Inside Vanilla*

Vanilla planifolia's botany, curing options and future market prospects

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Vanilla (*Vanilla planifolia* Andrews) is a climbing orchid indigenous to Mexico (F-1A). Vanilla was introduced to Europe by the Spanish Conquistadores in 1520, but commercial production of vanilla didn't begin for another 300 years with the discovery of hand pollination of the vanilla flower. In the wild, vanilla flowers are pollinated by insects.[1] When left on the vine, vanilla beans senesce, as manifested by yellowing and, next, by browning (F-1B), a process resembling commercial curing. However, the flavor quality of naturally cured beans is inferior.

In commerce, vanilla is propagated by cuttings and is cultivated in tropical regions. The plant requires three to four years to flower, and flowers once a year. The fruit (vanilla bean) is allowed to develop for eight to 10 months before harvesting. Worldwide production of vanilla beans is around 2,000t annually (US Department of Commerce and EUROSTAT).

Vanilla beans are harvested green, flavorless and subjected to a curing process for three to six months, depending on various curing protocols in different localities. The objective of the curing process is to develop the prized vanilla flavor and, in addition, to dry the cured beans for subsequent ethanolic-water extraction that renders the familiar vanilla extract.

This review will deal mostly with the curing process. Vanilla cultivation, biosynthesis, and economic aspects are discussed extensively in other reviews.[2-5] We do however provide information on the botany of the vanilla bean and how it may be related to the understanding of the curing process.

Botany of the Vanilla Pod

Two fruit regions: The syncarpous fruit of *V. planifolia* develops from an inferior ovary that eventually splits open along three lines at maturity, thus becoming a capsule. For the purposes of this study we have recognized two principal regions in the vanilla fruit: 1. The fruit wall, or "green" region, including the epidermis, ground and vascular tissues of the fruit wall. 2. The "white" region composed of the three parietal placentae (not including seeds), and the three bands of glandular hairs between them.

Climbing vanilla plant bearing green pod-like fruit. **F-1A**

*In memory of (Brigadier) General Edward Whitehall Rosenbaum, chairman of the board, David Michael & Co. Inc., a pioneer and visionary in vanilla research.

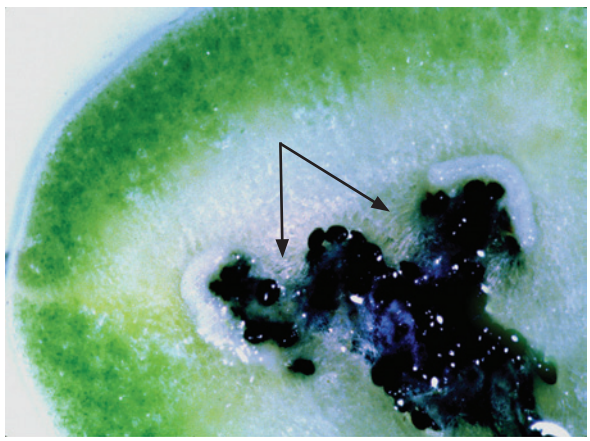
At a Glance

The fruit of the climbing orchid *Vanilla planifolia* (vanilla bean) is used for the commercial production of vanilla extract. Vanilla extract consists of numerous flavor compounds in addition to vanillin. These compounds lend a more complex flavor to natural vanilla than synthetic vanilla flavor. Development of the prized vanilla flavor in harvested green beans is dependent, however, on a curing process that is poorly understood.

The study presented here, as well as those of others, revealed that flavor precursors are found in the bean interior, in a placental region around the seeds. The hydrolytic enzymes that catalyze the release of the flavor precursors to flavor compounds (for instance glucovanillin to vanillin) are localized mostly in the outer fruit wall region. Other degradative systems may include polyphenol oxidases, peroxidases or other oxidative enzymes that catalyze an oxidative degradation of lipids or other cellular constituents to volatile flavor compounds. This perception suggests that the objective of killing — the first curing stage carried out by hot water scalding, freezing or by other methods — is to disorganize the bean tissue in order to create contact between substrates and their respective enzymes. Consequently, to preserve the viability of enzymes extreme killing conditions must be avoided. Sweating, a subsequent step in curing in which the enzyme-catalyzed production of flavor compounds occurs, may be best carried out at high humidity and temperatures (usually around 45°-55°C) that enhance the activity but do not lead to the denaturation of enzymes. The final curing steps, including drying and conditioning, aimed at drying the cured beans and the preservation of the formed flavor compounds, are accompanied in commerce by a substantial loss in vanillin or other flavor constituents. These stages in the curing processes might benefit from additional studies. Herein we also provide a review of the manufacturing and commercial use of various products obtained from cured vanilla beans.

Vanilla beans that have cured naturally on the vine, for 12, 15, 18 and 20 months after pollination. **F-1B**

Cross section (x 20) of freshly cut green vanilla bean. The figure shows an inner portion composed of seed (dark bodies). Arrows indicate a white placental tissue surrounding the seeds. Shown also are specialized hair cells as well as green outer fruit region. **F-2A**



The glandular hairs play a role in the biosynthesis of vanillin.

Fruit components: The green and white components comprise about 60 percent and 40 percent of the fruit weight, respectively. This weight ratio of the outer and inner portions appears to change, however, during early and advanced stages of pod development (F-2 A and B).

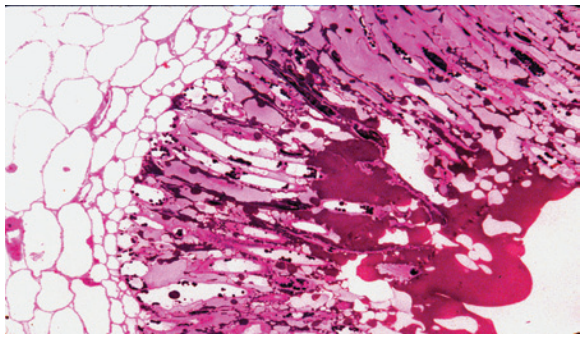
Fruit anatomy: The fruit's epidermis contains isodiametric ground epidermal cells, which lack prominent chloroplasts. Each epidermal cell contains a rhomboidal crystal of calcium oxalate and is bounded by thickened, pitted cell walls. Stomata are widely spaced. In some varieties, dozens of extra floral nectaries occur on the fruit. In other varieties, extra floral nectaries are entirely absent.

The fruit wall contains a ring of about 15 vascular bundles. The vascular bundles are unbranched, and each contains a strand of xylem and phloem with a sclerotic bundle sheath. The xylem consists of annular to helical and reticulate elements.

Tissue outside the ring of vascular bundles is composed of thin-walled parenchyma cells several times longer than wide. Each ground parenchyma cell in the

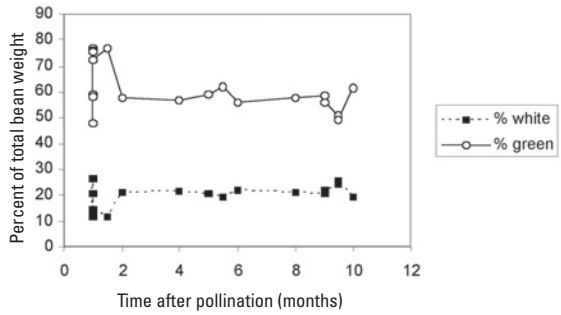
A magnified view (x 400) of cross section of green vanilla bean. The figure shows the senescing inter-placental hairs (right), which are part of the "white" tissue, containing enzymes in the vanillin biosynthetic pathway. Shown also are parenchyma cells in the fruit wall (left), which are part of the "green" outer fruit tissue, containing degradative enzymes.

F-2B



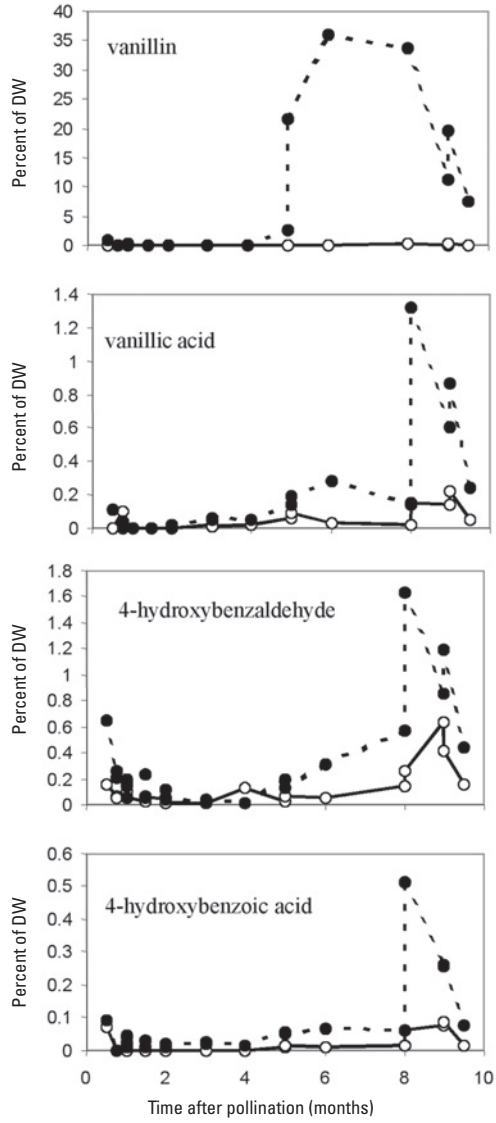
Changes in percentage of fresh weight of green (outer) and white (inner) tissue of vanilla pod during development on the vine.

F-3



Time course change in the content of various metabolites in the green outer tissue (solid lines) and the inner white tissue (dashed lines) of vanilla bean during pod development on the vine. Beans were harvested green at various stages of development. The various metabolites, present as glucosides, were hydrolyzed and the resulting aglycons determined as described previously (Podstolski et al., 2002).

F-4



outer fruit wall contains chloroplasts and occasional rhomboidal calcium oxalate crystals. Raphide "vessels" are abundant in the outer fruit wall and release mucilage-containing raphides when the fruit is cut, which is highly irritating if it contacts skin. No attempt was made to determine the development or structure of these large, complex cells, which are many times the length of ground epidermal cells, and contain tightly packed bundles of raphides if undisturbed.

Compared with the outer fruit wall, the wall tissue inside the ring of vascular bundles contains larger cells, with somewhat less abundant and smaller chloroplasts, making it much less green in freshly cut beans.

Pollination initiates fruit development: The inferior ovary of the non-pollinated vanilla flower has three weakly developed parietal placentae separated from each other by the smooth inner epidermis of the ovary. Pollination triggers the placenta to begin extensive branching, followed by ovule development.

Perhaps more important for the vanilla industry, are unusual glandular hairs that begin to develop

Just the Facts

Vanilla bean ripening period: eight to 10 months
Worldwide production of vanilla beans: 2,000 t/year
Curing process duration: three to six months
Combined US, French and German vanilla consumption: ca 80 percent total world imports
US vanilla import level: ca 50-60 percent total global vanilla production
Total US vanilla extract imports devoted to ice cream in US: 44 percent
Household vanilla extract consumption: 22 percent
Cola beverage vanilla extract consumption: 17 percent
Combined natural vanilla consumption in the chocolate and confections, alcoholic beverage, flavored yogurt, etc. segments: 17 percent
Percentage vanilla bean price increase in 2000 following Madagascar's Typhoon Huda: ca 500 percent
Estimated 2003 Madagascar vanilla crop: 40 percent (or more) below normal
Recent Indonesian vanilla price increase: 30-plus percent
2004 Vanilla bean prices: rock bottom, resulting in 30-50 percent reduction in the use of vanilla bean

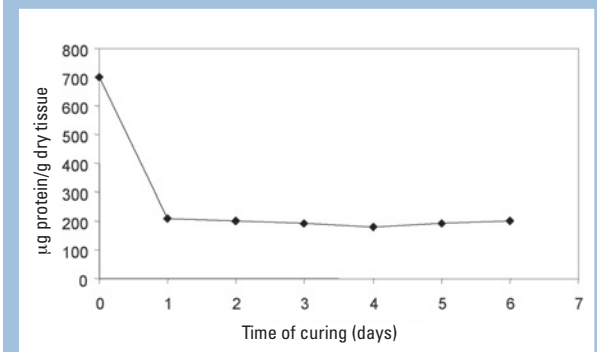
quickly in the regions between the placentae. Each hair is unbranched, and soon reaches a length of about 300 µm. Following pollination, large numbers of pollen tubes progress down the ovary. The pollen tubes move in three groups, each located in a narrow pocket at one side of each of the three placentae, flanked by the hairs. The hairs become cemented together during their development, and later break down, releasing their contents into the locule. The developing hairs have abundant endoplasmic reticulum, ribosomal structures, enlarged plastids containing lipid globules and other features that are the hallmarks of metabolically active cells.

Mature fruit: As the fruit develops, the inter-placental hairs develop thickened walls and a complex cytoplasm. Because of their size, number and thick walls the hairs are easily observed in transverse sections of vanilla fruits, as three lustrous white bands. Many seeds become appressed into the hairs in mature fruits. The three panels of hairs extend the

full length of the fruit. The cells contain abundant lipids, which are released into the locule and coat the seeds when the hair senesce later in ripening. The hairs develop complex cell walls, which cement the hairs together in mature beans.

Swamy suggested that vanillin is produced in these hairs, whose presence has been casually noted by most previous investigators.[6] This suggestion is confirmed by our work, showing that vanillin and related intermediates in the vanillin biosynthetic pathway accumulate in the inner white tissue of a developing vanilla pod, around the placental hairs (F-3). This information may be important for the understanding of and a rationale for the control of the curing process.

Changes in total protein content in vanilla bean during curing at 50°C. Total proteins were extracted periodically from bean tissue and estimated as previously described (Ranadive et al., 1983). **F-5**



Curing Process

Purpose of curing: When vanilla beans are harvested green, they lack in flavor. During bean development on the vine, for eight to 10 months, flavor precursors accumulate in the placental tissue surrounding the seeds in the inner core of the bean (F-4). However, flavor precursors, glucovanillin for instance, and enzymes that catalyze conversion of these constituents to final products are apparently sequestered in different regions in the vanilla pod. For example, estimation of β-glucosidase activity indicated that it was roughly 10-fold higher in the outer fruit wall than in the inner placental tissue and hair cells (see below). This was also confirmed by activity staining of a cross section of a vanilla pod (results not shown). These data, indicating that β-glucosidase is localized mostly in the pod outer region, suggest that in intact tissues of green beans hydrolytic enzymes, including β-glucosidase and perhaps other glycosyl hydrolases, are spatially separated from glucovanillin or other flavor precursors, which are localized in the fruit interior. The purpose of the curing process, then, is to create contact between flavor precursors and the enzymes that catalyze the hydrolysis of these compounds to flavor products, including vanillin and other flavor constituents. An additional objective is the drying of cured beans for the preservation of the formed flavor compounds. Vanilla flavor contains around 250 identified constituents; chief among them is vanillin.[7] Because the vanillin content in cured beans is a major criterion for bean quality, previous studies on the curing process focused on the production of vanillin from glucovanillin, the vanillin precursor. A major topic of the present manuscript will also entail vanillin formation during the curing of vanilla beans.

Traditional methods of curing: The curing process is comprised of four major stages: killing, sweating, drying and conditioning.

Killing: Modern methods of killing are based on the observation that, in the ancient Mexican method of curing, killing consisted of wilting the beans in the sun until they became brown.[8] Contemporary methods for killing vanilla beans include sun killing, oven killing, hot water killing, killing by scratching, and killing by freezing.[1] The stated purpose of the various killing methods is to bring about the cessation of the vegetative life of the vanilla bean and to allow contact between enzymes and substrates.[9,10] The most practical and most commonly used green bean killing methods are exposure to the sun, killing by oven heat or hot water killing.[2] In sun killing, a method originating from Mexico and practiced by the ancient Aztecs, the beans are exposed to direct sun under a dark cloth for a few hours for several days until the beans turn brown.[8,11] In oven killing, the beans are tied in bundles and rolled in blankets and placed in an oven at 60ºC for 36 to 48 h.[1,2] Hot water killing consists of placing the green beans in wire baskets and submerging them in hot water (60º-70ºC) for 3 min. A variation of this method consists of repeated submersion for 10 sec at a time at higher temperatures (80ºC).[1] If beans do not turn brown with killing by sun, oven or hot water, killing with hot water is repeated. Freezing — by dipping in liquid nitrogen or by holding beans for a few hours in a freezer (0º to -80ºC) — is yet another method of killing.[12] Our own experience and those of other studies indicate, however, that storage of frozen beans must be carried out at -70ºC or below to preserve the viability of enzymes that are involved in the curing process.[3,13]

Killing-induced browning in green beans is an index for cessation of vegetative life and, apparently, tissue disorganization. These conditions allow contact

between previously segregated substrates and enzymes present in different parts of the vanilla bean. This rationale is supported by the results showing that flavor precursors accumulate mainly in the inner white tissue of beans (F-5), whereas enzymes that catalyze their degradation to final flavor compounds might be present mostly in the outer green tissue. This reasoning is also supported by the observation that disruption of green bean tissue by mechanical means, using chopping or homogenation in water, initiates a rapid degradation of glucovanillin to vanillin.

Jones and Vicente evaluated various killing methods with respect to the quality of cured vanilla beans and found that killing by hot water gave the best product, with freezing second and scratching third.[14] Although the various killing methods achieve the same objective, namely, disruption of tissue organization and creation of contact between enzymes and substrates, killing by various methods may result in the selective survival of some enzymes, which catalyze different processes and at a different rates. Our studies, for example, show that the highest activity of β-glucosidase, peroxidase, polyphenol oxidase and protease was in heat-killed beans; on the other hand, killing by freezing resulted in a different enzyme profile.[13,15,16] Subsequently, killing by heat, scalding or by freezing may result in flavor with different attributes, since different enzyme complements may survive and catalyze the formation of different flavor spectra.

However, severe killing, using excessive heat for instance, may lead to a complete destruction of beneficial enzymes and, subsequently, an insufficient enzymatic activity to process the formation of flavor compounds. We believe that rapid killing by heat or freezing is more beneficial for bean quality because it minimizes the exposure time of useful enzymes to adverse conditions.

Sweating: After the killing process, beans are allowed to sweat. During this stage, killed beans develop the characteristic vanilla flavor, aroma and color. During the sweating stage, beans are held at high humidity and high temperature (45°-65°C) for seven to 10 days.[8,11] Traditionally, this process is carried out in sweat boxes in a closed room, but rarely in an oven.[2] The purpose of sweating is to retain enough moisture to allow enzymes to catalyze various hydrolytic and oxidative processes and allowing, perhaps, non-enzymatic reactions to occur. At the same time, some moisture is permitted to escape to reduce the water content sufficiently to prevent spoilage by microorganisms. The purpose of high temperatures during the sweating stage is to accelerate enzymatic and perhaps also non-enzymatic processes. High temperatures are achieved by wrapping killed and warm beans in various cloth materials, by densely stacking killed and warm beans in insulated containers, and by re-warming with exposure to the sun for a few hours each day during the sweating period. In some instances, the sweating beans are dipped daily in hot water.[1,8,10,16]

Estimation of β-glucosidase activity indicated that it was roughly 10-fold higher in the outer fruit wall than in the inner placental tissue and hair cells.

Drying and conditioning: At the end of a sweating period, beans are brown in color, and have developed most of the characteristic flavor and aroma of cured beans. However, at the end of this stage, beans contain about 60-70 percent moisture and are traditionally dried for protection against microbial spoilage and to stop any further enzymatic activity. At the end of the drying process, the moisture content in the beans reaches 25-30 percent of the bean weight.[2] It is possible that drying may also lead to the expulsion of volatile compounds, such as hexanal or other aldehydes, and other compounds that lend the "green" unripe note to vanilla flavor. Drying is the longest stage in the curing process. The most commonly used methods are sun and air-drying. These methods are occasionally supplemented by oven drying. Sun drying consists, traditionally, of spreading the beans on racks in the morning sun and transferring the sun dried beans to a shaded area in the afternoon. This protocol may be carried out daily for three months.[17] Theodose divided the process into rapid and slow drying.[10] In rapid drying, the beans are held in the sun for a few hours every day and then wrapped in cloths and placed indoors. This process is repeated for five to six days until the beans become supple, a sign of sufficient drying. In slow drying, the beans are placed on shelves in a well-aerated room and are moved outside to the sun every two to three days. This method of drying may last one month. Other workers proposed using drying equipment based on solar energy.[18,19] Theodose proposed to combine traditional drying with hot air drying to shorten the drying period.[10]

Drying is the most difficult stage in the curing process to protocol. Uneven drying may result from uneven bean size, differences in bean moisture content and from variable environmental conditions. The latter may include weather conditions during sun drying or from variations in the relative humidity during sun or air-dry-

Effect of different gas regimes on splitting[2] and browning[2] of green and yellowing vanilla beans.[1] Whole vanilla beans were held in sealed 20 L glass jars containing air or 100 percent oxygen, with or without ethylene (10 μl/L). **T-1**

Gas regime	Bean condition	Number of beans	Splitting Number of beans	Splitting %	Browning Number of beans	Browning %
Air	Green	85	18	21	5	6
	Yellowing	140	29	21	39	28
	Total	**225**	**47**	**21**	**44**	**20**
Air plus ethylene	Green	86	55	64	18	21
	Yellowing	133	18	14	45	34
	Total	**219**	**73**	**33**	**63**	**29**
Oxygen	Green	77	60	78	15	19
	Yellowing	119	30	25	53	44
	Total	**196**	**90**	**46**	**68**	**35**
Oxygen plus ethylene	Green	82	61	74	36	44
	Yellowing	117	41	35	60	51
	Total	**199**	**102**	**51**	**96**	**48**

[1] Mexican vanilla beans were harvested seven months after pollination and sorted by color (green and yellowing); [2] estimation of pod end splitting and pod browning was visual

ing. The drying stage is apparently critical to the development of flavor quality, but prolonged drying may lead to loss in flavor and vanillin content.

Bean appearance and suppleness are used by practitioners of the trade as an index for the moisture content. When beans are judged to have reached sufficient dryness, they are next placed in wooden boxes and held for an additional few months for conditioning. This stage may be viewed as a continuation of the drying process where additional moisture and volatiles may be lost.

Cellular and Metabolic Changes During Curing

Green or yellowing whole vanilla beans manifest little or no ethylene evolution, suggesting that the vanilla pod (bean) is probably a non-climacteric fruit. However, when held in oxygen, these beans produced some ethylene (results not shown). Balls and Arana (1942) observed that application of ethylene to green beans resulted in a brief upsurge in CO_2 evolution, followed by a continuous decline in CO_2 for the next 15 days.[20] This observation was confirmed by our own studies. Applied ethylene may also act as a maturing agent, although leading mostly to bean browning.[21] We observed that holding mature green beans in ethylene-containing air stimulated browning. Browning was further amplified when beans were held in oxygen and ethylene. Ethylene and oxygen also stimulated bean-end splitting (T-1). Interestingly, killing by scalding resulted in a similar response.[21] These results suggest that ripening processes in vanilla beans, as judged by the yellowing and subsequent browning, may be analogous to cellular processes induced by ethylene. In either case, naturally occurring browning in a senescing bean, as induced by ethylene or by killing, is associated with cellular disorganization. Importantly, this condition is manifested in the abolishment of compartmentalization in cells and tissues of vanilla beans. We observed, accordingly, that, in whole green beans, vanillin and other phenolic compounds are found in the bean interior, as evidenced by catechin staining. In killed beans, however, catechin staining shows that phenolic compounds have migrated

Recovery of close to a full yield of vanillin from glucovanillin (F-10) suggests that even residual enzyme activity may be sufficient to carry out nearly complete hydrolysis of glucovanillin to vanillin.

from the inner white portion and have occupied the entire tissue, further suggesting that killing leads to the organizational collapse. Catechin staining indicated, moreover, that killing by freezing was more thorough and uniform than killing by dipping beans in hot water at 65°C for 3 min.[22]

One consequence of killing is that glucovanillin, the vanillin precursor found mostly in the bean interior, namely, in placental tissues surrounding the seeds, may migrate and come in contact with glucovanillin hydrolyzing enzymes found in the outer pericarp tissue, and perhaps also with β-glucosidase found in the seeds (Jiang et al., 2000).[3,15,21-23] These conditions may also promote contact between other glycosyl hydrolases and their respective substrates previously sequestered in the tissue surrounding the seeds.

Cellular collapse may also lead to de-regulation in oxygen diffusion and activity in tissues because of an attendant destruction of biomembranes, which function as gas diffusion barriers.[24] Uncontrolled oxygen diffusion may, subsequently, initiate the onset of enzymatic and non-enzymatic oxidative processes. This metabolic fate is apparently manifested in bean browning, the hallmark of enzymatic and non-enzymatic oxidation, as discussed below. A third consequence of tissue disorganization is the unleashing of various hydrolytic enzymes — cell wall degrading enzymes for instance.

Killing-induced collapse of cellular organization may be accompanied by degradative processes, which are beneficial for vanilla flavor formation. It is not reasonable to assume, however, that cellular conditions in killed beans support synthetic processes.

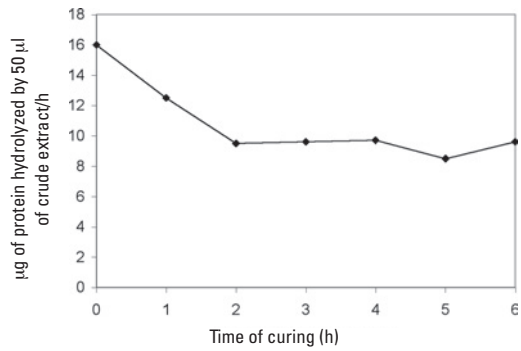
Changes in Enzymatic Activity During Curing

Protease activity: Killing and subsequent curing are associated with increased proteolytic activity in vanilla beans, as evidenced by rapid decline in the content of cellular proteins. Protein content decreased precipitously within 24 h, but remained constant afterward (F-6)**.** Wild-Altamirano showed that protease activity declines with bean development on the vine but remains steady when beans have matured.[25] During curing of harvested beans, protease activity declined within two days, to about 60-70 percent of the level found in fresh green beans, and remained steady afterward (F-6). Dignum et al. showed that, while severe scalding (80ºC for 30 min) led to de-activation of glucosidases and phenylalanine ammonia

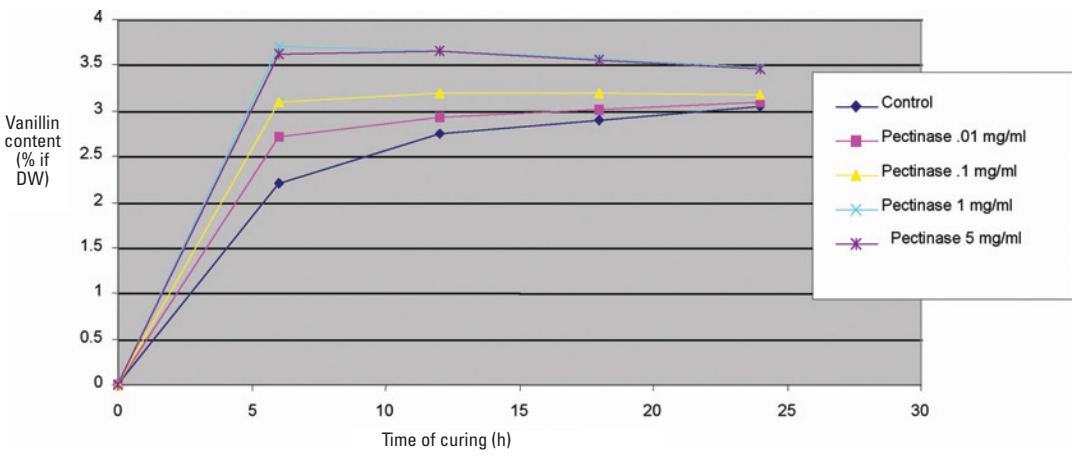
lyase, proteases survived this treatment.[26] Proteolytic degradation of bean proteins may result from the release of cellular proteases from various cellular compartments, vacuoles for example, where proteases are found in abundance. It is also likely that enhanced proteolytic activity is triggered by an abundance of denatured proteins, which are targeted for proteolysis by cellular proteases.[27] Denaturation of cellular proteins may occur during killing, by heat or freezing, and may also be caused by phenolic or other deleterious compounds, which were previously compartmentalized and released in killed bean tissues. Lipid peroxides formed in cured beans (F-11); it is possible that other oxidants also attack and denature cellular proteins.[27] A marked decrease in protein content after few hours of curing (F-5) suggests that the action of proteases diminished the capacity of enzymes to catalyze the formation of flavor compounds in curing beans. However, recovery of close to a full yield of vanillin from glucovanillin (F-10) suggests that even residual enzyme activity may be sufficient to carry out nearly complete hydrolysis of glucovanillin to vanillin.

Cell wall hydrolyzing enzymes: Ruiz-Teran et al. and other workers observed that addition of commercial preparations of cell wall degrading enzymes accelerated the hydrolysis of glucovanillin to vanillin in vanilla beans undergoing curing.[28-30] These results suggest that vanillin and related phenolic compounds may be trapped in the wall matrix and that wall hydrolysis may increase the accessibility of flavor precursors to their respective enzymes. We observed, accordingly, that addition of pure preparations of pectinase to chopped green beans accelerated the conversion of glucovanillin to vanillin. However, in control beans (no enzyme added), the vanillin content was roughly the same as in enzyme-treated beans at the end of the incubation period, although the rate of vanillin formation in control beans was slower (F-7). It appears, therefore, that the activity of endogenous wall hydrolyzing enzymes is sufficient to aid in the conversion of glucovanillin to vanillin, whereas applied enzymes merely accelerate the process. Additional studies are desirable to reveal whether the curing process is associated with a spontaneous wall degradation in cured vanilla beans and whether this process enhances the conversion of glucovanillin to vanillin and increases the extractability of released vanillin and other flavor constituents as

Changes in protease activity during curing of vanilla bean at 50°C. Fifty µl of crude extract represents 14.3 mg fresh weight of vanilla tissue. **F-6**

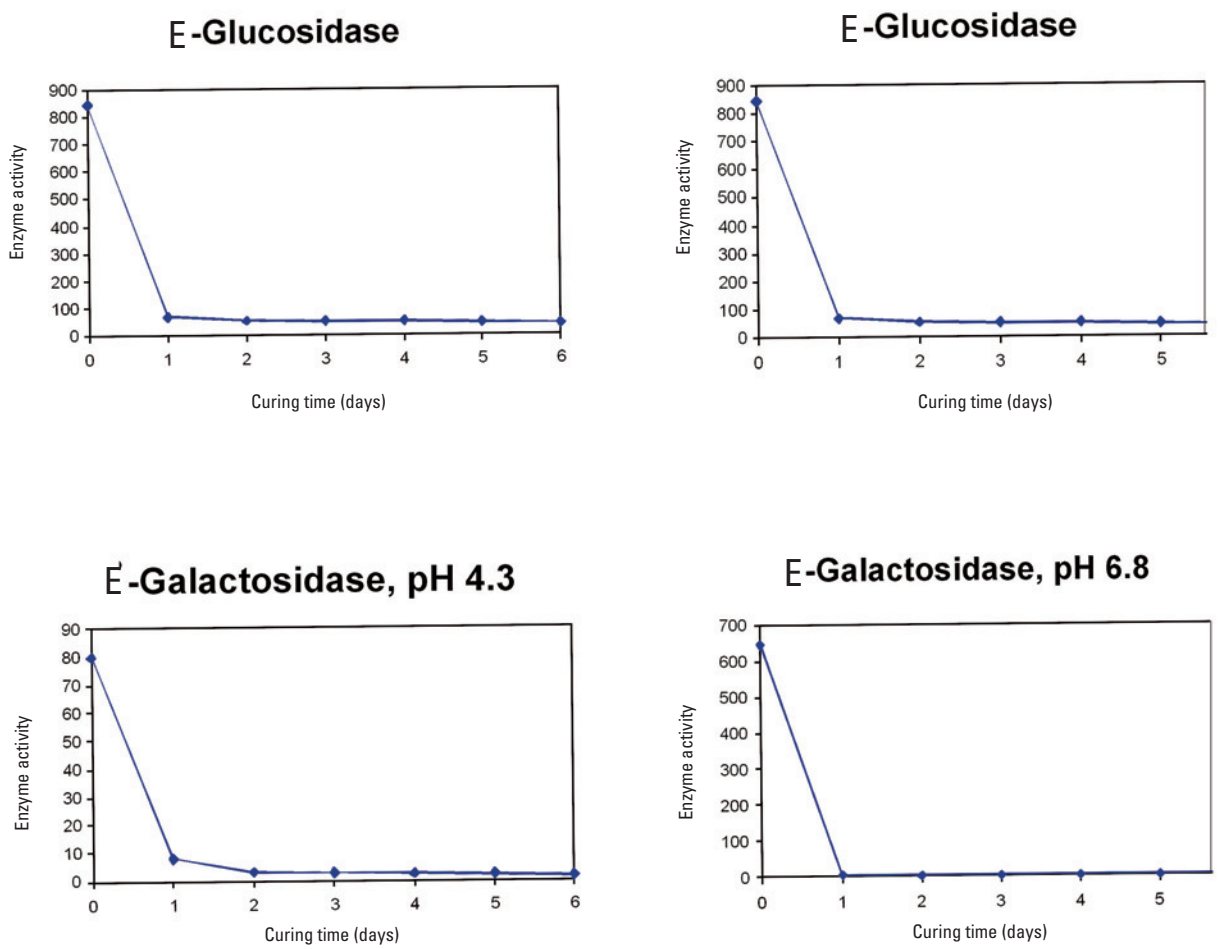


Effect of applied pectinase on changes in vanillin content in vanilla bean during curing at 50°C. Vanilla beans undergoing curing were supplemented periodically with pectinase as described before (Ranadive et al., 1983). Vanillin extraction and estimation was according to Havkin-Frenkel et al. (1996). **F-7**



Changes in the activity of glycosyl hydrolases in vanilla bean during curing at 50°C. Numbers in the body of the graph designate the initial enzyme activity at time 0.

F-8



Enzyme activity is defined as μg of p-nitrophenol/mL crude extract/h released from p-nitrophenyl-α-D-galactopyranoside, p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-β-D-galactopyranoside, for α-galactosidase, β-glucosidase, β-galactosidase, respectively. One mL of extract represents 286 mg FW tissue.

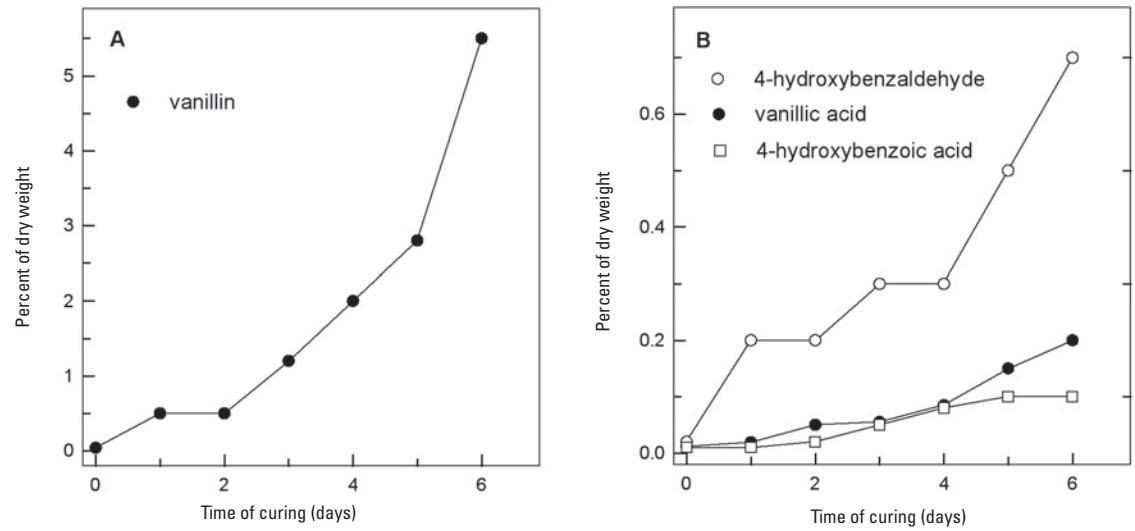
previously found (unpublished data).

Glycosyl hydrolases: According to Arana (1944), vanillin was first isolated from vanilla beans in 1858 by Gobley.[21] The formation of vanillin from glucovanillin during the curing process of vanilla beans has been first shown by Goris.[31] Other glycosyl conjugates of vanillin or other phenolic compounds containing mannose, galactose and rhamnose were found in trace amounts in the developing vanilla pod.[3,32-36] However, because of the importance of vanillin in vanilla flavor, the enzymatic hydrolysis of glucovanillin to vanillin is one of the most studied processes in vanilla beans. The rate of glucovanillin conversion to vanillin may be measured by the rate of disappearance of glucovanillin and an accompanying accumulation of vanillin, or by the activity of β-glucosidase in bean tissue as an inference for the hydrolysis of glucovanillin. β-Glucosidase activity is measured traditionally with the use of p-nitrophenyl-β-glucopyranoside or glucovanillin as substrates.

We found that green vanilla beans contain various glycosyl hydrolases, including α- and β-glucosidase, α- and β-galactosidase, and α- and β-mannosidase. F-8 shows changes in the activity of β-glucosidase and α- and β-galactosidase during curing of vanilla beans at 50ºC. However, activity of other glycosyl hydrolases (results not shown) tended to be low. Temperature optima for the enzyme activity were 50ºC for β-glucosidase, 55ºC for α-galactosidase and 60ºC for β-galactosidase in keeping with the temperature regimes used during the sweating stage of the curing process. To examine further the localization of β-glucosidase in green beans, we separated the green outer fruit region, placental tissue (without the seeds) and hair cells. We found that, when protected against proteolysis, β-glucosidase activity expressed

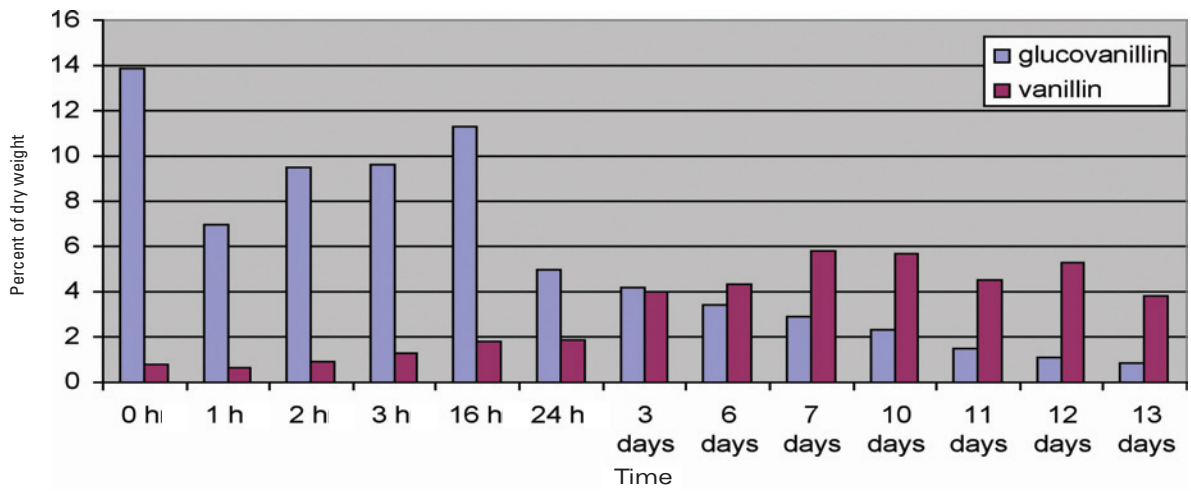
Changes in the content of vanillin (A), vanillic acid, 4-hydroxybenzaldehyde and 4 hydroxybenzoic acid (B) in whole vanilla bean during curing at 50°C.

F-9



Changes in the content of glucovanillin and in vanillin during time of curing of whole vanilla beans at 50°C.

F-10



as µg product/h/µg protein was as follows: 75.2 in green outer fruit tissue, 32.3 in the placental tissue and 11.1 in the hair cells. These results reinforce the need for proper killing to bring about the disorganization of bean tissue in order to establish contact between enzymes and their corresponding substrates.

The distribution of glucovanillin, along the longitudinal axis of green vanilla pods, was found to be as follows: 40 percent in the blossom end, 40 percent in the central portion and 20 percent in the stem end. This is in agreement with the observation by Childers et al. (1959) and other studies that vanillin crystals formed during curing appear mostly on the blossom end.[1] Accumulation of glucovanillin in vanilla pod developing on the vine ensues during the fourth month after anthesis. It then rises sharply for the next three months and levels off during the last stages of pod development.[37] Formed glucovanillin may be sequestered mostly in the inner white placental tissue around the seeds (F-4a).

The conversion of glucovanillin to vanillin during the curing process is shown in F-10. After eight days of curing at 50°C, the glucovanillin content decreased from an initial level of 14 percent to roughly 6 percent on dry weight basis. At the same period, the vanillin

Vanillin content in fresh and boiled whole vanilla beans supplemented with glucovanillin (GV) and β-glucosidase. **T-2**

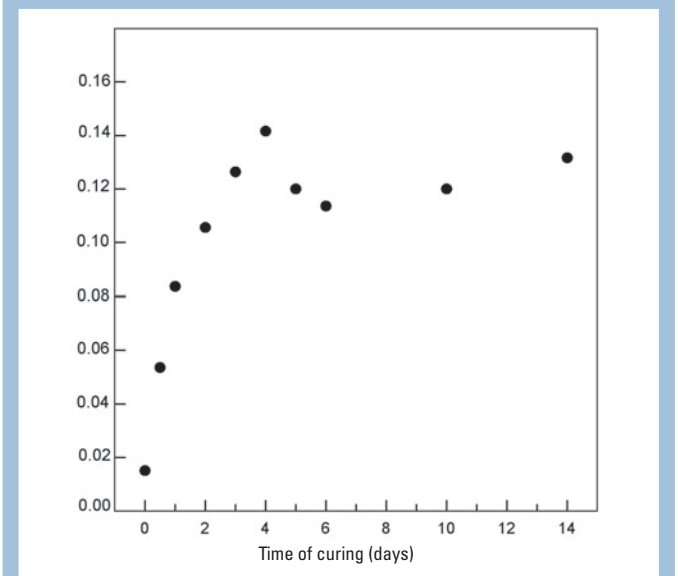
h of curing	Tissue condition	Compounds added	Vanillin % of DW
0	fresh tissue	none	0.0
24			1.8
48			1.9
0	fresh tissue	glucovanillin	0.0
24			2.2
48			2.5
0	fresh tissue	β-glucosidase	0.0
24			4.2
48			3.2
0	fresh tissue	β-glucosidase + GV	0.0
24			6.7
48			6.5
0	boiled tissue	none	0.0
24			0.0
48			0.0
0	boiled tissue	glucovanillin	0.0
24			0.0
48			0.0
0	boiled tissue	β-glucosidase	0.0
24			4.5
48			4.5
0	boiled tissue	β-glucosidase + GV	0.0
24			6.8
48			6.7

content liberated from glucovanillin rose to approximately 6 percent. The level of the two compounds leveled off afterward. The release of vanillin appeared to be accompanied by the accumulation of vanillic acid, p-hydroxybenzaldehyde and p-hydroxybenzoic acid (F-9b). This process was greatly accelerated in chopped beans, and was further enhanced in blender homogenized vanilla tissue (results not shown).

The accumulation of vanillin was accompanied by a marked decline in the activity of various glycosyl hydrolases, including β-glucosidase (F-8), a phenomenon that raised doubt regarding the efficacy of the enzyme to catalyzed hydrolysis of glucovanillin to vanillin. To explore this issue further, we examined the dependency of vanillin accumulation on enzymatic activity. T-2 shows that application of either glucovanillin or β-glucosidase led to an increase in the vanillin content in fresh green beans, and that vanillin content was increased further by the addition of both the substrate and the enzyme. However, when the activity of endogenous β-glucosidase activity was abolished by tissue boiling, production of vanillin ceased altogether and could not be restored even by the addition of glucovanillin. Conversely, vanillin accumulation was observed upon the addition of β-glucosidase to the boiled tissue and was enhanced further by the addition of glucovanillin. Collectively, these results suggest that conversion of glucovanillin to vanillin is predicated on the enzymatic activity of β-glucosidase, a conclusion confirmed by Dignum et al.[13] However, other studies suggest that conversion of glucovanillin to vanillin during traditional curing in Reunion approached only 40 percent of the hydrolytic capacity of β-glucosidase, though controlled curing under laboratory conditions resulted in the disappearance of almost 95 percent of the glucovanillin with a potential yield of vanillin of 5-7 percent (F-10).[17] These results suggest that curing under traditional field conditions, yielding between 1.5 to 3 percent vanillin on dry weight basis, may not exploit the full potential of the curing process or, alternatively, that the produced vanillin may have been lost during the prolonged drying and conditioning stages.[9,17,38,39]

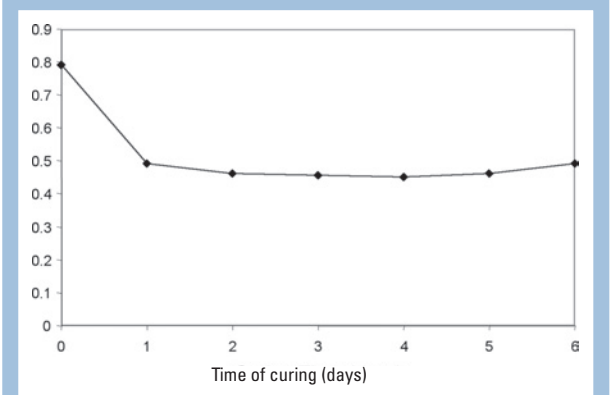
Change in the content of lipid hydroperoxides in bean tissue during curing at 50°C. Tissue increments of vanilla bean were removed periodically during curing for the estimation of lipid hydroperoxide as previously described (Eskin and Frenkel, 1976).

F-11



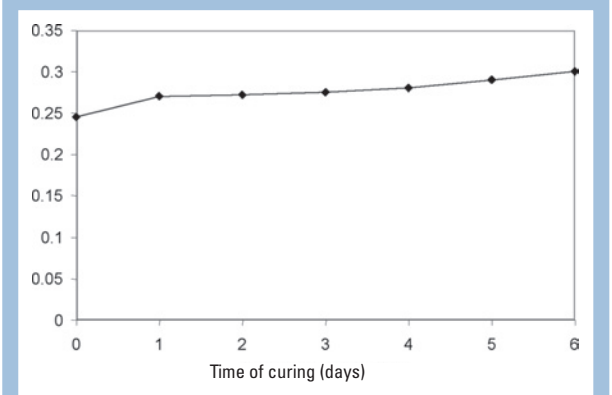
Changes in polyphenoloxidase (PPO) activity during curing of vanilla bean at 50°C. Fifty μl extract represent 14.3 mg of fresh weight vanilla bean tissue.

F-12A



Changes in peroxidase activity during curing of vanilla bean at 50°C. Fifty μl extract represent 14.3 mg of fresh weight vanilla bean tissue.

F-12B



Low activity of β-glucosidase in curing beans may result from proteolytic destruction of the enzyme, as well as denaturation by abundant phenolic compounds or oxidants. These conditions may influence the extraction and assay of the enzyme from cured beans. For example, determination of Km for β-glucosidase from vanilla beans, with the use of natural or synthetic substrates, yielded values around one order of magnitude higher than Km values for β-glucosidase in other organisms, suggesting that the enzyme in bean extract might have been in an abnormal state.[16] We found, by comparison, that when β-glucosidase was protected from proteolytic degradation (using protease inhibitors in the extraction and assay medium), Km values for the enzyme were lower than previously reported and comparable to those obtained by Hannum.[16,40] It is desirable to re-evaluate this issue by careful extraction and assay methods for β-glucosidase or other enzymes in vanilla beans.

Oxidative enzymes: Killing of vanilla beans is associated with de-greening and onset of browning reactions, found also during advanced senescence in other fruit, as well as during stress or disease injury in plant tissues.[41,42] Balls and Arana observed that various killing methods, including chemical, mechanical or heat stress, but not freezing stress, stimulated a temporary upsurge in carbon dioxide evolution, suggesting that the killing-induced respiratory upsurge may reflect the onset of oxidative processes in the killed pod.[8,20] This effect was mimicked by applied ethylene that led to a brief upsurge in CO_2 evolution in green beans, followed by a continuous decline in CO_2 for the next 15 days.[8,20] Arana observed that applied ethylene, though acting as a maturing agent, also led to bean browning.[9] Other studies, showing that ethylene-induced respiratory upsurge in plant tissues was accompanied by H_2O_2 accumulation, suggest that ethylene-induced browning may stem from ethylene-induced oxidative metabolism.[43] This view is supported further by the observation that stimulation of browning by ethylene is amplified by co-application of oxygen (T-1). These data infer that bean browning as occurring naturally at a terminal phase of senescence, or as induced by

ethylene, may reflect onset of oxidative conditions in vanilla beans. Because various stress conditions are associated with the accumulation of H_2O_2 and other reactive oxygen species (Kocsy et al., 2001), it is a reasonable assumption that stress conditions, employed in vanilla bean killing, may also lead to the onset of oxidative conditions manifested in browning of cured vanilla beans.[44]

Importantly, vanilla bean browning appears to be driven by enzymatic activity as evidenced, for example, by the inhibition of browning in beans subjected to excessive killing by prolonged or extreme heating, apparently because of enzyme denaturation.[45] This view is also supported by a study showing arrest of browning in green beans by autoclaving at 120°C and, furthermore, restoration of browning in autoclaved beans upon the addition of oxidative enzymes from fungal origin.[46] We observed, similarly, that, while killing by freezing resulted in typical browning in mature green beans, excessive boiling after freeze-killing resulted in the inhibition of browning, apparently because of heat denaturation of oxidative enzymes (results not shown).

Polyphenol oxidase (PPO) and peroxidase are the two major enzymes that may catalyze browning processes in killed vanilla beans.[39] PPO utilizes molecular oxygen to catalyze the hydroxylation of aromatic compounds, a reaction that is followed by subsequent oxidation of hydroxy groups to their respective quinones and by further polymerization of oxidation products. PPO-driven browning in vanilla pod may result mostly from the oxidation of tyrosine, caffeic and chlorogenic acid, as well as other phenolic compounds.[47] PPO activity, represented by a family of enzymes, is observed in injured or stressed plants, but not in intact plant tissues.[47] Peroxidase utilizes H_2O_2 and other hydroperoxides to catalyze the oxidation of various cellular substrates, including oxidation of aromatic compounds, bleaching of carotenoids, discoloration of anthocyanins or degradation of ascorbic acid.[47-50] Because peroxidase catalyzes the degradation of a wide array of cellular substrates, the role of the enzyme may be wider than just a contribution to browning. For example, peroxidase-driven peroxidation of unsaturated fatty acids, catalyzed by the heme group in the enzyme, may account for a marked increase in lipid hydroperoxides in killed vanilla beans (F-11), arising perhaps from the oxidation of abundant

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lipid bodies in placental hairs (results not shown).[51] Moreover, lipid peroxidation may be accompanied by the spontaneous propagation of lipid hydroperoxides that further amplify the oxidant effect of the enzyme.

Activity of peroxidase and PPO increases steadily during vanilla pod development, but in green beans the enzyme activity is apparently latent and not expressed.[15,25] Pod browning, as occurring naturally in senescence or induced by ethylene or various killing methods, appears to unleash the activity of these enzymes, apparently because of cellular disorganization. Several studies confirmed that activity of PPO remained high in vanilla beans following killing and during a subsequent curing stages or in tissue extracts of vanilla beans, although Jiang et al. observed low PPO levels in cured beans. We found that PPO activity decreased during curing by approximately 50 percent but remained steady and substantial (F-12A).[3,8,20,23,52]

By comparison, peroxidase activity actually increased with time of curing, suggesting that stress conditions and attendant proteolysis do not impair the enzyme activity (F-12B), in keeping with the observation that peroxidase activity is preserved even after dipping green beans in 80ºC for 20 min.[13] Peroxidase activity persisted well into the conditioning phase of the curing process and the enzyme activity was restored even after autoclaving at 120ºC, further indicating the enzyme is stable even under extreme conditions.[39]

PPO and peroxidase-catalyzed oxidation of aromatic compounds appear to be important in pod browning. However, oxidative processes may also entail an oxidative degradation of other cellular compounds, lipid peroxidation for example (F-11), which apparently give rise to volatile compounds including ketones, aldehydes, alcohols or hydrocarbons in cured vanilla beans.[7] We wonder whether the oxidation of lipids and perhaps other cellular constituents may also contribute to the development of the vanilla flavor during the curing process.

Vanilla Products: Types and Consumption

Cured vanilla beans are used for the extraction and the preparation of vanilla products. Maceration, percolation, countercurrent extraction or a batch process may be used to extract vanilla beans. For efficient extraction, the beans are chopped to pieces, about 1 cm in length. In some instances, the vanilla tissue is pulverized to fine powder under cooling to aid the tissue extraction.

The four basic types of vanilla products are the following: vanilla extract, vanilla oleoresin, vanilla absolute and vanilla powder/sugar. Each form has its typical organoleptic, physical, and functional attributes tailored by the choice of beans used for the process and the processing conditions. The vanilla extract is by far the most used vanilla product. Vanilla products are used in the food, dairy, confectionary, beverage, pharmaceutical and fragrance industries. In addition, the products in each category must meet the government regulations of the country where the products are manufactured or sold.

Vanilla extract: According to the US Food and Drug Administration regulation, vanilla extract is the solution, in aqueous ethyl alcohol, of the sapid and odorous principles extractible from vanilla beans. Ethyl alcohol content of such extract is not less than 35 percent by volume, and the extractible matter of one or more units of vanilla constituent. A unit of vanilla constituent is 13.35 oz of beans containing not more than 25 percent moisture, per gallon of finished extract. This definition constitutes roughly 1:10 ratio of bean material to solvent, respectively. This is the definition of one fold. In the United States, vanilla extract is sold on the basis of fold whereas, in other countries, France for example, extracts are sold on the basis of vanillin content.

Vanilla oleoresin: This product is obtained by extracting finely chopped beans with an aqueous ethanolic solvent or with other solvents. The solvent is then removed by evaporation rendering a final product that is a dark viscous liquid.

Vanilla absolute: Vanilla absolute is the highly concentrated form of a vanilla product, used mostly in the fragrance industry. A small quantity of vanilla absolute is used in the food and confectionery industry. It is also the most expensive vanilla product. At present, vanilla absolutes are prepared either by selective solvent extraction techniques or by the supercritical liquid CO_2 extraction. Solvents used in selective solvent processes are hydrocarbons (hexane, for example). The preparation and identity of this product is not as strictly regulated as that of vanilla extract.

Vanilla powder/sugar: Vanilla powder is made by mixing ground vanilla beans, vanilla oleoresin or concentrated vanilla extract with suitable sugar carrier ingredient. The final product is in essence sugarcoated vanilla extract.

Commercial vanilla products: In the United States, most of the cured vanilla is processed to obtain solvent extracts, which are used by food manufacturers and household consumers. In France, Germany and all of Continental Europe, the primary demand for domestic consumption is in the form of whole or powdered vanilla. Together, the United States, France and Germany consume about 80 percent of total world imports. The United States alone traditionally

imports and makes use of about 50-60 percent of the world's vanilla production.

Vanilla is used for flavoring ice cream, chocolate and other confectionery products, cola beverages, baked goods, alcoholic beverages, flavored yogurts, and numerous frozen desserts. Ice cream accounts for 44 percent of vanilla extract consumption in the United States. Household extract consumption accounts for 22 percent of vanilla extract, cola beverages for 17 percent, and 17 percent of natural vanilla is also used in chocolate and confections, alcoholic beverages, flavored yogurts and other products.[2] In the early 1980s, when the price of vanilla increased, the dairy industry opted to reduce the use of vanilla extracts, instead opting for less costly substitute flavors. With the decrease in vanilla price in 1990s, when the industry trends reverted back to the use of high quality products, vanilla extract was used in ice creams and gourmet foods. However, the percentage of vanilla extract used in households was reduced with the decline in home cooking. With the decrease in vanilla price in 1990s, industry developed high quality products, like premium ice cream and gourmet foods, which used vanilla extract (Rosskam, president and CEO, David Michael & Co. Inc., personal communication). For additional and more detailed information on this topic, also see Ranadive and Shankaracharya and Natarajan.[2,53]

The Future

The publication of this paper comes at the most tenuous time in the last 25 years for the growth and prosperity for producers and users of the world's most popular flavor. Madagascar, the main production region for vanilla beans, is beset by various disruptions. For example, in 2000 Typhoon Huda severely damaged the crop on the vine in Madagascar. Consequently, bean prices escalated by roughly 500 percent. Since the late 1970s, the food industry had been enjoying the lowest prices for vanilla extracts and the use of vanilla in food formulations and new products had been on a steady rise, creating a growing demand for vanilla beans. The abrupt and large increase in price has caused many companies to look for affordable substitutes. Some food companies simply switched from the use of natural vanilla to artificial vanilla while others looked at formulas with reduced vanilla content in combination with other characterizing flavors (for example, chocolate ice cream). Finally, new products that called for vanilla were delayed or canceled. The net effect of these changes has been estimated to reduce global demand by 20 percent. Unfortunately, the Madagascar vanilla market continued to maintain high prices for the 2001 crop. Prices for the 2002 crop also continued to remain high because of political instability. Unusually cool and wet weather in 2003 adversely affected flowering as well as curing of the vanilla crop. Insufficient flowering might create additional limitation to the industry, as the issue is not just price, but also having enough supply to meet demand. This is expected to lead to further escalation in the price of vanilla produced in Madagascar as well as other growing regions. Indonesia has already increased the price of vanilla by over 30 percent. Similar problems of supply and price occurred in the late 1970s, from which the industry was able to rebound. It is likely do so again. As a result, the use of vanilla decreased by 30-50 percent in the United States. The price of vanilla beans went down and at the same time new beans were getting into the market by new suppliers and new plantations.[54,55] In 2004, vanilla bean prices went down with further reduction in consumption (57, 58).

For the most part, the consumer is oblivious to global supply and price issues facing food manufacturers. All they know is that they love the taste of vanilla and that is unlikely to change. The question is whether love and desire for vanilla flavor will be satisfied by traditional natural vanilla or by synthetics.

Summary and Conclusions

The curing process of green vanilla beans is predicated on the disruption of cellular organization and the consequent unleashing of uncontrolled enzymatic and perhaps non-enzymatic processes. These reactions are beneficial to the development of the prized vanilla flavor. However, impaired enzymatic activity may arrest development of a full flavor complement. These impairment conditions may prevail during severe or excessive killing conditions. Appropriate curing, entailing controlled killing and a short sweating period around 50ºC, may provide sufficient enzyme activity for catalyzing the formation of vanillin and other flavor constituents and preclude the need for the application of commercial enzymes. Prolonged drying and conditioning may lead to a loss in formed flavor compounds, suggesting the exploration of new approaches to these stages of the curing process. Further work may determine whether oxidative conditions lead to enzymatic as well as non-enzymatic formation of flavor compounds in cured vanilla beans.

A periodic problem in supply and cost of vanilla emphasizes the need to gain the most out of available vanilla beans by an efficient curing process.

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