier of aroma compounds. Before any experiment, all

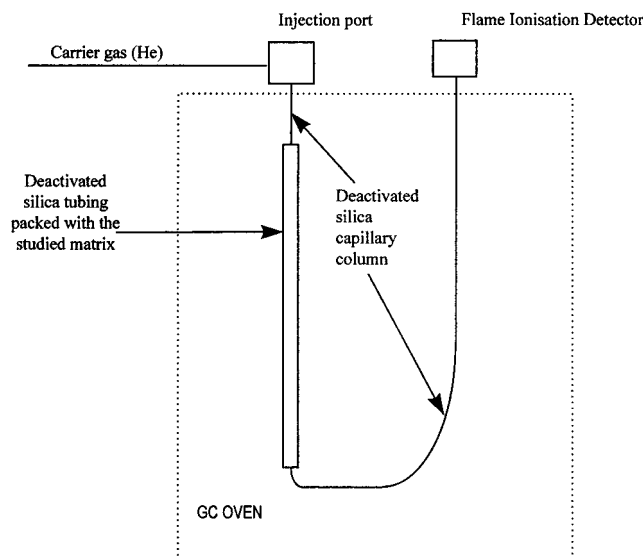


Figure 1. Design of inverse gas chromatography.

these materials have been equilibrated at 11% of relative humidity in a desiccator containing a saturated solution of lithium chloride (Acros Organics).

We selected flavor compounds that presented various functional groups and polarities and that covered a range of high boiling points (150–228 °C). They were also chosen for their close molecular weights (122.2 to 156.3 g/mol). Nonane and 1-octanol were obtained from Merck (Darmstadt, Germany); 2-octanone, decanal, phenylethyl alcohol, and ethyl hexanoate were from Aldrich (Steinheim, Germany); 1-octanethiol was from Elf-Aquitaine. For the determination of the influence of temperature on the starch structure and its consequences on flavor–starch interactions, we used natural 3,7-dimethyl-2,6-octadien-1-al (trivial name: neral) from René Laurent SA (Le Cannet, France).

Inverse Gas Chromatography. The setup for IGC was designed as shown in Figure 1 with a Carlo Erba 6000 Vega II gas chromatograph equipped with a flame ionization detector. The carbohydrate matrix, either starch or β -cyclodextrin, was packed in a deactivated silica tubing (4.5 mm i.d. and 23 cm long) linked to the injection and to the detection systems by a deactivated silica capillary column (i.d. 0.22 mm). The flow rate of the carrier gas (helium) was set at 20 mL/min for a head column pressure of 2.3×10^5 Pa. Small amounts (0.1 μ L approximately) of pure flavor compounds were injected separately in splitless mode with a 1 μ L syringe into the GC and were eluted isothermally. The retention times were determined by a Spectra-Physics SP4290 integrator. The specific retention volume V_g was calculated using eq 1, where j is the James and Martin compressibility factor, F (mL/min) is the carrier gas flow rate, T (K) is the temperature of the flow-meter, W (g) is the weight of stationary phase in the column, t_r (min) is the retention time of the injected flavor compound, and t_0 the retention time of not retained compounds.

$$V_g = j \frac{(t_r - t_0) F 273}{W T} \quad (1)$$

Results from IGC are usually represented in the form of a retention diagram, that is, a plot of $\text{Log } V_g$ against $1/T$. Figure 2 shows the theoretical diagram for a synthetic polymer (Chabert et al., 1973). Three parts can be distinguished on the graph: at low temperatures, the upper part of the curve (1) is a straight segment corresponding to the chromatographic equilibrium. The retention in this region results from the adsorption of the volatile compound on to the surface of the polymer. According to Arrhenius's law the slope of this straight segment is proportional to the enthalpy of adsorption ΔH (kJ/mol) of the injected compound on the surface of the stationary

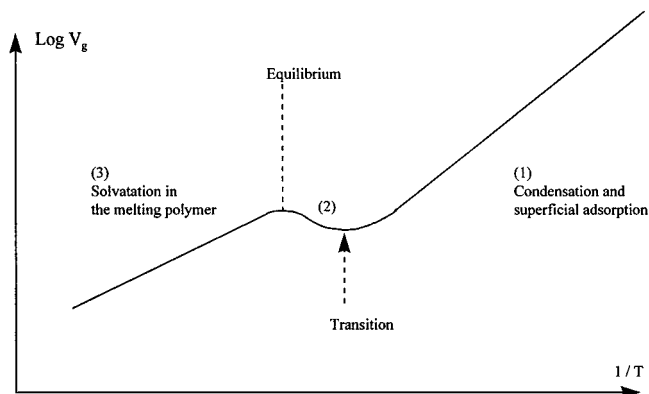


Figure 2. Retention diagram of volatile compounds on a synthetic polymer (in Chabert et al., 1973).

phase as given by eq 2, where T (K) is the temperature of the column and k a constant.

$$\text{Log } V_g = -\frac{\Delta H}{RT} + k \quad (2)$$

An increase in V_g defines a second region (2) where the temperature is high enough to enable macromolecular motions, which correspond to the glass transition. This segment represents a nonequilibrium absorption. The volatile molecules can diffuse in the bulk of the polymer and reach new retention sites. When temperature increases again, a new equilibrium is reached (3) that results from the absorption of the volatile molecules into the amorphous polymer phase.

Repeatability. The observed repeatability when replacing a column with a new packing of the same matrix was satisfactory. The coefficients of variation obtained for the retention times of neral at various temperatures on three different columns packed with the high amylose corn starch were indeed lower than 9% in any case. As for conventional GC, we may consider that the observed differences between the columns are mainly due to the column preparation care.

Heat Treatment of the Starch. The GC oven has been used to induce thermal treatments of the starch directly in the column. These treatments have been applied during 15 to 25 min at several temperatures ranging from 156 to 194 °C.

Differential Scanning Calorimetry. Samples of starch of approximately 40 mg were sealed in stainless steel pans and scanned from 20 °C to 190 °C at 10 °C/min in a Perkin-Elmer DSC7 previously calibrated with indium (mp 156.4 °C). An empty pan was used in the reference holder. The glass transition temperature was determined from the midpoint of the observed heat capacity change using the DSC7 software.

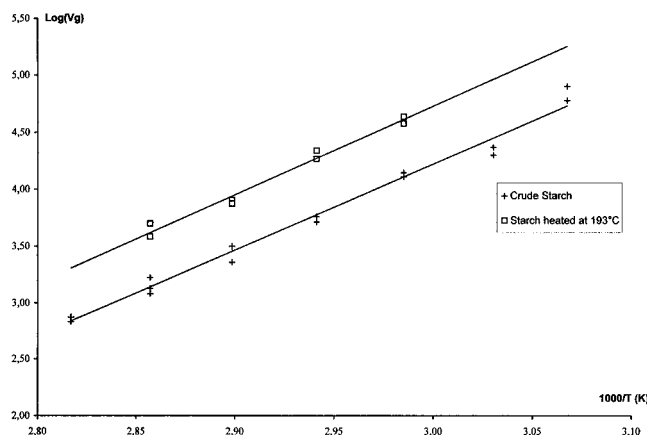
Dynamic Mechanical and Thermal Analysis. For this purpose, the starch samples were previously compressed into small tablets using a hydraulic press (10 tons during 5 min) and cut to obtain $9.5 \times 16.0 \times 1.4$ mm bars. To prevent dehydration, the bars were lubricated with Teflon immediately prior to the analysis. The DMTA measurements were carried out in a two-point bending mode with a Rheometric Scientific DMTA MkIII analyzer. The two cantilever points were 5 mm apart. The smallest available strain corresponding to a 10 μ m displacement was applied at a frequency of 1 Hz. The experiment was run from 60 °C to 230 °C at a heating rate of 5 °C/min. T_g was determined from the midpoint of the decrease in the storage modulus E' , as it is accompanied by a sharp peak in $\tan \delta$ (Kalichevsky et al., 1992).

RESULTS AND DISCUSSION

The retention times of the flavor compounds on wheat starch were compared (Table 1) to those obtained on β -cyclodextrin, which is known to be a good carrier material for aroma molecules (Kollengode and Hanna, 1997). Clearly, the retention of each injected compound on β -cyclodextrin at 100 °C is

Table 1. Comparison of the Retention Time, Indices, and Boiling Point of the Aroma Compounds

compounds	bp (°C)	retention times on wheat starch at 67 °C (min)	retention times on corn starch at 67 °C (min)	retention times on β -cyclodextrin at 100 °C (min)	retention indices on Carbowax 20M	retention indices on OV1
phenylethyl alcohol	220	15.6		>90	1859	1104
1-octanol	194	15.4	7.2	>90	1515	1061
decanal	207	9.5	4.4	32.5	1485	1188
1-octanethiol	197	1.9		4.6	1356	1112
2-octanone	175	1.3		6.2	1304	991
ethyl hexanoate	168	0.7	0.8	4.2	1223	983

**Figure 3.** Retention of neral on a column filled with corn starch.

indeed much higher than that on starch at 67 °C. However, alkanes are not retained on both matrixes.

These data also point out that the retention time is not only influenced by the boiling point of the compound into consideration. That is to say that interactions of some sort occur between the compound and the stationary phase. Furthermore, the comparison with the retention indices for these compounds on traditional GC stationary phases gives us some insights about the nature of these interactions. It can be noted that the retention times on wheat starch are ranked in the same order as the retention indices on Carbowax 20M which is a polar phase. Notably, we observed a preference retention of the alcoholic compounds. These data indicate that at such temperatures, the retention of these flavor compounds results from polar or hydrophilic interactions involving hydroxyl groups from the carbohydrate matrix. The fact that these interactions are likely to occur on the surface of the starch grains (adsorption) may explain the observed difference in retention time between starch and β -cyclodextrin.

In addition to this and as expected, we observed for neral a linear increase of $\text{Log } V_g$ as a function of $1/T$ within a range of medium temperatures [50–100 °C]. The corresponding enthalpy of interaction was -66.3 kJ/mol with high amylose corn starch and -71.6 kJ/mol with wheat starch. It should be noted that at such temperatures these values result both from the heat of vaporization of the neral condensed in the column and from the binding energy between neral and starch as we mentioned above. Using a similar technique, Maier (1969) obtained comparable values [40–54 kJ/mol] for the heat of adsorption of ethanol and ethyl acetate on small carbohydrates (glucose, lactose, and sucrose).

Effect of a Thermal Treatment. Although no retention measurements were possible at temperatures higher than 120 °C because injected compounds were immediately eluted, thermal treatments were applied to the starch alone at temperatures over 170 °C. Figure 3 shows the retention behavior of high amylose corn starch with neral, before and after a thermal treatment at 193 °C. A distinct shift appears on the retention diagram after the starch has been heated. Considering when cooling, the extrapolation of the third segment of the theoretical diagram for a semicrystalline polymer (Figure 2), we may attribute this shift to the glass transition of the starch. The screening of the treatment

Table 2. Experimental Values of T_g Obtained by IGC, DSC, and DMTA

	values of T_g (°C)		
	IGC	DSC	DMTA
wheat starch	173–183	146	not determined
corn starch	173–183	156	166

temperatures allowed us to locate the glass transition zone between 173 and 183 °C either for wheat and high amylose corn starch. No further change in the retention behavior was observed at higher temperatures. In addition to this, no structural change of the starch granules was observed under polarized light after these treatments.

This range of temperatures is comparable to the T_g that we obtained by DSC and DMTA (Table 2), although these techniques led to lower T_g . This can be explained by the higher degree of dehydration occurring during the IGC analysis. Water acts indeed as a plasticizer toward amylose and amylopectin chains of the starch and is known to dramatically affect its glass transition temperature. That is to say, the lower the water content is, the higher the T_g .

It is thus logical to obtain the lowest T_g using the DSC analysis during which the samples are tightly sealed and therefore least subject to dehydration. On the other hand, dehydration cannot be avoided with the DMTA, and the use of a dry carrier gas in IGC results in a complete dehydration of the sample.

It can be noted that we obtained the same T_g by IGC for the two kinds of starch. However, the width of the shift on the retention diagram was much larger for the high amylose corn starch (0.32 Log units) than for the wheat starch (0.08 Log units) which was barely larger than the standard deviation (0.05 Log units). Similarly with DSC, the heat capacity change at the glass transition was more important for the high amylose corn starch.

This result is in agreement with the data from the literature (Biliaderis et al., 1986; Kalichevsky et al., 1992) and may be attributed to the differences in the amylose content since only the bulk amorphous phase of the starch, that mainly consists of amylose, contributes to the heat capacity change associated with the glass transition.

In conclusion we can point out that IGC is a useful technique for testing the retention of aroma molecules on carbohydrates matrixes such as starches or maltodextrins and for evaluating the enthalpy of adsorption of these molecules. This method also allows checking the physical modification of the matrix and its possible consequences on the retention of aroma compounds. The main limit of this method concerns the low water activity of the matrixes, which are imposed by the experimental condition.

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