Mechanisms of Interaction Between Vanillin and Milk Proteins in Model Systems

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ABSTRACT:The effects of milk proteins on vanillin intensity reduction were studied in a phosphate buffer (pH 6.5) using urea and sodium dodecyl sulfate (SDS) as bond disrupting agents. The reduction of vanillin occurred immediately as the protein was introduced to the system. Bovine serum albumin (BSA) interacted more with vanillin than sodium caseinate (CAS) did. Heat treatment had no effect on vanillin reduction of the CAS system; however, free vanillin content was higher in the heated than nonheated BSA system. Hydrogen bonding appeared to be a major force for the interaction of vanillin and CAS. However, hydrophobic interaction seemed to be more important than hydrogen bonding in the vanillin and BSA system.

Keywords: vanillin, proteins, hydrogen bonds, hydrophobic interactions, flavor

Introduction

THE INTERACTION OF FLAVOR COMPOUNDS WITH OTHER COMPONENTS affects the perceived flavor of the foods. For example, the intensity of vanillin flavor in a food system can be reduced greatly by its interaction with other components, especially proteins. The interaction primarily occurs via the Schiff base formation, hydrogen bonding, and hydrophobic interaction. In liquid or high-moisture food systems, flavor compounds having aldehyde groups can bind covalently to the amino groups of proteins via the Schiff base formation (Damodaran 1996). However, flavor binding also may occur through the formation of ligands between polar-type flavor compounds and carbohydrates by hydrogen bonding (Whistler and Daniel 1985). The flavor bindings also may occur through hydrophobic interactions between nonpolar-type flavor compounds and hydrophobic peptide chains of protein molecules. For example, the reduction of flavor intensity of vanillin in low-fat ice cream mixes was attributed to the Schiff base formation (Graf and de Roos 1996). On the other hand, the interaction between vanillin and faba bean protein yielded low free energy, suggesting involvement of low-energy bondings such as hydrophobic interactions (Ng and others 1989a). Moreover, we observed that the Schiff base formation was slow in an aqueous model system and did not cause instant reduction of the vanillin flavor intensity (Chobpattana and others 2000). Therefore, we suspected that other forces such as hydrogen bonding and/or hydrophobic interaction are more likely the predominant forces in the vanillin-protein interaction.

The binding of vanillin with proteins is affected by the type and concentration of proteins as well as by heat treatment. McNeill and Schmidt (1993) reported that sodium caseinate (CAS) interacted more with vanillin than whey protein isolate (WPI) in sweetened drinks. Hansen and Booker (1996) found that whey protein concentrate (WPC) bound flavor more in an ice cream mix than CAS did. Heat treatment can alter the structure of proteins or lead to protein unfolding that may affect interactions with other food components. McNeill and Schmidt (1993) reported that a WPI treated with heat (85 °C, 10 min) caused significantly higher vanillin flavor intensity than an untreated isolate. However, the same heat treatment had no effect on the flavor intensity with CAS.

Our objectives were to investigate the importance of hydrogen bonding and hydrophobic interaction in the reduction of vanillin intensity in milk protein model systems and to understand the effect of different types, concentrations, and heat treatments of proteins on vanillin reduction.

Materials and Methods

Materials

All chemicals used in this study were of ACS analytical reagent grade unless otherwise specified. Vanillin, CAS, bovine serum albumin (BSA), gum arabic, sodium phosphate (mono and dibasic), urea, and sodium dodecyl sulfate (SDS) were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A). Food grade WPI (Provon 190) was obtained from Avonmore Waterford Ingredients, Inc. (Monroe, Wis., U.S.A.). Methanol (HPLC grade) and Centricon centrifugal YM-10 filter devices (Millipore Corp., Bedford, Mass., U.S.A.) were purchased from Fisher Scientific (Pittsburgh, Pa., U.S.A.).

Buffer and standard solution

Vanillin standard solution (500 ppm) and solution of CAS, WPI, BSA (6 and 12%), gum arabic (1%), urea (8 M) and SDS (0.5%) were prepared in 0.05 M phosphate buffer (pH 6.5) and held at 5 °C until used.

Reaction mixtures for studying reaction times

Two mL of the vanillin standard solution was pipetted into a 20-mL vial, followed by 5 mL of 6% CAS or BSA solutions, and 3 mL of phosphate buffer. The final volume (10 mL/vial) contained 100 ppm vanillin and 3% CAS or BSA. The control contained all but milk protein. The vials were placed immediately in an incubator (5 °C). A vial of each sample was removed from the incubator at 5, 15, 30, 60, and 120 min.

Reaction mixtures for studying protein concentrations

Two concentrations (3 and 6%) of CAS and BSA were prepared

using 6 and 12% milk protein stock solutions. The protein solutions were added to the vanillin following the same procedure used for the reaction times.

Reaction mixtures for studying heat treatment

To study the effect of heat treatment of proteins on their interactions with vanillin, the 6% CAS and BSA were heated in a water bath (Precision Scientific, Chicago, Ill., U.S.A.) at 68 °C for 30 min or 75 °C for 15 min with steady stirring. These temperatures were chosen to mimic the minimum recommended heating (≥ 68 °C for 30 min) for pasteurizing ice cream mixes (Morr and Richter 1988). Following the heat treatment, the solutions were cooled to room temperature (25 ± 1 °C) with flowing tap water. The final mixtures contained 100 ppm vanillin and 3% milk proteins. The control was prepared with the same procedure for samples, but no milk protein was added.

Reaction mixtures for studying bond disrupting

Urea was used as a hydrogen-bond disrupting agent, whereas SDS was used as a disrupting agent for hydrophobic interactions. Two mL of the vanillin standard solution was pipetted into a 20- mL vial, followed by 5 mL of 6% CAS or BSA solutions. After 1 h in a 5 °C incubator, 1 mL of urea and/or SDS standard solution was added plus additional buffer to make 10 mL for each vial. The vials were kept at 5 °C for 1 h before samples were taken. The final concentration of the mixture was 100 ppm vanillin, 3% milk protein, and 0.8 M urea or 0.05% SDS.

Reaction mixtures for studying different types of reactants

To study the effects of different reactants on vanillin interactions, the sample mixtures were prepared using CAS, BSA, WPI, and gum arabic solutions. The same procedures used for the reaction times were followed. The final mixtures contained 100 ppm vanillin, 3% CAS, BSA, or WPI or 0.1% gum arabic.

Sample treatment

All samples (10 mL each) of the reaction mixtures were held at 5 °C in an Equatherm incubator model 299-766 (Curtin Matheson Scientific, Houston, Tex., U.S.A.). Except for the study of reaction time, all were kept there for 120 min.

To isolate free (or unbound) vanillin from the protein matrix, centrifugal ultrafiltration was performed. Centricon YM-10 membrane filters were used for the ultrafiltration (10,000 nomimal molecular weight limit), and 1 mL of each sample was centrifuged (1240 x g) for 45 min at 5 °C using a Beckman J2HS centrifuge (Beckman Instruments, Inc., Palo Alto, Calif., U.S.A.). Approximately 0.5 mL of the ultrafiltrate was collected and used for the analysis of free vanillin.

High performance liquid chromatography (HPLC)

Free vanillin was determined with a Hewlett-Packard 1090A Series II HPLC equipped with a HP 1040 photodiode array UV-visible detector and a HP 9000 Series 300 ChemStation (Palo Alto, Calif., U.S.A.). Separation of the chemical components was achieved using a Spherisorb WP 10 µm C18 reversed-phase column (250 mm x 4.6 mm) with a C18 guard column (10 mm x 4.6 mm), both from Alltech Associates, Inc. (Deerfield, Ill., U.S.A.). The mobile phase was 60% methanol and 40% H_2O. The flow rate was 1.0 mL/min at 25 °C, and the injection volume was 20 µL. Retention times and on-line UV spectra were used for peak identification. Quantification was done by comparing peak areas of vanillin in control and reaction mixtures.

Statistical analysis

All treatments for each experiment were replicated 3 times. A randomized completed block design was used for most studies. For the bond disrupting study, a split plot design was employed with heat treatments of protein as a whole plot factor and bond disrupting agents as a subplot factor. Analysis of variance (ANOVA) and F-protected t-tests were performed using PROC Mixed in SAS (SAS Institute 1997). Significance of differences among means were defined at $p < 0.05$.

Results and Discussion

Effect of reaction time

Sharp and significant reductions ($p < 0.05$) of free vanillin occurred mostly within the first 5 min in the presence of CAS or BSA (Figure 1). This reduction was higher with BSA (about 30%) than with CAS (about 11%). No further significant vanillin reduction occurred after 15 min with either protein. Our previous work showed that the interaction of vanillin with amino acids/peptides took a considerable amount of time to form Schiff base compounds (Chobpattana and others 2000). Therefore, we suspected that the instant reduction of vanillin with CAS and BSA might have been caused by formation of low energy, weak bonds such as hydrogen bonds and/or hydrophobic interactions (Ng and others 1989a).

Effect of protein concentration

Protein concentration had a significant effect on the reduction of free vanillin content (Figure 2); it decreased as concentration of CAS or BSA increased from 0 to 6%. The reduction of free vanillin was significantly higher at 6% than at 3% with both proteins and was higher with BSA than with CAS. This indicates that the higher concentration of protein could interact more with vanillin.

These results suggest that the interaction of vanillin with proteins was concentration-dependent. Ng and others (1989a) reported that the percentage of bound vanillin increased as the faba bean protein concentration increased. Hansen and Heinis

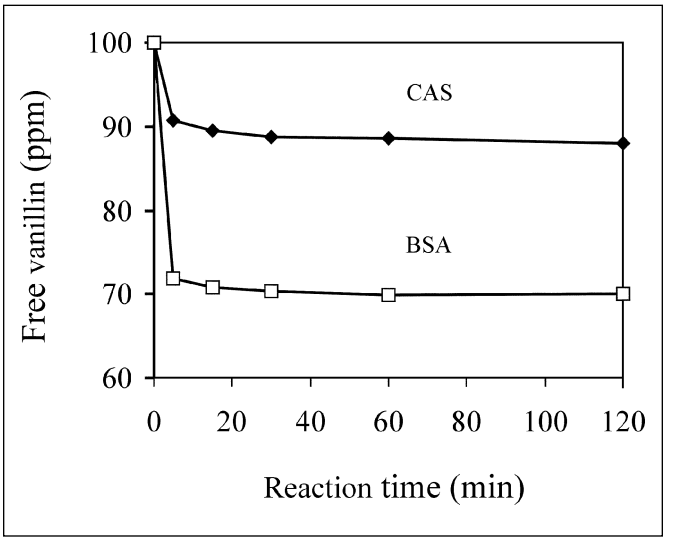

Figure 1—Effect of reaction time on vanillin reduction in a model system of 100 ppm vanillin and 3% sodium caseinate (CAS) or bovine serum albumin (BSA) in 0.05 M phosphate buffer (pH 6.5). Each data point represents the mean of 3 replications.

Table 1—Effect of heat treatment of proteins on vanillin reduction in a model system containing 100 ppm vanillin and 3% sodium caseinate (CAS) or bovine serum albumin (BSA) in 0.05 M phosphate buffer (pH 6.5)

	Free vanillin content (ppm)	
Treatment	**CAS**	**BSA**
Nonheated	88.30[a]	69.59[c]
Heated at 68 °C 30 min	88.16[a]	73.12[b]
Heated at 75 °C 15 min	87.92[a]	74.39[b]

[a,b,c] Means with different letters are significantly different ($p < 0.05$).

(1991) also reported that vanillin intensity, as determined by descriptive sensory analysis, was reduced in the presence of CAS or whey protein concentrate (WPC). However, no significant differences in intensity of vanillin occurred with increasing CAS concentrations from 0.12 to 0.50 %, but a significant reduction in vanillin intensity did occur with increasing WPC at the same levels. Our results showed that significant reductions of free vanillin occurred with increasing concentrations of both CAS and BSA. These reductions ranged from 10 to 34% for CAS and 30 to 51% for BSA when the proteins increased from 3 to 6%. This difference might have been due to the type and concentration of proteins as well as analytical methods (sensory analysis against HPLC).

Effect of heat treatment

The effects of heat treatment of proteins on the reduction of free vanillin appeared to be related to the type of proteins (Table 1). No significant difference occurred in vanillin reduction between nonheated and heated CAS ($p > 0.05$). However, small but significant difference of free vanillin (3.5-4.8%) occurred for nonheated and heat-treated BSA. No significant difference in vanillin reduction was observed with the 2 levels of heat treatment of BSA.

Ng and others (1989b) reported that vanillin intensity rated by a sensory panel was higher with heated faba bean protein than native proteins in a model system of 1200 ppm vanillin and 5% proteins. However, the results did not correlate with their HPLC measurements. McNeill and Schmidt (1993) reported that a sensory panel perceived more vanillin flavor in a heated WPI system than nonheated WPI, whereas no differences were found for vanillin flavor intensity between heated and nonheated CAS model systems. In addition, we observed no visual changes for the CAS system during heating, whereas the BSA system showed a cloudy and opaque appearance upon heating. It is well known that CAS is heat resistant to denaturation over a wide range of temperatures (Swaisgood 1996), whereas BSA is readily denatured by heat. De Wit and Klarenbeek (1984) reported that BSA was denatured most easily at 64 °C. Hayakawa and others (1992) reported that heat denaturation of BSA became apparent at 40 °C by fluorometric measurements. The increase of free vanillin in the heated BSA model system in our study may have been due to heat-induced structural changes, perhaps making some peptide chains of the protein unavailable to bind with vanillin. We postulate that the amino acid residues of denatured BSA could have interacted with each other, thus lowering the number of total residues available for binding with vanillin.

Effect of bond disrupting agents

The free vanillin contents of the vanillin-CAS model system were affected by the bond disrupting agents (Figure 3). Urea significantly increased the free vanillin content of the system ($p < 0.05$), whereas SDS did not cause significant increases. The free vanillin content with a combination of urea and SDS was not significantly different than that with urea only. These results suggest that hydrogen bonding appeared to be the major chemical interaction causing reduction of free vanillin in the CAS system. In addition, we observed that free vanillin contents of the nonheated and heated CAS were not significantly different ($p > 0.05$) within bond disrupting treatments (Figure 3).

However, in the BSA model system, the free vanillin content was significantly higher ($p < 0.05$) when urea or SDS was added than without bond-disrupting agents and it was slightly, but not significantly, higher with SDS (Figure 4). Addition of both urea and SDS yielded significantly higher vanillin content than either bond-disrupting agent alone. These observations suggest that

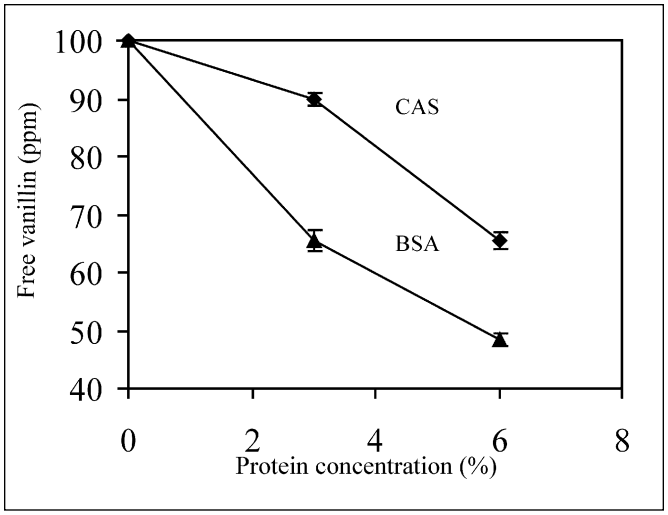

Figure 2—Effect of protein concentrations on vanillin reduction in a model system of 100 ppm vanillin and 3% sodium caseinate (CAS) or bovine serum albumin (BSA) in 0.05 M phosphate buffer (pH 6.5). Each data point represents the mean ± SD of 3 replications.

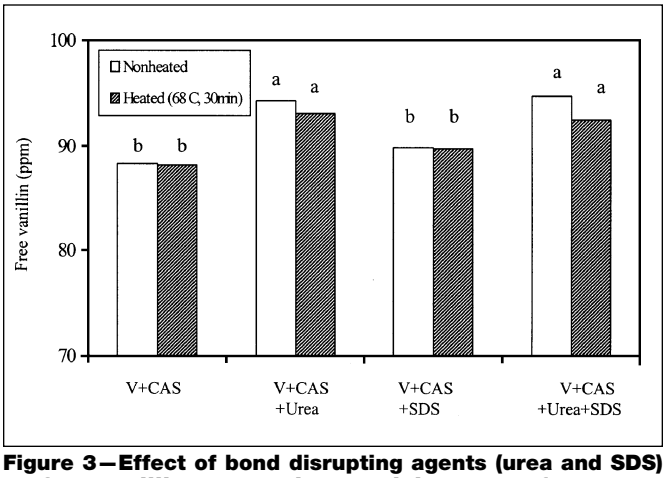

Figure 3—Effect of bond disrupting agents (urea and SDS) on free vanillin content in a model system of 100 ppm vanillin (V) and 3% sodium caseinate (CAS) in 0.05 M phosphate buffer (pH 6.5). Each bar represents the mean of 3 replications. SDS = sodium dodecyl sulfate. [ab] Different letters are significant ($p < 0.05$)

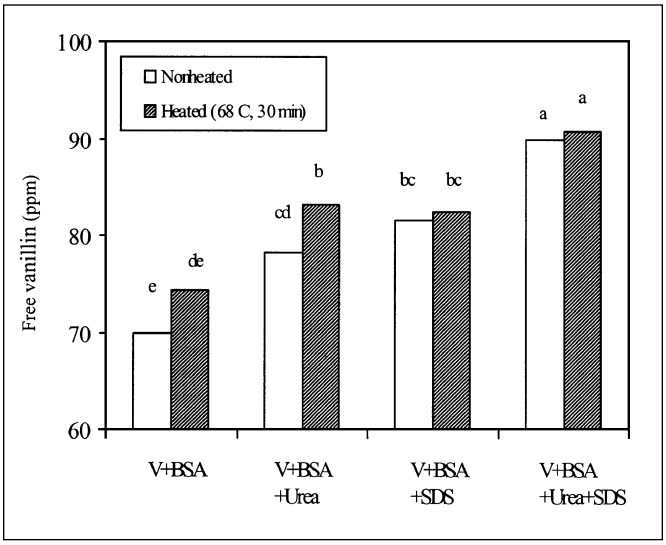

Figure 4—Effect of bond disrupting agents (urea and SDS) on free vanillin content in a model system of 100 ppm vanillin (V) and 3% bovine serum albumin (BSA) in 0.05 M phosphate buffer (pH 6.5). Each bar represents the mean of 3 replications. SDS = sodium dodecyl sulfate. [abcde] Means sharing the same letter are not significantly different ($p < 0.05$).

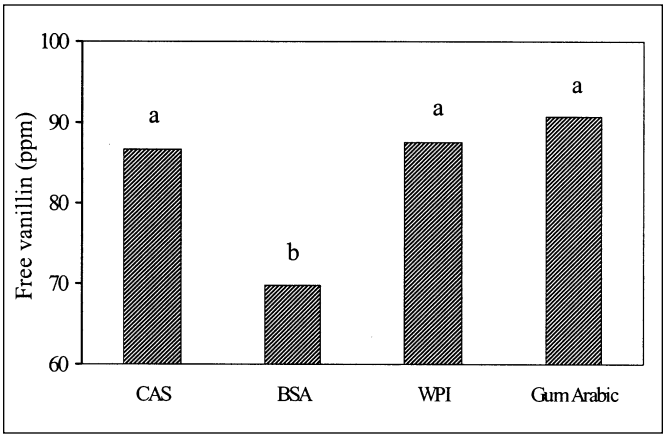

Figure 5—Effect of type of reactants on vanillin reduction in a model system of 100 ppm vanillin and sodium caseinate (CAS), bovine serum albumin (BSA), whey protein isolate (WPI) and gum arabic in 0.05 M phosphate buffer (pH 6.5). Each bar represents the mean of 3 replications. [ab] Different letters are significant ($p < 0.05$)

the interaction of vanillin and BSA involved both hydrogen bonding and hydrophobic interaction. Previous studies suggest that BSA is primarily hydrophobic in nature (O'Neill and Kinsella 1987). The tertiary structure of BSA reveals a hydrophobic core, which is accessible from the exterior of the protein molecule. Nevertheless, no significant difference in free vanillin contents was found between the nonheated and heated BSA systems with SDS, but for the addition of urea resulted in significantly higher vanillin content in the heated BSA than the nonheated.

Effect of type of reactants

The CAS, BSA, WPI and gum arabic all resulted in reductions of free vanillin (Figure 5). No significant differences in free vanillin content were found among CAS, WPI, and gum arabic, but the free vanillin was significantly lower ($p < 0.05$) with BSA than with all other systems. Because the CAS (MW ~ 23,000) has a lower molecular weight than BSA (~ 66,000), the CAS in the model system should have had more molecules than BSA at the same protein concentration (3%). However, the BSA might possess more binding sites that could interact with vanillin. Moreover, the BSA showed both hydrogen bonding and hydrophobic interaction with vanillin (Figure 4). According to the computed thermodynamic constants for the binding of carbonyl compounds to various proteins, BSA has more binding sites than β-lactoglobulin and native soy protein (Damodaran and Kinsella 1980; O'Neill and Kinsella 1987; Damodaran 1996). Damodaran and Kinsella (1980) reported that the reactions between ligand and proteins were dependent upon the structure of reactants and conditions of the interaction. Hansen and Heinis (1992) found that different flavor compounds such as benzaldehyde, citral, and limonene showed different degrees of interaction with CAS or WPC.

Gum arabic caused approximately 10% vanillin reduction (Figure 5). Gum arabic is primarily an acidic arabinogalactan polysaccharide, although it may contain polypeptide chains in variable amounts (BeMiller and Whistler 1996). Therefore, the dominant functional groups are hydroxyl and carboxyl groups that can interact with vanillin via hydrogen bonding.

Conclusion

THE CHEMICAL INTERACTIONS AND BONDINGS INVOLVED IN THE reduction of vanillin were significantly different with the different types and concentrations of proteins tested. This work provides further evidence that the instant reduction of free vanillin in aqueous protein systems might be caused by 2 major chemical forces, namely, hydrogen bonding and hydrophobic interaction. This finding may have significance for better understanding of the chemistry, as well as ways to minimize the potential loss of vanillin in food systems, especially nonfat systems, by disrupting the formation of weak binding forces or altering the type of proteins.

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