Optimization of Traditional Curing of Vanilla Beans

A reappraisal of sunning, drying and conditioning.

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Curing of vanilla beans is an art form, as well as a science. A number of recent advances in plant biochemistry and chemistry have clarified some stages of the curing operation and opened up opportunities for improvement in and control of the process. The most commonly practiced curing operation is the Bourbon traditional process includes the following stages.

Blanching Stage

This involves the immersion of the green vanilla beans in hot water, at 60°–65°C, for up to three minutes. This is typically conducted in metal containers heated over a wood fire. Blanch water temperature is frequently monitored using a thermometer. This hot water treatment removes adhering dirt and soil from the beans, inhibits vegetative growth and activates a number of plant enzymes which are important for the development of the flavor and color of the final product.[1]

Sweating, or Fermentation

The hot green beans are removed from the blancher and quickly transferred into large, closed, insulated wooden boxes and covered with wool blankets to prevent heat loss. Alternatively, beans may be wrapped in blankets in bundles, then stacked and covered with further insulating blanket layers. The beans are then fermented, or "sweated," for a maximum of two days, during which time the temperature remains in the region of 40°C.

The beans during this period turn from their initial green/yellow color to dark brown and become texturally supple. At the same time the flavor forming processes involving enzymatic hydrolysis of vanillin precursor(s), phenol browning and lipid oxidation occur. Moisture content at this stage is in the region of 80–85%.

Sunning

After sweating, the beans are sun-dried by spreading them out in single depth on tables or blankets on the ground for several hours a day, depending on weather conditions. Between these sessions, the beans are rewrapped in blankets and stored under cover to allow the sweating process to continue. Under favorable conditions sun-drying is typically completed after about two to three weeks, by which time water contents of 30–35%% are reached. During sunning further biochemical and chemical transformation continues with the concomitant water loss.

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Drying

To achieve a final water content in the beans of 25-38%% the pods are slow-dried, uncovered, indoors for about four to six weeks on wooden drying racks in airy warehouses. Additional chemistry, which is poorly understood, continues during this stage along with further water loss.

Conditioning, or Maturation

The vanilla beans are then ideally packed into grease-proof paper-lined wooden boxes for conditioning, which continues for several months after the drying process. During this maturation phase, the final well-balanced vanilla flavor profile develops.

More on Vanilla Curing

For further reading, see "Optimizing the Traditional Curing of Vanilla Beans" from Page 22 of the March 2015 edition of P&F, and "The Role of Lipids in Vanilla Beans and their Transformation during Curing" from Page 20 of the February 2014 issue of P&F; *www.perfumerflavorist.com/magazine/pastissues/*

A Closer Look at Aroma Compound Formation

A reappraisal of blanching and fermentation of traditionally cured vanilla beans has recently been conducted.[2] The remit of this review is to consider in more detail the important curing stages of sunning, drying and conditioning with respect to the biochemistry and chemistry occurring and how these relate to formation and transformation of key aroma and taste compounds.

The Curing Operations

The primary objectives for all traditional curing processes is to develop the characteristic flavor of the beans and to retain their visual integrity and appearance from the ripe green to the final brown, cured product. Sunning and drying bring about the overall process of moisture reduction of the fermented bean. Water content in the bean falls from an initial level in the fermented product at ca. 80–85% to a final value in the dried material from 25–38%, depending on the bean end use.[3] The beans, once these moisture levels are attained, are more resistant to microbial ingress and proliferation.

Water activity (a_w) of beans in the range of 38–25% moisture was determined to be 0.89–0.84.[4] Most spoilage bacteria are inhibited at aw below 0.90, while most molds require a value of ≤0.70. A combination of low water activity and high phenol content, of which the major representative is vanillin, seems to provide inhibitory conditions against microbial proliferation.[5] Natural vanillin exhibits strong antimicrobial properties against *Aspergillus flavus*, *A. niger*, *A. ochraceus* and *A. parasiticus* in laboratory media and fruit-based agar systems. The most resistant mold was *A. niger.* The vanillin inhibitory concentrations were in general lower than 2,000 ppm, and its influence was related to the fruit-base type.[6]

For most applications, however, final moisture levels in cured beans are around 20–25% and as such would not indicate a significant microbial hazard. Despite this, care must still be taken during the earlier, high moisture stages of curing, particularly if they are prolonged due to adverse weather conditions, to prevent mold growth and bean spoilage.

Sunning, Drying and Conditioning

Besides reducing moisture content, the sunning phase of curing represents an extension of the previous sweating stage. The drying phase probably marks a transitional period, which is intermediate between the biochemical dominance of sunning and the more chemical conditioning/maturation stage. Chemical transformations are likely to be the principal reactions during conditioning since most enzymatic activity has virtually ceased at this stage.

From a vanilla flavor perspective, the three major developmental initiatives that commence during the sweating stage of curing are the hydrolysis of glucovanillin and other glucosylated phenols, the hydrolysis and oxidation of complex lipids, and the oxidation of phenols to dimeric and polymeric pigments. These flavor changes are inextricably linked to the preceding major microstructural changes in individual cells, prominent among which are extensive disruption of membrane and organelle structures and loss of cellular antioxidant status.

Since the curing process is protracted, there are opportunities for both enzymatic and non-enzymatic reactions to occur. Among the key enzyme families in this context are lipases, glucosidases,

peroxidases and polyphenol oxidases. The non-enzymatic actives include reactive oxygen species such as the alkoxy radicals and lipid hydroperoxides.[7]

The key aroma impact compounds have been reported in traditionally cured Mexican vanilla beans.[8] These species are dominated by phenols, though aliphatic acids, unsaturated aliphatic aldehydes and C4 ketones make a significant contribution to the overall aroma impact of cured bean extracts.

The biogenetic pathways of these compounds, in conjunction with their link to the sweating and sunning stages of curing, can be instructive in defining the stages where these compounds are formed or lost by further transformation. Perez Silva et al. provided some insights into flavor compound formation and loss during the traditional curing operation.[9] To probe the stages and flavor pathways, samples of Mexican vanilla beans were subjected to curing via a seven-stage operation that took 90 days to complete. Beans were collected after each stage and analysis of the aroma compounds conducted (**T-1**).

It is important to note that these experiments only generate a snapshot of the levels of selected flavor compounds during the different stages of curing. It does not provide kinetic data on the relative rates of formation or loss nor the pathway, enzymatic or chemical for these same components. The Silva et al. study was conducted to follow the appearance and fates of some of the 23 key flavor entities, 13 of which were glycosylated, while the remaining 10 were not, against the background of the seven described curing stages.

The ripe green beans, stage S1, were devoid of virtually all flavor compounds found in the fully cured end-product. The major flavor precursors present in the undamaged ripe vanilla bean were the β-glucosides of a number of phenolic compounds of which vanillin was, in mass terms, the most abundant.[10] Selection of beans at the fully ripe stage assures the maximum level of phenol glucosides and probably triggers senescence and its manifestation in terms of cellular structure disruption and hydrolytic, lipolytic and oxido-reductase enzyme activation.

Moisture content of the ripe bean under the experimental conditions described was ca. 83%. It declined only slightly to 77% over the first 25 days of curing. During this initial period, conditions were conducive for enzymatic activity. Within this timespan, enzyme activities, depending on type, varied in stability. Under laboratory conditions, mimicking traditional curing, the highest enzyme activity was found in the ripe green bean. Functional β-glucosidase activity was lost after 24 hours following oven killing and sweating. Peroxidase, on the other hand, survived and retained ca. 20% of its maximum activity after about 29 days of curing.[11]

Between the 25th and 90th days following the commencement of curing, tissue moisture content fell from 77% to 27%. These later stages, featuring low water content, tend to exhibit limited or no enzyme activity, while the opportunity for chemical transformation is retained. Major vanilla bean structural changes occurred during the blanching and sweating stages, with comcomitant initiation of flavor formation.[2] Most of the glucosylated phenolic compounds underwent hydrolysis at S2, liberating the free phenols.

Most of the non-phenolic flavor compounds were generated in the initial sweating (S2) stage, and the subsequent early sun-drying/sweating stages (S3). The non-phenolics did not appear to maximize at comparable rates observed for the liberation of the glucosidically bound phenols. This is suggestive of more complex pathways of formation, which probably involve both enzymatic and chemical routes.

C4 Ketones and Alcohols

The C4 alcohols and ketones were absent in the green bean (S1). The origin of these oxygenated species was probably endogenous, though contribution by a microbial pathway cannot be

T-1. Relationship between curing stages and vanilla bean flavor, physical, biochemical and chemical changes (modified from reference 9)

Sample Stage	Treatment	Days Post Curing (% Water)	Physical Biochemical and Chemical Changes
S1	Green bean	0 (83)	Undamaged tissue
S2	Oven kill (60°C/48 hr) + sweating (48hr)	4 (82)	Biochemical activity, enzyme activation, cellular damage, glucoside hydrolysis, browning, flavor formation begins
S3	Sun dry (3*) + sweating (24hr) (3**)	10 (nd)	Biochemical/chemical flavor formation continues
S4	Sun dry (6*) + sweating (24hr) (6**)	25 (77)	Chemical/biochemical flavor changes continue
S5	Sun dry (9*) + sweating (24hr) (9**)	42 (nd)	Chemical/biochemical flavor changes continues
S6	Sun dry (10*) + sweating (24hr) (10**)	60 (nd)	Chemical biochemical flavor formation continue
S7	Conditioning	90 (27)	Chemical flavor changes continue

*Sun-drying cycles of 2-4 hr each
**Sweating cylcles of 24 hr each

excluded. The mechanism of formation this C4 family of molecules in plants has been determined. A key precursor in their genesis is acetolactate. Its derivation was from the amino acid threonine via threonine deaminase through the key C3 metabolite pyruvate. This latter compound, which can be derived by a number of routes, can add an acetyl group, via acetohydroxy acid synthase, to form 2-acetolactate. Removal of carbon dioxide from this compound by the appropriate decarboxylase leads to 3-hydroxy-butanone.[12] Reduction of this carbonyl compound via the NADH-dependent acetoin reductase, or meso-2,3-butandiol dehydrogenase, produces 2,3-butandiol.[13]

As an example of this C4 family, 3-hydroxy-butanone, maximized at S2 at a level of 50 ppm dry weight, declines by 92% to 2.5 ppm dry weight at the end of the conditioning stage. In curing, the most rapid decline was during stages S4 to S7. The loss may be due to the inactivation of the biosynthetic pathway and/or interconversion between this >C=O compound and its reduced derivative, namely butan-2,3-diol. The latter compound is known to participate in acetal formation by reacting with vanillin to form vanillin-2,3-butandiol acetal, a known component of cured vanilla beans. This chemical conversion occurs readily at room temperature. Gas chromatographic analysis of traditionally cured Madagascan vanilla beans exhibited two peaks corresponding to two or three of the isomers of 2,3-butandiol, namely the chiral 2R,3R, and 2S,3S and the achiral or meso 2R,3S forms. Thin-layer chromatography of the same vanilla beans confirmed the presence of the 2,3-butandiol acetal(s) of vanillin.

Acetic Acid

Acetic acid is virtually absent in the green bean, but appears and maximizes at S2. At S7, the compound then declines to a level of about 15% of its highest value. The major decline occurs between stages S2 and S3. The C2 compound probably originates by endogenous processes, but may also arise via microbial routes. Acetic acid in mass terms is one of the major components of cured vanilla beans. The C4 homologue, butanoic acid, occurs to a much lower extent. Acetic acid, principally as the CoA derivative, is a key intermediate in plant anabolic and catabolic processes. It can arise by hydrolysis of acetyl CoA. It is formed from pyruvate via the pyruvate dehydrogenase complex. Pyruvate itself is generated by glycolysis or other carbohydrate metabolic processes. Acetic acid is also the end-product of β-oxidation of even-numbered, long-chain, linear fatty acids—a process that occurs in plant peroxisomes.[13,14] Under hypoxic or anoxic conditions that may occur during sweating of vanilla beans, pyruvate catalyzed by pyruvate decarboxylase can be converted to acetaldehyde. Under appropriate conditions, the 2C aldehyde can be oxidized to acetic acid.[2] In addition, it is possible that some of the acetic acid may be lost due to esterification reactions and/or evaporation. Equally plausible is the loss of acetic acid by microbial degradation, via the citric acid cycle, to carbon dioxide and water. Both *Bacilli* and *Acetobacter*, known to be involved in acetate metabolism, were present in beans and contact materials during vanilla curing.[15]

Guaiacol

Of the phenols, guaiacol was the only one reportedly found in the free form and absent as the glycoside.[9,10] Odoux et al., however, reported the presence of the glucoside of guaiacol in ripe vanilla beans.[16] Evaluation at 50°C of the aroma compounds found over the first 21 hours of fermentation of chopped ripe green vanilla beans demonstrated that after one hour of incubation there was no guaiacol present, despite the fact that free vanillin, liberated from the glucoside, was readily observed. Vanillin continued to accumulate after three, seven and up to 22 hours, when the experiment was terminated. This was consistent with its gradual liberation from the glucoside during this period. This situation was the reverse of that exhibited for N-heterocyclic compound indole, which maximized after one hour of fermentation and declined between three and seven hours and was absent after 22 hours. The formation of indole results from the transient activation of a secondary metabolic pathway involving indole-3-glycerophosphate lyase, which results in liberation of indole.[17]

Guaiacol, on the other hand, was absent up to seven hours and appeared only after 22 hours of incubation. The detail of the precursor role of the guaiacol glucoside, if any, and its mechanism of accumulation in the free form are not clear and will require further experimental testing. Therefore, the presence of guaiacol as the glucoside remains an open question. In this current study, however, guaiacol maximized at S2 at 650 ppm dry weight of beans. After this stage its content decreased exponentially to a steady level of 16 ppm between S5 to S7. The mechanism and pathway of the loss of this phenol is discussed below.

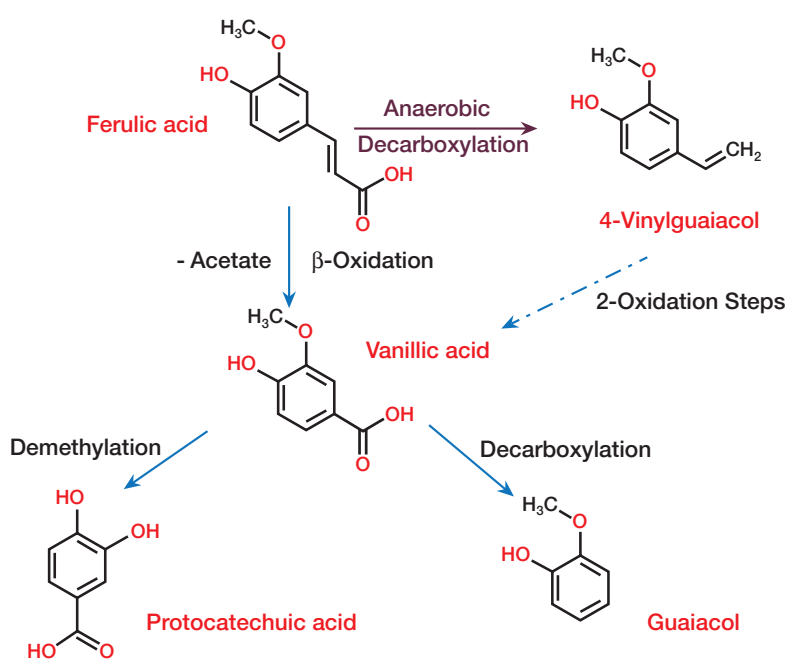
There are a number of routes for the formation of guaiacol in plants. A biosynthetic route has been reported in Solanum lycopersicum by methylation from the *o*-dihydroxy phenol catechol. A tomato O-methyltransferase (CTOMT1) with homology to a Nicotiana tabacum catechol OMT was cloned from *S. lycopersicum* cv. M82 and expressed in *Escherichia coli*. The recombinant CTOMT1 enzyme preferentially methylated catechol, producing guaiacol. Knockdown of CTOMT1 resulted in significantly reduced fruit guaiacol emissions. CTOMT1 overexpression resulted in slightly increased fruit guaiacol emission, which suggested that catechol availability might be limiting guaiacol production. The above data supports the proposition that CTOMT1 is a catechol-O-methyltransferase that produces guaiacol in tomato fruit.[18] In vanilla beans it is possible that the precursor of guaiacol could be *o*-catechol or its β-D-glucoside. Alternatively, guaiacol can be formed from vanillic acid by thermal or enzymatic decarboxylation. Vanillic acid itself can be derived from ferulic acid by anaerobic decarboxylation to 4-vinyl guaiacol. Upon oxidation of the vinyl double bond, the latter compound can form vanillin, which upon further oxidation of the aldehyde group realizes vanillic acid. Vanillin released from the glucoside during S2 can also act as a source of vanillic acid. Ferulic acid can alternatively be aerobically deacetylated by a β-oxidation process to directly produce vanillic acid and, as a result, guaiacol by decarboxylation (**F-1**).[19]

The observed loss of guaiacol described in F-1 can occur by a number of routes. Evaporation is a possibility, but there is no supporting evidence available for this proposition. Enzymatically, guaiacol is susceptible to oxidation by peroxidases. Lignin peroxidase in the presence of hydrogen peroxide as the hydrogen acceptor converts guaiacol to the diperoxide tetraguaiacol.[20] Other studies have demonstrated the conversion of guaiacol by C-C coupling, in the presence of horseradish peroxidase/hydrogen peroxide to guaiacol dimers and trimers.[21,22,23] In addition, the reaction of guaiacol with iron III and chromium VI afforded similar C-C dimers and trimers to those reported above.[24] The oxidative route via peroxidase/hydrogen peroxide presents a plausible route for the loss of guaiacol, particularly considering the known stability of peroxidase in vanilla beans.[11]

Vanillin

The well-known oxidation of vanillin to the C-C linked dimer dehydro-divanillin is catalyzed by horseradish peroxidase/hydrogen peroxide.[25] Vanillin has only the *o*-position available for H-abstraction, so this compound mainly realizes the dimeric compound. Under the same enzyme catalysis, the more reactive guaiacol carries both *o*- and *p*- hydrogen atoms, making available para-para and para-ortho coupling and leading to dimers and trimers, respectively. Alternative enzymatic oxidation pathways are available for vanillin and guaiacol via polyphenoloxidase, with dioxygen as the hydrogen acceptor. Indeed, the browning reaction that occurs during the sweating stage of vanilla curing is dioxygen-dependent. Anaerobic conditions during the sweating stage of curing prevents the normal browning process and realizes higher content of vanillin since further oxidation of this phenol is limited compared to sweating under aerobic conditions.[26]

F-1. Aerobic transformations of ferulic acid by *Rhodotorula rubra* (modified from reference 19)



During the curing process hydrogen peroxide may be limiting. As a result, peroxidase/hydrogen peroxide might not be active and could be replaced by polyphenoloxidase/dioxygen oxidation of phenols carrying hydrogen atoms in the *o*- or *p*-position of the aromatic ring. A polyphenoloxidase has been isolated from ripe vanilla pods.[27] Substrates for the purified enzyme include the *o*-diphenols catechol and 4-methylcatechol. This enzyme oxidizes the *o*-diphenols via semi quinones to *o*-quinones. These reactive compounds can react further to generate brown polymeric products. There is also the opportunity for the intermediate semiquinones to revert back to their original reduced *o*-diphenol state by coupled oxidation with other mono- and diphenols. In the case of the monophenol vanillin, the likely product is the dimer "dehydrodivanillin." For guaiacol, the expected products would be the dimer and trimers indicated above. Therefore, there are parallels between the degradatitive loss of vanillin and guaiacol, probably sharing in common an enzymatic oxidative process utilizing peroxidase/hydrogen peroxide and/or a polyphenoloxidase/dioxygen with the reaction pathway dependent on the availability of the two different hydrogen acceptors. The variances observed in the rate of loss of both phenols may also reflect, in part, the rapidity of the glucosidase hydrolysis of the respective phenol glucosides and the further degradation of the liberated phenols. In the case of vanillin, the accumilation of the free phenol is a slow process that maximizes at S4 and declines slowly to S7. During this period there remains residual vanillin glucoside, which seems to serve as a continuous reservoir of the free phenol. Only after S4 does the rate of vanillin depletion appear to exceed that of vanillin glucoside hydrolysis. For guaiacol, there was no information available on the loss of the glucoside, if present, so that there is little glucoside reservoir to mitigate the oxidation of this phenol.

Methyl Cinnamate

Methyl cinnamate followed a pattern of maximization and depletion similar to that observed with guaiacol. This methyl ester was first detected at S2 at 104 ppm dry weight and decreased rapidly during subsequent curing phases. The present authors' own studies demonstrated the presence of methyl cinnamate at 50°C one hour after commencement of fermentation of chopped ripe vanilla beans of Indian origin.1 Subsequent monitoring of the fermentation for three, seven and 22 hours showed virtually no change in the level of this ester. This is suggestive of the presence of the cinnamate ester in the ripe vanilla bean or, less likely, its formation during the first hour of curing.

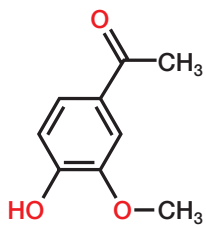
The Mexico study did not detect the ester as S1, but it was present at a maximum of 104 ppm dry weight at S2, four days after commencement of curing. After this stage it declined at a comparable rate to that of guaiacol. It fell rapidly by S3, which was 10 days from the start of curing. The two sets of data taken together suggest that methyl cinnamate is present in the ripe bean and that it remains at this level for up to about four days from the initiation of curing. Decline after this time is probably reflective of hydrolysis of the ester by chemical and/or enzymatic routes.

In plants, cinnamic acid is synthesized from phenyl alanine via phenylalanine ammonia lyase. This carboxylic acid, as the CoA ester, is then converted to the methyl ester via *p*-coumarate/cinnamate carboxyl methyltransferase, employing S-adenosyl methionine as the methyl donor.[28,29] Loss of methyl cinnamate from vanilla beans during curing could be via evaporation, further transformation by introduction of a para phenolic hydroxyl group, hydrolysis of the ester linkage via esterase activity or by combinations of the three.

Acetovanillone

Acetovanillone (F-2) is a key aroma compound in cured vanilla beans. It appears to be mostly present as the glucoside in the green bean, and only as the free phenol in the cured bean. Like most of the other phenols in vanilla beans, this compound is probably biosynthesised during the development/early maturation stages of the green bean, glucosylated prior to maturation, then released by hydrolysis of the glucoside during the sweating and sunning phases of curing.

F-2. Acetovanillone



A soluble enzyme acetovanillone synthase, molecular mass ca. 79kDa, extracted from tobacco cell cultures synthesized acetovanillone (apocynin) from feruloyl-CoA in the presence of NAD+. The biosynthetic pathway was via a CoA-dependent β-oxidation of the appropriate hydroxycinnamic acid.[30] This route parallels the known β-oxidation pathway for degradation of longer chain fatty acids that occurs in plant peroxisomes.[31]

The C7 and C10 Mono- and Di-unsaturated Aldehydes

The C7 and C10 mono-and di-unsaturated aldehydes maximise at stages S4 to S5 i.e. 25 to 42 days after commencement of curing. This is significantly later than the timescale of phenols release from their glucosidic precursors. At these later stages moisture contents are ca.75 and 55% respectively.

The hydrolysis of complex membrane lipids and the formation of reactive oxygen species provide the environment for oxidation of the hydrolyzed unsaturated lipids. After such a prolonged period following the initiation of curing enzymatic activity, lipoxygenase and peroxidase activity would, in particular, be expected to have decreased, though not be entirely eliminated. These residual enzyme activities offer routes to unsaturated aldehydes, but the likelihood of a non-enzymatic auto-oxidative processes involving oleic and linoleic acids can similarly account for relatively delayed formation of the unsaturated aldehydes.[32]

The Branched and Odd-number Short-chain Fatty Acids

The concentration of the branched chain and odd number short chain fatty acids in the different curing stages were not reported in the above study. These compounds included the C5 linear valeric acid and the two-branched chain C4 and C5 compounds, isobutyric and isovaleric acids, respectively. Valeric acid can be formed via C2 (as malonyl CoA) addition to propanoyl CoA via the fatty synthase present in plastids, or via β-oxidation of longer-chain odd-carbon-number fatty acids in peroxisomes.[33,31] The branched chain acids, namely isobutyric and isovaleric acids, more likely arise from the appropriate amino acids. In the case of isobutyric acid, the parent amino acid is probably valine, while the potential precursor of isovaleric acid is the amino acid leucine.

There are a number of enzymatic and chemical routes from amino acid parents to specific short-chain fatty acids. A pathway via the Ehrlich reaction is capable of catalyzing the oxidative deamination of amino acids. The sequence of reactions is first a deamination, followed by a decarboxylation step.[34] Using phenylalanine as an example, the first step is transamination by an amino acid transferase onto 2-oxoglutarate to produce phenylpyruvate and glutamate, respectively. The corresponding decarboxylase converts phenylpyruvate to the Cn-1 compound phenylacetaldehyde. In the presence of a dehydrogenase, the aldehyde may be reduced to the alcohol phenylethanol; in the presence of an aldehyde oxidase, the aldehyde can form phenylacetic acid. The corresponding ester can be produced by further reaction of the CoA derivative of the carboxylic acid with an alcohol catalyzed by an alcohol:acyl transferase.[35]

There are chemical options for the removal of ammonia and carbon dioxide from an alpha-amino acid to produce an aldehyde or ketone with one less carbon atom than the starting amino acid. This is the Strecker reaction, known since 1862, which is catalyzed by a family of Strecker reagents.[36] The generally held belief is that Strecker catalyst formation is as the result of the Maillard reaction, and that it requires elevated temperature conditions in excess of 95°C and the presence of an amino acid and reducing sugars.[37,38]

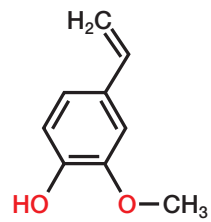
Strecker reagents are present in plant tissues as intermediates or products of the action of known oxidoreductase enzymes.[39,40] Such origins permit Strecker reactions to occur at ambient temperatures. Dignum et al. proposed that the enzymes peroxidase and polyphenoloxidase, both present in ripe green vanilla pods, might play a role in Strecker degradation of amino acids.[41]

Thus, routes to odor-important aldehydes such isobutyric and isovaleric acids are possible under ambient conditions in fermenting/sunning vanilla beans, catalyzed by Strecker reagents from the appropriate alpha-amino acids. This hypothesis may be tested by the POD/hydrogen peroxide and/or PPO/molecular oxygen oxidation of catechol in the presence of the amino acids such as valine and leucine.

The Full Development of Vanilla Aroma

The conditioning or maturation phase is the final stage of curing. It represents a period of more than 30 days when final moisture content in the processed beans reaches about 20–27%.This stage appears to promote full development of the final vanilla aroma. The reactions that occur during this phase of curing, however, are not well described or understood. However, it is likely that enzyme activity, pivotal during fermentation and part of the drying stage, will probably be limited or non functional, with chemistry dominating.

F-3. 2-Methoxy-4-vinylphenol



The work of Perez-Silva et al. demonstrated major changes in a number of aroma compounds during curing.[9] These included acetic acid, the C4 ketones, guaiacol, vanillin and a number of related phenols originally present as glucosides. Many of the other key compounds listed by Perez-Silva et al. showed little activity or change during curing.[8] Many of these latter compounds were gravimetrically insignificant, but flavor-active. As such, they were not considered in the genesis study, but remain important in vanilla flavor.[9] Among those listed were acetovanillone, methy cinnamate and the vinylphenols, namely 4-vinylguaiacol and 4-vinylphenol. The likely origin of acetovanillone and methyl cinnamate have been considered above. The probable pathway to the vinyl phenols, as exemplified by 4-vinylguaiacol, is via decarboxylation of ferulic acid under enzymatic catalysis or thermal conditions.[19]

An alternative pathway to 4-vinylphenols from phenolic α,β-unsaturated carboxylic acids under mild conditions has been demonstrated. This reaction pathway utilizes readily available secondary amines such as the amino acid proline. This compound adds to ferulic acid under mild conditions, affording the corresponding β-amino acid which, by further amine and carbon dioxide elimination, leads to 2-methoxy-4- vinylphenol (**F-3**) as the single product.[42]

By analogy, 4-vinylphenol can be similarly derived by decarboxylation of *p*-coumaric acid. Compounds of the vinyl phenol type probably arise at least in part by further transformation of precursors, including ferulic acid, which probably exists as the glucoside. The liberated phenol can be modified as described above to give the final volatile phenol. This situation probably applies to all of the stable glucosylated phenols post-hydrolysis. On liberation, these phenols are substrates for enzymatic and/or chemical reactions leading to volatile and non-volatile simple and complex molecules.

Non-volatiles

Most consideration in curing has been directed to the key aroma compounds. Some of the non-volatile compounds merit consideration because they might compliment the aroma compounds by contributing taste and mouthfeel characteristics of the cured vanilla beans, in addition to their defining role in the formation of brown pigments.

The rapid decline in the content of the 2-hydroxybutanone may be due to inactivation of its biosynthetic pathway and/or interconversion between this >C=O compound and its reduced derivative, butan-2,3-diol. The latter compound is known to participate in acetal formation by reacting with vanillin to form the non-volatile vanillin-2,3-butandiol acetal, a known component of cured vanilla beans. This chemical conversion occurs readily at room temperature (see above). Vanillin-2,3-butandiol acetal, which has vanillalike character, has been applied in flavor formulations.

Polymerized phenols are the other complex but non-volatile family of compounds found in cured vanilla beans. During the normal sweating stage of curing the green to brown color transition of the vanilla bean occurs. This observed browning is a dioxygen-dependent process and probably involves enzymatic and, perhaps, non-enzymatic oxidation of phenols.[26] Enzyme-catalyzed oxidation of phenols occurs in the presence of peroxidase(s), which generally have a requirement for hydrogen peroxide as the hydrogen acceptor. Ortho-diphenols in the presence of peroxidase and hydrogen peroxide in the peroxidatic mode produce reactive *o*-quinones, which can react further to produce "brown" polymers. Polyphenol oxidases on the other hand, especially catalase or laccase, utilize dioxygen directly in this role. The polyphenol oxidases include catechol oxidase, which oxidizes *o*-diphenols to *o*-quinones, and laccase, which oxidizes *o*-diphenols to *o*-quinones and can oxidize *p*-diphenols to *p*-quinones. These *o*- and *p*-quinones are highly reactive and can participate further to generate brown pigments by dimerization and polymerisation reactions.[43] In addition, as indicated above, some intermediates and products in oxidoreductase reactions can act as Strecker reagents. In this context, a polyphenol oxidase and a cell-wall-bound peroxidase have been isolated from ripe vanilla pods.[44,45]

A group of non-volatile, oxidized, phenolic compounds were recently isolated from traditionally cured Madagascan vanilla beans. These compounds represented a family of simple dimeric and other more complex coupled phenols that contributed to the mouthfeel/velvety mouth-coating character of vanilla bean extracts. Of the seven isolated compounds identified, six have not been previously reported as velvety mouth-coating tastants in vanilla beans.[46]

These phenol oxidation compounds described by Schwarz and Hofmann were almost exclusively absent from the ripe green bean but appeared in the final cured product.[46] Mechanistically, these compounds probably arise primarily by C-C radical coupling of the appropriate single and mixed mono-phenols mediated by peroxidases/hydrogen peroxide and/or polyphenoloxidases/dioxygen.

Another potential reaction that may occur in an overlapping manner between drying and conditioning of vanilla beans is the photochemical transformation of phenols. Much of the traditional curing of vanilla beans involves exposure of the beans to sunlight as part of the drying regime. During sun drying there is the possibility of photo-induced chemical transformation of flavor molecules mediated by direct or photosensitised reactions. Exposure of beans to light, often intense, occurs during the sunning/drying phase of curing for some parts of a 40–50-day period. Vanillin in ethanol solution, exposed to sunlight, is readily decomposed to dehydro-divanillin.[47] The reaction is probably a carbon-based free radical process. This same vanillin dimer is also readily formed by enzymatic oxidation of vanillin by horseradish peroxidase/hydrogen peroxide.[25]

Summary

The three stages of sunning, drying and conditioning complete the curing operations of vanilla beans following the earlier key functions of fermentation, or sweating, and drying. There is transitional overlap between all of the vanilla curing processes, although major select activities occur within each of the phases. The earliest stages of fermentation/sweating and and sunning are the periods when most changes occur. Primary among these are:

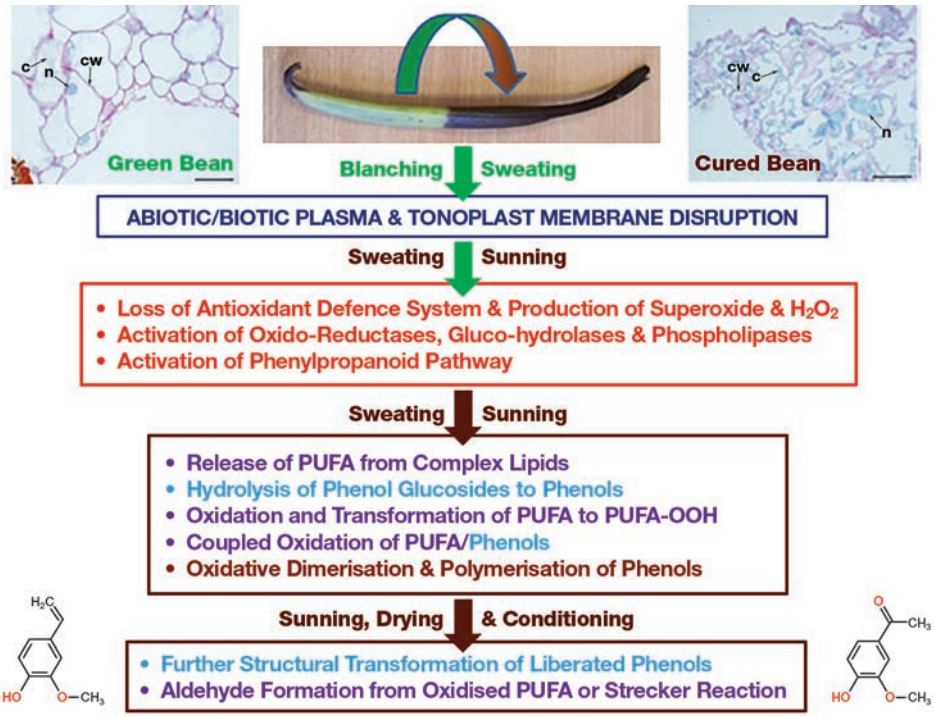
- Extensive loss of cellular and organelle integrity.
- Activation of hydrolytic, glucolytic, lipolytic and oxidoreductase enzymes
- Liberation of phenols from their β-D-glucosides.
- Oxidation of liberated phenols by dimerization and polymerization to form simple dehydro-dimers, trimers and complex polymeric colored pigments.
- Formation of a number of non-phenolic compounds that appear to be produced and transformed at rates slower than that observed for the hydrolysis of the phenol glucosides. This may be suggestive of more complex enzymatic/chemical pathways involved. These early stages of curing are generally associated with high enzymatic activity.

The later operations of sunning, drying and conditioning seem to facilitate the further transformation of many of these earlier-formed compounds. This leads to the development of the whole spectrum of flavor molecules found in the cured bean. In most cases, the aroma compounds identified in the early part of curing show significant decline during the last three stages. Water content is decreased, enzyme activity is low or non-existent, and chemistry is probably dominant. The chemistry involved in these transformations can be conjectured, but has not been clarified extensively.

The only group of compounds that maximized at the later stages of curing—between 25 and 42 days—were the C7 and C10 mono-and di-unsaturated aldehydes. At these stages, moisture contents are between ca. 55% and 75%. Enzyme activity would be limited after such a prolonged period, with decreased peroxidase activity. This enzyme offers routes to unsaturated aldehydes, though non-enzymatic auto-oxidative processes involving oleic and linoleic acids can similarly account for these compounds.[32]

Butan-2,3-diol is known to participate in acetal formation by reacting with vanillin to form vanillin-2,3-butandiol acetal, a known component of cured vanilla beans. This chemical conversion occurs readily at room temperature. The origin of guaiacol by hydrolysis of its glucoside, or its formation by another route, is unclear and will require further experimentation to elucidate.

F-4. Pathway for structural changes, enzyme activation and flavor formation during traditional curing of vanilla beans (modified from reference 7)



4-Vinylguaiacol and 4 vinylphenol are important aroma compounds of cured vanilla beans. 4-Vinylguaiacol can be derived by decarboxylation of ferulic acid under enzymatic catalysis or thermal conditions. In addition, 4-vinylphenols can be produced from phenolic α,β-unsaturated carboxylic acids through a mild pathway using readily available secondary amines such as proline. 4-Vinylphenol can be similarly derived from *p*-coumaric acid. This latter route could occur at the later stages of curing by chemical intervention, rather than by enzymatic means.

Compounds of the vinyl phenol type probably arise at least in part by further transformation of precursors that probably exist as glucosides. The liberated phenols, derived from the appropriate precursor, can be modified as described above to give the final volatile phenol. This situation applies to potentially all of the glucosylated phenols, which in the free form are substrates for enzymatic and/or chemical reactions leading to non-volatile simple and complex molecules.

Strecker reactions can lead to odor-important aldehydes such isobutyric and isovaleric acids. These reactions can occur under ambient conditions, employing catalysts derived from the activity of specific oxidoreductases or their products in fermenting/sunning vanilla beans by Strecker catalysis from the appropriate alpha-amino acids.

It may be that the final stages of curing bring about the formation of gravimetrically insignificant flavor-active compounds. The nature of these compounds is not fully established, though it is likely that the key compounds are numbered among the 26 identified by Perez-Silva et al.[8] **F-4** outlines the principal

pathway for structural changes, enzyme activation and flavor formation during traditional curing of vanilla beans. The detail of the pathways involved in the final stages of the formation of cured vanilla bean aroma and mouthfeel/taste actives has yet to be fully clarified, while the role of chemistry in these transformations is at the moment mostly conjecture. Hopefully this review will provide some insights into experimental approaches that may be employed to clarify the pathways involved.

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This paper is dedicated to all of the men and women vanilla farmers who work tirelessly, against great odds, to bring the vanilla bean to fruition over a nine-month period.

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