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Effect of Flavor Compound Chemical Structure and Environmental Relative Humidity on the Binding of Volatile Flavor Compounds to Dehydrated Soy Protein Isolates

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Influence of flavor compound chemical structure, including functional group and stereochemistry, and environmental relative humidity (RH) on the binding of volatile flavor compounds to dehydrated soy protein isolates (SPIs) was evaluated by inverse gas chromatography. Binding of selected volatile flavor compounds differed slightly between SPIs of different origins. Results showed that the flavor compound chemical structure greatly determined its binding potential to SPIs. Binding of nonpolar flavor compounds (hydrocarbon) to soy proteins was attributed mainly to nonspecific van der Waals dispersion forces and was not affected by adsorbed water. The more polar flavor compounds (ester, ketone, aldehyde, and alcohol) exhibited both specific (hydrogen bonding, dipole forces) and nonspecific interactions, and their binding with soy proteins was greatly impaired by adsorbed water in the extremely low humidity region (approaching 0% RH). Further water uptake in the 30 to 50% RH region did not significantly affect the binding of polar compounds, although sorption of alcohol compounds (when present at high levels) further increased.

KEYWORDS: Soy protein isolate; volatile; flavor compound; flavor binding; chemical structure; relative humidity; inverse gas chromatography

INTRODUCTION

As an excellent protein source providing many health benefits, as well as possessing various functionalities, soy offers many desired properties to a food system. Thus, there is a great market potential for soy food products. However, the flavor problems associated with soy foods have greatly affected consumer acceptance of soy products (*1*−*5*) and hence have hindered the expansion of the soy food market.

The interaction of flavor compounds with soy proteins has a great impact on the flavor of the final food product since soy proteins can strongly bind with certain flavor compounds. This not only may retain off-flavors but also may cause flavor imbalance, making it challenging to properly flavor soy products (*3*, *4*, *6*). The interaction can be affected by every step along the processing line, from initial ingredient selection to processing and storage of the final product. In addition, flavor retention by soy proteins can greatly influence the rate of flavor release and hence affect flavor perception when the food is eaten (*7*). Therefore, knowledge of the mechanisms of flavor binding by soy proteins is essential for optimization of processing and storage conditions as well as for the development of new products with controlled flavor retention and release properties. A good understanding of the flavor binding behavior of soy protein also is valuable for the development of soy products with highly acceptable flavor properties.

Investigation of interactions between volatile compounds and soy proteins had been conducted mainly in aqueous soy protein systems by the use of static headspace and equilibrium dialysis techniques (*8*−*11*). Hydrophobic interactions were suggested to be responsible for the binding of volatiles by soy proteins in solution (*8*−*11*). In contrast, even though a large variety of low-moisture soy products (i.e., cookies, nutrition bars, and cereal-based products) exist, little information is available concerning the binding of volatile flavor compounds by soy proteins in the solid state (*12*, *13*). In general, a low-moisture product has a long shelf life, during which flavor quality changes may occur as a result of flavor migration and/or binding (*14*). In addition, the storage relative humidity (RH) level greatly affects the shelf life of low-moisture products, and the exchange of moisture with the environment can have a great impact on the flavor quality of a product by affecting the flavor retention/release properties of the food system (*7*). Therefore, knowledge of flavor−soy protein interactions in semi-dry food systems is important. To our knowledge, no study has been reported in the literature discussing the influence of relative humidity on the interactions between volatile flavor compounds and soy proteins in the solid state.

Inverse gas chromatography (IGC) is an excellent method for the study of binding of volatile compounds by nonvolatile solid food substances (*12*, *13*, *15*−*17*). The advantages of IGC

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Table 1. Heats of Adsorption [$-\Delta H_s$ Values ± Standard Deviation (kJ mol^{-1})] and Sorption Constants [S Values ± Standard Deviation (nmol g^{-1} Pa^{-1})] Determined for Individual Volatile Probes on Different Commercial SPIs at 0 and 30% RH[a]

		0% RH			30% RH		
		hexane	hexanal	hexanol	hexane	hexanal	hexanol
$-\Delta H_s$ (kJ mol^{-1})[b]	SPI 1	28.3 ± 0.4	43.0 ± 1.7	68.4 ± 2.1	27.5 ± 0.9	35.9 ± 2.1	54.4 ± 1.8
	SPI 2	29.6 ± 0.8	56.0 ± 0.3	80.7 ± 0.9	30.1 ± 0.0	42.6 ± 0.5	66.9 ± 0.4
	SPI 3	29.3 ± 0.4	79.5 ± 1.5	96.1 ± 4.6	28.8 ± 1.5	47.6 ± 1.3	72.6 ± 0.4
S (nmol g^{-1} Pa^{-1})[c]	SPI 1	0.29 ± 0.02	16.3 ± 1.9	120 ± 6	0.31 ± 0.02	4.11 ± 0.13	27.7 ± 0.2
	SPI 2	0.18 ± 0.01	8.20 ± 0.76	117 ± 8	0.18 ± 0.00	3.18 ± 0.05	36.3 ± 0.1
	SPI 3	0.14 ± 0.01	12.6 ± 0.1	196 ± 6	0.14 ± 0.00	3.42 ± 0.14	42.4 ± 0.6

[a] Average from two columns and a total of four replicated measurements; each column was prepared with soy isolates from a different production batch. [b] Based on data determined at 30, 35, and 40 °C. [c] Data determined at 35 °C.

over traditional equilibrium methods include its simplicity, speed, and accuracy (*18*−*19*). Because of its extreme sensitivity, IGC is an excellent tool for the measurement of adsorption of flavor compounds at very low levels to closely simulate conditions encountered in real foods.

In our previous study, we developed a rapid and sensitive inverse gas chromatographic system for the investigation of the binding of volatile flavor compounds to soy protein isolate in the solid state (*20*). A unique attribute of our IGC system was the precise control of the carrier gas relative humidity, which enabled the evaluation of the influence of relative humidity on the flavor binding properties of dehydrated soy proteins. The objective of the present study was to apply IGC to investigate the effect of flavor compound chemical structure, including functional group and stereochemistry, and environmental relative humidity on the binding of volatile flavor compounds by dehydrated soy protein isolates. Thermodynamic parameters of adsorption and sorption isotherms were determined.

MATERIALS AND METHODS

Volatile Compounds. Analytical grade (>97% purity) hexane, 1-hexene, limonene, ethyl butyrate, 2-hexanone, hexanal, *trans*-2-hexenal, 1-hexanol, *cis*-3-hexen-1-ol, and *trans*-2-hexen-1-ol were obtained commercially (Aldrich Chemical Co.; St. Louis, MO).

Soy Protein Isolates. Three representative soy protein isolates (SPIs) were obtained from three different commercial sources, and they were labeled as SPI 1 (Archer Daniels Midland Co.; Decatur, IL), SPI 2 (Protient Inc.; St. Paul, MN), and SPI 3 (Cargill Soy Protein Solutions; Minneapolis, MN). Protein, lipid, moisture, and ash contents of these SPIs were determined with standard AOAC methods (*21*−*24*). Prior to use, a freshly received sample was sieved to obtain particle sizes between 129 and 145 μm.

Column Preparation. Sieved SPI was packed into a deactivated glass tube (17.8 cm × 4 mm i.d.; Supelco; Bellefonte, PA) using the procedure described previously (*20*). Each column was connected to the IGC system and conditioned to the desired temperature and RH level under carrier gas for 48 h prior to experiments. Whenever the temperature was changed, the column was reconditioned for at least 12 h to ensure that the new equilibrium condition was established.

IGC Measurement. Inverse gas chromatography (IGC) is a molecular probe technique that can be used to study volatile−nonvolatile interactions, which is done by preparing the IGC column with the nonvolatile material being studied and injecting a series of known amounts of volatile probes of defined properties. The fundamental parameter determined from an IGC experiment is the retention volume, from which heat, free energy, and entropy of adsorption can be determined, and hence, the surface chemistry of the solid substance as well as the thermodynamic properties of the sorbate−sorbent system can be assessed. The IGC instrument used in the present study was modified from a conventional GC (6890 Series; Agilent Technologies, Inc.; Palo Alto, CA) equipped with a flame ionization detector (FID). Desired RH conditions were readily created and precisely controlled by mixing dry and wet (saturated with water vapor) helium gas flows at proper ratios. The configuration of the instrumentation and the methodology for conducting IGC experiments have been described in detail (*20*).

Influence of SPI Origin. To evaluate and compare the flavor binding properties of soy protein isolates of different origins, three different SPIs (SPI 1, SPI 2, and SPI 3) obtained from three different commercial sources were included in this part of study, and their interactions with selected volatile probes (hexane, hexanal, and 1-hexanol) were evaluated at two different relative humidity (RH) levels (0 and 30%). For each SPI evaluated, columns were prepared with soy isolates from two different production batches to ensure that representative data were obtained for each SPI studied.

Effect of Flavor Compound Chemical Structure and Relative Humidity. Interactions between the 10 volatile flavor compounds and SPI 1 were measured under both dry (0% RH) and humidified (30, 40, and 50% RH) conditions. This allowed for the evaluation of both the effect of flavor compound chemical structure and the effect of RH on flavor−soy protein interactions. The binding of selected volatile probes (hexane, hexanal, and 1-hexanol) to SPI 1 at 8% RH was also measured to further examine the influence of very low moisture content on their interactions.

Data acquisition and peak area integration were achieved using HP Chemstation software (Agilent Technologies). Measurements were performed on two different columns for each RH−SPI set studied, with the mean values were reported. Statistical analysis (*t*-test or ANOVA; $p \leq 0.05$) was conducted in data analysis.

RESULTS AND DISCUSSION

Since all commercial soy protein isolates contained a small amount of nonprotein substances, only apparent measurements are implied throughout the discussion in this paper.

Flavor Binding Properties of Soy Protein Isolates of Different Origins. Compositional analysis results showed that the protein contents for these three SPIs were 93.0, 93.9, and 91.1% ($N \times 6.25$; dry basis) for SPI 1, 2, and 3, respectively; while the lipid contents were 4.1, 2.3, and 2.9% (dry basis) for SPI 1, 2, and 3, respectively.

At both RH levels evaluated (0 and 30% RH), a similar pattern was observed in terms of the relative interaction potential of the three volatile probes to SPIs (**Table 1**). Under each RH condition studied, relative binding strengths and amounts of sorbate uptake were always observed in the following order for individual SPIs: 1-hexanol > hexanal > hexane. This may reflect the similar chemical composition of these SPIs.

On the other hand, under each humidity condition evaluated, the absolute interaction potential of individual volatile probes was not the same across the SPIs (**Table 1**), suggesting that differences exist among the three SPIs. A slight difference exists in the lipid content between the three SPIs. Therefore, nonpolar flavor−lipid interactions may influence the apparent interaction forces observed. However, heat of adsorption data showed that the most polar compound (1-hexanol) interacted most strongly

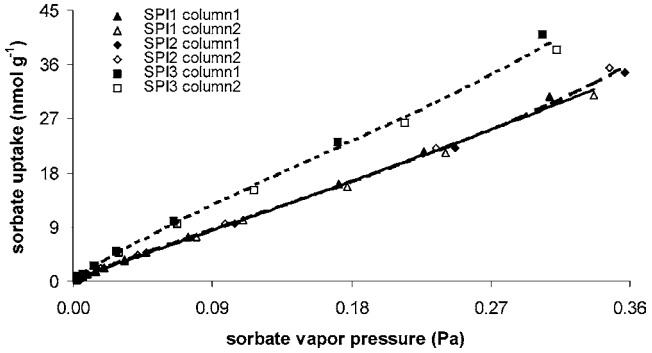

Figure 1. Sorption isotherms determined for 1-hexanol with different commercial SPIs at 35 °C and 0% RH.

with the SPIs. In addition, binding forces determined for individual volatile compounds across the three SPIs were not in direct agreement with their lipid content (**Table 1**). These suggest that flavor−lipid interactions did not play a predominant role in the overall binding strengths observed.

Sorption constant *S* (determined from the initial slope of the sorption isotherm; **Table 1**) reflects both the interaction strength (indicated by ΔH_s) and the number of binding sites. The differential sorption constants determined for individual volatile probes among the three SPIs may be the result of different binding strengths (as was reflected in the ΔH_s values; **Table 1**) only or in combination with a different number of binding sites available on individual SPIs. For example, at 0% RH and 35 °C, the interaction of 1-hexanol with SPI 1 was weaker than with SPI 2 (68.4 ± 2.1 vs 80.7 ± 0.9 kJ mol^{-1}; **Table 1**), but the sorption constant of 1-hexanol to SPI 1 was comparable to that of SPI 2 (120 ± 6 vs 117 ± 8 nmol g^{-1} Pa^{-1}; **Table 1**). These data imply that although binding forces between 1-hexanol and SPI 1 were weaker, a greater number of binding sites was available on SPI 1 than on SPI 2, resulting in comparable sorption of 1-hexanol to SPIs 1 and 2. The higher sorption constant determined for 1-hexanol on SPI 3 than to SPI 1 or SPI 2 (196 ± 6 vs 120 ± 6 and 117 ± 8 nmol g^{-1} Pa^{-1}; **Table 1**) may be due to the stronger binding forces involved (96.1 ± 4.6 vs 68.4 ± 2.1 and 80.7 ± 0.9 kJ mol^{-1}; **Table 1**) alone or in combination with a greater number of binding sites. **Figure 1** compares the sorption isotherms determined for 1-hexanol with the three SPIs, which displays the relative binding potential of 1-hexanol to individual SPIs within a wider range of sorbate activity. As seen, the IGC method is very sensitive and can detect subtle differences in surface sorption characteristics among the three SPIs.

Such differences in terms of binding site energy levels and number of available binding sites among these three SPIs may reflect their different surface physicochemical properties. As a biopolymer, the physicochemical and hence functional properties of soy protein are determined by many factors including raw materials and processing history (*25*, *26*). These three SPIs obtained from three different manufacturers were made by different methods including membrane processing (SPIs 2 and 3) and traditional extraction (SPI 1) techniques. Although the chemical compositions of these SPIs are similar, their physicochemical properties and hence functionalities may be quite different because of variation in processing such as the amount of heat and chemical treatments they received (*25*, *26*). On the other hand, even within a single manufacturer, SPIs having different functional properties are made by properly adjusting individual processing steps. In fact, a large variety of SPIs for different applications are commercially available (*25*, *26*). Therefore, it is not surprising that differences exist among SPIs

Table 2. Heats of Adsorption [$-\Delta H_s$ Values ± Standard Deviation (kJ mol^{-1})] Determined for Individual Volatile Probes at Different Relative Humidities (RHs)[a]

volatile probe	0% RH	8% RH	30% RH	40% RH	50% RH
hexane	28.3 ± 0.4	29.7 ± 0.9	27.5 ± 1.0	29.3 ± 2.3	28.7 ± 2.3
1-hexene	27.5 ± 0.6	*b*	30.2 ± 1.7	28.7 ± 2.0	28.1 ± 2.2
limonene	47.3 ± 0.0	*b*	46.6 ± 1.2	46.5 ± 0.4	47.7 ± 0.9
ethyl butyrate	43.2 ± 1.1	*b*	38.9 ± 0.1	40.0 ± 0.8	39.8 ± 2.2
2-hexanone	44.1 ± 2.0	*b*	37.6 ± 2.0	39.5 ± 1.2	39.9 ± 1.6
hexanal	43.0 ± 1.7	41.6 ± 0.9	35.9 ± 2.1	38.7 ± 1.5	39.4 ± 1.6
trans-2-hexen al	50.8 ± 2.2	*b*	41.1 ± 1.8	42.4 ± 0.7	41.3 ± 1.0
1-hexanol	68.4 ± 2.1	63.4 ± 1.4	54.4 ± 1.8	58.5 ± 0.2	54.9 ± 1.5
trans-2-hexen-1-ol	92.5 ± 3.2	*b*	56.3 ± 1.3	57.7 ± 1.4	55.2 ± 0.7
cis-3-hexen-1-ol	84.8 ± 1.6	*b*	53.7 ± 1.7	55.1 ± 2.5	53.8 ± 0.4

[a] Average of two columns and a total of four replicated measurements; based on data determined at 30, 35, and 40 °C. [b] Not determined.

in terms of their absolute flavor binding potential. Interactions between selected volatile compounds and soy proteins having different degrees of denaturation had been examined in both liquid (9, *27*) and dry (*13*) systems, and it was found that the extent of binding varied among these soy proteins as a result of their degree of denaturation. Additional studies are needed to further examine the relationship between surface physicochemical properties and flavor binding properties of dehydrated soy proteins. This may provide useful information for ingredient development such that more varieties of SPIs having unique flavor binding properties (decreased or enhanced retention for specific flavor compounds) are available for specific applications.

Although the absolute flavor binding capacities of the three SPIs were not the same, they showed similar flavor binding patterns. Therefore, within the scope of this study, only SPI 1 was selected to further evaluate the influence of flavor compound chemical structure and environmental relative humidity on the binding of volatile flavor compounds to dehydrated soy proteins as discussed next.

Effect of Flavor Compound Chemical Structure and Relative Humidity on Binding. A total of 10 volatile probes were selected in the present study. These compounds differ in volatility and polarity, representing several different groups of volatile flavor compounds (hydrocarbon, ketone, aldehyde, ester, and alcohol) commonly found in foods. In addition, some of these compounds were suggested to be responsible for the off-flavors of soy products.

Effect of Flavor Molecule Functional Group on Binding. On the basis of the heat of adsorption data determined at 0% RH (**Table 2**), it was apparent that the binding strengths of the selected volatile probes to soy proteins were related to the chemical classes to which they belong. That is, hydrocarbons (including limonene) interacted most weakly and alcohols interacted most strongly with SPI 1, with the ester, ketone, and aldehyde compounds interacting with SPI 1 with similar forces. These differences can be attributed to the different functional groups these compounds carry. For hydrocarbons, probably mainly nonspecific interactions (van der Waals dispersion forces) were involved as suggested by the weak binding forces observed (**Table 2**). For the ester, ketone, aldehyde, and alcohol compounds, both specific (hydrogen bonding, dipole forces) and nonspecific interactions may be involved based on the functional groups they carry. The strongest interaction forces observed for the three alcohols (**Table 2**) suggest that high-energy hydrogen bonding and/or more than one hydrogen bond was involved. This is possible because of the electron donor and acceptor nature of the hydroxyl group. Other thermodynamic parameters

Table 3. Sorption Constants [*S* Values ± Standard Deviation (nmol g^{-1} Pa^{-1})] Determined for Individual Volatile Probes at Different Relative Humidities (RHs)[a]

volatile probe	0% RH	8% RH	30% RH	40% RH	50% RH
hexane	0.29 ± 0.02	0.25 ± 0.01	0.31 ± 0.03	0.25 ± 0.00	0.25 ± 0.00
1-hexene	0.24 ± 0.00	*b*	0.24 ± 0.00	0.23 ± 0.00	0.22 ± 0.00
limonene	24.6 ± 0.3	*b*	24.8 ± 0.5	24.9 ± 0.4	24.4 ± 0.3
ethyl butyrate	8.68 ± 0.18	*b*	3.58 ± 0.05	3.03 ± 0.07	2.92 ± 0.01
2-hexanone	11.4 ± 0.3	*b*	3.92 ± 0.01	3.40 ± 0.09	3.27 ± 0.00
hexanal	16.3 ± 1.9	3.78 ± 0.01	4.11 ± 0.18	3.65 ± 0.09	3.53 ± 0.01
trans-2-hexenal	30.7 ± 0.5	*b*	8.55 ± 0.20	7.47 ± 0.16	7.30 ± 0.08
hexanol	120 ± 6	46.9 ± 0.4	27.7 ± 0.4	27.0 ± 0.6	26.9 ± 0.1
trans-2-hexen-1-ol	235 ± 9	*b*	28.5 ± 0.3	29.7 ± 0.1	32.8 ± 1.2
cis-3-hexen-1-ol	168 ± 3	*b*	23.6 ± 0.4	24.3 ± 0.3	27.5 ± 0.7

[a] Average of two columns and a total of four replicated measurements. [b] Not determined.

determined (data not shown) were in good agreement with the heat of adsorption data. The importance of the functional group on the binding of volatile flavor compounds to soy protein in the dry state (*12*) or in solution (*8*−*11*) had been previously suggested. Aspelund and Wilson (*12*) also reported the involvement of van der Waals forces or even hydrogen bonding in the binding of volatiles to dehydrated soy protein.

Effect of Flavor Molecule Stereochemistry on Binding. Both ΔH_s and *S* values determined at 0% RH (**Tables 2** and **3**) show that binding of 1-hexene to SPI 1 was similar to that of hexane, suggesting that the presence of a double bond did not have a significant influence on the binding of weak interacting compounds (such as alkanes). However, retention by SPI 1 was higher for both *trans*-2-hexen-1-ol and *cis*-3-hexen-1-ol than 1-hexanol (**Tables 2** and **3**), indicating that the presence of a double bond together with a strongly interacting functional group (such as hydroxyl group) could have a significant influence on flavor−soy protein interaction probably through altering the molecular stereochemistry as explained next. On one hand, molecule rigidity increases due to the restricted rotation of the double bond, which enhances the exposure of the hydroxyl group and hence its interaction potential with soy proteins. The shorter the distance between the double bond and the hydroxyl group, the more rigid the hydroxyl end of the molecule becomes and hence the easier it is for the molecule to form a hydrogen bond with soy protein. On the other hand, the position of the double bond influences the molecular stereostructure, which in turn has an influence on binding. The three-dimensional structures of the three alcohols reveal that *cis*-3-hexen-1-ol is relatively more spheric in shape. This steric hindrance can reduce its accessibility to polar binding sites on soy proteins as compared with the other two alcohols. The combination of the previous two aspects (molecular rigidity and stereostructure) determines that *trans*-2-hexen-1-ol will bind more tightly and adsorb to a greater degree to SPI 1 than the other two alcohols (**Tables 2** and **3**). Meanwhile, the binding strength and sorption constant of *cis*-3-hexen-1-ol to SPI 1 were slightly higher than those of 1-hexanol (**Tables 2** and **3**), but their retentions by SPI 1 were not significantly different at the higher sorbate activity region as seen in the sorption isotherms determined (**Figure 2**), reflecting the counteracting contributions of molecular rigidity and stereostructure in *cis*-3-hexen-1-ol to its interactions with soy proteins.

Similarly, *trans*-2-hexenal interacted more strongly and adsorbed more to SPI 1 than hexanal (**Tables 2** and **3**), which is partly due to the fact that the carbonyl group of *trans*-2-hexenal is more exposed allowing for greater interaction. In addition, the existence of a conjugated double bond in the *trans*-2-hexenal molecule further enhances molecular rigidity as well as electron density of the carbonyl end, facilitating its binding to soy proteins as was supported by the experimental data.

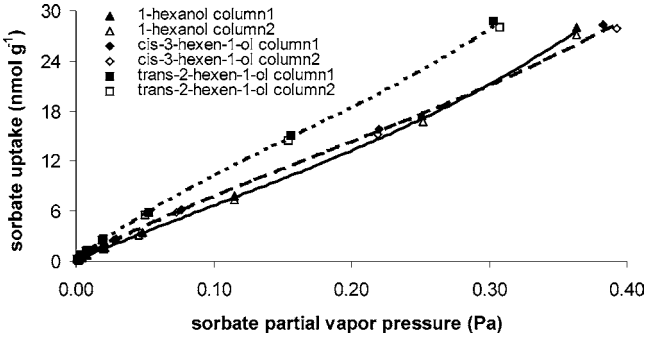

Figure 2. Sorption isotherms determined with SPI 1 for 1-hexanol, *trans*-2-hexen-1-ol, and *cis*-3-hexen-1-ol at 35 °C and 0% RH.

Under each humidified condition evaluated (30, 40, and 50% RH), the relative binding strengths and sorption constants (**Tables 2** and **3**) of these volatiles to SPI 1 still follow the same trend as observed at 0% RH. That is, hydrocarbons (including limonene) bound most weakly and to the least extent, followed by the ester, ketone, and aldehyde compounds and then alcohols. This indicates that under humidified conditions, the functional group still makes a substantial contribution to the interactions with soy proteins. However, differences caused by functional group were less profound as compared with those observed under dry conditions. In addition, the contribution of molecular stereochemistry to the binding of polar flavor compounds to SPI 1 was overshadowed in the presence of moisture (**Tables 2** and **3**). These findings indicate the great impact water may have on flavor−soy protein interactions, as will be discussed further in the following sections.

Effect of Relative Humidity on Binding. Both thermodynamic and sorption data suggest that the binding of hexane, 1-hexene, and limonene (**Tables 2** and **3**) to SPI 1 is not affected by the presence of water, which further supports their weak interactions with SPI 1.

On the other hand, for the other relatively polar volatile probes (ethyl butyrate, 2-hexanone, hexanal, *trans*-2-hexenal, 1-hexanol, *trans*-2-hexen-1-ol, and *cis*-3-hexen-1-ol), their interaction strengths (**Table 2**) with SPI 1 decreased when the RH level was increased from 0 to 30%, suggesting that competition for high-energy binding sites between flavor compound and water exists. Because of its very high polarity, water can readily and tightly bind with soy proteins, and once it occupies a high-energy polar binding site, it is not readily displaced. As such, the lower sorption constants determined for these compounds (**Table 3**) could be attributed to the weakening of binding forces and a decrease in the number of binding sites as well. In fact, interaction potentials of both hexanal and 1-hexanol with SPI 1 were diminished even at 8% RH (**Tables 2** and **3**), which further reveals the much higher affinity of water to SPI 1. When the RH level was further increased from 30 to 40 and 50%, no significant difference was observed in their binding potentials to SPI 1 as reflected in both ΔH_s and *S* values determined (**Tables 2** and **3**), suggesting that their interactions with soy proteins were relatively weak and limited. However, sorption isotherms determined for the three alcohols showed gradual increasing sorption at the high sorbate activity region when the RH level was increased from 30 to 40% and then to 50% RH, as is seen in **Figure 3** using *trans*-2-hexen-1-ol as an example. This probably could be attributed to the occurrence of water−alcohol interactions via hydrogen bonding and/or dipole forces,

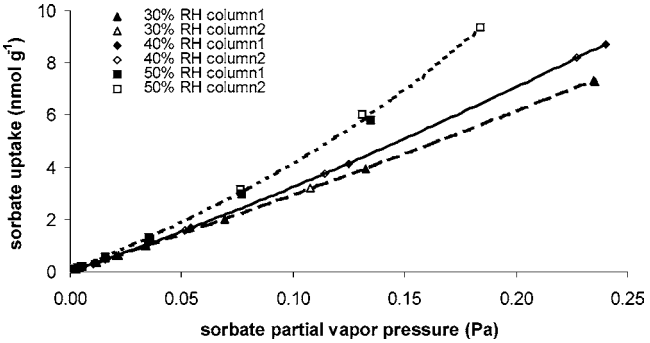

Figure 3. Sorption isotherms determined with SPI 1 for *trans*-2-hexen-1-ol at 35 °C and at different relative humidities (RHs).

which is likely to occur for flavor compounds that have a relative high polarity and when both water and flavor compounds are present at relatively high levels. In addition, as the water sorption further increased, plasticization may occur such that the protein structure becomes less rigid, leading to increased flavor−protein interactions (*28*). It should be made clear, though, that it may not be appropriate to directly compare adsorption on surfaces containing adsorbed water (ternary system) to adsorption on dry surfaces (binary system). However, the comparisons we made here can help to show the impact of environmental RH on the flavor binding properties of SPI 1.

The previous data imply that product formulation and RH of the storage environment are critical for low-moisture soy products since their flavor binding properties and hence product flavor quality may change as a result of a change in water activity. In contrast to its importance, the impact of moisture on the flavor binding behavior of hydrophilic food ingredients in low-moisture food systems was scarcely addressed in the literature partly due to the difficulty of obtaining humidity control with the previously developed methods. Thanh et al. (*29*) studied the interactions between volatile (acetone, ethyl acetate, diacetyl, 2-propanol, *n*-hexanol, and benzaldehyde) and nonvolatile compounds (carbohydrates and caseinate) in the presence of water and found that the sorption of volatiles may be increased or decreased when the RH level was increased from 11 to 32%, depending on the nature of both the volatile compounds (such as volatility and polarity) and the substrates. Seuvre et al. (*28*) evaluated the interactions between selected aroma compounds (2-nonanone and D-linalool) with β-lactoglobulin as a function of the state of protein hydration and found that sorption of these two compounds was very low and did not significantly vary in the low water activity region (A_w < 0.43). Boutboul and others (*15*) used an IGC technique to compare the retention of selected volatiles (1-hexanol, 2-hexanol, octanal, ethyl hexanoate, and limonene) by starch under dry (0% RH) and humid (56% RH) conditions and found that retention was higher, especially for 1-hexanol, under humid conditions. However, in their study, only one humidified condition was examined. In the present study, flavor−soy protein interactions under several different humidity conditions (0, 8, 30, 40, and 50% RH) were evaluated and compared, with both thermodynamic and sorption data determined, giving better insight into the possible flavor−soy protein interaction mechanisms involved as well as providing a better picture of their interactions within a wider range of sorbate concentrations.

Results from this study demonstrated that the chemical structure of volatile flavor compounds greatly determined its binding with dehydrated soy proteins and that relative humidity had a substantial influence on the interaction potential of polar flavor compounds. In contrast to the hydrophobic interactions suggested to be responsible for the binding of volatiles to soy protein in solution, nonspecific interactions (for nonpolar flavor compounds) or in combination with specific interactions (for polar flavor compounds) are responsible for the binding of volatiles to soy proteins in the dehydrated state. The combination of thermodynamic and sorption data not only can quantitatively describe the binding strengths and hence possible binding forces involved but also can reveal any subtle changes in the surface sorption properties of soy proteins in the presence of water, providing better insight into the interaction mechanisms.

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