JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Inverse Gas Chromatographic Evaluation of the Influence of Soy Protein on the Binding of Selected Butter Flavor Compounds in a Wheat Soda Cracker System

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Binding of selected volatile butter flavor compounds to wheat vs soy-containing crackers was studied by inverse gas chromatography (IGC), sensory evaluation, and equilibrium sorption measurements. IGC data showed greater binding of γ-butyrolactone and butyric acid to both types of crackers than either diacetyl or hexanal, possibly due to the involvement of stronger binding forces such as hydrogen bonding and even ionic forces in the case of butyric acid. The presence of soy proteins did not affect binding of diacetyl and hexanal but increased binding of strongly interacting compounds γ-butyrolactone and especially butyric acid, probably through enhanced matrix polarity. In agreement with the IGC data, sensory evaluation results showed that the headspace aroma intensities were similar between the two diacetyl-flavored crackers, while they significantly differed between the butyric acid-flavored crackers. In addition, equilibrium sorption measurement data showed that binding of butyric acid was higher in the soy-containing cracker, but sorption of diacetyl to the two crackers did not significantly differ.

KEYWORDS: Soy; soda cracker; butter flavor; flavor binding; inverse gas chromatography; sensory study; equilibrium sorption measurement

INTRODUCTION

Flavor−ingredient interactions in foods directly impact flavor quality by influencing flavor retention during processing and storage as well as affecting the rate and extent of flavor release during food consumption. Strong binding of certain flavor compounds by soy proteins not only can cause retention of off-flavors but also may result in unbalanced flavor profiles in formulated soy foods. This has made it challenging to properly flavor soy-containing products (*1*−*3*).

Considerable research has been conducted to better understand flavor−soy protein interaction mechanisms (*4*−*13*). However, most of these studies used single ingredient or simple aqueous buffer systems, which do not closely resemble real foods. In fact, most food products are complex mixtures consisting of various types of ingredients, which further complicates favor−matrix interactions and, hence, the disposition of individual flavor compounds in the food system. Therefore, results obtained from a simple model system may not be directly applied to real foods. In the case of product reformulation, modification of the flavoring system is usually required. In such cases, a method that can directly measure flavor binding in real foods instead of in an individual ingredient is extremely desirable, which certainly can aid in the efficient design of a flavoring system to ensure optimum product flavor quality.

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We have developed a rapid and sensitive inverse gas chromatography (IGC) method to study binding of volatile flavor compounds to solid food substance (*14*) and have applied this method to characterize flavor−soy protein interactions under low-moisture conditions (*15*). The objective of the present study was to evaluate the possibility of applying this IGC method to study flavor−matrix interactions in a real food system. In the present study, we applied IGC to evaluate the influence of soy protein in a wheat soda cracker system on the binding of selected volatile butter flavor compounds. Binding of selected butter flavor compounds by the crackers was also measured by both sensory evaluation and equilibrium sorption measurements, and the results were compared to further evaluate the potential usefulness of the IGC data.

MATERIALS AND METHODS

Volatile Compounds. Standard volatile compounds diacetyl (butane-2,3-dione), butyric acid, γ-butyrolactone, hexanal, 2-ethyl butyric acid, and pentane-2,3-dione of analytical grade (>99% purity) were purchased from Aldrich Chemical Co. (St. Louis, MO). Food grade (>99% purity) diacetyl and butyric acid used in crackers prepared for sensory study were obtained commercially from Aldrich Flavors and Fragrances (St. Louis, MO).

Soda Cracker Ingredients. A representative soy protein isolate (SPI; protein, >90% (db); fat, <4%; and moisture, <6.0%) was provided by Archer Daniels Midland Co. (Decatur, IL). Other ingredients including flour (Pillsbury Best all purpose flour, The Pillsbury Co., Minneapolis, MN), shortening (Crisco all-vegetable shortening, The J.

10.1021/jf060538+ CCC: $33.50
Published on Web 06/23/2006

Table 1. Cracker Formulations

	wheat cracker	soy-containing cracker
flour (g)	100.0	75.0
SPI (g)	0.0	25.0
salt (g)	2.0	2.0
baking powder (g)	2.4	2.4
shortening (g)	11.2	11.2
water (g)	59.2	64.0
butyric acid (g)[a]	0.46	0.46

[a] Only added in formulation of butyric acid-flavored crackers prepared with method A.

M. Smucker Co., Orrville, OH), baking powder (Clabber Girl double-acting baking powder, Clabber Girl Co., Terre Haute, IN), and salt (Morton salt, Morton International., Inc., Chicago, IL) were purchased at local supermarkets.

Preparation of Plain Soda Crackers. Plain soda crackers (no flavoring added) were prepared by first combining flour, salt, and baking powder in a mixing bowl, cutting in shortening, and then gradually adding water to form a dough. The dough was rolled out on a floured surface to 1−2 mm thick, cut into 3 cm (o.d.) disks, pricked with the tines of a fork 3−4 times, then placed on an ungreased baking pan, and baked at 350 °F till lightly brown. The formulations used to prepare the dough are given in **Table 1**. The baked crackers were allowed to cool completely at room temperature, then kept in brown glass bottles with Teflon-lined caps, and stored at room temperature until analysis. The day after the crackers were made, water activities of the crackers were measured (at 25 °C in triplicate) with a series 3TE AquaLab water activity meter (Decagon Devices, Inc., Pullman, WA).

Preparation of Flavored Soda Crackers. Flavored soda crackers were prepared using two different methods (methods A and B). In method A, butyric acid-flavored crackers were prepared in a similar manner as the plain crackers, except that a known amount of butyric acid was added to water when making the dough (**Table 1**). With method B, freshly prepared plain crackers were ground and sieved to 40/50 mesh sizes. Then, 10 μL of an aqueous flavor solution (containing 1.311 mg of butyric acid or 2.877 mg of diacetyl) was added to 30 g of the cracker powder placed inside a 250 mL Teflon-lined screw cap glass bottle. The bottles containing the cracker and flavoring were gently shaken on an Orbital shaker (model DS-500; VWR Scientific Products, Buffalo Grove, IL) for 24 h to reach equilibrium (preliminary studies showed that no significant difference was found in flavor uptake among crackers equilibrated for 4−48 h). Sensory evaluation and equilibrium sorption measurements were conducted immediately after the flavored crackers were prepared.

Flavor−Cracker Interactions Measured by IGC. IGC is a dynamic gas phase technique that can be applied to study volatile−nonvolatile interactions. Through measurement of thermodynamic parameters of adsorption and sorption isotherms, the surface chemistry of a solid material and the thermodynamic properties of the sorbate−sorbent system can be evaluated.

To prepare the IGC column, freshly prepared plain crackers were ground into powders and sized to obtain 100/120 mesh particles and then packed into a deactivated glass tube (17.8 cm × 4 mm i.d.; Supelco; Bellefonte, PA) using the procedure described previously (*14*). Each column was connected to the IGC system and conditioned under carrier gas to the desired temperature and relative humidity (RH) conditions for 48 h prior to experiments. Whenever the temperature was changed, the column was reconditioned for at least 16 h to ensure that the new equilibrium condition was established.

Thermodynamic parameters of adsorption and sorption isotherms were determined using the procedures described before (*14*). Interactions between the four volatile flavor compounds and the two soda cracker matrices at an RH level of 15% were measured at 30, 35, and 40 °C. For each type of cracker evaluated, measurements were performed on two different columns, each packed with cracker obtained from a different batch. The reported data are mean values. Statistical analysis (*t*-test; $p \leq 0.05$) was conducted to analyze the data.

Perceived Cracker Headspace Aroma Intensities Evaluated by Sensory Evaluation. Prior to sensory evaluation, 2.0 g of the flavored cracker (prepared with method B) was transferred to a sniffing bottle (125 mL Nalgene Teflon FEP wash bottle with siphon tube removed from the cap; Fisher Scientific, Pittsburgh, PA). Each bottle was covered with aluminum foil and labeled with a three-digit random number. The perceived aroma of the cracker headspace was evaluated using the 2-AFC with warm-up method developed by Thieme and O'Mahony (*16*).

During the "warm-up" procedure of the 2-AFC test, panelists sniffed back and forth between a pair of samples labeled "A" and "B" (one was wheat cracker, and the other was soy-containing cracker) until they could detect a difference between them and wrote down the nature as well as the direction of the difference between the two samples (for example, "B is more buttery than A"). The sensory attribute described by the panelists was then used in the instructions for the subsequent sample testing, during which they evaluated each pair of the test samples and indicated the one that was stronger in the aroma attribute identified during the warm-up phase of the test. Panelists were instructed to sniff a water bottle between every sample during both the warm-up and the actual sensory testing. Eight replicated pairs of samples were prepared for each flavor compound (diacetyl and butyric acid) studied, and each compound was tested on a single day over a two-day period using the same panelists.

A total of 30 panelists (21 females and nine males; 19−55 years old) participated in this study. They were recruited from faculty/staff and graduate students by sending out e-mail solicitations. Panelists were compensated for their participation. Data of the 2-AFC tests were analyzed by β-binomial statistics using the IFPrograms software (version 7.3; The Institute for Perception, Richmond, VA) with the null probability of 0.5. Approval for all sensory studies was obtained from the Institutional Review Board at the University of Illinois (IRB Protocol Number 05352).

Flavor Binding by the Crackers Determined by Equilibrium Sorption Measurement. Solvent extraction followed by gas chromatography (GC) analysis was used to determine the amount of flavor uptake by the crackers at equilibrium. To prepare the extract, 3.0 g of flavored cracker powder was diluted with 20 mL of deodorized distilled−deionized water in a 50 mL test tube, spiked with an internal standard solution (containing 51.0 μg of pentane-2,3-dione and 88.0 μg of 2-ethyl butyric acid in methanol). The cracker solution was acidified to pH 2 with 1 M aqueous HCl, and then, 5 mL of ether was added to the solution. Prior to extraction, the solution was gently agitated and vented for about 1 min. Then, the water/ether solution was extracted for 10 min and subsequently centrifuged (IEC HN-SII Centrifuge; Damon/IEC Division, Needham Heights, MA) at 3200 rpm for 10 min to break the emulsion. The top organic layer was collected and kept at −20 °C until analysis. Extractions were preformed in triplicate for each cracker−flavor set evaluated. Extract (1 μL) was injected into a GC-MS system consisting of an HP 6890 GC/5973N mass selective detector (MSD; Agilent Technologies, Inc.) using the cold-splitless mode (initial temperature, −50 °C; ramp, 12 °C/s; final temperature, 260 °C; final hold time, 10 min; splitless time, 1 min; and vent flow, 50 mL/min). Separations were performed on a Stabilwax-DA column (30 m × 0.25 mm i.d.; 0.5 μm film; Restek Corp.). Helium was the carrier gas at a constant flow of 1.0 mL/min. The GC oven temperature was programmed from 35 to 225 °C at a rate of 8 °C/min with initial and final hold times of 5 and 30 min, respectively. MSD conditions were as follows: capillary direct interface, 280 °C; ionization energy, 70 eV; mass range, 35−300 amu; EM voltage (Stune + 200 V); and scan rate, 5.27 scans/s. The amount of flavoring retained in individual cracker systems was determined by internal standard calibration using 2-ethyl butyric acid and pentane-2,3-dione as the internal standard for butyric acid and diacetyl, respectively.

RESULTS AND DISCUSSION

When developing the soda cracker prototypes, three commercial SPIs were obtained and screened for their suitability for use in making soda crackers. One SPI that gave the most acceptable sensory characteristics (flavor/taste and texture;

Table 2. 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 Flavor Compounds on Wheat and Soy-Containing Crackers at 15% RH

	diacetyl	hexanal	γ-butyrolactone	butyric acid
		$-\Delta H_s$ (kJ mol^{-1})[a]		
wheat cracker	29.6 ± 0.2	35.7 ± 0.4	45.4 ± 0.2	57.3 ± 2.5
soy-containing cracker	29.1 ± 0.4	35.6 ± 0.1	49.3 ± 0.2	69.3 ± 0.9
		S (nmol g^{-1} Pa^{-1})[b]		
wheat cracker	9.07 ± 0.10	65.8 ± 1.5	438 ± 11	1160 ± 94
soy-containing cracker	9.46 ± 0.03	67.7 ± 0.0	477 ± 7	2182 ± 6

[a] On the basis of data determined at 30, 35, and 40 °C. [b] Data determined at 35 °C.

evaluated by an internal panel) to the finished soy-containing cracker was selected for this study. Flour substitution at a level of 25% (weight basis; **Table 1**) was chosen such that the soy-containing cracker not only had acceptable sensory quality but also contained 6.25 g of soy protein per serving (30 g), which qualifies it as a healthy snack according to the Food and Drug Administration-approved health claim (*17*). Four volatile butter flavor compounds, diacetyl, hexanal, γ-butyrolactone, and butyric acid, each representing one of the major chemical classes (ketone, aldehyde, lactone, and fatty acid) found in butters (*18*, *19*), were selected in the present study to assess the butter flavor binding potential of the two soda crackers.

Flavor Binding Measured by IGC. A humidity level of 15% RH was selected for IGC experiments to closely simulate the water activity (a_w) conditions of the crackers that we prepared (a_w of wheat and soy-containing crackers, 0.103−0.190 and 0.143−0.178, respectively, at 25.0 °C). Because both crackers represent multicomponent systems, apparent measurements representing individual systems as a whole are implied throughout the discussion.

For the soy-containing cracker, enthalpies of adsorption (ΔH_s) determined for diacetyl, hexanal, γ-butyrolactone, and butyric acid were 29.1 ± 0.4, 35.6 ± 0.1, 49.3 ± 0.2, and 69.3 ± 0.9 kJ mol^{-1}, respectively (**Table 2**). The heat of adsorption was also determined for butanal (24.7 ± 0.1 kJ mol^{-1}). On the basis of these values and with volatile compound carbon chain length being taken into account, the relative binding strengths of these compounds with soy-containing crackers from weak to strong were as follows: aldehyde < diketone < lactone < acid. The observed stronger binding of diacetyl to the soy-containing cracker than butanal (29.1 ± 0.4 vs 24.7 ± 0.1 kJ mol^{-1}; **Table 2**) could be attributed to the fact that, as a diketone, diacetyl contains two interaction centers (carbonyl oxygens) while butanal contains only one. The much stronger binding observed for γ-butyrolactone as reflected in the much higher ΔH_s values determined (49.3 ± 0.2 kJ mol^{-1}; **Table 2**) suggests the involvement of hydrogen bonding, which is likely due to the high accessibility of the oxygens dictated by its relative planar (four-carbon ring) molecular configuration. The even higher ΔH_s values determined for butyric acid (69.3 ± 0.9 kJ mol^{-1}; **Table 2**) indicate the involvement of much stronger interaction forces such as hydrogen bonding or even ionic bonds since it has the capability of interacting with both polar and charged functional groups. A similar trend in terms of the relative binding forces of these four compounds with the wheat cracker was observed (**Table 2**). Other thermodynamic data (data not shown) as well as sorption data (**Table 2**) also support the above findings.

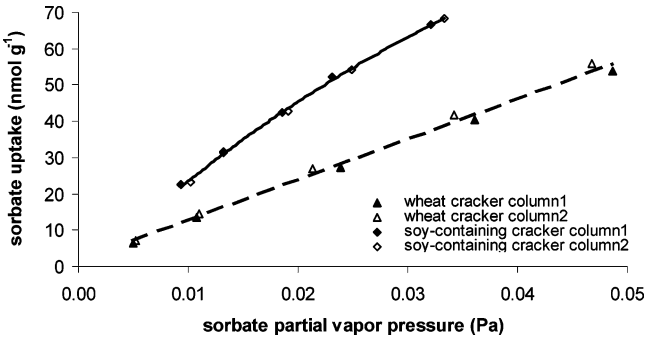

Figure 1. Sorption isotherms determined for butyric acid on wheat cracker vs soy-containing cracker at 35 °C and 15% RH.

These two crackers contain approximately 10% lipid (**Table 1**), and nonpolar flavor−lipid interactions may have an influence on the apparent interaction forces observed. However, as seen from the above discussion, heat of adsorption data showed that the most polar compound (butyric acid) interacted most strongly with the cracker matrices (**Table 2**), suggesting that mechanisms other than nonpolar flavor−lipid interactions play a predominant role in the overall binding strengths observed.

In our previous study (*15*), we demonstrated the importance of the volatile flavor compound chemical structure on flavor−soy protein interactions. The above results further illustrate the importance of flavor compound chemical nature in flavor−matrix interactions in real foods. A comparison was further made across the two cracker systems for each volatile flavor compound studied to examine the influence of food ingredients on flavor−cracker interactions as discussed below.

Both thermodynamic and sorption data (**Table 2**) suggest that diacetyl interacted to about the same extent with the two cracker systems. As compared with starches (the major components of wheat flour), SPI contains relatively higher amounts of polar and charged functional groups. Incorporating SPI to the cracker should have enhanced the system polarity and, hence, the interactions with those relative polar compounds such as diacetyl. However, the relatively weak and comparable binding of diacetyl to both crackers suggested that under the humidity level studied (15% RH), diacetyl cannot compete with water for polar binding sites. As a result, only weak binding forces were involved and the inclusion of soy did not enhance the binding potential of diacetyl with the cracker matrix. Similar to diacetyl, binding of hexanal to the two cracker systems was not significantly different (**Table 2**). We had previously demonstrated that in the presence of moisture interactions between hexanal and SPIs were relatively weak (*15*); therefore, it is not surprising to find that inclusion of soy did not enhance hexanal−cracker interactions. γ-Butyrolactone showed slightly higher binding with the soy-containing cracker than with the wheat cracker (**Table 2**). This indicates that at 15% RH γ-butyrolactone molecules can compete with water to some extent for polar binding sites, which further suggests the involvement of relatively strong binding forces. Heats of adsorption data suggest that butyric acid interacts more strongly with the soy-containing cracker than with the wheat cracker (69.3 ± 0.9 vs 57.3 ± 2.5 kJ mol^{-1}; **Table 2**). The greater influence of soy protein on the binding strength of butyric acid to the cracker indicates that at 15% RH, butyric acid can compete with water for binding sites to a greater extent and that the inclusion of soy did increase system polarity and, hence, facilitated greater binding of polar flavor compounds. The determined sorption isotherms (**Figure 1**) also showed that a

significantly higher amount of butyric acid was bound to the soy-containing cracker than to the wheat cracker. Furthermore, the Langmuir isotherm determined with the soy-containing cracker system (**Figure 1**) indicates that binding sites of different energy levels exist. Meanwhile, the linear isotherm observed with the wheat cracker reflects the relatively homogeneous surface nature of the wheat cracker.

δ-Decalactone, together with diacetyl, butyric acid, and hexanal, are some of the odorants that are important to the overall flavor of butters (*18*−*20*). γ-Butyrolactone instead of δ-decalactone was chosen in the present study because their molecular structures are similar, as well as the fact that γ-butyrolactone has a relatively high volatility, which enables feasible IGC measurement of flavor binding under the low-temperature conditions (30−40 °C) used in this study. The differential binding potential of γ-butyrolactone, and especially butyric acid, between the two cracker model systems indicates that the sensory attributes of the two crackers may be impacted as a result of differential flavor binding. To examine this possibility, two of the four volatile butter flavor compounds, diacetyl and butyric acid, were selected and their bindings with the two crackers were evaluated by sensory evaluation as discussed below.

Flavor Binding Evaluated by Sensory Evaluation. In this part of the study, a 2-AFC with a warm-up method was used to determine whether a difference exists between the headspace aroma intensities of flavored wheat vs soy-containing crackers. The warm-up procedure was included to preliminarily observe the main difference between the two (diacetyl or butyric acid) flavored crackers in terms of their headspace aroma characteristics. Terms such as "buttery/creamy" and "cheesy/pungent/stinky" were given by the panelists to describe the perceived major difference between the diacetyl and the butyric acid-flavored crackers, respectively. Results showed that the perceived headspace aroma intensities were not significantly different between the two diacetyl-flavored crackers ($p < 0.2232$; estimated probability of the data, 0.5333; and power of the test, 18.8%). Meanwhile, significant differences were observed between the butyric acid-flavored crackers ($p < 0.0015$; estimated probability of the data, 0.6333; and power of the test, 91.5%).

The perceived aroma intensity of a food upon sniffing is largely determined by the type and amount of volatile flavor compounds present in the gas phase (headspace) above the food. Furthermore, the amount of a specific volatile aroma compound present in the headspace is mainly dictated by its distribution between the food matrix and the headspace, which not only is determined by the physicochemical properties of the volatile compound itself but also is affected by flavor−matrix interactions (*21*−*23*). Therefore, by measuring the perceived specific headspace aroma intensity, binding of the added flavorant by the food matrix can be indirectly assessed. In the present study, because the same amounts of flavor compound (diacetyl or butyric acid) were added to the two crackers and equilibrated before any measurements were taken, the similar headspace aroma intensities of the two crackers suggested similar binding of diacetyl to the crackers, while the significantly different perceived odor intensities between the crackers indicated that binding of butyric acid to the two crackers differed. As such, general agreement was found between sensory evaluation and IGC data. A third technique, which determined flavor binding by direct measurement of flavor uptake at equilibrium, was also conducted to further evaluate the soundness of the above IGC and sensory evaluation results.

Table 3. Level of Added Flavorant Retained in Crackers Determined by Equilibrium Sorption Measurement

	method A[a]	method B[b]	
	butyric acid (ppm)	butyric acid (ppm)	diacetyl (ppm)
wheat cracker	613 ± 55	28.8 ± 3.0	14.5 ± 2.7
soy-containing cracker	759 ± 68	35.6 ± 4.9	16.4 ± 2.1

[a] Flavor added prior to baking. [b] Flavor added after baking.

Flavor Binding Determined by Equilibrium Sorption Measurements. The amounts of butyric acid retained in the crackers prepared with method A were determined to evaluate flavor binding/retention during cracker preparation. Results showed that a higher level of butyric acid was retained by the soy-containing cracker than by the wheat cracker (**Table 3**). This supports the IGC data in that butyric acid is bound more tightly and interacts to a greater extent with the soy-containing cracker (**Table 2** and **Figure 1**). We also attempted to compare the retention of diacetyl by the two crackers during the baking process. However, a preliminary study showed that when diacetyl was added at low levels, it had little impact on the flavor of the finished product since the majority of diacetyl was lost during baking due to its high volatility. On the other hand, when diacetyl was added at high levels, it reacted with other ingredients (via Maillard reaction) as evidenced by a discoloration observed as well as the lack of a "buttery" note in the finished crackers. Therefore, binding of diacetyl was evaluated only with crackers prepared with method B as discussed below.

Flavor binding/sorption via interaction with added flavoring (diacetyl or butyric acid) was determined with crackers prepared using method B. Again, results agreed well with the IGC data. That is, a higher amount of butyric acid was bound to the soy-containing cracker than to the wheat cracker, while the uptake of diacetyl by these two different cracker types was not significantly different (**Table 3**). The flavored crackers used in this part of the study were prepared in the same way as those used in sensory studies. Furthermore, the amount of flavor compound present in the headspace is in reverse proportion to that present in the food matrix. Therefore, the results of equilibrium sorption measurements agree well with the sensory data.

Binding or sorption of volatile flavor compounds by nonvolatile food substances can have positive or negative impacts on the flavor of a food system. Strong interactions may be beneficial in that it may protect the flavor compounds from loss during processing and storage of the food and then release the bound flavors when the product is consumed. On the other hand, strong retention of a key aroma-active compound can suppress the primary flavor impact of a product or even cause flavor imbalance. In addition, strong retention of flavor compounds having undesirable flavors can cause off-flavors. Therefore, when developing a new formulation or using new ingredients, knowledge of which flavor compounds will bind and to what extent is essential for efficient design of flavoring systems to ensure that the desired flavor quality can be obtained.

In the present study, flavor−cracker interactions measured by IGC were in good agreement with data determined from sensory studies and equilibrium sorption measurements. As compared with the other two methods used, IGC measurements are relatively simpler and more convenient to conduct and can generate data rapidly. Results of this study indicate that IGC could be a potentially useful tool to rapidly measure flavor−

food matrix interactions in complex real foods under low moisture conditions, which may provide useful information aiding in design of appropriate flavoring systems as well as prediction of sensory impact.

Flavor binding, retention, and release are closely related phenomena, and they play important roles on actual sensory impact. However, more work is still needed to further evaluate the potential of relating IGC data to flavor release and sensory impact under conditions simulating actual food consumption. Information obtained from such studies could provide a better understanding of the underlying mechanisms of flavor binding, release, and ultimate flavor impact and their interrelationships.

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Received for review February 23, 2006. Revised manuscript received May 8, 2006. Accepted May 21, 2006. Funding for this study was provided by the U.S. Department of Agriculture (NRI Competitive Grants Program, Project 2005-35503-16234).

JF060538+