Adsorption of Off-flavor Compounds onto Soy Protein: A Thermodynamic Study

Thomas G. Aspelund* and Lester A. Wilson

Heats of adsorption, free energy of adsorption, and entropy of adsorption for the reversible adsorption of off-flavor compounds onto soy protein isolate were measured by using gas chromatography. Homologous series of alcohols, aldehydes, ketones, hydrocarbons, and methyl esters were used to ascertain the effect of functional group and chain length on adsorption. Triplicate samples were run at 80, 90, and 100 °C, and the resulting data were analyzed by statistical linear analysis. The heats of adsorption of aldehydes, ketones, and methyl esters were not statistically significant from each other but were significantly different from the hydrocarbons and the alcohols. The hydrocarbons adsorbed the weakest and the alcohols adsorbed the strongest onto dry soy protein. The free energy of adsorption and energy of adsorption both were negative and supported the heat of adsorption data. The functional group of the ligand plays a significant role in binding of flavor compounds to soy protein in the dry state.

Soybeans have been used in the world food supply for many generations, especially in the Eastern or Oriental cultures. Soy protein and soybean foods are presently becoming more acceptable in the American and European food markets (Honig et al., 1979). However, the flavor of soybeans is believed to be one of the major limiting factors to their use in human foods (Hammonds and Call, 1972; National Agricultural Research Policy Advisory Committee, 1974; Johnson, 1976; Meyer and Williams, 1976). This was recently reinforced by the National Science Foundation when they assigned the top priority in processing and utilization research to maximizing the acceptance of soy in human foods via the identification and removal of undesirable flavors (Milner et al., 1978).

The undesirable flavors referred to in these articles are the "green" and "beany" flavors that are developed by lipoxygenase activity or autoxidation of oils in raw crushed and full-fat flours (Rackis et al., 1979). These flavors interact with the soy protein and remain in other soy products, such as flours, concentrates, and isolates (Meyer, 1970; Kalbrener et al., 1971; Eldridge, 1978; Smith and Circle, 1978; Wolf and Cowan, 1975). When these soy products are used as extenders (in hamburger) or fabricated foods (e.g., soy analogues), these off-flavors may be released, thus decreasing the consumer acceptability of these products (Twigg et al., 1977; Ashraf and Snyder, 1981). Although there have been numerous studies identifying the products from lipoxygenase activity in soy (Arai et al., 1970; Goosens, 1975; Sessa, 1979), few have attempted to determine the mode of interaction and release from soy protein systems (Beyeler and Solms, 1974; Franzen and Kinsella, 1974; Gremli, 1974; Aspelund and Wilson, 1979; Crowther et al., 1981; Damodaran and Kinsella, 1981a,b). The earlier studies of Franzen and Kinsella (1974) and Gremli (1974) in aqueous systems did not obtain basic thermodynamic data nor evaluate their results statistically. This precludes making predictions concerning the mode and strength of binding of flavors on soy. Before the work of Aspelund and Wilson (1979) and Crowther et al. (1981), no statistical analysis of flavor binding data has been reported.

Better understanding of the binding and release of off-flavors from soy in aqueous and dry systems is essential to the development of methods or processes to remove or minimize this flavor and increase the acceptability of soybean products in the marketplace. The current empirically derived methods of removing or preventing off-flavor development in soybeans (extraction of lipids, extraction of off-flavors, heating to inactivate lipoxygenase and/or drive off undesirable flavors) have improved soy protein utilization, but they have not solved the soy protein flavor problem. Further insights into the mechanisms involved in flavor binding and their release from soy protein could shed further light into preventing this flavor problem from occurring and thus significantly improve the utilization and profitability of soybeans.

In addition, the results from this study could aid in the retention of desired flavors in soy protein products. This area also could significantly improve the utilization of soy protein in human foods (Schutte and Van Der Ouweland, 1979; Lyon, 1980).

Recently, the adsorption and binding of flavor volatile onto dry soy protein has been investigated to learn more about the formation of off-flavors by interactions taking place on the protein's surface (Aspelund and Wilson, 1979; Crowther et al., 1981). The strengths of adsorption are determined by the thermodynamic function of heats of adsorption, which can be determined by gas chromatography. Green and Pust (1958) were the first to use gas chromatography to determine thermodynamic functions; other worker such as Gale and Beebe (1964) and Kiselev and Yashin (1969) used this technique to determine heats of adsorption of flavor molecules on such absorbents as carbon black, bone mineral, porous alumina, and silica gel. These workers derived equations for the determination of the heats of adsorption and found their results to compare favorably to the more traditional calorimetric technique. Simplicity, speed, and accuracy (in most cases) are the advantages of using the chromatographic technique over other techniques for the determination of thermodynamic functions (Gale and Beebe, 1964). McMullin et al. (1975) used this method with lactose, and Aspelund and Wilson (1979) demonstrated that the method could also be used with soy protein. More recently Ehler et al. (1979) and Crowther et al. (1981) have used the method with lactose, sucrose, glucose, and processed soy isolate.

The purpose of this study was (1) to learn more about the flavor compounds, and how they adsorb onto dry soy protein, (2) to determine the strengths of adsorption of flavors onto soy protein by heats of adsorption data, (3) to determine the modes of adsorption by using this information and other thermodynamic data including Gibb's free energy and entropy of adsorption, and (4) to use statistical analysis to determine the significance of the data.

EXPERIMENTAL SECTION

Selection of the Soy Isolate. Soy protein isolate Edi-Pro A was obtained from Ralston Purina. Edi-Pro A is a spray-dried isoelectric protein isolate. Preliminary

Department of Food Technology, Iowa State University, Ames, Iowa 50011.

0021-8561/83/1431-0539$01.50/0

investigations were carried out (1) to determine if the isolate would make a good column packing material, (2) to study the affect of heating on the soy protein isolate's structure while in the gas chromatograph column, and (3) to investigate the preexistence of any flavor compounds in the selected soy isolate.

Observations of the isolate's ultrastructure were made through a JSM 35 (JEOL) scanning electron microscope. Each sample was prepared for observation by coating the brass sample stub with silver metallic paint (for adhesion of sample and electrical contact) and then sprinkling the isolate on the painted surface and drying. The samples were coated with carbon and gold before being viewed in the microscope.

Samples of the isolate were sent to Micromeritics Instrument Corp. of Norcross, GA, for surface area determinations. The surface areas of the Edi-Pro A replicate samples were very precise, with an average of 0.2019 m^2/g ± 0.0011 m^2/g. There was no difference in surface area for samples degassed at 30 or 100 °C.

Qualitative headspace analysis was then done on Edi-Pro A at various storage temperatures to determine if any flavor compounds preexisted in the soy or if any could be formed by various chemical reactions, thus interfering with the adsorption studies. One-hundred-milliliter reaction vials were filled to approximately one-third their volume (14.5–18.0 g) with Edi-Pro A, sealed with Teflon-coated rubber septa and standard aluminum seals (Supelco, Inc.), and stored at 21, 45, or 80 °C. Fifty-microliter headspace samples were analyzed by gas chromatography after 1 h, 24 h, and 5 days by using a 6-ft 10% Carbowax 20M on 80/100 Chromosorb W AW packed glass column and a 3% SE-30 on 80/100 Supelcoport (Supelco, Inc.) packed glass column.

On the basis of surface area determinations, observations under the light and electron microscope, preliminary results with packed columns, and qualitative headspace analysis of the isolate, Edi-Pro A was found to be acceptable for this study.

Flavor Compounds. The flavor compounds used in this investigation were a homologous series of alcohols, aldehydes, ketones, hydrocarbons, and methyl esters. These compounds are fairly volatile at room temperature and allow carbon number and functional group affects to be studied in the relationship of the adsorption onto dry protein. Also, some of these compounds (e.g., *n*-hexane, 1-pentanol, 1-hexanol, 1-heptanol, 2-pentanone, and 2-heptanone) are considered to be possible contributors to soy's off-flavor problems (Arai et al., 1970; Goosens, 1975; Wolf and Cowan, 1975; Eldridge, 1978; Sessa, 1979).

Preparation of the Soy Column. Approximately 1.5 g of Edi-Pro A was packed into a silanized glass, 3-ft GC column (2-mm i.d.) by using a Millipore vacuum pump and tapping the column with a pencil to avoid any gaps or voids in the packing material (use of a vibrator caused the isolate to become too densely packed, reducing flow rate and increasing the pressure across the column). The exact mass of isolate per column was determined to be 1.4, 1.4, and 1.5 g for columns 1, 2, and 3, respectively. Glass wool plugs were placed in both ends of the column, and the column was initially conditioned overnight at 80 °C with a nitrogen flow rate of 20 mL/min.

Gas Chromatography. The analyses were performed on a Varian 3740 gas chromatograph equipped with dual FID detectors. The injector and detector temperatures were maintained at 150 °C. Nitrogen flow through the column was 20 mL/min, and detector gas flows were 30 mL/min for hydrogen and 300 mL/min for air. The output signal from the chromatograph was fed through a Varian CDS 111 electronic integrator to a Linear 242 recorder.

Methods. Through additional preliminary investigations (quantitative headspace analysis), it was determined that the flavor compounds would be stored in 5-mL reaction vessels sealed with Teflon-coated septas and standard aluminum seals (Supelco, Inc.). The 5 μL of the compound's headspace volatiles at room temperature was collected with a 50-μL Hamilton gas-tight syringe and injected onto the soy column. Three replications of each compound were injected at each column temperature. The adsorption study consisted of obtaining retention times for each compound at 80 °C and then increasing the column temperature to 90 °C. The following day, the study was repeated, obtaining the retention times at 90 °C. The column temperature was increased to 100 °C, and the study was completed upon obtaining the retention times at 100 °C. The nitrogen flow rates were frequently monitored and kept constant (a prerequisite for the heat of absorption determination) at 20 mL/min at all three temperatures. This adsorption study was repeated on three separate columns.

The retention time of each compound was corrected for dead time in the column by subtracting the retention time of methane (a nonadsorbent gas).

Through the quantitative analysis of headspace volatile by using vapor-pressure data at room temperature, it was determined that the quantity of each compound per 5-μL injection was in the range of 10^{-6} g for the lower molecular weight ketones, aldehydes, and hydrocarbons and around 10^{-9} g for the larger molecular weight alcohols and aldehydes. The methyl esters were in the range of 10^{-7}–10^{-8} g/injection. Such minute quantities of compounds per injection was a prerequisite for this type of study.

RESULTS AND DISCUSSION

The soy headspace analysis samples stored at low temperatures (21 and 45 °C) showed virtually no compounds present in the headspace; however, the samples stored at 80–100 °C had several (7–13) compounds in the headspace, with the tentative identification of *n*-hexane and *n*-pentane. All these compounds were in such small quantities that, upon conditioning of the soy isolate in the GC column at 80 °C for 6–8 h, removal of the compounds was achieved, and none could be detected at the GC's most sensitive setting of 1×10^{-12} A/s.

Through observations under the light microscope, Edi-Pro A appeared to be composed of fairly spherical protein particles. Figure 1 shows a scanning electron micrograph of the isolate under low and high magnifications (104× and 1040×, respectively). The ultrastructures of soy isolates have been described by Wolf and Baker (1975), and from their descriptions, Edi-Pro A was considered as a representative sample of isolate with no unusual features. Figure 1A,B represents Edi-Pro A before the adsorption studies, while Figure 1C,D represents Edi-Pro A after the adsorption studies. Comparing Edi-Pro A before and after any heat treatment (Figure 1), no noticeable heating effects on the protein's structure were observed. This was supported by visual and experimental observations as well.

The heat of adsorption ($-\Delta H$) is calculated from the formula derived by Gale and Beebe (1964), which states $\ln T_{cor} = -\Delta H/(RT) + C$ at constant flow rate. When the natural log of the compounds corrected average retention time ($\ln T_{cor}$) is plotted against the inverse of the absolute temperature ($1/T$), a linear relationship should exist, and the slope of that line multiplied by the gas constant (R)

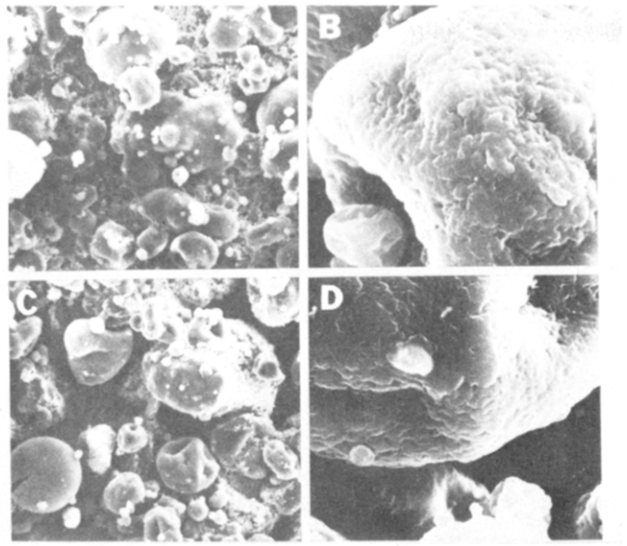

Figure 1. Scanning electron micrographs of Edi-Pro A. (A) Edi-Pro A before the adsorption studies at low magnification (104×). (B) Edi-Pro A before the adsorption studies at high magnification (1040×). (C) Edi-Pro A after the adsorption studies at low magnification (104×). (D) Edi-Pro A after the adsorption studies at high magnification (1040×).

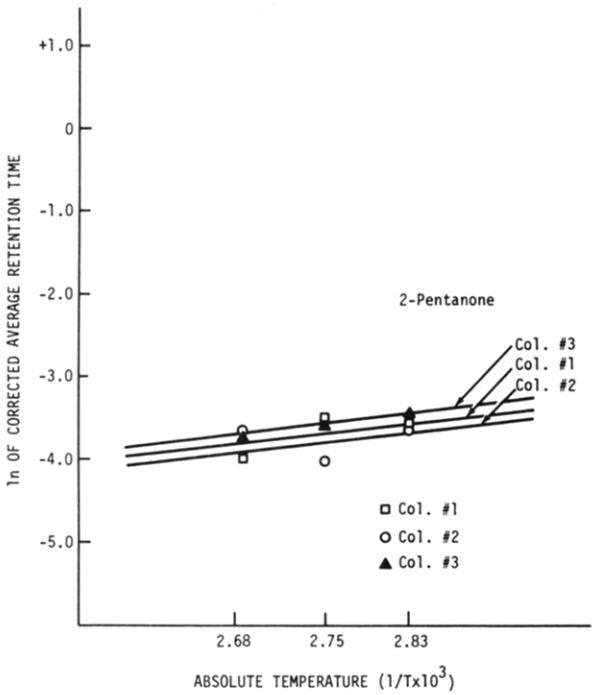

Figure 2. Linear regression lines and observed points for 2-pentanone.

will give the heat of adsorption for that compound onto soy protein.

To state with confidence that the $-\Delta H$'s calculated are valid, a statistical linear regression analysis was run on all the data. The average corrected retention time for each compound at each temperature for each column was plotted against the inverse of the absolute temperature. Linear regression lines were fit to the observed values, the slopes were determined for each line (one for each column), and an average or estimated slope was given on each compound. The statistical analysis gave the following information: (1) the significance level at which the regression fit the data, (2) the estimated slopes and standard errors, (3) the significance of the slopes being greater than zero, (4) any significant temperature affects, and (5) any

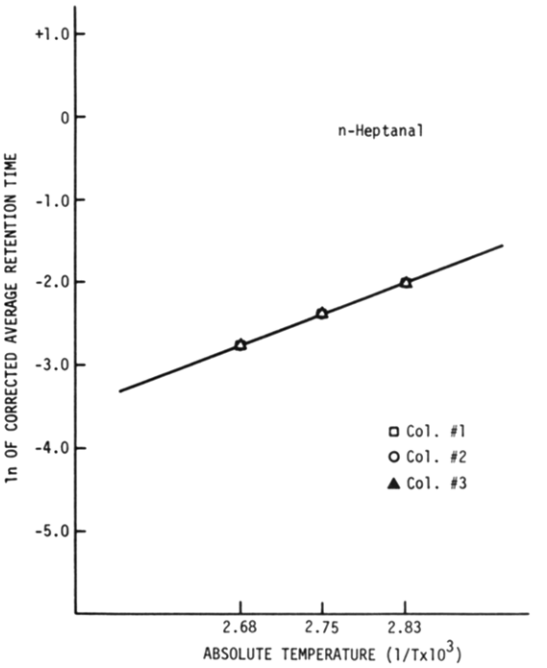

Figure 3. Linear regression lines and observed points for 1-heptanal.

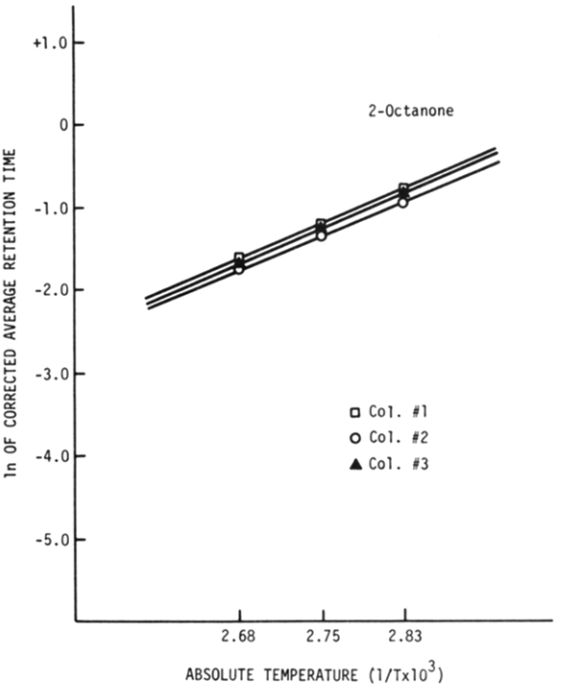

Figure 4. Linear regression lines and observed points for 2-octanone.

significant variations within columns or replicates.

Several of the low molecular weight compounds (2-butanone, 2-pentanone, 1-butanal, 1-pentanal, *n*-hexane, *n*-heptane, and *n*-octane) proved to be nonsignificant (<95%) by the statistical analysis. For these compounds, the regression analysis did not fit the data, the slopes were not significantly greater than zero, and no temperature affects were observed. Figure 2 shows the regression lines for 2-pentanone to represent the compounds that were shown to be nonsignificant. Figure 3 (1-heptanal) represents the majority of the flavor compounds for which the regression was significant (>95%); the slopes were significantly greater than zero, temperature affected the adsorption, and the estimated slope is valid. Figure 4 (2-

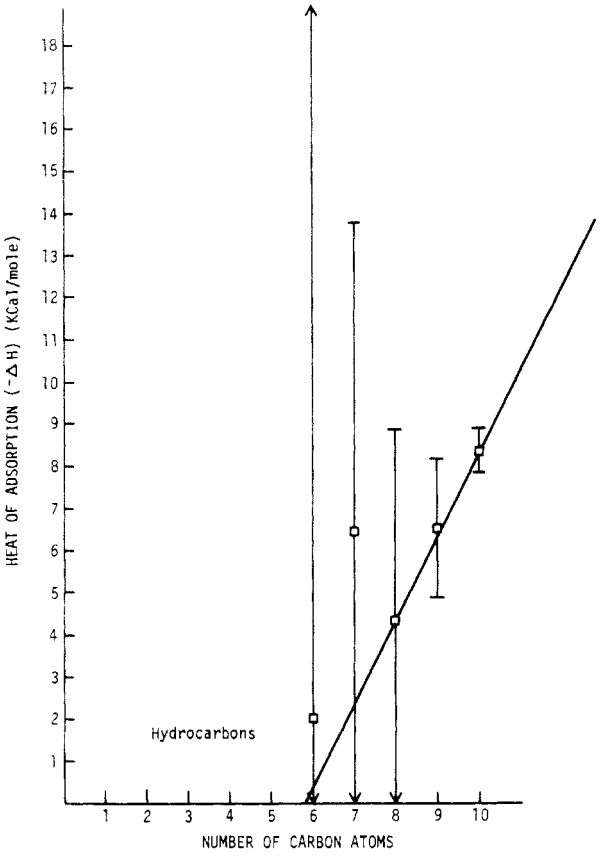

Figure 5. Effect of carbon number on ΔH for the hydrocarbons. Vertical bars denote three standard deviations (99% confidence limit).

octanone) represents the single significant compound that showed significant column or replicate variation. Further statistical analysis was made on 2-octanone, and from an F-test ratio, it was determined that it was indeed variations between columns that caused the problem. A mass temperature interaction was then run on this compound, and it was discovered that the mass differences between columns had no affect on the variation. For 2-octanone only, the estimate slope is invalid, and individual slopes (for each column) must then be used to determine the heat of adsorption (see Table I). This column variation could be explained by the lower volatility of 2-octanone in combination with slight packing variations between columns under the conditions of this study.

For the seven compounds shown by statistics to be nonsignificant, it was observed that their retention times were very close to that of methane (the nonadsorbent gas). Slight injection errors result in large standard errors as determined by the statistical analysis. It is believed that the column temperature used was too high to make valid measurements concerning the retention times of these compounds.

Kiselev and Yashin (1969) stated that, if heat of adsorption values were plotted against the carbon number, a fairly linear relationship should exist within a homologous series. Figures 5–9 give the heats of adsorption, including 3 times the standard error for each compound to signify the range in which $-\Delta H$ lies with a 99% confidence limit in such a plot. It is obvious from these figures that the nonsignificant compounds from the aldehydes (Figure 6), ketones (Figure 7), and hydrocarbon (Figure 5) series fell off the plot; however, the range of their standard errors show that each compound could have followed this linear relationship. Because judgments concerning these compounds could not be made, extrapolated values would be

Table I. Thermodynamic Quantities Determined for Soy Isolate at 90 °C

compounds[a]	V_S, mL/m²	$-\Delta H$, kcal/mol	$-\Delta G$, cal/mol	$-\Delta S$,[b] cal/(mol K)
n-nonane	3.75	6.52	952	15.32
n-decane	8.39	8.36	1530	18.79
2-hexanone	3.29	6.04	857	14.26
2-heptanone	7.62	8.11	1470	18.29
2-octanone[c]	18.73	10.61	2110	23.38
1-hexanal	3.14	8.89	825	22.18
1-heptanal	6.17	9.61	1310	22.81
1-octanal	12.54	13.52	1820	32.44
methyl pentanoate	3.58	6.71	915	15.95
methyl hexanoate	8.16	8.19	1510	18.35
methyl heptanoate	19.81	10.48	2160	22.91
methyl octanoate	46.52	12.56	2760	26.93
1-butanol	5.77	10.45	1260	25.29
1-pentanol	10.99	11.25	1730	26.18
1-hexanol	31.24	13.89	2470	31.40
1-heptanol	70.95	18.06	3070	41.22
1-octanol	165.52	16.39	3690	24.94

[a] Statistically significant compounds with a 99% confidence limit ($n = 9$). [b] ΔS values were calculated from the Gibbs-Helmholtz equation ($\Delta G = \Delta H - T\Delta S$). [c] For 2-octanone, the average slope cannot be used for ΔH determinations. The individual slopes to be used are as follows: column 1 = 10.05 kcal/mol; column 2 = 10.42 kcal/mol, column 3 = 11.37 kcal/mol.

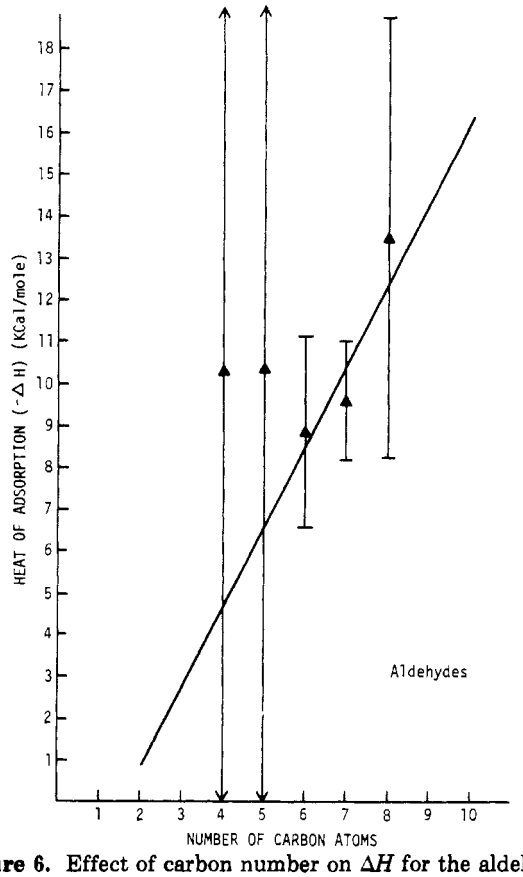

Figure 6. Effect of carbon number on ΔH for the aldehydes. Vertical bars denote three standard deviations (99% confidence limit).

the most accurate ΔH values for the nonsignificant compounds (Kiselev and Yashin, 1969). Table II gives a summary of the ΔH values for the significant compounds, as well as extrapolated values for the nonsignificant ones and for compounds not studied in this investigation.

The plots shown in Figure 5–9 also were done by McMullin et al. (1975), and Ehler et al. (1979) in their

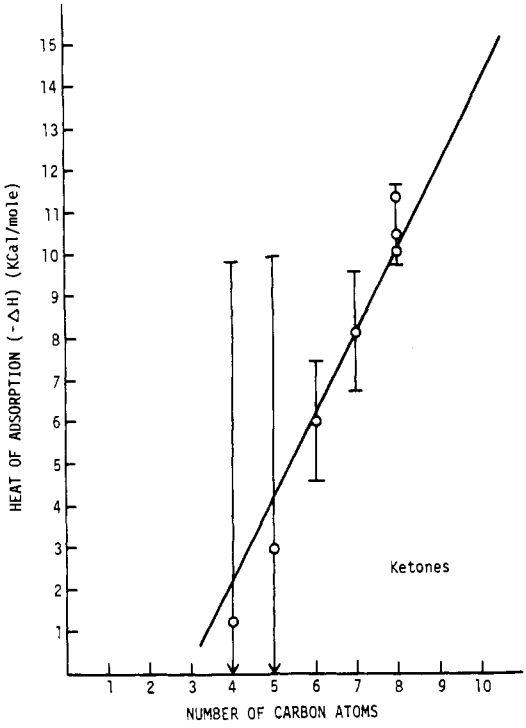

Figure 7. Effect of carbon number on ΔH for the ketones. Vertical bars denote three standard deviations (99% conference limit).

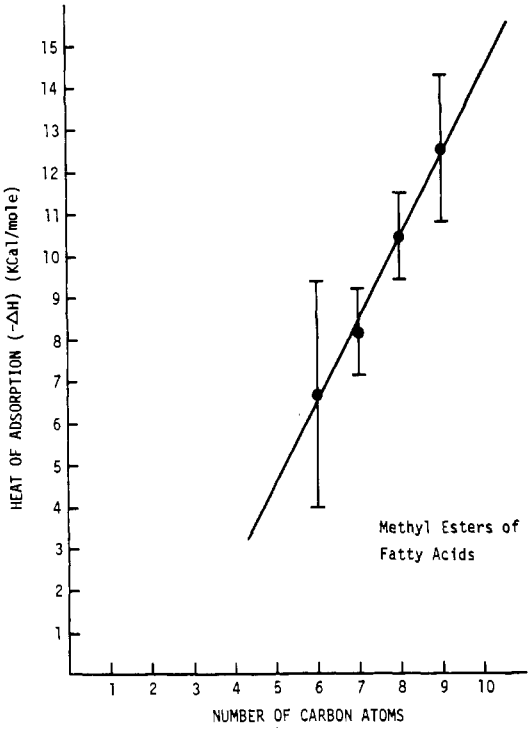

Figure 8. Effect of carbon number on ΔH for the methyl esters. Vertical bars denote three standard deviations (99% confidence limit).

study of adsorption of flavor molecules onto lactose, sucrose, and glucose. The plots showed similarity in one major respect in that the heats of adsorption for the alcohols with a given number of carbon atoms were significantly higher than for the other compounds with a similar number of carbons. This was also observed by Crowther et al. (1981) using heat-treated Edi-Pro A. The hydrocarbons showed the least heat of adsorption values, whereas the ketones, aldehydes, and methyl esters pos-

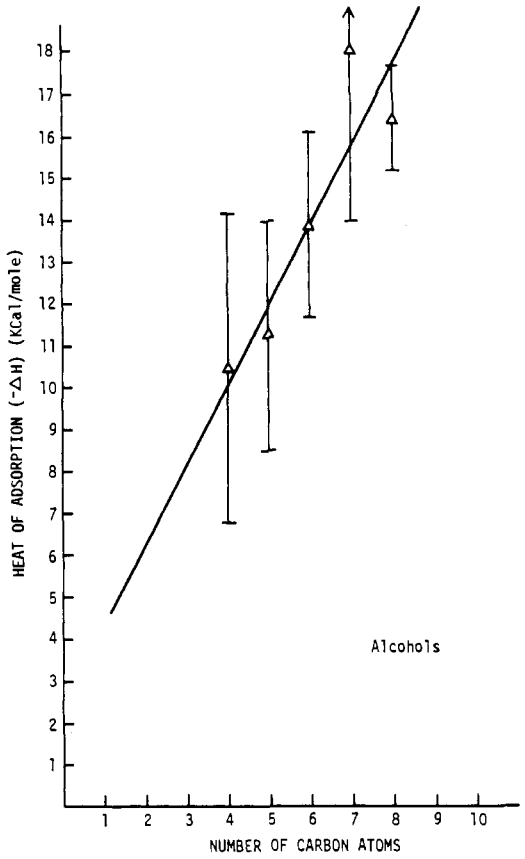

Figure 9. Effect of carbon number on ΔH for the alcohols. Vertical bars denote three standard deviations (99% confidence limit).

sessed intermediate adsorption strengths onto both lactose and soy protein.

Kiselev and Yashin (1969) classified adsorbates as group A, B, C, or D, depending on the compounds peripheral electron-density distribution. The hydrocarbons are classified as group A adsorbates, group B adsorbates would be the ketones, aldehydes, and esters, and the alcohols would be group D adsorbates.

Adsorbents have also been classified by Kiselev and Yashin (1969) and are of three types, I, II, or III. A type I adsorbent is one in which its surface bears no functional groups and allows for nonspecific type reactions only. Type II adsorbents contain localized positive charges on the surface, and type III are adsorbents with localized negative charges; soy protein with the availability of a variety of functional groups would be classified as both a type II and a type III adsorbent in which nonspecific and specific interaction may occur. The sum of the nonspecific and specific interactions would be a measure of the strength of adsorption and, thus, of the heat of adsorption.

It is assumed that the hydrocarbons (having the lowest heats of adsorption for a given number of carbon atoms) interact with soy protein with nonspecific (van der Waals) interactions only. From Table II, it is observed that the group B adsorbates (ketones, aldehydes, and methyl esters) interacted with soy, having heats of adsorption that were about 6–8 kcal/mol higher than the heats of adsorption for hydrocarbons with an equal number of carbon atoms (with the exception of some of the low molecular weight compounds). These group B adsorbates all contain oxygen atoms with lone pairs of electrons that could interact with positively charged functional groups on soy protein and possibly a hydrogen bond. Hydrogen bonds have energies of formation in the range of 3–10 kcal/mol but generally 5 kcal/mol (MacKenzie, 1962). Therefore, the ketones,

Table II. Statistically Significant (95%) and Extrapolated Heats of Adsorption on Edi-Pro A

compounds	carbon no. 4	5	6	7	8	9	10
ketones	2.3[a]	4.3[a]	6.04	8.11	10.61[b]	12.1[a]	14.1[a]
methyl esters	2.1[a]	4.6[a]	6.71	8.19	10.48	12.56	14.7[a]
aldehydes	4.7[a]	6.6[a]	8.89	9.61	13.52	14.3[a]	16.3[a]
alcohols	10.45	11.25	13.89	18.06	16.39	19.6[a]	21.6[a]
hydrocarbons	0[a]	0[a]	0.4[a]	2.4[a]	4.3[a]	6.52	8.36

[a] Extrapolated values. [b] Average of three slopes for 2-octanone.

Table III. Temperature Dependence of Gibb's Free Energy and Entropy of Binding for Six Carbon Flavor Compounds on Soy Protein Isolate[a]

compound	temp, °C	$-\Delta G$, cal/mol[b]	$-\Delta S$, cal/(mol K)[b]
1-hexanal	80	989	22.36
	90	825	22.18
	100	540	22.38
2-hexanone	80	1014	14.23
	90	857	14.26
	100	731	14.23
1-hexanol	80	2750	31.54
	90	2470	31.40
	100	2110	31.56
methyl pentanoate	80	1100	15.91
	90	915	15.95
	100	773	15.92

[a] 99% confidence level. [b] Average from three columns and nine replications.

aldehydes, and methyl esters interact immediately onto soy protein showing both nonspecific (van der Waals forces) and specific interactions, possibly forming one hydrogen bond.

From Table II it also is evident that the ΔH's for the alcohols are an additional 5–7 kcal/mol (9–10 kcal/mol for heptanol) higher than the ΔH's for the group B adsorbates with the same number of carbon atoms. This is in the range of a second hydrogen bond. The proton of the alcohol group could be attracted to a negatively charged functional group existing on the soy protein; the oxygen of the alcohol group could then be attracted to a positively charged functional group existing on the soy proteins, thus forming two hydrogen bonds. The alcohols interact with soy protein with both nonspecific (van der Waals) and specific interactions, possibly forming two hydrogen bonds.

That the heats of adsorption for similar compounds on lactose, sucrose, glucose (McMullin et al., 1975; Ehler et al., 1979), and soy protein are similar reflects the polar nature of their exposed binding sites. However, it is to be expected that, when these compounds are in an aqueous solution, a reordering of the binding affinities will occur (that is, the alcohols may not be bound to the same extent due to their interactions with water).

The Gibb's free energy (ΔG) can be determined from its relationship to the specific retained volume (V_S) at equilibrium: $-\Delta G = RT \ln V_S$ (Sawyer and Brookman, 1968). The specific retained volume (V_S) was calculated from the formula derived by Sawyer and Brookman (1968), which states $V_S = (T_{cor})$(flow rate)/[(surface area)(mass of the packing material)] at a constant flow rate. The Gibb's free energy (ΔG) for each of the statistically significant compounds (Table I) is negative, which indicates that the adsorption process occurs spontaneously. The ΔG values were found to increase (become more negative) with increasing chain length (Table I), by an average of 578 cal/methylene group in the chain. This is remarkably similar to the value of 600 cal/CH_2 obtained by Damadaran and Kinsella (1981) using an aqueous equilibrium dialysis system. The ΔG values also decreased with increasing temperature (Table III), indicating that higher temperatures are less favorable to adsorption, which is characteristic of a physical adsorption process. The alcohols' free energy values were more negative (−4 to −1 kcal/mol) than those of the other compounds (−2.5 to −0.5 kcal/mol), indicating that the alcohols are more likely to bind under these conditions.

Entropy (ΔS) is a measure of the degree of randomness or disorder within a system. A loss of a molecules' translational motion will result in a decrease in entropy (Morowitz, 1971). The entropy of adsorption is negative for all the absorbed compounds (Table I). While the alkanes, esters, ketones, and aldehydes had entropys in the range of −30 to −15 cal/(mol K), the alcohols had values that were much more negative [−40 to −25 cal/(mol K)]. This indicates that they have lost more freedom of motion than the other compounds studied. These data are consistent with the proposed binding hypothesis of two hydrogen bonds forming for alcohols. The data also indicate that ΔS is not a function of temperature (Table III).

The negative Gibb's free energy for all the flavor compounds studied implies that the interaction of these compounds with dry soy protein is spontaneous. The thermodynamic data also show that the binding reaction is being driven by the enthalpy of adsorption in gaseous systems. This emphasizes the importance of the ligand's functional group in the binding of flavors to soy protein. Likewise, Crowther et al. (1981) observed that the adsorption coefficient (K) decreased with heat treatment of Edi-Pro A. They postulated that as the protein denatured more nonpolar regions were exposed, decreasing both the solubility and availability of polar binding sites.

The thermodynamic data can only give an indication of the mode of binding by flavor compounds inasmuch as soy protein is not a crystalline substance. However, these data combined with the determination of the number and type of binding sites can help to solve the flavor problems of soy.

Registry No. Nonane, 111-84-2; decane, 124-18-5; 2-hexanone, 591-78-6; 2-heptanone, 110-43-0; 2-octanone, 111-13-7; 1-hexanal, 66-25-1; 1-heptanal, 111-71-7; 1-octanal, 124-13-0; methyl pentanoate, 624-24-8; methyl hexanoate, 106-70-7; methyl heptanoate, 106-73-0; methyl octanoate, 111-11-5; 1-butanol, 71-36-3; 1-pentanol, 71-41-0; 1-hexanol, 111-27-3; 1-heptanol, 111-70-6; 1-octanol, 111-87-5.

LITERATURE CITED

Arai, S.; Noguchi, M.; Kaji, M.; Kato, H.; Fujimaki, M. *Agric. Biol. Chem.* **1970**, *34*, 1420.

Ashraf, H. L.; Snyder, H. E. *J. Food Sci.* **1981**, *46*, 1201.

Aspelund, T. G.; Wilson, L. A. "World Soybean Research Conference-II"; Carlin, F. T., Ed; Westview Press: Boulder, CO, 1979; Vol. II, p 32.

Beyeler, M.; Solms, J. *Lebensm.-Wiss. Technol.* **1974**, *7*, 217.

Crowther, A.; Wilson, L. A.; Glatz, C. E. *J. Food Process Eng.* **1981**, *4*, 99.

Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1981a**, *29*, 1257.

Damodaran, S.; Kinsella, J. E. *J. Agric. Food Chem.* **1981b**, *29*, 1249.
Ehler, K. F.; Bernhard, R. A.; Nickerson, T. A. *J. Agric. Food Chem.* **1979**, *27*, 921.
Eldridge, A. C. "Soybeans: Chemistry and Technology"; Smith, A. K.; Circle, S. G., Eds.; Avi Publishing Co.: Westport, CT, 1978.
Franzen, K. L.; Kinsella, J. E. *J. Agric. Food Chem.* **1974**, *22*, 675.
Gale, R. L.; Beebe, R. A. *J. Phys. Chem.* **1964**, *68*, 555.
Goosens, A. E. *Food Process. Ind.* **1975**, *44*, 20.
Green, S. A.; Pust, H. *J. Phys. Chem.* **1958**, *62*, 55.
Gremli, H. A. *J. Am. Oil Chem. Soc.* **1974**, *51*, 95A.
Hammonds, T. M.; Call, D. L. *Chem. Technol.* **1972**, *2*, 156.
Honig, D. H.; Warner, K. A.; Seike, E.; Rackis, J. J. *J. Agric. Food Chem.* **1979**, *27*, 1383.
Johnson, D. W. "World Soybean Research Proceedings of the World Soybean Research Conference"; Hill, L. D., Ed.; Interstate Printers and Publishers, Inc.: Danville, IL, 1976; p 1014.
Kalbrener, J. E.; A. C.; Moser, H.; Wolf, W. J. *Cereal Chem.* **1971**, *48*, 595.
Kiselev, A. V.; Yashin, Yy. I. "Gas-Adsorption Chromatography"; Plenum Press: New York, 1969.
Lyon, L. *Food Prod. Dev.* **1980**, *14*, 58.
MacKenzie, C. A. "Unified Organic Chemistry"; Harper and Row: New York, 1962.
McMullin, S. L.; Bernhard, R. A.; Nickerson, T. A. *J. Agric. Food Chem.* **1975**, *23*, 452.
Meyer, E. W. "Proteins as Human Food"; Lawrie, R. A., Ed.; Avi Publishing Co.: Westport, CT, 1970; p 346.
Meyer, E. W.; Williams, L. D. "World Soybean Research Proceedings of the World Soybean Research Conference"; Hill, L. D., Ed.; Interstate Printers and Publishers, Inc.: Danville, IL, 1976; p 904.
Milner, M.; Scrimshaw, N. S.; Wang, D. I. C. "Protein Resources and Technology: Status and Research Needs"; Avi Publishing Co., Inc.: Westport, CT, 1978; pp 288, 290.
Morowitz, H. J. "Entropy for Biologists"; Academic Press: New York, 1971.
National Agricultural Research Policy Advisory Committee "National Soybean Research Needs"; National Soybean Research Coordinating Committee: Washington, DC, 1974.
Rackis, J. J.; Sessa, D. J.; Honig, D. H. *J. Am. Oil Chem. Soc.* **1979**, *56*, 262.
Sawyer, D. T.; Brookman, D. J. *Anal. Chem.* **1968**, *40*, 1847.
Schutte, L.; Van Der Ouweland, G. A. M. *J. Am. Oil Chem. Soc.* **1979**, *56*, 289.
Sessa, D. J. *J. Agric. Food Chem.* **1979**, *27*, 2340.
Smith, A. K.; Circle, S. J. "Soybeans: Chemistry and Technology"; Avi Publishing Co.: Westport, CT, 1978.
Twigg, G.; Kotula, A. W.; Young, E. P. *J. Anim. Sci.* **1977**, *44*, 218.
Wolf, W. J.; Baker, F. L. *Cereal Chem.* **1975**, *52*, 387.
Wolf, W. J.; Cowan, J. D. "Soybeans as a Food Source"; CRC Press: Cleveland, OH, 1975.

Received for review July 14, 1982. Revised manuscript received December 30, 1982. Accepted January 10, 1983. Portions of this paper were presented at the World Soybean Research Conference-II, March 26–29, 1979, Raleigh, NC. This work was supported by the Iowa State University Research Foundation and the Iowa Agriculture and Home Economics Station (Journal Paper No. J-10721, Project 2164, a contributing project to the North Central Regional Research Project NC-136).

Characterization of the Main Secondary Components of the Liquid Sugars from Cane Molasses

Gerardo Palla

The main nonsugar components of liquid sugars from cane molasses have been identified as phenolic and phenylpropanetriolic glucosides coming from the lignin material of cane during juice extraction and processing. The glucosides have been recovered by column absorption using nonionic polymers, have been fractionated through a silica gel column, and have been characterized by GC–MS, ^{1}H NMR, and ^{13}C NMR techniques.

In a previous work (Palla, 1982) we reported an efficient method to recover minor nonsugar organic colored components of sugar juices and syrups obtained by cane molasses demineralization. We reported also the structure of some phenolic compounds that we considered the main components of the sugar color. We have continued the work on the characterization of the nonsugar components, and we can now give an exhaustive description of the minor components of this type of sugar syrup. To this purpose we examined the silica gel chromatographic fractions of the raw organic material recovered from a wide series of liquid sugars; we found the greatest amount of nonsugars was composed by phenyl glucosides (10–20%), phenylpropanetriol glucosides (40–60%) and minor amounts of other phenolic derivatives. A fraction with high molecular weight has also been detected (10–30%). The characterization of the compounds has been made by MS, GC–MS, ^{1}H NMR, and ^{13}C NMR and by reference to synthetic standards such as phenols, phenyl glucosides, and phenylpropanetriols.

Istituto di Chimica Organica dell'Universita', 43100 Parma, Italy.

EXPERIMENTAL SECTION

Recovery and Fractionation. The recovery of the colored nonsugar material has been made following the absorption method described for nonionic polymers (Palla, 1982); in this way 1.2 g of colored material (80% of the total nonsugars) has been recovered from 300 g of liquid sugar sample (70% of dry matter, 99.3% of total sugars). The material recovered was dissolved in methanol and filtered. The amount dissolved (1.05 g) was chromatographed through a silica gel column (1.5 × 70 cm, Kieselgel 60 Merk), eluted with ethyl acetate and methanol (7/3 v/v). A control on the column effluent showed eight main chromatographic bands: A (9 mg, R_f 0.78, pale yellow), B

0021-8561/83/1431-0545$01.50/0