Ripe vanilla beans (bean length ca.16–18 cm) showing yellowing at distal extremity.

Vanilla Curing

The senescent decline of a ripe vanilla bean and the birth of vanillin

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Cured vanilla beans

Vanilla bean curing, whether occurring naturally on the vine or practiced at curing stations, is a facet of plant senescence. This process is an actively regulated and genetically controlled development that includes slow and ordered breakdown of selected tissue structures with associated membrane leakage, degradation of chlorophyll and proteins and extensive lipid peroxidation (due to enhanced production of oxygen free radicals); the oxygen free radicals are, in fact, the primary mediators of oxidative damage during plant senescence.[1] Dropping of leaves from plants in the fall is the most common example of plant senescence, which entails massive mobilization and recycling of nitrogen, carbon and minerals to other parts of the plant.[1]

Human intervention is required to pollinate the vanilla flower that eventually results in formation of immature pod or bean. The bean ripening process—which is visually characterized by yellowing of the pod at the distal extremity—takes seven to nine months. The ripe bean, then, begins to split at the end of the pod and starts "browning" at the same location.

If the ripe vanilla bean is left on the vine, the senescence process occurs naturally. However, traditional curing takes a different route that begins with plucking of ripe vanilla beans from the vine; this method accelerates the senescence process and is known as curing. The curing process, which takes about 150 days, refers to a series of human interventions to transform an aromaless, ripe vanilla bean (containing ca. 80% water) into an aromatic, dark brown, 20–30% water-containing cured vanilla bean.

The Curing Process

The Aztec emperor Montezuma II introduced natural vanilla to European conquerors in the early 16th century;

Traditional curing of vanilla beans **F-1**

the latter soon became interested in its cultivation. Nevertheless, hand pollination did not replace natural vanilla pollination—which takes place via the melipona bee—for the next 300 years.[2] However, once it did, in the neighboring regions of Mexico, the crop flourished, and it continues to do so even today. Vanilla is now grown in a climatic belt, bordered by the Tropics of Cancer and Capricorn, north and south of the equator, respectively. In 2006–2007, the major Bourbon vanilla producing areas in Madagascar, Reunion and the Comoro Islands, accounted for about two-thirds of the world production.[3] The other major vanilla producing areas include India, Uganda, Indonesia, Papua New Guinea and Mexico. Today, vanilla is a highly prized crop and one of the most important and globally embraced materials in the fragrance and flavor industry.

Traditional vanilla curing involves the following sequential steps, with slight variations depending on the geographical location.*

- ***Blanching, wilting or "killing" stage*:** Generally entails, though doesn't exclusively involve, immersion of ripe beans in hot water (60–65°C) for 2–3 minutes.
- ***Sweating stage:*** The beans are wrapped in blankets and stored in closed wooden boxes, where they are fermented for about 48 hours.
- ***Sunning stage:*** Here, the beans are spread out on blankets in the sun for 1–3 hours, then rewrapped in blankets and kept in closed wooden boxes for the rest of the day. This cycle is repeated 10–20 times, depending on local weather conditions.
- ***Drying stage:*** Beans are placed indoors, on racks, at ambient temperatures for 4–6 weeks.
- ***Conditioning stage:*** The beans are bundled and packed, according to their lengths, in closed boxes at ambient temperature for more than eight weeks.

The outline of a typical traditional curing operation is depicted in **F-1.**

For the Bourbon curing process in Madagascar, average vanillin contents in the cured beans are about 1.80 g/100 g dry weight.[3] A deeper analysis, however, highlights a number of deficiencies in the traditional curing operation. First, despite being long-established, the process is not well understood in terms of the biochemistry, enzymology and chemistry of flavor formation during the different stages of curing. Moreover, the important stage of "sunning" is subject to temperature/time fluctuation, due to dependency in weather; this eventually leads to variations in product quality, including vanillin content. Other limitations of a traditional vanilla curing process include: few opportunities to vary, control or direct the curing process; poor economics due to labor-intensive operations; and substandard crop hygiene resulting from excessive handling and often lack of clean working surfaces.

*For more details, visit *www.orchidsasia.com/vanillacuring.htm*

The Science Behind the Vanilla Curing Process

Recent studies on vanilla curing have focused on better understanding the factors controlling the enzymatic transformation of the primary precursor glucovanillin to vanillin and glucose (see **F-2**). Here, a few key questions arise. What are the tissue structural factors which influence this conversion? Which enzymes are involved in the transformations? What is the efficiency of the glucovanillin to vanillin conversion? What is the fate of vanillin when liberated from the glucoside? How do the other non-phenolic aroma compounds arise?

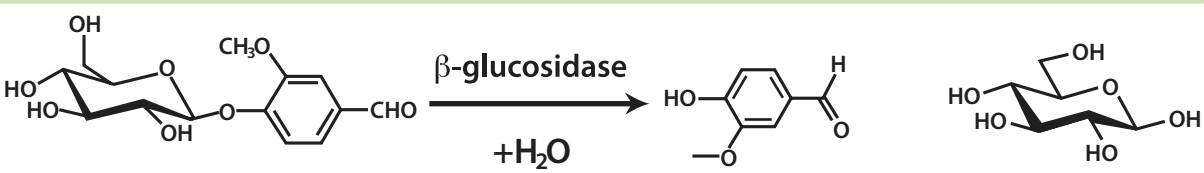
Some recently published work has thrown light on some of these questions. Contemporary studies on factors affecting the hydrolysis of glucovanillin suggest that only one endogenous glucosidase is involved in the formation of vanillin from the precursor glucovanillin.[4] Anatomical studies show that vanilla beans have a triangular cross section with a central cavity containing seeds. Each angle surrounding the seeds is lined with tubular papillae, or hair cells, whereas the cavity is bordered by a placental region, or lamellae (**see F-3**).

Glucovanillin and β-glucosidase are present in the central part of the fruit; the β-glucosidase activity is associated with the cytoplasm and/or periplasm of the mesocarp and endocarp cells, and to a lesser extent with the papillae.[5] Although the site of glucovanillin has not been determined, it is believed to be present in the vacuole due to its analogy with the disposition of other secondary metabolites.[6]

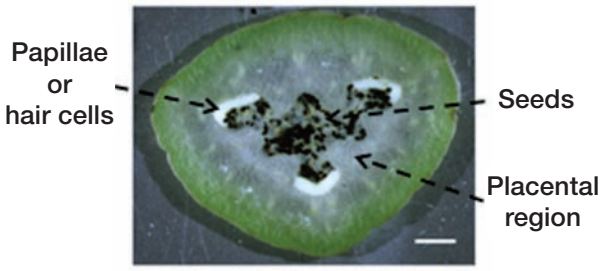
Therefore, the efficiency of the transformation of glucovanillin to vanillin depends on decompartmentalization of the enzyme and its substrate, and on the stability of the glucosidase during the initial stages of the curing process. Decompartmentalization refers to the breakdown or disruption of the sub-cellular organization of cellular structures.

The effects of different pre-treatments—such as natural senescence, pre-freezing/thawing and hot water blanching—on the cellular disassembly of whole vanilla beans have been the focus of several studies. The intent has been to evaluate the degree of tissue disruption, the extent of glucoside hydrolysis and activity of the endogenous β-glucosidase during these treatments.[7] These findings revealed that the extent of cellular disruption was proportional to the degree of glucovanillin hydrolysis. Therefore,

Enzymatic conversion of glucovanillin to vanillin **F-2**



Cross section of a mature vanilla bean (bar = 0.2 cm) **F-3**



when monitored by light and electron microscopy, natural senescence and freezing/thawing were found to result in maximum cellular disassembly, whereas the effects of hot water blanching were found to be relatively less significant.

Also, while untreated ripe beans showed less than 5% glucovanillin hydrolysis, the related β-glucosidase activity was high, at ~1000 nkatal/g fresh weight (1 nkatal of β-glucosidase activity is the amount of enzyme that hydrolyzes 1 nmole of substrate per second at pH 7.0 and 40°C). Similarly, the fruit undergoing natural senescence exhibited more than 95% glucovanillin hydrolysis, even when the residual β-glucosidase activity was undetectable. Similar observations were made for glucovanillin conversion and enzyme activity for beans that were frozen at -18°C for three days and then thawed. It is worth noting that freezing is reported to have a detrimental effect on the activity of the vanilla bean β-glucosidase.[8] Beans treated with hot water blanching at 60°C for 2 min resulted in less than 50% glucovanillin hydrolysis and no measurable β-glucosidase activity after the fermentation step. In addition, the degeneration of cellular structure following water blanching, on microscopic examination, was less pronounced than that observed following natural senescence or freeze/thaw. These studies suggest that the limiting factor in hydrolysis of glucovanillin is the extent of cellular disruption; this provided that β-glucosidase activity is not lost by pre-treatment of ripe beans at temperatures in excess of 85°C.

It is hypothesized that β-glucosidase and glucovanillin are segregated within the cell in undamaged tissue and that damage to cellular integrity causes catalyst to come in contact with substrate. This in turn results in the formation of vanillin, and related loss of β-glucosidase activity. Further analysis indicated that oxidation products developed in the brown bean during fermentation did not interfere with the activity of the β-glucosidase.[7] These observations suggest that the intervention prior to fermentation is critical to effect significant tissue damage and consequent high yield of vanillin from glucovanillin. It is important in the context of these observations to note that the degree of hydrolysis of glucovanillin is dependent on the combined pretreatment and fermentation stages. Although both natural senescence and freeze/thaw are very effective in tissue structure disruption, these methods are not extensively applied for practical and economic reasons. This creates a need for consideration of other tissue protocols that produce tissue damage. Interestingly, senescence shares some common features with other stress-related inducers, including heat, cold, and plant hormone application such as ethylene or jasmonates.[9]

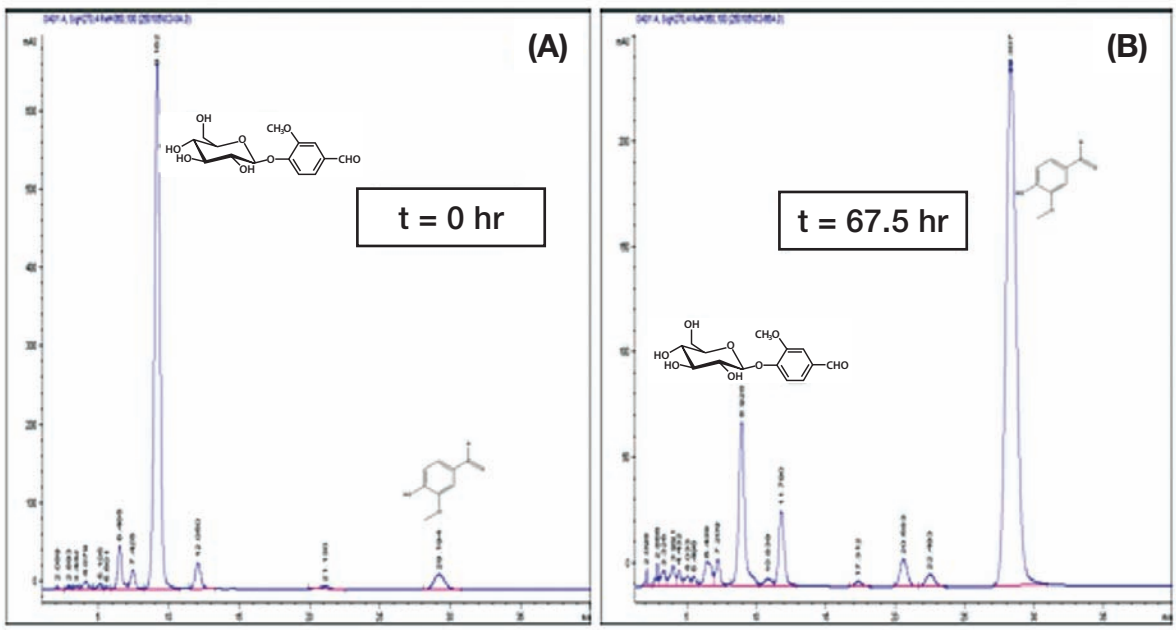
The final level of vanillin in cured vanilla beans depends on a number of factors, including:

- The initial content of glucovanillin in the ripe vanilla bean
- The extent of tissue damage engineered prior to fermentation
- The extent of conversion of glucovanillin to vanillin during fermentation
- The temperature/time variables during the fermentation process
- The further transformation of vanillin following its release from the β-D-glucoside during fermentation and subsequent drying and conditioning stages

Some of our own work has been focused on a few aspects of glucovanillin to vanillin conversion. Using tissue pretreatment protocols, in conjunction with defined fermentation conditions and extraction procedures, it is feasible to monitor the initial concentration of glucovanillin in ripe, green vanilla beans and follow the progress and the extent of conversion to vanillin during the fermentation phase of curing.

F-4 shows a high pressure liquid chromatogram (HPLC) of a solvent extract of ripe vanilla beans (A)

Glucovanillin to vanillin conversion during fermentation of ripe vanilla beans; extract of ripe vanilla beans (A); ripe vanilla beans fermented at 50°C for 67.5 hr (B) **F-4**



and the same beans after blanching pretreatment, then fermentation for 67.5 hr at 50°C (B).

Measurements of glucovanillin levels in ripe vanilla beans ranged from 35–45 mMoles/100 g dry weight of tissue depending on their geographical origin. Typically, 90% glucovanillin to vanillin conversion can be achieved during fermentation, under optimum conditions, so that vanillin content at the end of this process can be in the region of 32–41 mMoles. This corresponds to vanillin content from 4.9–6.2g/100 g dry weight. Final vanillin levels at the end of the total curing process are lower than these values, probably due to further transformation of this reactive phenol.

Vanillin is formed during the early stages of fermentation of vanilla beans, and accumulates over time. A similar situation probably applies for the other glucosidically bound phenols such as guaiacol and *p*-cresol. Interestingly, the N-heterocyclic compound indole appears at the early stages of fermentation and reaches a maximum after about 1 hr, but unlike vanillin, it disappears after about 7 hr of incubation. **F-5** shows a series of GC/MS snapshots of the transient formation of indole, and accumulation of vanillin and guaiacol during fermentation of ripe vanilla beans.

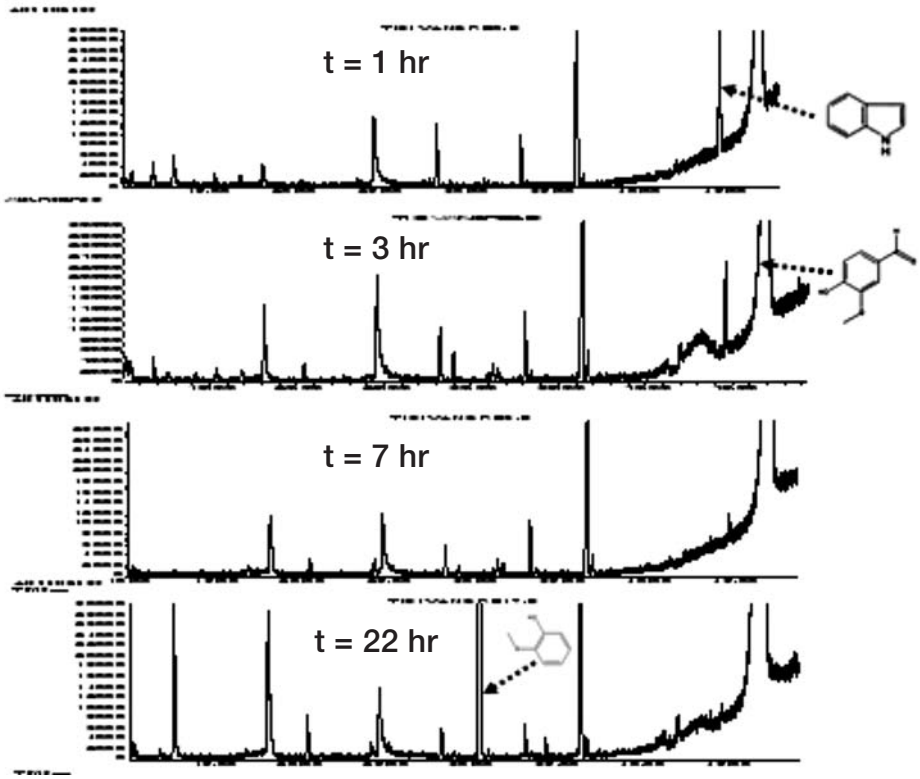
Indole is an intermediate in the final stages of the primary metabolic pathway starting from indole-3-glycerophosphate and catalyzed by tryptophan synthase to the amino acid tryptophan. Mechanical damage of tissues, elicited by herbivores, activates a transient secondary metabolic pathway, via indole-3-glycerophosphate lyase, liberating free indole.[10] Indole in many plants serves as a component of a volatile cocktail that signals the natural enemies of feeding herbivores.[11]

The flavor of vanilla extracts is complex, and a number of publications have highlighted the different aromas in extracts of the cured vanilla bean. More than 250 compounds from a number of geographical origins have been identified in vanilla beans. These include aromatic and aliphatic aldehydes and ketones, alcohols, acids and esters, as well as the phenols, depending on the method of extraction.[12,13] The recent work of Perez-Silva et al. has identified and quantified 65 compounds in extracts from cured Mexican vanilla beans using GC/MS.[13] In addition, using GC-O assessment, a group of panelists was able to perceive 26 aroma-active compounds. Ten of the compounds detected in this study were phenols; interestingly, three of these—namely 4-methylguaiacol, guaiacol and acetovanillone, at concentrations of 3.8, 9.3 and 13.7 ppm, respectively—were considered in organoleptic terms to be as odor-potent as vanillin. The latter was present in the extracts at about 5,000 times their concentration. In addition, there were a number of other odor-potent aromatic esters, aliphatic acids and aldehydes that contributed to the total aroma profile.

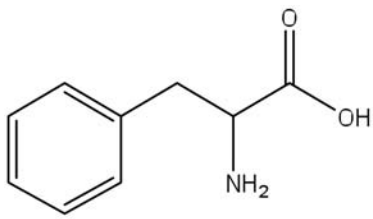
It is of interest to consider the biochemical and/or chemical origin of these families of compounds. The principal routes to some of the molecules present include:

- The phenylpropanoid secondary metabolic pathway from phenylalanine, for example to vanillin, guaiacol, methyl cinnamate
- The polyunsaturated fatty acid/lipoxygenase pathway to C6-C10 aldehydes and alcohols, such as (E,Z)-2,4-decadienal
- Intermediary metabolism, including acetic and butanoic acid formation
- Glycolysis/pyruvate/acetolactate pathway to generating the C4 dioxy compounds, including acetoin and 2,3-butanediol

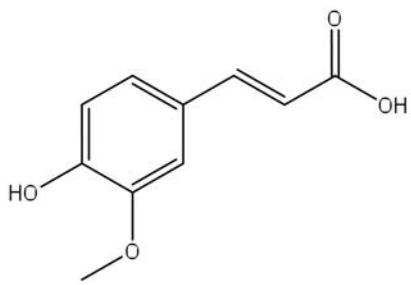
Transient formation of indole and accumulation of guaiacol and vanillin during the early stages of fermentation of ripe vanilla beans of Indian origin **F-5**



L-amino acid phenylalanine **F-6.1**



Ferulic acid **F-6.2**



The phenylpropanoid pathway is the major biosynthetic route to the phenolics.[14] The key compound in this pathway is the L-amino acid phenylalanine (**see F-6.1**). Among the products of this complex pathway are the lignans and lignins, and their precursor compounds, including caffeic and ferulic acid (**see F-6.2**).

The aliphatic acids probably arise by two alternative pathways. For the branched chain acids, they are probably generated biochemically from the corresponding Cn+1 amino acids. The route is via oxidative deamination of the corresponding amino acid—for example, leucine followed by decarboxylation to 3-methylbutanal, and finally oxidation by an aldehyde dehydrogenase to produce isovaleric acid.[15]

Acetic acid, in quantitative terms, is one of the major components of cured vanilla beans, whereas the C4 homologue butanoic acid occurs to a much lesser extent. The C2 compound, principally as the SCoA derivative, is a key intermediate in both plant anabolic and catabolic processes. It can arise by hydrolysis of acetyl SCoA formed via glycolysis or other carbohydrate metabolism. Alternatively, acetic acid is the end product of β-oxidation of even-numbered-long chain fatty acids—a process that takes place in the peroxisomes of plant cells.[16,17]

The biosynthetic origin of the C4 compounds 2,3-butanediol and the related 3-hydroxy-2-butanone in plants occurs via the pyruvate/acetolactate pathway. Pyruvate from glycolysis and its hydroxyethyl-thiamine pyrophosphate form 2-acetolactate, catalyzed by acetohydroxyacetate synthase or acetolactate synthase.

Decarboxylation of 2-acetolactate, catalyzed by acetolactate decarboxylase, leads to 3-hydroxy-2-butanone.[18] Reduction of the single carbonyl group of 3-hydroxy-2-butanone via the NADH dependent acetoin reductase or meso-2, 3-butanediol dehydrogenase produces 2,3-butanediol.[16,a]

Additional aroma compounds may well be products of transformation of proteins, peptides, amino acids, reducing sugars, polysaccharides and lipids, with or without the concerted activities of enzymes. A summary of some of the potential precursors, pathways and key aroma compounds found in cured vanilla beans is shown in **F-7**.

Curing is an accelerated senescence process in which the structural integrity and functional organization of the vanilla bean is compromised and where organized chaos reigns. When the identities of the key aroma compounds are known and their pathways of formation defined, it is then, in principle, possible to constrain and direct the curing process to optimize particular biosynthetic elements—for instance, enhance flux through the phenylpropanoid pathway to generate vanillin-type phenols. A key enzyme in such an activation process is phenylalanine ammonium lyase (PAL).[19]

PAL initiates the transformation of phenylalanine in plant tissues. This enzyme is present in plastids along with the enzymes involved in the formation of tyrosine and tryptophan.[17] Phenylalanine serves as the precursor of key secondary metabolites such as lignin, lignans and the flavonoids, while tryptophan is transformed to the indolic phytoalexin secondary metabolite, camalexin. These pathways are induced by environmental signals, including wounding and microbial, and bacterial and fungal incursion. The buildup of camalexin is strongly correlated with up-regulation of genes of the *Arabidopsis* tryptophan biosynthetic pathway.[20]

A number of other reaction pathways appear to be important contributors to the chemistry and biochemistry of curing, but the understanding of their role in flavor formation still remains to be determined.

Of particular interest in this context, however, is the further oxidative transformation of vanillin and related phenols, potentially mediated by the vanilla bean endogenous oxido-reductases viz., peroxidase and polyphenoloxidase.[21-23] The compounds formed by these enzymes, including the "brown" pigments, of mostly undefined structure, are characteristic of the fermentation stage of curing and are responsible for the dark color of cured vanilla beans and extracts. Non-enzymatic oxidative polymerization of phenols can contribute additionally to chromophore formation.

It has also been observed that about 50% of the vanillin potential inherent in the glucoside in the ripe vanilla bean is lost during the totality of the curing process.[24,b] The glucovanillin level in the mature bean is a reliable indicator of the potential for vanillin formation. Theoretically, every 2.06 g of glucovanillin in the green bean can realize 1 g of vanillin via complete hydrolysis of the glucoside.

As previously indicated, factors that can affect vanillin yield include:

- The extent of tissue disruption. The efficiency of this process in conjunction with appropriate time/temperature conditions for the fermentation stage of curing are important in maximizing hydrolysis of the phenolic glucosides.
- Further transformation of the liberated vanillin, and the other phenolics, by both enzymatic and chemical mediated processes.
- Potential reaction of the aromatic aldehyde as well as the phenolic hydroxyl group of vanillin. Although mechanisms and products are not well established, this area could be a target of future interest from the perspective of flavor formation.

Chemical reactions may play a role, particularly during the later parts of the curing process, when extended temperature/times are employed, especially during "sunning," drying and conditioning. In the presence of reducing sugars and a source of nitrogen, the formation of volatile products via the Maillard or related reactions may occur.[25] However, knowledge in this area is limited. Lipid oxidation could also participate in flavor formation by the classical oxylipin pathway, and also by autoxidation processes.[26]

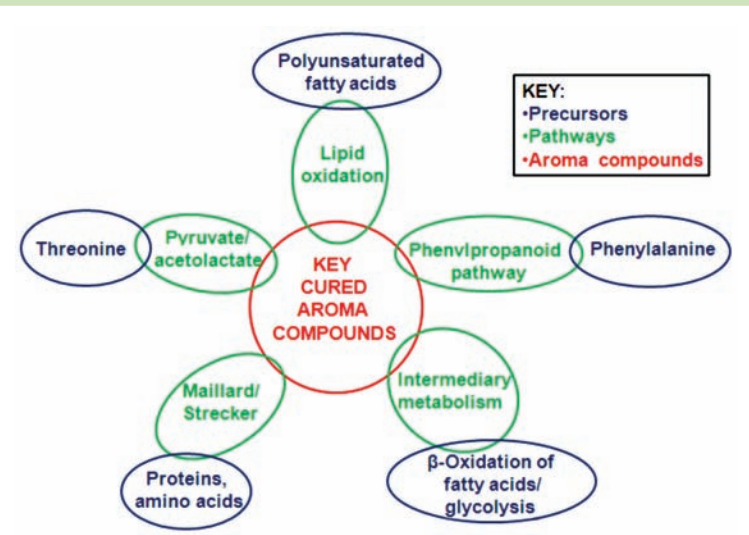
The role of microorganisms in flavor formation remains an open question and merits further consideration.[27] Nevertheless, most recent studies suggest that at least the early part of curing, i.e. the fermentation stage, is primarily executed by endogenous enzymes in the ripe vanilla bean.

It is clear that curing of vanilla beans is a very complex, multicomponent process. To better understand the formation of flavors, both phenolic and non-phenolic, a more detailed dissection of the senescence process needs to

[a] *www.msu.edu/~narayan/2-3-Butanediol.pdf*

[b] *www.cirad.fr/en/actualite/communiqué.php?id=683*

Potential precursors and pathways to key aroma compounds in cured vanilla beans **F-7**



be considered. This activity, coupled with in-depth analysis of the biochemistry and chemistry of the different stages of the curing process, will realize controlled flavor formation.

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