Modeling Flavor Release Using Inverse Gas Chromatography–Mass Spectrometry

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Inverse gas chromatography (IGC)–mass spectrometry was used to determine the extent of flavor release from a food matrix as a function of moisture uptake. At the surface of a solid, components with higher binding affinities can exchange with and replace components with lower binding affinities. As a low moisture baked product absorbs moisture from the air, flavor is lost from the matrix as water molecules exchange with the flavor molecules. The amount of flavor lost over time can be modeled using this approach to determine the onset of flavor release and total amount of flavor release as well as the identity of the released components and their relative order of exclusion from the matrix.

Keywords: *Inverse gas chromatography; flavor release; modeling*

INTRODUCTION

Inverse gas chromatography (IGC) is typically considered a single-solute chromatographic technique. The shape, height, width, and total area of the eluting peak is used to determine the absorption and desorption characteristics of the probe, or binding component, with the solid adsorbent. The difference between the peak shape of the injected probe with and without the sample tube packed with the solid adsorbent results in the sorption isotherm (*1–3*). The sorption isotherm represents the "partition coefficient" between the probe's concentration in the solid adsorbent matrix to that of the gas phase for the adsorption and desorption cycle. IGC is a dynamic measurement in that the adsorption and desorption phenomena are determined in real time. The advantages over static measurements are that the adsorption and desorption of a component or mix of components can be determined in one experiment rather than several. IGC is therefore suited to the study of equilibrium processes associated with changes on the surface of the sample.

The interaction of the probe with the solid matrix and flavor components present on the surface of the matrix was studied. The relationship between water adsorption and subsequent flavor release was modeled in terms of mass of water introduced, water vapor pressure, and temperature. The thermodynamic parameters $\Delta G°$ and $\Delta H°$ are used to theoretically explain the observed interactions (*4*).

The adsorption of water is one proposed cause of flavor loss from a solid matrix. There is an amount of excess or unbound surface area that water can bind to. Once the water is adsorbed to this area, water will compete for other binding sites with organic molecules already adsorbed on the surface of the solid adsorbent. Due to the excess proportion of water to flavor compounds, and if the thermodynamics of water binding to the surface are favorable, the flavor compounds are excluded from the surface. It was possible to determine the amount of water needed to bind to the excess surface area, corresponding to an onset of increased flavor release from the sample. The amount of flavor release increased as the sample adsorbed water until all available surface-bound flavors were excluded. The actual amount of surface area covered by the flavor molecules is thought to be small, such that the point at which the water will maximally compete with the flavor molecules is thought to be at the water monolayer value for the sample matrix. For a homogeneous matrix, each compound will have a distinct binding energy and will desorb differently. A heterogeneous matrix will have varied binding energies for a given compound, and it will be hard to differentiate any of the desorption processes. The use of mass spectrometry in selected ion mode (MS/SIM) allows for the independent detection of several flavor compounds and the ability to monitor their desorption independently from the sample matrix (*5*).

For a heterogeneous surface, the surface energy is a sum of the binding energy of all binding sites, $\gamma_s = \gamma_s^d + \gamma_s^p$, both those characterized as dispersive, γ_s^d (van der Waals forces) and those characterized as polar, γ_s^p (dipole interactions). Each flavor component adsorbed on the surface has a specific binding energy with each site on the surface. At equilibrium, the binding potential of a flavor component bound to the surface, u_s, and the water in the gas phase, u_g, is the same: $u_s = u_g = u°(T) + RT \ln(P_{i(\text{atm})})$ and $\Delta G° = u_s - u°(T)$, where $P_{i(\text{atm})}$ is the water vapor pressure in atmospheres (*6–8*).

The theoretical basis for equilibrium isotherms is the assumption that equilibrium between the probe in the vapor phase and on the matrix surface is achieved during the course of the experiment. This allows for the calculation of the free energy of adsorption, $\Delta G°$, where $\Delta G° = RT \ln K = RT \ln(P_i/P_0) = \Delta G° - T\Delta S°$, where P_i is the water vapor pressure in the carrier gas and P_0 is the water vapor pressure of saturated air (*9*). The same

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can be said for water in the vapor phase and the flavor component adsorbed on the sample surface.

The partitioning of the released flavor component into the carrier gas stream also needs to be considered. If the partition coefficient for the released flavor component is low, the overall rate of desorption is reduced. It is possible to have a concentration of water vapor in the carrier gas stream that will reduce the partitioning of a flavor component and minimize its loss due to moisture gain. The process involves the competition of the water and flavor molecule for surface area and the rate of partitioning of either water or the flavor component into the carrier gas stream. An increase in the amount of water vapor will increase the probability of its successfully competing for the binding sites. If at the increased water vapor pressure the partitioning coefficient decreases, it will increase the overall thermodynamic value ΔG. There will be a point at which the rate of flavor loss is at a maximum over a range of water vapor pressures: $\Delta G = (RT \ln K_{pi})_{flavor} + [RT \ln(P_i/P_o)]_{water}$. At that point the partition coefficient, K_{pi}, is at a maximum. That point can be found by varying P_i/P_o at constant T.

MATERIALS AND METHODS

Instrumentation. Instrumentation consists of an Agilent 6890 gas chromatograph interfaced to an Agilent 5973 mass selective detector (MSD). A splitless injector is used for the introduction of the probe (water) along with either a flame ionization detector or MSD to monitor the effluent. The column comprised $^1/_{16}$ in. stainless steel tubing ~30 cm long connected to a $^1/_4$ in. i.d. × 5 cm aluminum tube packed with ~100 mg of the solid adsorbent. The aluminum tube is connected to another 30 cm length of $^1/_{16}$ in. stainless steel tubing and attached to a $^1/_{16}$ in. tee allowing flow of the effluent into the flame ionization detector and the MSD. A 1 m length of 0.25 mm i.d. uncoated fused silica capillary tubing (Hydroguard FS, Restek Corp.) is used to restrict the flow into the MSD to 2 mL/min. Column flow rates are 20−60 mL/min with the injector temperature at 125° C and the oven temperatures varying from 30 to 70 °C. Injection volumes varied from 20 to 100 μL. The MSD was used in the SIM to measure the absorption/desorption of several desorbed components simultaneously from the sample. Ions monitored by SIM were predetermined on the basis of flavor compounds known to exist in the sample. Data were collected using standard chromatography (Turbochrom Workstation version 6.1, Perkin-Elmer Corp.) and mass spectrometry software (ChemStation software, rev. B, Agilent).

Sample Preparation. Samples were ground with a mortar and pestle and sieved to obtain a particle size range between 50 and 70 mesh (CE Tylor Inc., Mentor, OH). Sample particle size is important because the surface area and amount and rate of flavor release vary with particle size. Samples were protected from moisture and stored in an airtight container until used. Samples were used as received and not dried; initial moisture and relative humidity measurements were made.

RESULTS AND DISCUSSION

Methodological parameters were established using a buttery flavored cracker. Water was used as the probe to compete with organic compounds already adsorbed onto the solid matrix, the cracker. A typical response curve is shown in Figure 1 and demonstrates that peak height represents the maximum vapor pressure that can be achieved by the probe and that the area of the peak is proportional to the mass of probe injected. This allows for the determination of the vapor pressure as a function of peak height. The ratio of $(P_i/P_o) \times P_{v,water} = P_{v,i}$, where
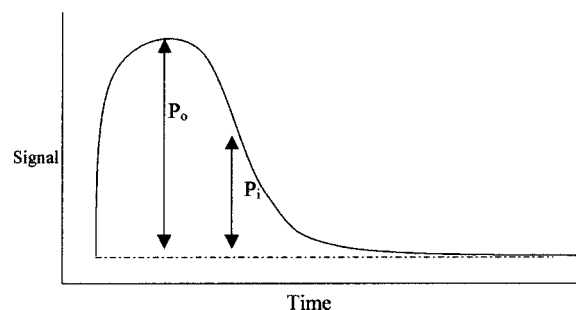
Figure 1. Typical response for water from an IGC experiment. Peak area is proportional to mass of the probe. P_o is the maximum peak height, and P_i is the signal height at time i.
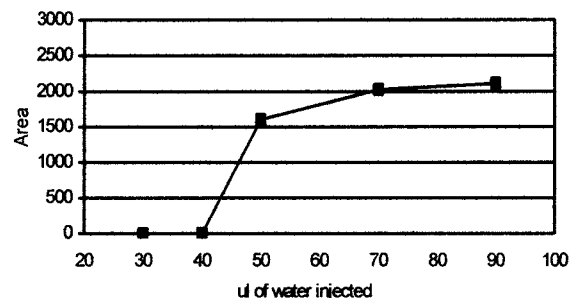

**Figure 2.** Total flavor released at 50 °C, 40 mL/min air, and 100 mg sample size.

$P_{v,water}$ is the vapor pressure of pure water at the temperature of interest and $P_{v,i}$ is the actual water vapor pressure of the carrier gas at time i.

A series of injections of water are made to determine the amount of water needed to bind to the excess surface area on the matrix. The excess surface area is thought to be filled at the inflection point in the curve. This value correlates with the moisture value at the inflection point. At that point, the water is competing for binding sites on the surface and flavor compounds are excluded from the surface and removed from the matrix by the carrier gas stream. Additional volumes of water do not increase the total amount of flavor removed because there is a finite amount of surface area for either the water or flavor to bind. To determine the actual amount of water that binds, a blank is analyzed along with the sample and the sample area is subtracted from the blank to yield the amount and rate of water absorption (*10*). In a separate set of experiments, the water monolayer value was determined to be 4.5% at a P_i/P_o ratio of 0.42. A correction is required to account for any moisture initially present in the sample. The ratio of maximum peak height of the blank to actual height of the sample gives the quantity P_i/P_o or relative humidity.

Figure 2 demonstrates the amount of water needed to bind to the excess surface area and the effect of subsequent amounts of water resulting in a release of organic compounds from the matrix. Approximately 4% of the water volume injected was adsorbed by the matrix. The total ion signal is due to several components (see Table 1). To determine the individual effects of water on the release of these organic compounds, MS/SIM was used to follow the desorption of four separate ions representing six compounds. The ions monitored are not unique to these compounds but do provide a reasonable marker. Figure 3 shows the desorption isotherms measured using MS/SIM. The desorption rates are distinguishable and different for the various
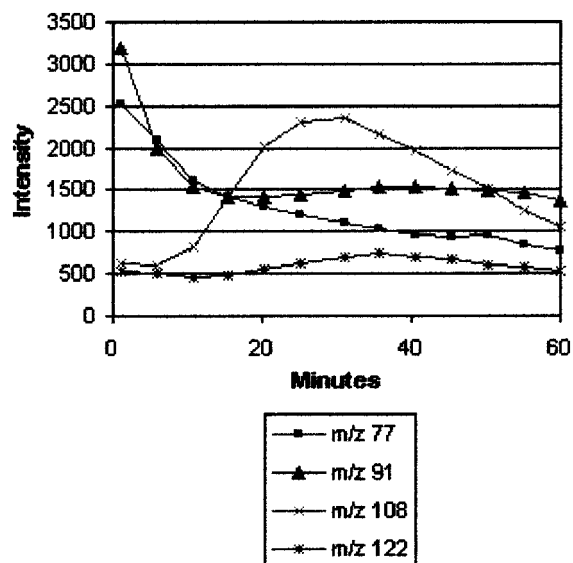
Figure 3. Flavor desorption isotherms: 50 °C, 40 mL/min He, and 150 mg sample weight.
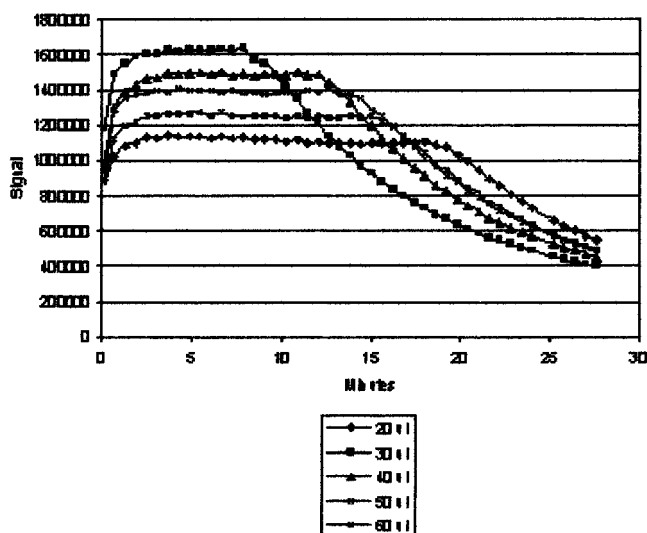

Figure 4. Water isotherms generated at 50 °C and 40 mL/min He using MSD (*m/z* 18).

Table 1. Compounds and *m/z* Ratios Monitored

m/z ratio	compound
77	benzaldehyde
91	tropyllium ion
108	dimethylpyrazine
122	ethylmethylpyrazine

ions monitored. Dimethylpyrazine (*m/z* 108) is desorbed more quickly than ethylmethylpyrazine (*m/z* 122), which indicates that moisture adsorption will affect and change the flavor profile and perceived flavor. This demonstrates the ability of IGC-MS/SIM to follow successfully the competitive adsorption of water and desorption of multiple compounds. Equilibrium between the probe, water, in the vapor phase and the sample is not achieved during the chromatographic run time. In Figure 3, the sudden jump in the *m/z* 77 ion profile is thought to be due to a structural collapse or phase change due to the amorphous structure of the sample. There will be a point at which the rate of flavor release is at a maximum; this will occur when ΔG is at a minimum. Figures 4 and 5 show that regardless of the amount of water injected, the maximum release of acetic
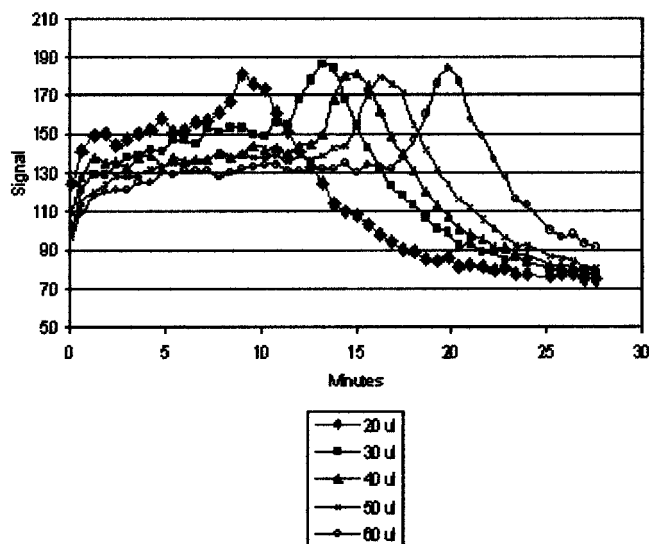
Figure 5. Resulting desorption of acetic acid generated at 50 °C, 40 mL/min He, and 100 mg sample weight using MSD (*m/z* 60).

Table 2. P_i/P_o Ratio at Maximum K_{pi} for Acetic Acid

	20 μL of water	30 μL of water	40 μL of water	50 μL of water	60 μL of water
50 °C/40 mL	0.910	0.920	0.939	0.946	0.909
50 °C/60 mL	0.881	0.865	0.900	0.939	0.924

acid occurs at an average P_i/P_o of 0.91 (CV = 2.8%), $\Delta G = (RT \ln K_{pi}) = [RT \ln(P_i/P_o)]$. There appears to be a relationship between K_{pi} and P_i/P_o (Table 2) such that the relative rates and amounts of flavor release can be modeled using the vapor pressure of water and temperature.

SAFETY

There are no specific safety issues associated with these experiments.

ABBREVIATIONS USED

IGC, inverse gas chromatography; IGC-MS, inverse gas chromatography−mass spectrometry; IGC-MS/SIM, inverse gas chromatography−mass spectrometry, selected ion mode; MSD, mass selective detector.

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