

Chemical and Biochemical Changes Occurring During the Traditional Madagascan Vanilla Curing Process

Effects on the sensory qualities of cured beans

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During the past few years, several reviews have been published on specific aspects of vanilla research and in particular on chemical changes that happen during the traditional curing process.^{1,2,3} This labor- and time-intensive process is of utmost importance in determining the ultimate sensory quality of the cured beans. The final product of this process is the cured, dry bean that has not only become shelf-stable but has also developed the familiar vanilla flavor, the latter having been formed as a result of very many chemical and enzymatic reactions that occur concurrently during the curing process.

Every vanilla-growing country has developed its own specific curing process, but all of these generally consist of four main steps, namely killing/blanching, sweating, drying and conditioning.⁴ Killing is very often achieved by submerging the freshly picked green beans in hot water for a short time (e.g. 55–60°C for about 3 min) during which the vegetative phase is stopped.

In the sweating step, the hot beans are kept wrapped in blankets, thus maintaining a temperature of about 45–50°C for one or two days. During this period endogenous enzymes are active and the beans develop their characteristic brown color.

During the remaining months of the process, the beans undergo passive drying until they have a dry substance level of about 70–75%, thereby becoming microbiologically shelf-stable. Many (bio)chemical reactions take place in the course of this long production process, some of which result in the formation of low molecular weight, flavor-active compounds that together contribute toward the final flavor of the cured bean.⁵

The main biochemical reaction occurring during the curing process is the hydrolysis of the glucovanillin (the β -glucoside of vanillin), which is catalyzed by the β -glucosidase endogenously present in the green bean.⁶ A certain amount of hydrolysis can also occur before the green bean is picked from the vine, whereby the degree of hydrolysis depends upon how ripe the bean actually is before being picked.

The fate of glucovanillin and vanillin have been studied during the various steps of the traditional curing process as performed on the island of Réunion.⁴ In this present publication, the findings made during the traditional curing process performed during the 2005 season in Madagascar are described, and, in particular, an attempt is made to explain how some of the vanillin losses occur during the process.

Results and Discussion

The vanillin content of traditionally cured beans is often ~2% relative to the dry matter of the beans, while its original potential in the green beans often amounts to some 5–6%.⁴ This data indicates that less than 50% of the total vanillin originally present in the green beans—both in the form of free vanillin and glucovanillin—actually survives the overall curing process.

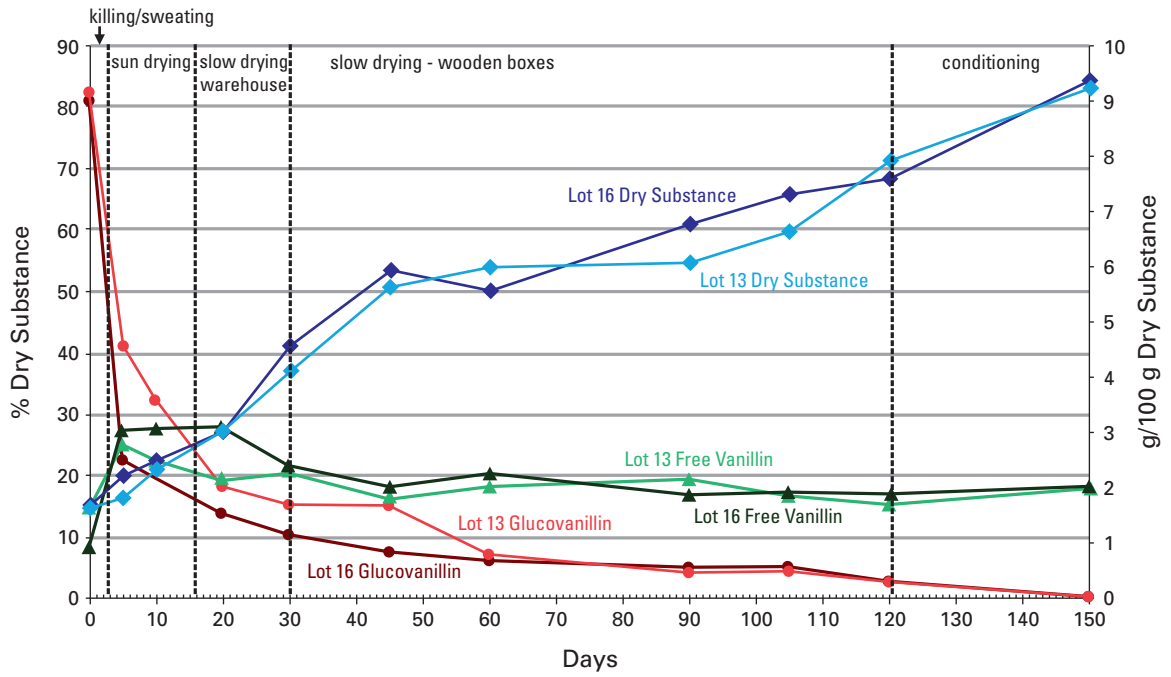
There has been one very detailed study published so far that has investigated how the levels of glucovanillin and vanillin behave during the curing process typically used on Réunion, which is somewhat different from the traditional Madagascan process.⁴ This study confirmed the more or less efficient conversion of the glucovanillin into vanillin during the complex procedure, which actually includes two killing and two sweating steps. An approximate 60% loss in the original total vanillin concentration of ~5.5 g per 100 g dry substance was determined during the many steps of the curing process.

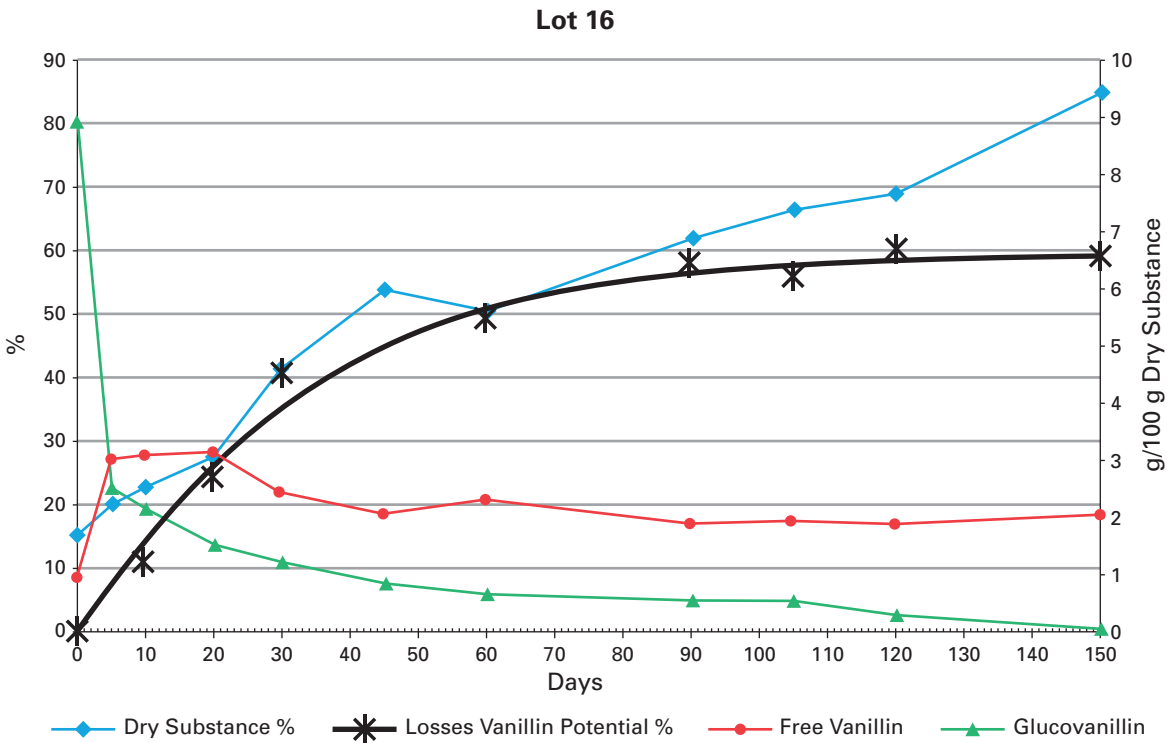
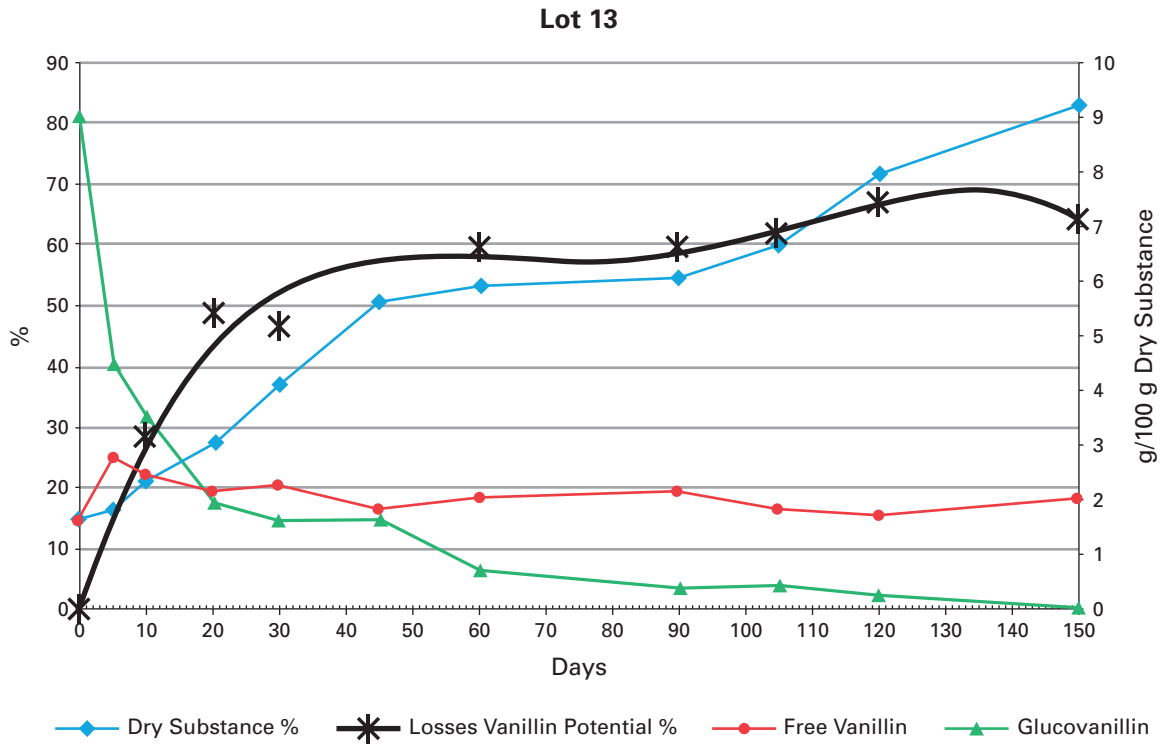
The killing/sweating steps and the conditioning step were described as being crucial in the overall process since they involve both the liberation of vanillin from the precursor, glucovanillin, and also the stability of the vanillin during the subsequent processing steps. During the killing/sweating steps, the hydrolysis of the glucovanillin was incomplete. During the drying stage, the concentration of total vanillin (both the glucosylated and free forms) decreased substantially while the concentration of free vanillin remained steady. From the data reported, it was not possible to determine which form of the vanillin—i.e. whether the aglycone or the glucosylated form—was actually lost during the process.

As part of the ongoing R&D program directed toward process optimization, it was decided to study

the Madagascan curing process and to interpret the findings in light of what was known from the literature. To this end, trials were performed in our Madagascan facilities and under the conditions traditionally used for curing vanilla. Samples of the beans were taken at well-defined times and analyzed for glucovanillin, vanillin, p-hydroxybenzaldehyde, vanillic acid and p-hydroxybenzoic acid via HPLC. Dry substance matter was determined by drying of the finely cut samples to constant weight in an oven. Such measurements were performed in duplicate and the concentrations of the various analytes were expressed in terms of g per 100 g dry matter.

The typical time profiles found for the two separate 400 kg lots investigated are shown in **F-1**. The results show that there is a very rapid decline in the concentration of glucovanillin during the killing and sweating steps (first two days), which is caused by hydrolysis catalyzed by the endogenous β -glucosidase. Even though the initial hydrolysis is rapid—about 90% hydrolysis is achieved after 20–30 days—the cleavage of the remaining 10% requires very many weeks before it is complete. As would be





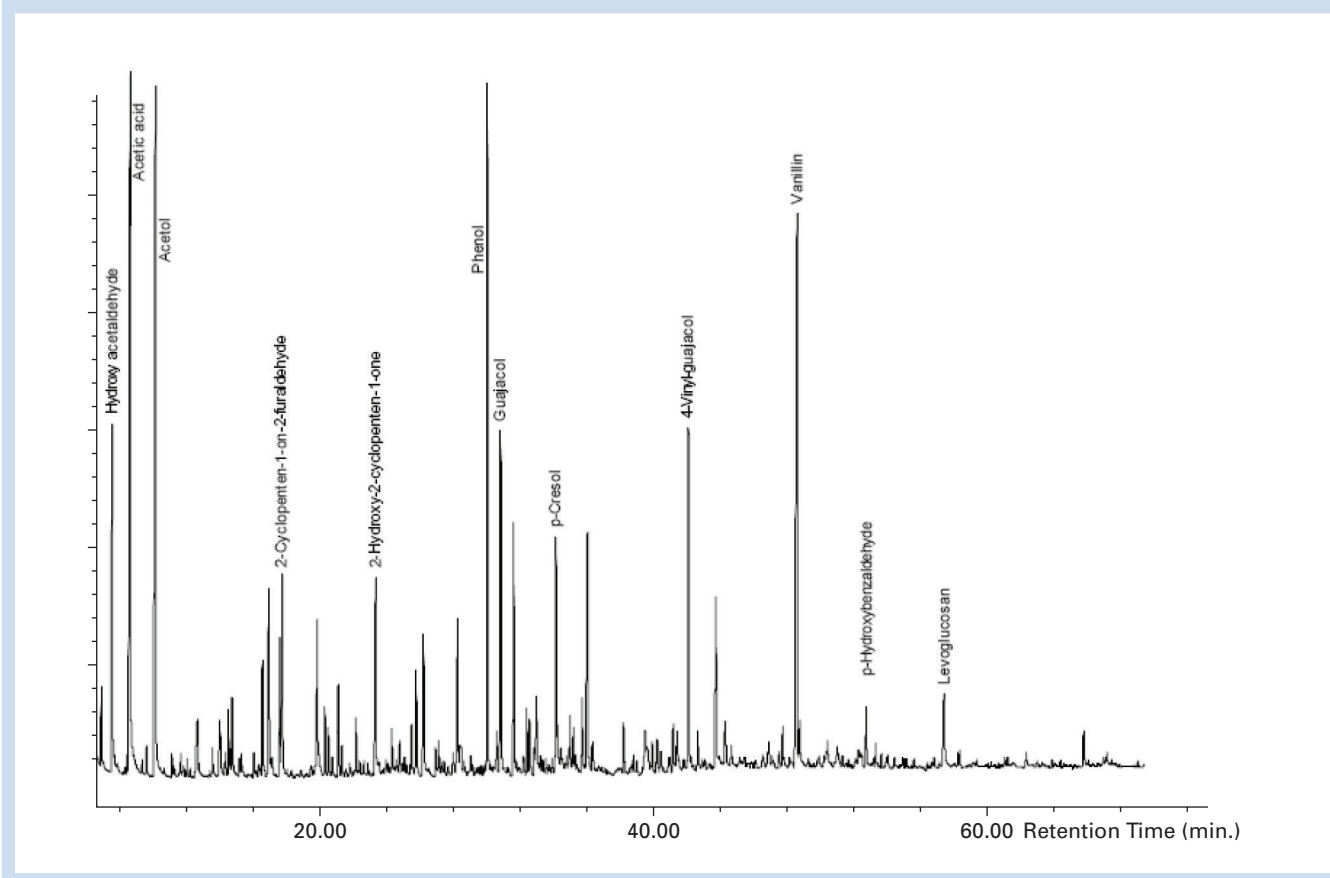
expected, the concentration of free vanillin in the beans increases as the level of the glucovanillin declines, and the highest value detected was approximately 3.2 g per 100 g dry substance for lot 16 after 20 days. As can also be seen in **F-2**, both vanillin concentration profiles go through a poorly defined maximum during the first 20–30 days, then decline slightly and end up at a value of about 2.0 g per 100 g dry substance.

Theoretically speaking, the initial concentration of approximately 9 g glucovanillin (**F-1**) is equivalent to about 4 g of free vanillin and the approximate 2 g vanillin actually found, meaning that some 50% of the original vanillin has been lost during this process. The calculated time profiles for the vanillin losses are also shown in **F-2** for both lots investigated. As can be seen, the vanillin losses are greatest during the first 30–40 days of the process, but the data does not allow further conclusions as to whether these losses are due to vanillin or glucovanillin being lost.

Vanillin is a very reactive molecule and losses can theoretically be due to quite a number of different reactions, both chemical and physical, whereby the latter type would presumably be mostly via sublimation.⁷ Losses resulting from chemical reactions would include the formation of Schiff's bases with low molecular weight amines naturally present in the beans, and also vanillin destruction via its participation in the Maillard reaction. This latter reaction is very probably the reason for the very rapid decline in the vanillin level of fermentation broths, which in so-doing can also turn quite dark brown.⁸

The possibility of analytically detecting vanillin that may have reacted during the curing process with polymeric materials present in the beans such as lignin was also investigated. In order to check this possibility, the residues obtained after exhaustive ethanolic extraction of both green beans and traditionally cured brown beans were subjected to pyrolysis GC followed by MS experiments.⁹ Under the flash pyrolysis conditions (approximately 6 mg of the finely ground, dry extraction residue was treated at 470°C in a stream of nitrogen), the sample was thermally degraded and the volatile organic material collected quantitatively as an oil in a cold trap.¹⁰ The extraction residue obtained from the green beans that was treated in exactly the same way represents the genuine blank sample. The ethanolic extraction of the fresh green beans was performed very soon after harvest (within a few hours) so that any free vanillin that may have been present in the green beans did not have the opportunity to undergo the putative reaction with lignin.

Under the experimental conditions used, there was very little difference in the yields of pyrolysis oil obtained from the two extraction residues obtained from the normally cured bean and the green bean. In both cases, the yield was approximately 50%, and this value was very reproducible. However, the results of GC analyses of the two pyrolysis oils differed considerably and especially so with respect to their



vanillin contents. The pyrolysis oil from the residue from the normally cured beans contained about 800 times more vanillin than the corresponding oil from the green bean residue. A typical GC trace obtained for the pyrolysis oil from normally cured beans is shown in **F-3**, and this not only shows the presence of vanillin as one of the larger peaks but also *p*-hydroxybenzaldehyde. The ratio found for these two compounds averaged at approximately 9:1;

this corresponds very well with the average values found over the years for the beans themselves, namely in the range 10–20:1.¹¹ This finding should not really be that surprising, since if you expect vanillin to undergo reactions during the curing process then the *p*-hydroxybenzaldehyde presumably also reacts in an analogous fashion.

Isotopic analyses were performed on the pyrolysis oil obtained from the residue from the traditionally cured beans; the results are shown in **T-1** and **T-2**. The values found for vanillin correspond very well

IRMS analysis of vanillin

T-1

Origin	$\delta^{13}\text{C}_{\text{V-PDB}} [\text{‰}] \pm 0.7\text{‰}$	$\delta^2\text{H}_{\text{V-SMOW}} [\text{‰}] \pm 5\text{‰}$	$\delta^{18}\text{O}_{\text{V-SMOW}} [\text{‰}] \pm 1\text{‰}$
Vanillin ex pyrolysis oil	-20.4	-96	+13.2
Vanillin ex cured beans (<i>planifolia</i>) (n = 15, literature data)	-21.5 to -19.2	n.d.	n.d.
Vanillin ex cured beans (Bourbon) (n > 70, Symrise data)	-22.0 to -19.0	-115 to -52	+6.7 to +12.4
Vanillin ex lignin (n = 7, Symrise data)	-28.4 to -27.3	-195 to -178	+5.9 to +9.5

IRMS analysis of p-hydroxybenzaldehyde

T-2

Origin	$\delta^{13}\text{C}_{\text{V-PDB}} [\text{‰}] \pm 1.0\text{‰}$	$\delta^2\text{H}_{\text{V-SMOW}} [\text{‰}] \pm 10\text{‰}$	$\delta^{18}\text{O}_{\text{V-SMOW}} [\text{‰}] \pm 1\text{‰}$
pHB ex pyrolysis oil	-18.6	+63	n.d.
pHB ex cured bean (n = 38, Symrise data)	-19.8 to -15.0	+25 to +164	+6.9 to +12.7

with those already known for natural vanillin obtained by extraction of cured vanilla beans (*V. planifolia*) and differed significantly from the values for nature-identical vanillin obtained by the chemical degradation of lignin.¹² The isotopic data found for

the p-hydroxybenzaldehyde present in the pyrolysis oil also corresponded very well with both the literature values and the Symrise internal values for p-hydroxybenzaldehyde obtained extractively from cured vanilla beans (T-2).

Calculations based on the yield of the pyrolysis oil obtained and its vanillin level have shown that between 15% and 20% of the total free vanillin present in the cured beans used in these investigations were present as covalently bound vanillin and as such were consequently not sensorially active.

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