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Inverse Gas Chromatographic Method for Measurement of Interactions between Soy Protein Isolate and Selected Flavor Compounds under Controlled Relative Humidity

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An inverse gas chromatographic (IGC) method was developed to study the binding interactions between selected volatile flavor compounds and soy protein isolate (SPI) under controlled relative humidity (RH). Three volatile probes (hexane, 1-hexanol, and hexanal) at very low levels were used to evaluate and validate system performance. On the basis of the thermodynamic data and the isotherms measured at 0% RH, 1-hexanol and hexanal had higher binding affinities than hexane, which could be attributed to hydrogen-bonding interactions with SPI. At 30% RH, 1-hexanol and hexanal were retained less than at 0% RH, indicating possible competition for binding sites on the SPI surface between water and volatile probe molecules. Results showed that the thermodynamic data determined were comparable to the available literature values. Use of IGC allowed for the rapid and precise generation of sorption isotherms. Repeatability between replicate injections and reproducibility across columns were very good. IGC is a potentially high-throughput method for the sensitive, precise, and accurate measurement of flavor−ingredient interactions in low-moisture food systems.

KEYWORDS: Soy protein; flavor; binding; relative humidity; inverse gas chromatography

INTRODUCTION

Flavor is an important factor in determining food product acceptance. Despite the well-recognized health benefits associated with the consumption of soy foods (*1*, *2*), these products have not received widespread acceptance. This is mainly due to a number of flavor problems associated with soy foods (*3*−*5*). Aside from the occurrence of off-flavors, soy proteins can bind with certain flavor compounds, making it difficult to properly flavor soy products (*3*−*6*). Therefore, understanding the nature of the binding of flavor compounds by soy proteins is essential for the development of formulated soy products with acceptable flavors.

Interactions of aroma compounds with soy proteins have been investigated mainly in aqueous soy protein systems by use of static headspace and equilibrium dialysis techniques (*7*−*11*). Although these studies provide useful information such as binding constants and the number of binding sites, they lack the necessary sensitivity to enable study of the problem under conditions that simulate real food systems. Furthermore, the above methods are unsuitable for the study of dry and semidry food systems.

Few studies were found in the literature concerning flavor−soy protein interactions in low-moisture systems (*12*, *13*). In these two studies, valuable thermodynamic data of adsorption were determined for selected volatiles under dry conditions; however, the influence of water on the retention of volatiles by soy proteins was not evaluated. In contrast to the abundance of water sorption isotherms reported for various food ingredients, isotherms describing the sorption of volatile compounds on food materials are very limited (*14*), and to our knowledge volatile sorption isotherms on soy proteins have not been reported.

Inverse gas chromatography (IGC) was applied in the present study to investigate the interactions of selected flavor compounds with soy protein isolate (SPI). In contrast to conventional GC, a nonvolatile substance is the subject of interest in IGC. By preparing the GC column with the nonvolatile material being studied and injecting a series of known amounts of volatile probes of defined properties, valuable information on the surface chemistry of the solid substance and the thermodynamic properties of the sorbate−sorbent system can be obtained (*15*−*19*).

The advantages of IGC over the traditional equilibrium methods include simplicity, speed, and accuracy (*16*, *17*). Due to the extreme sensitivity provided by the detector, IGC is capable of measuring adsorption of flavor compounds at very low levels (nanograms to micrograms per gram) to closely simulate conditions encountered in real foods. In addition, IGC is suitable for the study of dry and semidry materials. IGC has become a powerful tool for the characterization of surface properties of polymeric materials and has been shown to have wide application in many disciplines (*14*−*22*).

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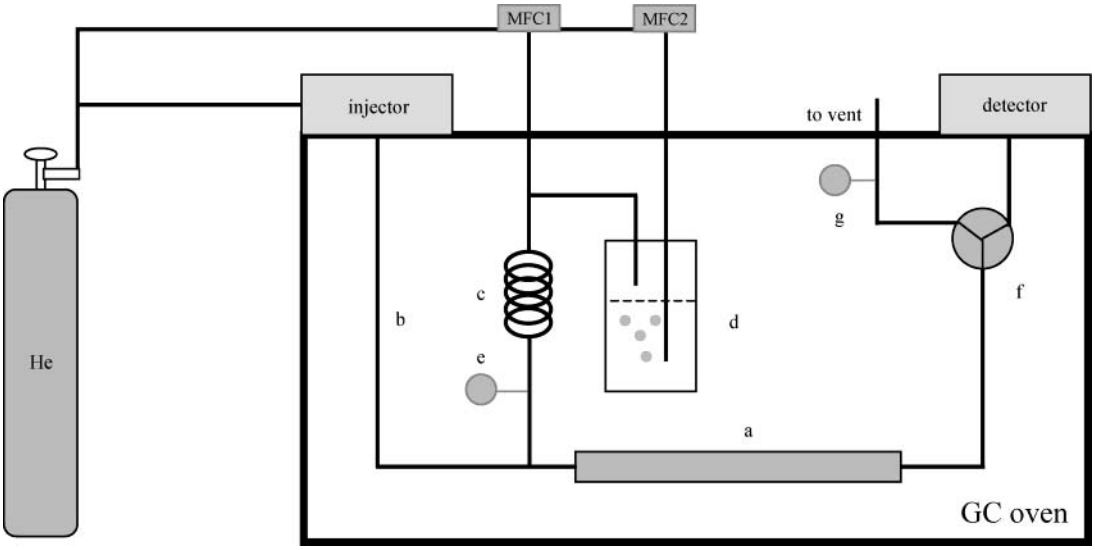

Figure 1. Schematic diagram of the inverse gas chromatography system used in this study; components: (a) IGC column; (b) capillary flow restrictor; (c) mixing loop; (d) water bubbler; (e) pressure gauge; (f) three-port valve; (g) temperature/relative humidity probe.

The objective of our study was to develop a rapid and sensitive IGC method for the study of flavor−soy protein interactions under controlled relative humidity. Thermodynamic data and sorption isotherms of selected volatile probes were determined to evaluate and validate system performance.

MATERIALS AND METHODS

Volatile Compounds. Hexane, hexanal, and 1-hexanol of purity >98% were obtained from Aldrich Chemical Co. (St. Louis, MO).

Soy Protein. A representative commercially available SPI (ProFam 974; protein content > 90%; fat content < 4%) was provided by Archer Daniels Midland Co. (ADM; Decatur, IL). Freshly received sample was sieved to obtain particle sizes between 129 and 145 μm.

Column Preparation. The sieved SPI sample was packed into a deactivated glass tube (17.8 cm × 4 mm i.d.; Supelco, Bellefonte, PA) under vacuum and with the aid of a mechanical vibrator. Both ends of the column were plugged with deactivated glass wool and stainless steel screens. Each column contained ~0.76 g of SPI and was ~12 cm in length. Prior to an experiment, each new column was connected to the IGC system and conditioned under carrier gas for 48 h to remove any volatile contaminants. Whenever the temperature was changed, the column was reconditioned for at least 12 h to ensure that the new equilibrium condition was established.

IGC Instrument. A conventional GC (6890 series; Agilent Technologies, Inc., Palo Alto, CA) equipped with a flame ionization detector (FID) was modified for IGC. A schematic diagram of the apparatus is shown in **Figure 1**. Ultrahigh-purity helium was used as carrier gas. The packed column (a) filled with SPI sample was connected to the injector using a deactivated fused silica capillary column (b, Hydrogard, 2 m × 50 μm i.d.; Restekm Bellefonte, PA), which merged with the carrier gas at the head of the packed column. The relative humidity of the carrier gas was controlled by mixing (c) dry and humidified (obtained by passing dry helium through a bottle (d) filled with distilled water) carrier gas in the proper ratio. Flow rates of the dry and humidified carrier gases were each independently controlled by mass flow controllers (MFC1 and MFC2, respectively; Pneucleus Technologies, LLC, Hollis, NH). A digital pressure gauge (e, PTC Electronics, Inc., Wyckoff, NJ) was installed upstream of the packed column to measure the column head pressure. Uncoated Silcosteel tubing (10 cm × 1.02 mm i.d.; Restek) was used to connect the end of the packed column to a three-port valve (f, Valco Instruments Co. Inc., Houston, TX), which was included so that column exit flow could be directed to either the FID or a temperature/RH probe (g, HMP42; Vaisala, Woburn, MA) prior to experiments to ensure that the desired conditions were established.

Other connections were made using 1.02 mm i.d. Silcosteel tubing (Restek). All essential components of the system and the connection tubing were housed inside the GC oven (**Figure 1**), which provided accurate (±0.2 °C) temperature control as verified by a calibrated external temperature probe (HMP42; Vaisala). Injector and detector temperatures were set at 250 °C, and the GC oven was operated under isothermal conditions (30, 35, or 40 °C). Carrier gas flow rate was set at 20 mL min^{-1} for all experiments. A computer program (HP FlowCalc 2.0; Agilent Technologies, Palo Alto, CA) was used to calculate the required inlet pressure based on the actual column head pressure under specific experimental conditions. Injections were achieved by using a multipurpose sampler (MPS2; Gerstel Inc., Baltimore, MD).

In a typical experiment, a known amount of neat flavor compound was injected with a 0.5 μL syringe (SGE Inc., Austin, TX) using split injection mode. To calculate the thermodynamic parameters of adsorption, trace amounts of the volatile probes were injected to approach infinite dilution. From the retention times recorded, the net retention volume V_N (mL) was calculated (*16*, *22*) as

$$V_N = (t - t_0)F_c \quad (1)$$

where t (min) and t_0 (min) are the retention times of a probe and of a nonretained compound (such as methane), respectively, and F_c (mL min^{-1}) is the corrected carrier gas flow rate:

$$F_c = F \times \frac{T_c}{T_f} \times \frac{P_f}{P_o} \times j \quad (2)$$

where F (mL min^{-1}) is the exit flow rate at the column outlet (atmosphere); T_c and T_f (K) are the temperatures of the column and the flow meter, respectively; P_f (psi) is the pressure in the flow meter; P_o (psi) is the column outlet pressure; and j is the James and Martin compressibility factor, which is calculated as

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

where P_i (psi) is the column inlet pressure.

Specific retention volume V_g (mL g^{-1}) was defined as

$$V_g = V_N \times \frac{273}{T_c} \times \frac{1}{m_s} \quad (4)$$

where m_s (g) is the mass of the stationary phase.

Enthalpy (ΔH_s) and free energy (ΔG_s) of adsorption were given by (*16*, *22*)

$$-\frac{\Delta H_s}{R} = \frac{\partial(\ln V_g)}{\partial(1/T)} \quad (5)$$

$$\Delta G_s = -RT_c \ln K_p \quad (6)$$

where R is the universal gas constant and K_p is the partition coefficient, calculated as

$$K_p = V_N/V_p \quad (7)$$

where V_p (mL) is the volume of the stationary phase, which can be calculated from the following equations:

$$V_p = V - V_0 \quad (8)$$

$$V_0 = t_0 F_c \quad (9)$$

where V (mL) is the volume of the column and V_0 (mL) is the interstitial volume.

Then the entropy of adsorption ΔS_s (kJ mol^{-1} K^{-1}) can be estimated according to the following equation:

$$\Delta S_s = \frac{\Delta H_s - \Delta G_s}{T} \quad (10)$$

To measure the sorption isotherms, increasing amounts of the volatile probes were injected. Sorption isotherms were constructed on the basis of the method of Kiselev and Yashin (*16*). Sorbate partial vapor pressure p (Pa) and the corresponding sorbate uptake N_a (μmol g^{-1}) were calculated from the following equations:

$$p = \frac{NRT_c h}{F_c A_p} \quad (11)$$

$$N_a = A_{ads} N/A_p m_s \quad (12)$$

where N (μmol) equals the moles of the probe injected; h (pA) is peak height; A_p (pA min) is the peak area; A_{ads} (pA min) is the area bounded by the retention time of a nonretained peak, the peak height, the diffused side of the peak, and the x-axis (time).

The sorption constant S (μmol g^{-1} Pa^{-1}) was determined from the initial slope of the isotherm:

$$N_a = Sp \quad (13)$$

Whenever RH is used in this paper, average relative humidity ($\overline{RH}$) is inferred due to the pressure gradient that exists along the column. $\overline{RH}$ was calculated as

$$\overline{RH} = RH_o/j \quad (14)$$

where RH_o is the relative humidity measured at the column outlet at atmosphere and j is the James and Martin compressibility factor.

Data acquisition and area integration were achieved using HP Chemstation software (Agilent Technologies). Experiments were performed in duplicate and on three (at 0% RH) or two (at 30% RH) different columns, with the average values reported. Statistical analysis (Student t test) was performed to evaluate the effect of RH on volatile probe retention ($p \leq 0.05$).

RESULTS AND DISCUSSION

Evaluation and Validation of System Performance. Results showed excellent repeatability between replicate injections and very good reproducibility across columns. In general, variation was <3% between replicate determinations on the same column and <6% between different columns. For example, **Figure 2** shows isotherms determined for hexanal on three different columns. High sensitivity and excellent precision are essential for measurement of flavor−ingredient interactions, because volatile compounds are generally present in foods at only trace levels.

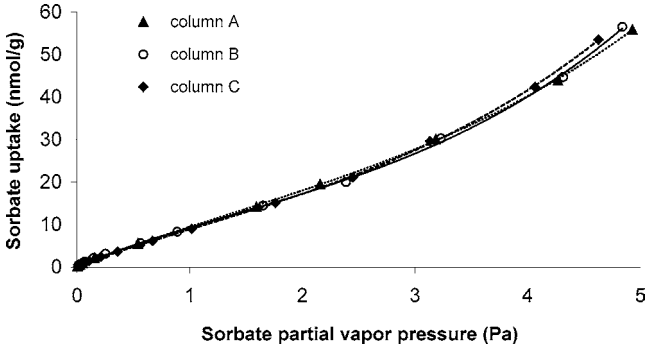

Figure 2. Comparison of isotherms determined for hexanal at 35 °C and 0% RH on three different columns.

Table 1. Comparison of $-\Delta H_s$ and $-\Delta G_s$ Values Determined in This Study with Those Published on Soy Protein Isolates Using Similar Methods at 0% Relative Humidity

parameter	compound	this study, on ProFam 974	ref *12*, on EdiPro A	ref *13*, on EdiPro A
$-\Delta H_s$ (kJ mol^{-1})	hexanal	43.0	37.2	46.4
	1-hexanol	68.4	58.1	69.8
$-\Delta G_s$ (kJ mol^{-1})	hexanal	5.8[a]	4.1[b]	*c*
	1-hexanol	12.4[a]	11.5[b]	*c*

[a] Determined at 40 °C. [b] Determined at 80 °C. [c] Data not available.

Table 2. Comparison of $-\Delta H_s$ Values (Kilojoules per Mole, ± Standard Deviation) Determined for Individual Compounds at 0 and 30% Relative Humidity

RH	hexane	hexanal	1-hexanol
0%[a]	28.3 ± 0.4	43.0 ± 1.7	68.4 ± 2.1
30%[b]	27.5 ± 1.0	35.9 ± 2.1	54.4 ± 1.8

[a] Average from three columns and a total of six replicated measurements. [b] Average of two columns and a total of four replicated measurements.

Thermodynamic data determined under dry conditions were compared with available literature values. The ΔH_s values determined for hexanal and 1-hexanol (**Table 1**) were similar to literature values determined for SPIs using similar methods (*12*, *13*). The ΔH_s values determined herein (−28.3 to −68.4 kJ mol^{-1}) were within the same order of magnitude as those previously determined for polar substances such as lactose (−48.6 and −73.4 kJ mol^{-1} for hexanal and 1-hexanol, respectively; *23*) and cellulose (−47.0 and −57.1 kJ mol^{-1} for heptane and octane, respectively; *18*). Also, the ΔG_s values determined for hexanal and 1-hexanol at 40 °C were comparable to published values determined on SPI at 80 °C (*12*; **Table 1**).

Results also demonstrate the rapid and high-throughput nature of this method for sorption isotherm measurement. Depending on the volatile probe and the conditions being studied, the amount of time required to obtain an isotherm is generally only a few hours. It is well-known that with conventional methods (such as use of desiccators) usually days or even weeks are required to obtain a water sorption isotherm. Even though sorption isotherms of volatile compounds determined on food materials are scarcely found in the literature, it can be estimated that a large amount of time would be required to measure a volatile sorption isotherm if traditional methods are used. In addition to being time consuming, conventional methods for

Table 3. $-\Delta G_s$, $-\Delta S_s$, and S Values (± Standard Deviation) Determined for Individual Compounds at 0 and 30% Relative Humidity

		hexane		hexanal		1-hexanol	
	temp (°C)	0% RH[a]	30% RH[b]	0% RH[a]	30% RH[b]	0% RH[a]	30% RH[b]
$-\Delta G_s$ (kJ mol^{-1})	30	0.07 ± 0.06	0.10 ± 0.05	6.87 ± 0.15	6.05 ± 0.07	14.1 ± 0.2	11.1 ± 0.1
	35	0.46 ± 0.10	0.39 ± 0.05	6.50 ± 0.10	5.60 ± 0.00	13.4 ± 0.1	10.4 ± 0.1
	40	0.76 ± 0.07	0.84 ± 0.08	5.80 ± 0.10	5.15 ± 0.07	12.4 ± 0.1	9.60 ± 0.0
$-\Delta S_s$ (kJ mol^{-1} K^{-1})	30	93.6 ± 1.3	90.7 ± 2.6	119 ± 5	98.5 ± 6.8	179 ± 6	143 ± 6
	35	93.4 ± 1.7	90.4 ± 2.8	119 ± 5	98.4 ± 6.9	179 ± 7	143 ± 6
	40	92.9 ± 1.4	90.4 ± 2.7	119 ± 5	98.2 ± 7.0	179 ± 6	143 ± 6
S (nmol g^{-1} Pa^{-1})	30	0.38 ± 0.01	0.38 ± 0.05	19.8 ± 2.4	5.28 ± 0.39	187 ± 7	39.5 ± 0.3
	35	0.29 ± 0.02	0.31 ± 0.03	16.3 ± 1.9	4.11 ± 0.18	120 ± 6	27.7 ± 0.4
	40	0.24 ± 0.01	0.24 ± 0.03	11.6 ± 0.8	3.33 ± 0.12	78.8 ± 4.3	19.4 ± 0.3

[a] Average from three columns and a total of six replicated measurements. [b] Average of two columns and a total of four replicated measurements.

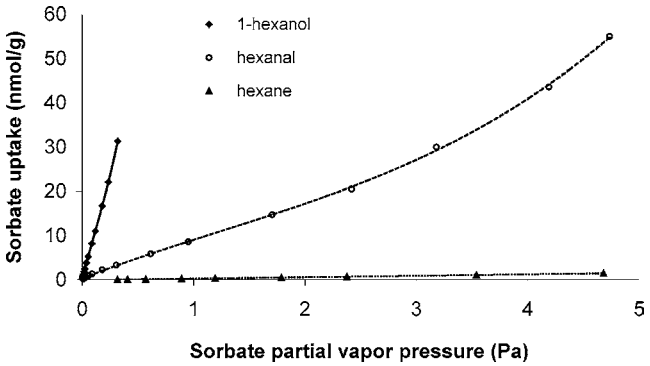

Figure 3. Comparison of isotherms determined for three volatile probes at 35 °C and 0% RH.

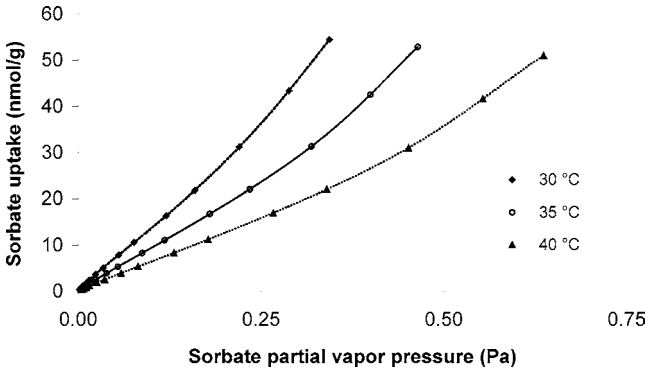

Figure 4. Comparison of isotherms determined for 1-hexanol at 0% RH at different temperatures.

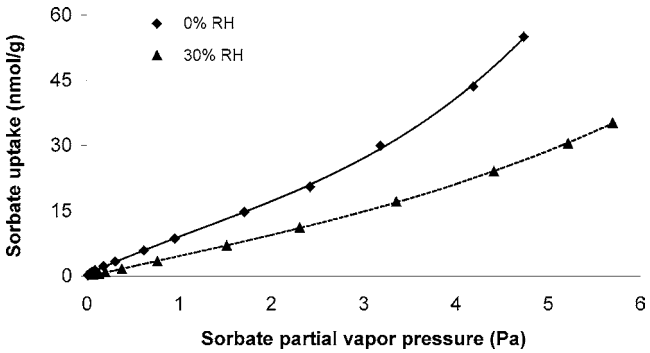

Figure 5. Comparison of isotherms determined for hexanal at 35 °C and 0 or 30% RH.

measuring isotherms lack sensitivity and accuracy, especially at the very low sorbate concentration region, where data are generally not easily obtainable.

Thermodynamics of Adsorption. At infinite dilution, only absorbate−absorbent interaction is important and the enthalpy of adsorption (ΔH_s) reflects the strength of interaction between absorbate and absorbent (*16*). On the basis of the determined ΔH_s values, hexane had the lowest and 1-hexanol had the highest binding affinity to SPI at both RH conditions evaluated (**Table 2**). Because these three sorbates have the same carbon number, the observed difference in their binding affinities to SPI must be attributed to the different functional groups they carry. For hexane, possibly only weak forces (e.g., van der Waals force) were involved, resulting in the weak affinity observed, as is generally the case for saturated hydrocarbons. However, hydrogen bonding was likely to be involved at 0% RH in the case of both hexanal and 1-hexanol as suggested by the differences in their ΔH_s values over that of hexane, which are comparable to the energy of forming a hydrogen bond (10−40 kJ mol^{-1}; *23*). The much higher ΔH_s values obtained for 1-hexanol suggest that either hydrogen bonding of higher energy or more than one hydrogen bond was involved. Similar trends in binding potentials for the above compounds to polar macromolecules have been reported (*12*, *13*, *23*).

By comparing the ΔH_s values determined for each compound at different RHs, it was found that an increase of RH from 0 to 30% did not affect the binding of hexane to SPI. However, retention of hexanal and 1-hexanol by SPI was less favorable at 30% RH (**Table 2**), which may be attributed to the competition of binding sites on the SPI surface between these volatile probes and water molecules. Because water is highly polar in nature, it can readily interact with some of the high-energy polar binding sites on the SPI surface through the formation of hydrogen bonds, resulting in decreased availability of binding sites for flavor compounds and hence the less favored retention observed.

Other thermodynamic parameters determined also agree with the above findings. As shown in **Table 3**, the negative ΔG_s values determined for hexanal and 1-hexanol indicated the spontaneous nature of the adsorption process; the more negative ΔG_s values determined for 1-hexanol further suggest its higher affinity to SPI. The ΔS_s values determined for 1-hexanol were more negative that those for hexane and hexanal (**Table 3**), indicating that it had lost more freedom of motion than the other compounds, which is consistent with the above observation that much stronger interactions were involved between 1-hexanol and SPI. However, it should be noted that apparent measurements were implied in the above discussion and hereafter on the binding affinities of volatile probes to soy protein. This is due to the fact that commercial SPIs usually contain a small amount of lipid (<4% in the SPI used in this study), which may have some influence on these interactions.

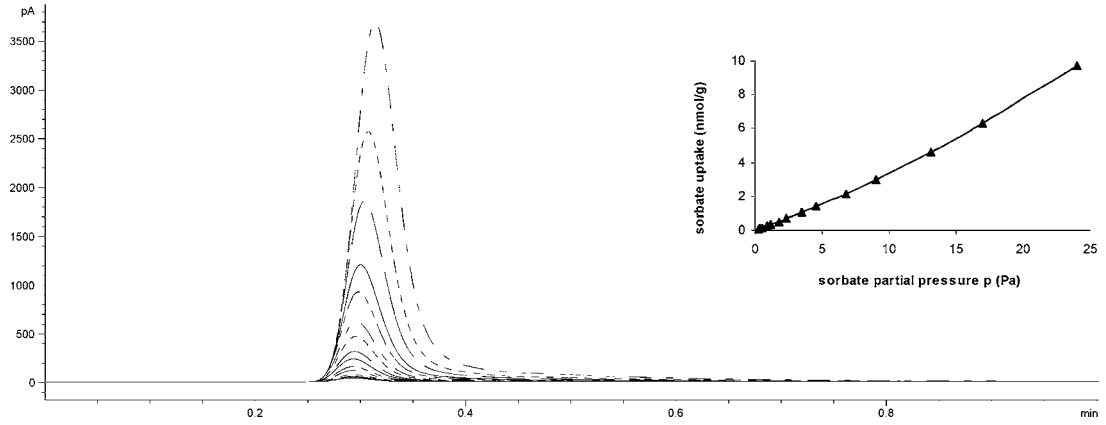

Figure 6. Superimposed chromatographic peaks obtained with increasing mass of sorbate and the corresponding sorption isotherm determined for hexane at 35 °C and 0% RH.

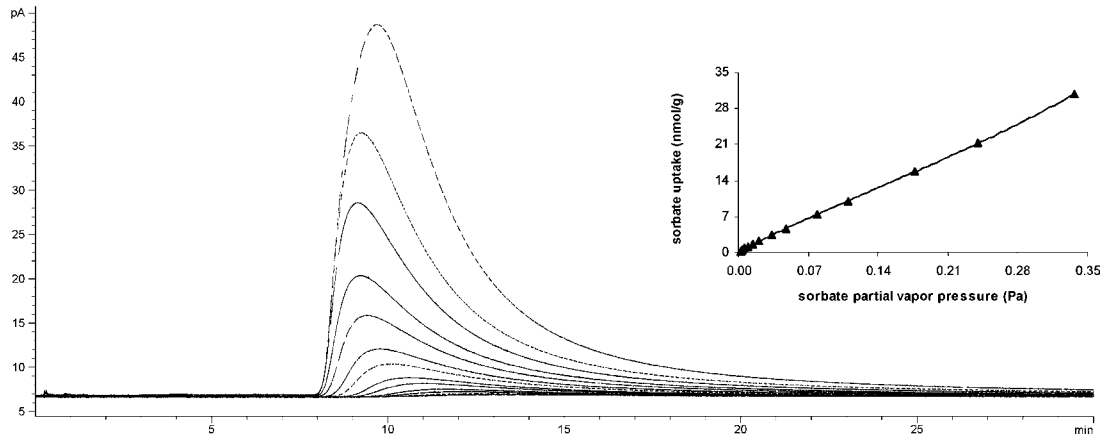

Figure 7. Superimposed chromatographic peaks obtained with increasing mass of sorbate and the corresponding sorption isotherm determined for 1-hexanol at 35 °C and 0% RH.

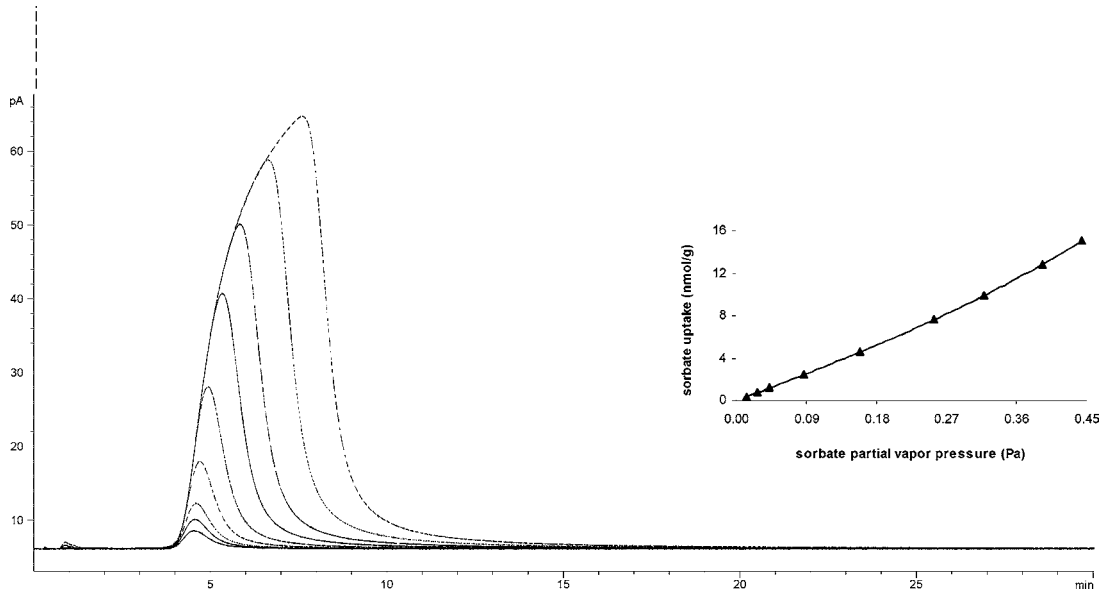

Figure 8. Superimposed chromatographic peaks obtained with increasing mass of sorbate and the corresponding sorption isotherm determined for 1-hexanol at 35 °C and 30% RH.

Sorption Isotherms. Figure 3 shows the comparison of the isotherms determined at 0% RH and 35 °C for individual compounds. Hexane exhibited a type III isotherm according to the BET classification, indicating weak adsorption. Meanwhile, type II isotherms were observed for hexanal and 1-hexanol (**Figure 3**), which suggests their moderate binding affinities to SPI. In addition, the concave profile in the low sorbate vapor pressure region, characteristic of type II isotherms, indicates that binding sites of different energy levels exist. **Figure 4** is a comparison of the isotherms determined for 1-hexanol at

different temperatures. As expected, adsorption was less favored at higher temperature, which is characteristic of a physical adsorption process (*12*).

The sorption isotherms also reflect the influence of water on the binding affinities of volatile compounds to SPI. At 30% RH, type III instead of type II isotherms (as was the case at 0% RH) were observed for hexanal and 1-hexanol. The less favored retention of hexanal by SPI at 30% RH versus that at 0% RH is clearly seen by plotting the isotherms determined at both conditions on the same curve (**Figure 5**). Similar findings were found for 1-hexanol. Meanwhile, retention of hexane at 0 and 30% RH did not differ.

Sorption constants (*S*) determined from the initial linear portion of the isotherms also demonstrate the differential binding affinities of the volatile probes to SPI (**Table 3**) and were consistent with the other data discussed above.

The chromatographic peak profiles observed also correlate well with the sorption isotherms determined. For example, peak maxima of hexane increased as mass of sorbate increased, correspondingly, a type III isotherm was determined (**Figure 6**). On the other hand, peak maxima obtained for 1-hexanol at 0% RH initially decreased with increased mass of sorbate, then became constant, and then increased as sorbate vapor pressure further increased, which is characteristic of substances exhibiting type II isotherms (**Figure 7**); however, only weak interaction was indicated at 30% RH (**Figure 8**).

At infinite dilution, nearly symmetrical peaks were always observed for hexane, whereas slightly tailed peaks were observed for both hexanal and 1-hexanol at 0% RH. The observed tailing suggests that some of the absorbate molecules were strongly retained (*16*, *24*), resulting from the heterogeneous soy protein surface as is common for biopolymers. Tailed peaks were observed by other researchers for 1-hexanol and octanal on polar materials (*13*). However, peak tailing may also indicate the existence of kinetic processes such as bulk diffusion. Considering the fact that relatively low temperatures (30−40 °C) were used in the current study, at which soy protein exists in its glassy state (the reported T_g for dry soy protein is 103−160 °C; *25*, *26*), diffusion was expected to be very slow and negligible, with surface adsorption being primarily responsible for retention.

The results of this study demonstrate that sorption isotherms correlate well with the thermodynamic data. Together they provide useful information on the thermodynamic aspects of flavor−soy protein interactions, which may be useful in real-world applications. For example, information on the relative binding strengths of individual flavor compounds to food ingredients may facilitate the selection and design of appropriate flavoring systems in specific food applications. However, it should be made clear that adsorption under humidified conditions may not be directly comparable to adsorption under dry conditions. When water is present, interactions may occur between flavor compounds and the water sorbed onto SPI. Furthermore, it is of interest to know how the adsorption behavior of a flavor compound is affected at other humidity levels. Additional studies are currently underway to further evaluate the effects of humidity and volatile probe structure on flavor−soy protein interactions.

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