Optimizing the Traditional Curing of Vanilla Beans

A reappraisal of blanching and fermentation methods.

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Traditional curing of vanilla beans is a process steeped as much in history as science. However, recent work by a number of authors has begun to unravel some aspects of the chemistry and biochemistry of the individual stages the curing operation.[1] The principal components of the traditional curing operation, the most common method of which is the Bourbon process, include:

Blanching: This involves the immersion of the green vanilla beans in hot water, at 60°–65°C for ca. 3 min. This is typically conducted in metal containers heated over a wood fire. Temperature control may be monitored using a thermometer. This process washes the beans free of adhering dirt and soil, 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, stacked in a large collection 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, but varies in the region of 40°C. During this period, the beans lose their initial green color and turn 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 occurs.

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

Drying: To achieve final water content of 20–25% in the beans, the pods are slow-dried on uncovered wooden drying racks in airy warehouses. Additional chemistry, which is poorly understood, continues during this stage, along with further water loss.

Conditioning: Ideally, the vanilla beans are then 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.

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The remit of this review is to consider in more detail the important blanching and fermentation stages of curing with respect to the biochemistry and chemistry occurring, as well as consideration of routes to optimize the processes involved.

The Curing Operations

The primary objectives for all traditional curing operations are to develop the characteristic flavor of the bean and to retain the visual integrity, stability and appearance of the final brown cured product.

Blanching

Blanching is the first important stage of the curing operation. As mentioned, the process removes adhering dirt and soil and inhibits vegetative processes. This hot water treatment also activates a number of plant enzymes, which are important for the development of the flavor and color of the final product, and brings about changes to cell membrane structures and integrity. The commonest, most practical blanching procedure employed is hot water immersion of the beans. Water blanching conditions vary, but in general water temperatures of 60°–65°C

For further reading, see "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/*

are employed for periods of 2–3 min. Variations on these temperature/time conditions have been tested.

The upper temperature limit for blanching tends to be ca. 80°C, with an exposure time of about 10 seconds. Beyond this temperature/time regime, the risk of enzyme inactivation is significant. Marziezcurrena et al. predicted that at pH 6.0, approximately the pH of the ripe vanilla bean, and at 60°C/3 min in hot water, 50.6% of the endogenous β-glucosidase activity was lost.[2] At the same pH and 70°C/3 min, the loss predicted was 83.5%.

The kinetic parameters for the thermal inactivation of β-glucosidase isolated from vanilla beans were described by Marquez and Waliszewski.[3]

Maximum enzyme activity was observed at pH 6.5 and 38°C. When hot water blanching was applied, the enzyme activity was modified. At pH 6.0 and 60°C for 3 min, 51% of activity was lost. At the same pH and 70°C for 90 sec and 80°C for 30 sec, loss of enzyme activity was 60% and 48%, respectively. When ripe vanilla beans were heated in an oven at 60°C for 36–48 hours, the calorifico process commonly employed in Mexico, all β-glucosidase activity was lost.

Traditional hot water blanching alone is not efficient enough to facilitate intimate contact of the separately compartmentalized β-glucosidase and glucovanillin. As indicated above, elevated temperatures may result in the loss of endogenous β-glucosidase activity. These deficiencies, in conjunction with the current sweating conditions employed, are probably responsible for the low (<50%) conversion of glucovanillin to vanillin during traditional curing.[4]

A key target in any curing operation would be the maximization of cell membrane damage and retention of high endogenous β-glucosidase activity, whilst maintaining the visual integrity of the beans.

A number of methods, including freeze-thaw, microwave, ultrasonic and mechanical tissue damage, have been considered as alternatives to hot water immersion. Most of these procedures have some practical limitations.

Among these alternative contenders, freeze-thaw offers potential in terms of extensive cellular membrane disruption and glucovanillin conversion efficiency.[4] Freezing induces a type of water-deficient stress in plant cells since the chemical potential of ice is less than that of liquid water. Freezing-induced cellular dehydration is the most widespread cause of damage in plants.[5] Ice formation in plants is initiated in the intercellular spaces, with the result that cellular water moves down the water potential gradient, across the plasma membrane and toward the cellular ice. Alternatively, rapid freezing can result in the formation of ice crystals in the cytoplasm, resulting in organelle destruction and cell death. There is also evidence that oxidative stress, as well as protein denaturation, may occur in response to freezing. The generation of reactive oxygen species (ROS) is a consequence of oxidative stress, which may further compound cellular damage and cell death.[6] Oxidative stress also occurs during senescence and tissue wounding, whether by physical damage of tissue or as part of the systemic response as plants to chewing by herbivores.[7]

Freezing/thaw pre-treatment of ripe beans was demonstrated as a route to increasing the vanillin content in vanilla beans compared to that which could be obtained by traditional hot water pre-treatment. The length or rate of freezing appeared not to be significant in relationship to vanillin yield, so freezing could be employed for a minimum of one day and up to 90–100 days. Typical bean treatment involves freezing at -18°C for five days

prior to fermentation at temperatures in the range of 25°–60°C, in a closed chamber, for three to five hours. Alternatively, beans were frozen at -18°C for three days, then fermented at 30°C for three hours. Extraction of the latter treated product realized 4.7% vanillin compared to conventionally cured beans, with vanillin content between 2.0–2.5%. This represented a conversion of glucovanillin, present in the starting beans at 36 mmol/100 g dry weight, of 86%, compared to 46% for beans cured by hot water blanching followed by fermentation under the same conditions.[8]

Mechanical bruising by passing green beans between a rubber roller in combination with an enzyme cocktail of added cellulase, pectinase and β-glucosidase and incubation for four days realized 4.25% vanillin. Freeze-thaw as an adjunct to enzyme addition and incubation for four days realized ca.7% vanillin. Freeze-thaw per se realized 6% vanillin. On this basis, freeze-thaw is an effective method +/- enzymes for vanillin formation; at 7% vanillin, this method compares most favorably with traditional curing, where levels of vanillin were ca. 2–3%.[9]

Microwaves have been applied more for the extraction of cured vanilla beans. Extraction of commercially cured vanilla beans using focused microwave-assisted extraction resulted in a 62-fold decrease in extraction time and an increase in vanillin and para-hydroxybenzaldehyde concentrations of 40–50% with respect to the official Mexican extraction method.[10]

Flash détente, popularized by French firm Pera, is an evolution of the traditional thermovinification method in the processing of grapes for wine production.[a] Crushed and destemmed grapes are rapidly heated to 80°–90°C. The heated grape must is then piped to a vacuum flash cooling system in which the temperature is rapidly lowered to 30°–32°C. This flash cooling causes instant and extensive intercellular structural damage in grape skins, thus favoring better color extraction and avoiding the appearance of jammy characteristics, which often appear in classically thermovinified wines.

DIC short-time thermic treatment is a process similar to flash détente.[b] It involves a very short heat treatment of just a few seconds, followed by a sudden pressure drop to vacuum levels. Claimed features of this process include:

- Reduction of drying time and consequent prevention of product degradation.
- Rapid and controlled evaporation of water entrained within the product.
- Texturing of the product induced by swell drying.
- Open product texture facilitates improved extractability of volatiles and non-volatiles.
- Inhibition of enzymatic activity.
- Microbiological and biological decontamination of the products.

This process appears to have value in potentially modifying the structural integrity of plant materials. In the case of vanilla beans, this could permit extensive cellular destructing and potentially facilitate interaction between the gluco-phenols and β-glucosidase and other enzymes, as well as inhibiting microbial proliferation. These two technologies merit further evaluation for vanilla bean processing, especially regarding the structural changes that occur and the possible negative effects of the physical treatment on endogenous enzyme activity. In principle, the flash détente and DIC approach could produce positive effects in terms of tissue disruption, similar to those of the freeze/thaw process. The practicality of setting up such technologies in a curing facility would need to take into consideration cost/product quality advantages and throughput versus the current cheap and facile hot water blanching procedure. Presented with an inefficient blanching process, combined with 24–48 hours of sweating at undefined and unregulated temperatures, it is hardly surprising that low glucovanillin conversions are achieved.

This situation is exacerbated by the reported instability of the endogenous β-glucosidase.[11]

Sweating, or Fermentation

At this stage of curing, the hot green beans, after removal of the water bulk by draining, are usually bundle-wrapped in blankets and transferred into large, closed, insulated wooden boxes, which are then covered with wool blankets to limit heat loss. Alternatively, the beans are bundle-wrapped in blankets, stacked under cover and further covered with additional insulating blankets. The beans are then fermented, or sweated, for two to three days, during which time the temperature is reported to remain in the region of 40°C. There is no real temperature control practiced during the operation. During this period, the beans lose their initial green color and turn brown, becoming supple and initiating the flavor-forming processes.

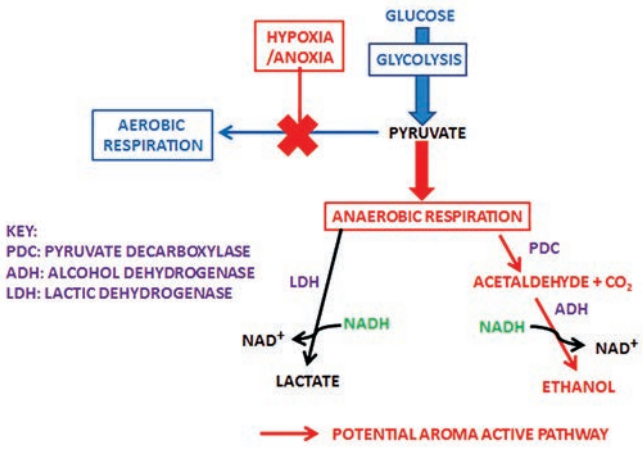
The biochemistry that occurs during the sweating stage of curing is complex and is triggered by the abiotic stress of elevated temperature during the blanching phase. An early consequence is the hydrolysis of the cell membrane complex lipids, followed by the formation of ROS and the oxidative burst, which initiates extensive oxidation of the mostly hydrolyzed membrane polyunsaturated fatty acids. These changes upset cellular organization, resulting in a catastrophic cascade of destruction involving activation of hydrolytic and oxidoreductase enzymes, degradation of chlorophyll and loss of the cellular antioxidant defense system. From a vanilla flavor perspective, the three major developments are the hydrolysis of glucovanillin and other glucosylated phenols, the oxidation of complex lipids, and the separate or linked oxidation of phenols to dimeric and polymeric brown compounds. 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, β-glucosidase, peroxidases and polyphenol oxidases. The non-enzymatic actives include ROS such as the alkoxy radicals and lipid hydroperoxides.[12]

The sweating process of vanilla curing is surprisingly limited to a maximum timescale of 24–48 hours, yet the reported conversion of glucovanillin to vanillin is <50%.[4] It would seem reasonable to extend the fermentation stage to achieve greater hydrolysis of the phenol glucosides, though this is not common practice, and the known instability of the β-glucosidase is a further complicating factor.[11] The reason for this limited fermentation timescale merits consideration.

The standard procedure for sweating, or fermentation, as described above, is to wrap the blanched beans in blankets, then stack them in mounds under further blanket cover. Alternatively, the hot blanched beans can be placed in large, deep wooden boxes with a covering lid. The beans can be

[a] *www.pera.fr/france/telecharg/flash_detentee.pdf*
[b] *www.abcar-dic.com/index.php?id_site=2&id_page=14*

F-1. Aerobic and anaerobic pathways in plant tissues; modified from reference 6

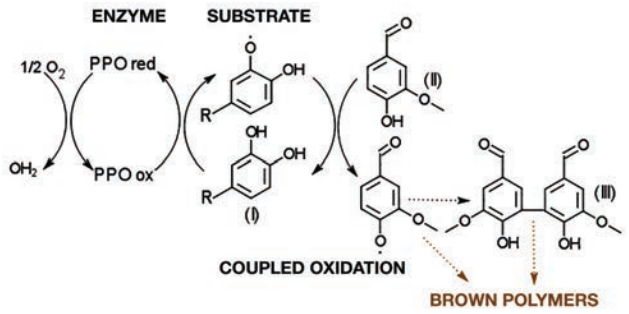


wrapped in bundles using wool blankets or merely placed, without wrapping, in the box. The depth can be anything up to 1.5–2.0 m, with box dimensions of the order of 2 m x 2 m x 1.5 m width, depth and height, respectively, with a volume of about 6 m^3. The boxes are usually overfilled beyond the top before the lid is put in place. In addition, some settling by compression occurs. This compacting helps by allowing limited ingress of air, but detracts by effecting closer packing of the bean mass. It has been observed that the bottom layer of beans incubated under these conditions for up to 48 hours are usually wet from percolating water and, with limited drainage, are usually slimy, smell unpleasant and often exhibit visual evidence of microbial infestation. Over the same timescale, about 5% of the total bean mass may remain green in color, rather than turning brown. If these green beans are exposed to air, the color changes to brown within a few hours. Based on the above information, the retained green state described is probably due to localized hypoxic conditions.

Vanilla beans respire at the ripe stage, and as such have an oxygen demand. Although a climacteric has not been clearly demonstrated, it is established that ethane gas can initiate senescence in the vanilla pod.[13] It appears that there is a gradual depletion of molecular oxygen during the fermentation stage. This is exacerbated by the close packing of the beans in the fermenter unit. As time progresses, hypoxic and, perhaps, anoxic conditions begin to dominate over the normal aerobic environment. The switch to anaerobic metabolism realizes the possibility for proliferation of anaerobic microorganisms and the appearance of molecules associated with anaerobiosis. The short-term acclimatization to hypoxic or anoxic conditions stimulates glycolytic transformation of glucose to pyruvate, which, via its intermediate acetaldehyde, is converted under fermentative conditions to the principal end product ethanol. Both of these carbon compounds are volatile and odor-characterizing. An alternative reductive process converts the common intermediate pyruvate to lactate. The depletion of NAD+ that occurs during glycolysis is recovered by oxidation of NADH during anaerobic respiration (**F-1**).

During the normal sweating stage of curing, the green to brown color transition of the vanilla bean was shown to be a molecular oxygen-dependent reaction. Under anaerobic conditions, this color change does not occur. Despite this, the hydrolysis of phenol glucosides is not inhibited. Indeed, in this situation, as measured by vanillin accumulation, the hydrolytic process appears to be enhanced. Beans subjected to anaerobic conditions will remain green even after ca. seven days of incubation. These anaerobic beans, if subjected to an aerobic atmosphere, will rapidly revert to the characteristic brown color after about four hours of exposure.[14] This observed browning is a molecular oxygen-dependent process, and probably involves enzymatic and perhaps non-enzymatic oxidation of phenols. Enzyme-catalyzed oxidation of phenols occurs in the presence of peroxidase(s), which generally have a requirement for hydrogen peroxide as the hydrogen acceptor. In the presence of peroxidase and hydrogen peroxide in the peroxidatic mode, ortho-diphenols produce reactive o-quinones, which can react further to produce "brown" polymers. Peroxidase can also operate in the oxidatic mode against selected substrates such as dihydroxyfumaric acid, and has the ability to reduce molecular oxygen to reactive oxygen species such as the superoxide anion. The superoxide ion in turn regenerates hydrogen peroxide and provides a cyclic pathway in which only catalytic amounts of hydrogen peroxide are required, with molecular oxygen appearing as the primary cosubstrate consumed.[15] On the other hand, polyphenol oxidases, especially catalase or laccase, utilize molecular oxygen 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

F-2. Potential polyphenol oxidase-dependent coupled oxidation of phenols



o- and p-quinones are very reactive and can participate further to generate brown pigments by dimerization and polymerization reactions.[16]

A polyphenol oxidase has been isolated from ripe vanilla pods.[17] Substrates for the purified enzyme, molecular weight ca. 34 kDaltons as a monomeric form, were the o-diphenols catechol and 4-methylcatechol. This study demonstrated that the enzyme utilizes the oxidation of mono-and diphenols to catalyze the cooxidation of various cellular substrates, probably including vanillin. Other cosubstrates present in the bean could include the o-diphenols tyrosine, caffeic and chlorogenic acids, as well as the mono-phenols vanillin and ferulic acid. Optimum pH for the enzyme was 3.0 for 4-methylcatechol and 3.4 for catechol. Optimum temperature was 37°C, but with a broad activity range of 20°–50°C. The enzyme was thermo-stable at 65°C and retained up to 90% of its activity after heating for 20 min. The high activity at low pH is suggestive of a vacuolar site for the enzyme. The most potent enzyme inhibitor was the m-diphenol 4-hexylresorcinol, followed by ascorbic acid. A possible mechanism for a coupled oxidation process is shown in **F-2**, which depicts a polyphenol oxidase-type reaction.

The initial reaction involves the oxidation of an o-diphenol (I); in this example, depending on the R-structure, it could be catechol, methyl catechol, tyrosine, caffeic or chlorogenic acid. The potential enzyme is a polyphenol oxidase of the catecholase type with molecular oxygen as the hydrogen acceptor. The oxidized phenolic product from the polyphenol oxidase reaction is the semi-quinone of compound (I). This semiquinone can be reduced back to the original o-diphenol via a coupled reaction, with the concomitant oxidation of a further mono-phenol such a vanillin (II), or ferulic acid or an o-diphenol such as the enzyme substrates (I) listed above. The vanillin oxidation product, the phenoxy radical, may be stabilized by conversion to the unsubstituted carbon-based radical on the carbon atom adjacent to the carbon-carrying phenolic hydroxyl group. This radical can dimerize to form dehydrodivanillin (III) and react further to form polymerized products with other phenols. In addition the semiquinones produced from the polyphenol oxidase reaction on compounds, (I) can also interact further with other phenols to form polymeric "brown" pigments. This proposed mechanism fits with the known dependence of ripe vanilla bean browning on molecular oxygen. In the absence of O_2 in such substrate/enzyme systems, the polyphenol oxidase may be trapped in the oxidized state until O_2 becomes available. Addition of O_2 to this system would allow the reduction of the enzyme and the sequence described above to occur, resulting in the browning reaction with or without the coupled reaction. In the presence of hydrogen peroxide as the hydrogen acceptor and peroxidase in the peroxidatic mode, vanillin is rapidly dimerized principally to dehydrodivanillin (III).[18] That browning does not occur in the absence of molecular oxygen suggests that peroxidase is not involved in the browning process unless hydrogen peroxide is limiting these conditions.

The fermentation problem therefore may be one of limitation of the timescale of the traditional sweating stage imposed by the restriction of access of molecular oxygen to the respiring vanilla beans. The consequences may be insufficient time to facilitate extensive conversion of glucovanillin to vanillin, which reaches values of <50%; this would occur in conjunction with limited decompartmentation of β-glucosidase and glucovanillin. The described instability of the vanilla β-glucosidase is probably also a factor.[11] In addition, the switch to anaerobic metabolism can realize off-flavors not observed under aerobic conditions.

The solution, therefore, may be a combination of making sufficient molecular oxygen available throughout the fermentation process and suitably perturbing the cellular structure of the intact bean. These options could be achieved by:

- Control of the hot water blanching procedure to ensure an initial higher activity of the endogenous β-glucosidase or institution of other cellular "damaging" interventions such as freeze-thaw that may not significantly compromise the hydrolytic enzyme, whilst facilitating greater loss of cellular membrane integrity.
- Operate the fermentation reaction under conditions in which oxygen is not limiting.
- Introduction of temperature/time control during the fermentation stage of curing to optimize the conditions conducive to flavor formation.

These approaches could permit longer, controlled, fermentation times on more cellular-compromised structures, potentially delivering improved flavor character, higher yields of vanillin and good bean appearance during traditional curing.

Summary

Traditional curing of vanilla beans is a process with efficiency limitations in terms of the final vanillin content. The curing process is dominated by hydrolytic and oxidative reactions, which play a key role in the formation and transformation of the principal flavor compounds. Opportunities exist to improve this process by a number of interventions, which are based on knowledge of the inherent biochemistry and chemistry, particularly as these relate to the important steps of blanching and sweating or fermentation.

Interventions to improve the process include:

- Management of the blanching operation or other interventions to deliver extensive cellular disruption.
- Control of the fermentation stage of curing to ensure sufficient availability of molecular oxygen to maximize optimum flavor and vanillin content, whilst minimizing anaerobically driven off-flavor formation.
- Imposition of appropriate time/temperature conditions to deliver process control.

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