

Material properties

The Physics and Chemistry of Vanillin

Research sheds light on practical ways to prevent losses in vanillin during production

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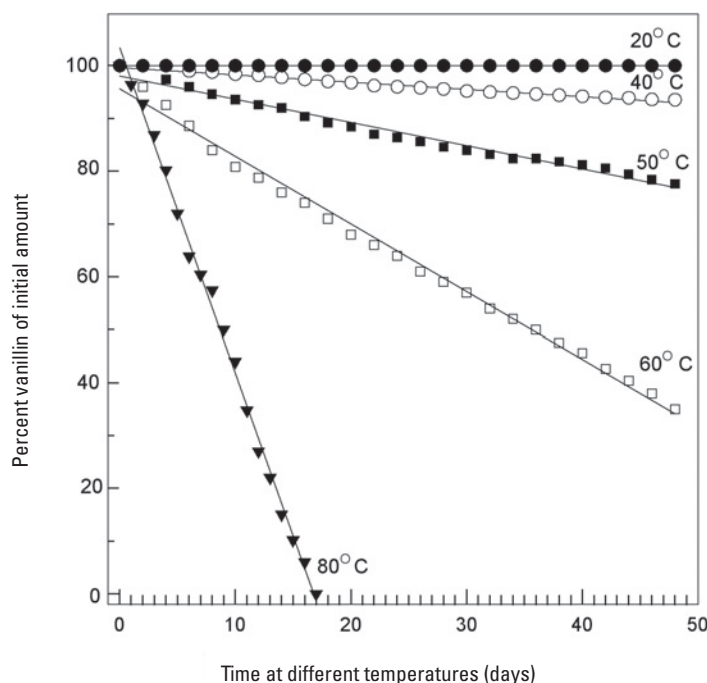
Vanillin (3-methoxy-4-hydroxybenzaldehyde), a major flavor constituent of vanilla flavor, accumulates as glucovanillin, a parent compound of vanillin, in the interior of the vanilla pod, where it is synthesized by specialized cells.¹ Synthesis of glucovanillin ensues when the vanilla pod has reached its maximum size and continues at a rapid rate for three to four months, then tapers off gradually for an additional few months. At the end of the vanilla bean development, glucovanillin is found in the central cavity of the pod in rather large abundance.² Mature beans then are subjected to a curing process, which releases the free form of vanillin from glucovanillin and enables the development of a compound that makes up the prized vanilla flavor.³

However, only a fraction of the vanillin produced by green beans may be recovered by a curing process. We found that the content of vanillin may reach around 7-8 percent, on a dry weight basis, using an appropriate curing protocol (results not shown). In commercial practice, however, curing usually yields 2.5-4.5 percent vanillin or less, on a dry weight basis, of cured beans.⁴ This corresponds roughly to 1.75-2.1 percent of vanillin in cured beans containing 30 percent moisture. An additional portion of vanillin, released by the curing process, might be lost when cured beans are extracted for the preparation of vanilla extract.⁵ Another fraction of vanillin is lost and appears to fade away with time when vanillin, introduced in pure form or in vanilla extract, is added to foods or other materials. An understanding of the physical and chemical reactivity of vanillin may provide practical tools for preventing losses in vanillin from the time the mature vanilla bean is harvested and cured through the residency period of vanillin in foods or other products. In the present work, we put forth a conjecture suggesting that the behavior of vanillin in an aqueous environment might shed some light on this problem.

Aggregate and Soluble State of Vanillin

Crystals grown from vanillin and soluble in organic solvents exist in several polymorphic forms.⁶ Vanillin I, obtained by slow cooling of dissolved vanillin, is a monoclinic crystalline state,⁷ although earlier observations suggested an orthorhombic crystal structure.^{6,8} Vanillin II, crystallized as rods and needles, is formed when vanillin crystallizes by cooling rapidly from an organic solvent.⁶ Vanillin (3-methoxy-4-hydroxybenzaldehyde) and iso-vanillin (3-hydroxy-4-methoxybenzaldehyde) molecules are linked in dry crystals by intermolecular hydrogen bonds between OH and OH and by intramolecular hydrogen bonding between OH and OMe groups.⁸ Hydrogen bonds linking neighboring molecules lead to the formation of linear macromolecular chains in a crystal lattice.^{7,9} In *O*-vanillin (2-hydroxy-3-methoxybenzaldehyde), formation of hydrogen bonds involves both intermolecular and intramolecular hydrogen bonds between OH and aldehyde groups.^{10,11}

Hydrogen bond-driven structuring of vanillin in linear macromolecular chains may evolve further to form a supermolecular three-dimensional array, linked by means of π - π stacking interaction of aromatic rings.¹² Thus, the tendency of vanillin or other aromatic compounds to form multimolecular structures by hydrogen bonding, stemming partially from thermodynamic reasons,¹³ may be reinforced and amplified by aromatic π - π stacking interactions.¹⁴ The combination of these forces results in the formation of vanillin clusters or aggregates.



Dry crystalline vanillin (500 mg.) was held in an aluminum dish at different oven temperatures, shown in the body of the graph, and weighed daily. Changes in the amount of vanillin are presented in the graph as percentage of vanillin of the initial amount.

The structure of formed vanillin clusters is preserved in aqueous solutions containing low percentages of alcohol or other organic solvents, apparently because the hydrophobicity of organic solvents enhances aromatic stacking.¹⁵⁻¹⁷ However, when vanillin is exposed to mostly an aqueous microenvironment, there might be a diminution in π - π stacking interactions. Moreover, water molecules enveloping vanillin crystals may form hydrogen bonds with the compound and, thereby, compete with intrahydrogen and interhydrogen bonds that link neighboring vanillin molecules in a cluster. Hence, an aqueous environment might enhance the solubility of vanillin and, in consequence, break up the multimolecular structure of vanillin aggregates. Nonetheless, because log D, the distribution coefficient of compounds between aqueous and other solvents, is high for vanillin at acid pH, vanillin has a low propensity to partition from an aqueous environment onto other solvents at acid conditions.¹⁸ However, the distribution coefficient is diminished greatly in alkaline conditions and, in consequence, the tendency to partition onto an aqueous phase is increased as conditions become progressively alkaline. In general terms, then, acid conditions conserve vanillin in the aggregate state, whereas alkaline conditions disperse vanillin from its aggregate form onto the surrounding water.

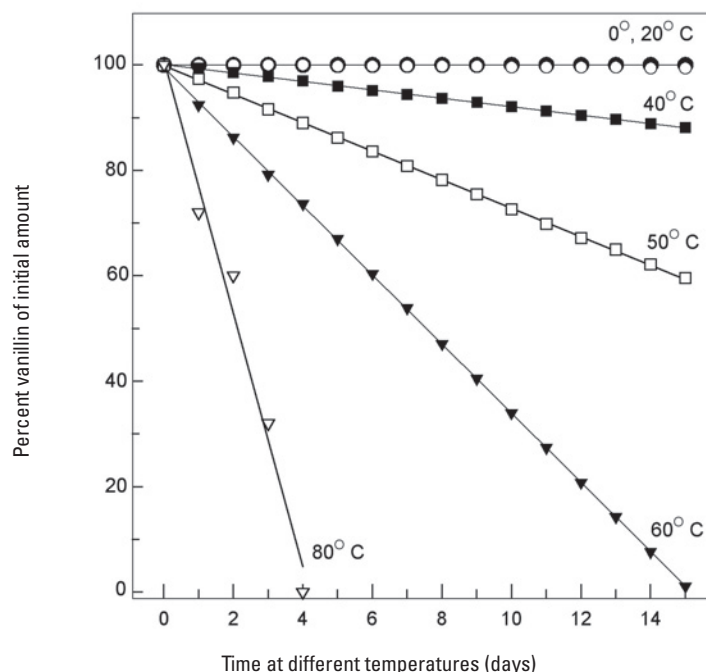
Hydration Mediates Solubility and Escape Tendency of Vanillin

We tested this information by examining conditions that modify water solubility of vanillin and, subsequently, retention or loss of the compound.

Effect of temperature: F-1 shows that dry crystalline vanillin is not readily volatile at 40°C (104°F) or below. Disappearance of vanillin, apparently by sublimation, is enhanced above 40°C and is amplified progressively with an increase in temperature. This pattern persists even in a vacuum (F-2). Although vacuum conditions accelerate the escape tendency of vanillin, temperature conditions around 40°C persist as a breaking point from slow to fast phase in vanillin disappearance. These results are at variance with the observation that the vapor pressure of vanillin increases in a monotonic linear fashion with an increase in temperature from 12°-60°C, inferring that vanillin volatility is a first order function of temperature.⁹ Our studies suggest that 40°C is a delineation point between low and high vanillin volatility. Apparently there is

Rate of disappearance of dry crystalline vanillin, held under 0.1 atmospheric pressure and at different temperature regimes

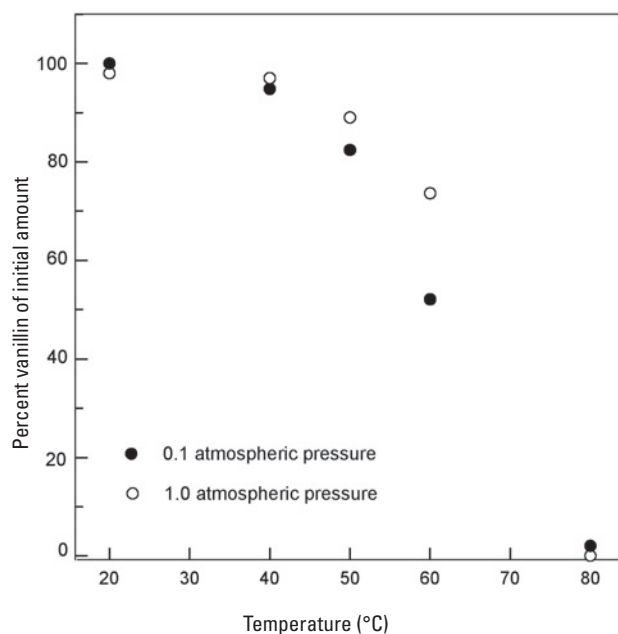
F-2



Dry crystalline vanillin (500 mg.) was held in an aluminum dish in sealed desiccators, kept at 0.1 atmospheric pressure. The desiccators were kept at different oven temperatures, shown in the body of the graph, and the vanillin weighed daily. Changes in the amount of vanillin are presented in the graph as percentage of vanillin of the initial amount.

Percentage of dry crystalline vanillin remaining after being held at 1.0 or 0.1 atmospheres and at different oven temperatures for 18 and four days, respectively

F-3



sufficient energy to break hydrogen or other bonds linking neighboring vanillin molecules and, consequently, release of vanillin in the free volatile state (F-3).

The effect of temperature on escape tendency of vanillin also was tested on vanillin in solution. Vanillin was dissolved in an organic solvent (methanol), and the solution was subjected to various temperature regimes. F-4 shows, as expected, that methanol volatilized at a progressively higher rate with an increase in temperature, and disappeared almost completely at the end of 24 h. By comparison, there was little or no change in the content of vanillin at the various temperature regimes. These results, indicating that the solvent was volatilized preferentially, suggest that affinity between the methanol solvent and the solute (vanillin) is broken readily by heat energy, causing methanol — but not vanillin — to volatilize. Methanol predominantly forms two hydrogen bonds, whether in a state of pure liquid or as oligomers in the presence of other solvents — CCl_4 for example.¹⁹⁻²¹ Methanol also can form hydrogen bonds with aromatic rings, though of moderate strength.^{22,23} Hence, methanol-vanillin hydrogen bonding may be weak and broken by heat. Moreover, methanol may strengthen attraction between neighboring molecules in a vanillin cluster, because hydrophobicity of methanol may enhance aromatic π - π stacking interactions.

A different picture emerges when vanillin is solubilized in water held at alkaline pH (F-5). As with

methanol, water volatility was enhanced with an increase in temperature. However, the rate of disappearance of vanillin in water, unlike methanol, was related closely to that of the solvent. A high correlation coefficient value (0.9826) between disappearance of water and that of vanillin (top of F-5) suggests co-evaporation of water and vanillin. The previous results indicate that heat energy could not volatilize vanillin from methanol, although it did lead to the evaporation of the solvent. However, the present results indicate that heat energy leads to a covolatilization of water and vanillin. Apparently, heat energy could not disengage a strong affinity between a water solvent and the vanillin solute. It is a reasonable assumption that resistance to breakage of water-vanillin linkage apparently stems from the efficacy of the solvent and solute to form strong hydrogen bonding. A likely scenario suggests that water, invading the vanillin cluster where vanillin molecules nestle in a multimolecular array, forms hydrogen bonds with vanillin molecules. Hydrated vanillin molecules then are carried away from a vanillin aggregate and driven off by heat as guest molecules complexed to water (F-6).

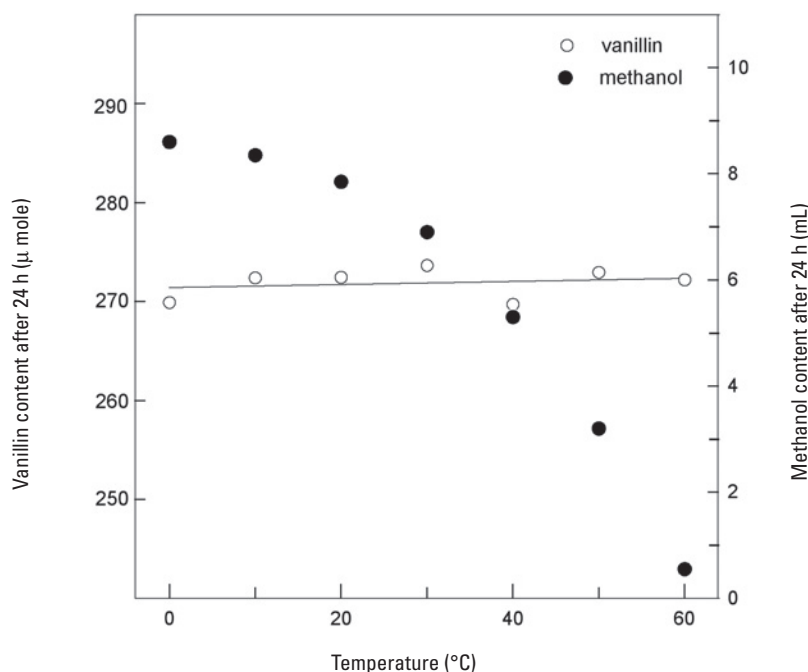
Effect of acid-base conditions: Solubilization of vanillin in water creates acid conditions around pH 3.3 (Figure 7) arising from a hydrogen exchange capacity of a hydroxyl group in vanillin.²⁴ Titration of vanillin with a base, either KOH or NaOH, resulted in a

characteristic titration curve with a pK (pH at midpoint titration curve) around 7.5 (F-7), which is in keeping with other observations.²⁵ Vanillin is, then, a weak electrolyte resembling the behavior of other aromatic compounds and is capable of acting as a buffer by virtue of weak dissociation of hydrogen ions.²⁵ Enhanced hydrogen ion dissociation, induced by alkaline conditions and, subsequently, the presence of vanillin in an ionized state, may account for the solubility of the compound in basic environments. However, solubility also may result from cation binding to the π (electron) face of aromatic structures, termed cation- π interaction. Cation- π interaction by an electrostatic attraction, though noncovalent, is viewed as a process lending polarity to hydrophobic matrices and an additional force that might further increase the water solubility of vanillin upon the introduction of cations.²⁶

We made use of acid-base conditions to mediate the water solubility of vanillin and examine how temperature regimes might influence the retention or escape tendency of vanillin. Creation of alkaline conditions

Content of vanillin or methanol in vanillin-methanol solution held at different temperature regimes for 24 h

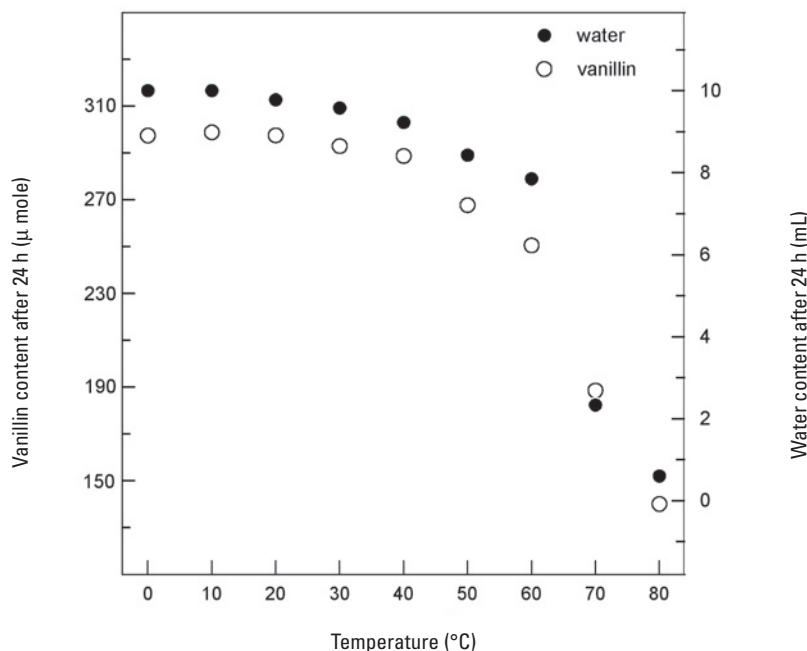
F-4



Ten mL methanol containing 500 μ mole vanillin was held in test tubes at 0°C, 10°C, 20°C, 30°C, 40°C, 50°C and 60°C. At the end of 24 h, the volume of the remaining methanol was recorded and brought to the original volume of 10 mL. The content of vanillin then was determined by HPLC.

Content of vanillin or water in vanillin-water solution adjusted to pH 8.0 and held at different temperature regimes for 24 h

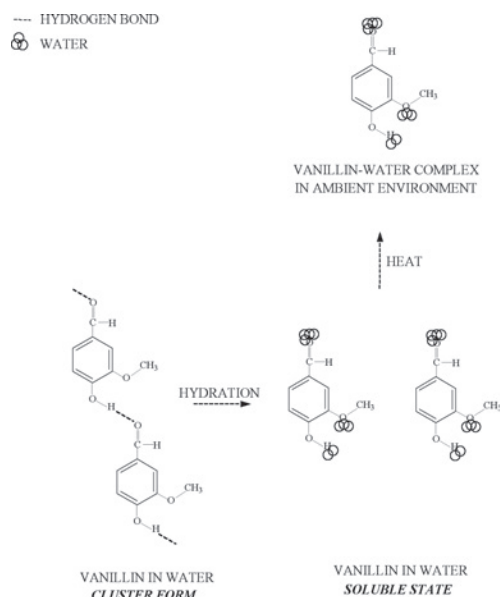
F-5



Ten mL water containing 500 μmole vanillin was adjusted to pH 8.0 and held in test tubes at 0°C, 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C and 80°C. At the end of 24 h, the volume of the remaining water was recorded and brought to the original volume of 10 mL. The content of vanillin then was determined by HPLC.

Content of vanillin or water in vanillin-water solution adjusted to pH 8.0 and held at different temperature regimes for 24 h

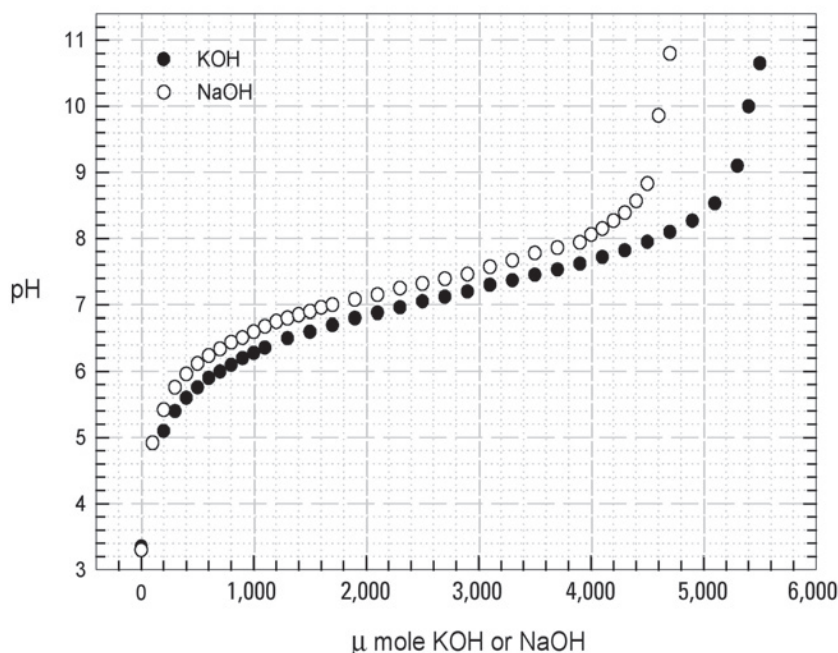
F-6



Surrounding water invades a cluster where vanillin molecules are linked in a multimolecular array and form hydrogen bonds with vanillin molecules. Hydrated vanillin molecules then are carried away from a vanillin cluster and driven off by heat as guest molecules complexed to water.

to enhance water solubility of vanillin revealed that temperature-induced escape of vanillin and water was correlated highly, as shown previously (F-5), apparently because of heat-driven escape of vanillin-water clusters (F-6). In contrast, the escape pattern of vanillin and water was dissimilar in acid conditions (F-8). Water loss by evaporation was characteristically in proportion to increase in temperature, whereas vanillin content remained constant and began to diminish only at high temperatures (70°C and above). It is a reasonable assumption that acid conditions, which arrest vanillin ionization, attenuate vanillin-water interaction, and, subsequently, water does not interact readily with — and scavenge vanillin molecules from — vanillin aggregates. This insight may be important for regulating escape or retention of vanillin in aqueous solutions.

In the growing vanilla pod, formed glucovanillin is immersed in acid conditions native to plant tissues. Acid conditions in plant tissues and, in addition, acidity stemming from vanillin's own activity may oppose solubility and escape of vanillin released inadvertently from glucovanillin. Because a considerable amount of vanillin is lost to the ambient environment during the curing process of vanilla beans, presumably due to vanillin interaction with escaping water, it is worth examining whether alteration in acid-base conditions may account partially for this phenomenon. Furthermore, because water solubility of vanillin



or other aromatic compounds is increased greatly by interaction with ammonia, release of ammonia in diseased or rotting beans also may contribute to loss of vanillin.²⁶ A similar rationale also may be relevant to losses in vanillin applied to foods or other materials.

Water Structuring Mediates Vanillin Chemistry

Carbonyl group is prone to attack by various reagents, notably nucleophiles.²⁷ The carbonyl group in aldehydes, including vanillin, also tends to form acetals by reacting with primary alcohols under mild acid conditions.^{28,29} Stability studies indicate that vanillin tends to form acetals in the presence of methanol or other primary alcohols, leading to the depletion of vanillin throughout a period of days.^{6,30} However, because water is a byproduct of acetal formation, its addition can prevent or reverse formation of acetals.²⁷

A more problematic reaction is the tendency of carbonyl groups to react with and form Schiff base with primary amines.³¹ Although Schiff base formation is hindered by acid conditions, as well as high water activity, subsequent molecular rearrangements — generally termed Maillard reaction (nonenzymatic browning) — render this reaction irreversible. Thus, amine bearing native compounds in foods or other materials could react with vanillin, giving rise to a Maillard reaction and resulting in discoloration and, moreover, attendant depletion of vanillin.

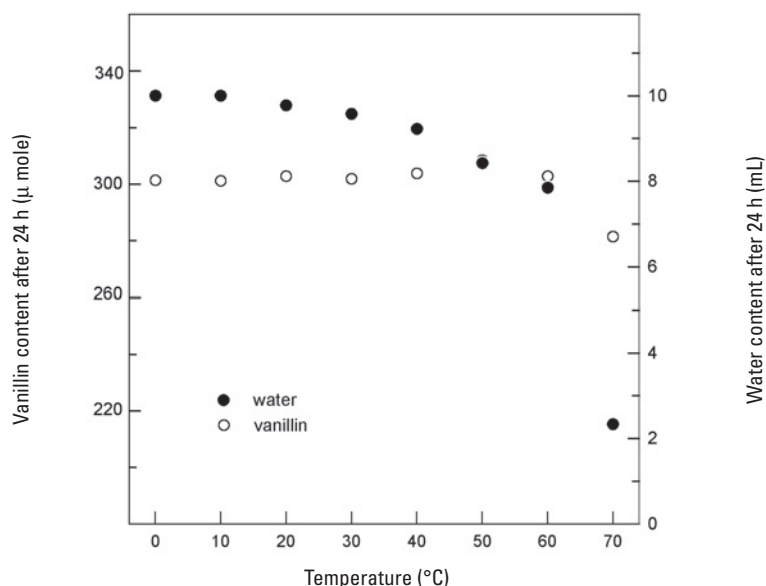
The Maillard reaction between food materials and aromatic flavors (vanillin, for instance) is driven by hydrogen bonding and by hydrophobic interactions that

contribute to weak noncovalent interactions between reactants.^{32,33} An additional perspective, however, is the effect of vanillin aggregation on the solubility and reactivity of the compound in aqueous media. Because vanillin is prone to form multimolecular structures by hydrogen bonding and by aromatic π - π stacking interaction, clustering by vanillin molecules presents a hindrance to solubility and, subsequently, chemical reactivity of the compound in aqueous media.

Because water is the most structured liquid, by virtue of strong intermolecular hydrogen bonding, breakage of the water hydrogen bond network might lead to increased vanillin solubility and subsequent chemical reactivity.³⁴ Accordingly, reinforcement of water structuring by the addition of small amounts of alcohol decreases the solubility of vanillin, whereas disruption of water structure by higher alcohol concentrations greatly enhances solvent-solute interfacing and increased solubility of vanillin.^{15,35} An effect on the hydrogen bond network in water may arise also from ion-water interactions. Ions with low-charge density display ion-water interactions that are weaker, relative to water-water interaction. This ion family, termed chaotropes, disrupts the hydrogen bond network in water. Ions with

Content of vanillin or water in vanillin-water solution adjusted to pH 2.0 and held at different temperature regimes for 24 h

F-8



Ten mL water containing 500 μ mole vanillin was adjusted to pH 8.0 and held in test tubes at 0°C, 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C and 80°C. At the end of 24 h, the volume of the remaining water was recorded and brought to the original volume of 10 mL. The content of vanillin then was determined by HPLC.

high-charge density, termed kosmotropes, exhibit strong ion-water interactions relative to water-water interactions and reinforce water structuring.^{36,37} We used cesium ion (Cs^+), a strong chaotrope, to examine whether disruption of hydrogen bond and, hence, increased water-vanillin interfacing, will stimulate vanillin-lysine Schiff base formation and a consequent Maillard reaction.³⁶ F-9 shows that, under stringent conditions consisting of acid pH, there was only a trace amount of Maillard products. However, the presence of Cs^+ greatly stimulated the reaction in a concentration-dependent manner, even in acid conditions (Figure 10). The presence of Li^+ , a strong kosmotrope, depressed the accumulation of Maillard products, even though the reaction barely was observable in the absence of Li^+ (F-10). Other authors showed, in a similar approach, that the addition of urea or SDS (hydrogen bond-disrupting agents) resulted in enhancement of vanillin-protein Schiff base formation.³³ Thus, manipulation of water structuring also may influence rate of reactions, particularly under stringent conditions.

Applications

The previous discussion outlines molecular features that might determine the

fate of vanillin during various handling stages of the compound.

The propensity of vanillin to form aggregates through the combined forces of hydrogen bonding and aromatic stacking presents a hindrance to vanillin solubility in water. The tendency of vanillin to aggregate, resulting in hindrance to vanillin solubility and reactivity, is strengthened further by reinforcing the structure of surrounding water through the addition of small amounts of organic solvent or the presence of kosmotropes, as well as by acidification. Under these conditions, limited vanillin solubility might prevent complexation to, and escape of, vanillin with water. Limited solubility appears also to hinder the chemical reactivity of vanillin. This may be important for preserving the substance during various handling stages of cured vanilla beans or in the prevention of vanillin chemical reactivity that might result in the depletion of vanillin in foods or other materials.

In contrast, enhanced vanillin solubility through disruption of water structure in the presence of chaotropes or alkaline conditions may promote the escape of vanillin and the reactivity of the compound, particularly the formation of Schiff base and subsequent Maillard reaction. The presence of transition metals, even in trace amounts, further stimulates the process.³⁸ These conditions, although usually undesirable, might be exploited to drive vanillin onto the ambient environment when necessary.

The involvement of ammonia or amines deserves special consideration. Rotting and disease liberate

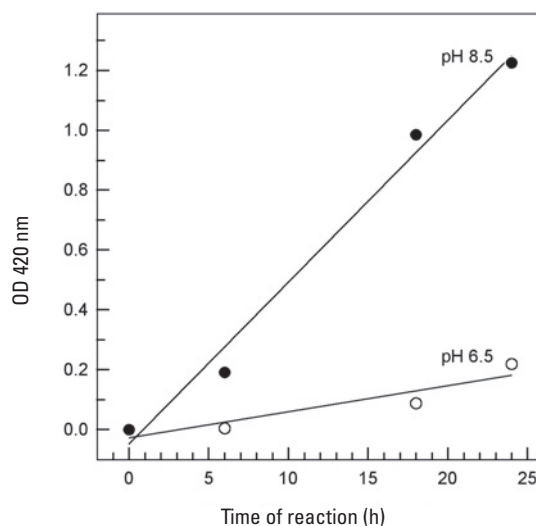
ammonia from degraded proteins in vanilla beans. Ammonia, in turn, may raise the pH and can function as a reactant in the Maillard reaction.³¹ Moreover, ammonia is a strong chaotrope which could enhance greatly the solubility of vanillin.³⁶ These conditions may combine to promote the reactivity of vanillin in, and escape of the compound from, aqueous solutions.

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Intensity of vanillin-lysine Maillard reaction at pH 6.5 and 8.5

F-9

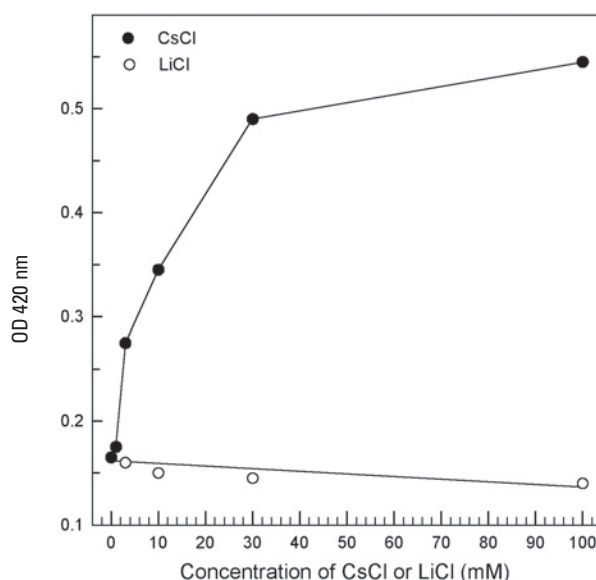


Solutions containing 5 mM vanillin and lysine were adjusted with NaOH to pH 6.5 or 8.5 and held at 65°C for 24 h. Samples were drawn periodically for estimating the Maillard reaction intensity by spectroscopy at 420 nm.

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Intensity of vanillin-lysine Maillard reaction in the absence and presence of different concentrations of CsCl or LiCl

F-10



Solutions containing 5 mM vanillin and lysine, kept at pH 6.5, were supplemented with 0, 1, 3, 10, 30 and 100 mM of CsCl or LiCl and held at 65°C for 24 h. Samples were drawn at the end of 24 h for estimating the Maillard reaction intensity by spectroscopy at 420 nm.



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