Green Vanilla Bean Quality

Understanding the parameters of the vanilla bean brings about potential for control, management and flexibility in process operation and in the quality of the final product

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The defining driver for the global expansion and proliferation of the vanilla industry from its origins in Mexico occurred around 1841 when Edmond Albius, on the French-controlled island of Reunion, conducted hand pollination of the vanilla flower in a practical way, thus opening up vanilla cultivation on a large scale.[1]

Early reports of vanilla curing in the 15th century were recorded when the Aztecs conquered the Totonac people of Mexico and developed a liking for the vanilla bean—*black flower*—for flavoring chocolate drinks.[2]

The vanilla bean curing process, in essence, converts a ripe, green/yellow, unflavored vanilla bean into the readily recognized aromatic brown pod and its alcohol/water extract of commerce.

The main vanilla growing areas in the world occupy a belt essentially bounded by the Tropics of Cancer and Capricorn, north and south of the equator, respectively. Major producing countries are Madagascar and the Comoros Islands, India, Uganda, Papua New Guinea, Indonesia and Mexico. Madagascar is by far the principal producer of cured vanilla beans.[3]

Contemporary knowledge of the detailed physiology, anatomy, biochemistry and chemistry of curing, especially as they relate to taste and aroma, are at best limited. Why indeed should industry concern itself about this lack of information?

Understanding these parameters of the vanilla bean brings about opportunity for control, management and flexibility in process operation and in the quality of the final product. The principal elements of any plant raw material are:

1. The genetic nature of the tissue
2. The nurture encompassing geographical origin of the raw material including the soil, climate and growing conditions
3. The maturity state of the tissue at harvest
4. The processing operation(s) to generate the final product state

Flexibility, therefore, in vanilla products is encompassed by the above four elements. In terms of genetic base there are essentially three species of the genus *Vanilla* of commercial significance, namely *Vanilla planifolia*, *Vanilla tahitensis* and *Vanilla pompona,* the former being, by far, the dominant species of commerce. The geographical origin encompasses the soil, climatic and growing conditions, and as such these define the principal six origins indicated above.

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Harvest maturity is a very important factor and frequently equates with the development of a particular trait, e.g. color development associated with the biosynthesis and deposition of the carotenoid lycopene and flavor formation during tomato fruit maturation.[4]

The overall quality of the cured vanilla bean is dependent on the properties of the incoming raw material and the nature of the curing operation. The curing process, including solvent extraction, is the common set of parameters by which the final state of the raw material can be flexibly manipulated and directed.

The above elements in combination generate the global flavor spectrum of the vanilla bean (**F-1**). It is to defining the state of the incoming raw material that is the focus of this short review.

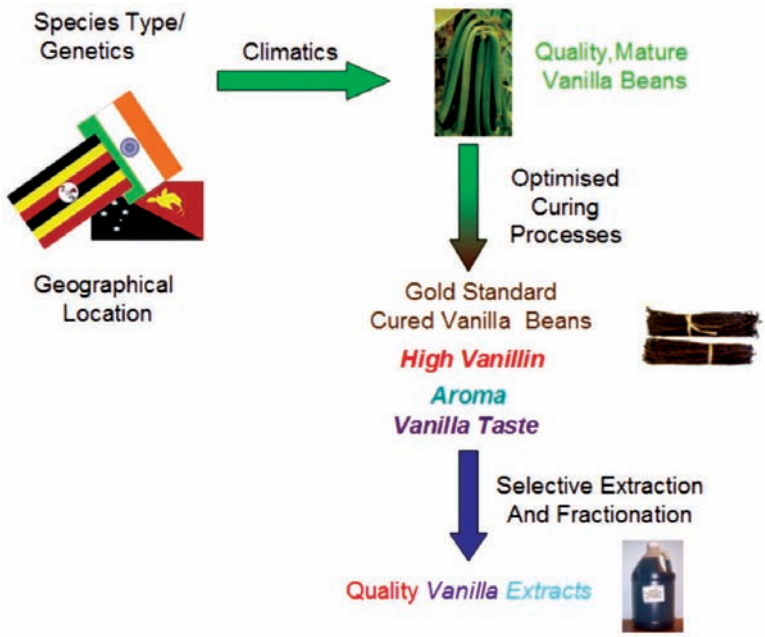
Vanilla Bean Raw Material Quality

There are two key truths with regard to vanilla quality:
1) good quality in can deliver good quality out, and
2) bad quality in always delivers bad quality out.

Vanilla beans are, in general, handled and processed differently than other food raw materials, in part because the whole operation is steeped in tradition. Ripe beans are often harvested by small farmers who have limited

knowledge of crop management and the necessary supplementary resources. In many cases there is poor hygiene practice, limited control of process operations, including curing, resulting in variable final product quality. A move to better management of the crop at the farmer level in conjunction with selection of only good quality and ripe raw materials could significantly contribute to improved quality. In addition good hygienic process operation in conjunction with regular quality control could raise the standard and particularly the value of the final cured vanilla bean and associated solvent extract. Much work has been carried out in recent years to improve the quality of the beans received from vanilla farmers. The focus of these efforts has been to offer training to farmers in the management of the crop with particular emphasis on the pollination process and care during the nine-month post-pollination period required for maturation. Farmer cooperatives have facilitated this process and in addition have provided social, health and educational support by conducting programs on HIV/AIDS, schooling, finance management, malaria prevention, and female emancipation. Such work has been supported by overseas government aid programs as well as participation by multinational companies and local vanilla farmers and curers, particularly in Uganda.[a,b,c]

Raw material quality and processing of vanilla beans **F-1**



It is outside the scope of this review to discuss the agronomic aspects of vanilla growing; such information is summarized in other publications.[5]

Selecting fully matured vanilla beans is not a straightforward task. First of all, there is a requirement for farmers to have suitable experience in recognizing ripe vanilla pods. Such recognition in most cases is conducted by visual observation of characteristic surface yellowing at the distal extremity of the bean (**F-2**).

Ripe vanilla beans **F-2**

For beans that are green in color and virtually mature, however, there are often difficulties in distinguishing this group from immature beans from the next season's crop. This is a particular problem in areas like Uganda where there are two maturation periods, generally around December–January and June–July, in the same year. This is further confused by the fact that beans of the same maturity may exhibit differing "greenness" as the result of unequal sun exposure.

This dilemma can be resolved for green beans by examination of longitudinal sections cut through the placental region. Such sections by visible observation readily exhibit the state of maturity of the bean in a pronounced manner (**F-3**).

Placental color is white in immature beans from the next season crop for a two-season/year product. Immature beans from the current crop show yellowing of the placenta. Ripe beans exhibit a more pronounced yellowing. At the final cured stage, the placental region is a dark, chocolate brown color.[6,7] It is interesting to note that mature beans subjected to freezing for ca.18 hours at about -20°C then thawed exhibited a similar white appearance in the placental region to that seen in unripe beans. Such low temperature cycling can be

[a]Danish International Development Agency (*DANIDA*), an organization inside the Ministry of Foreign Affairs of Denmark, set up to provide humanitarian help and assistance in developing countries

[b]Firmenich SA, Rue de la Bergère 7, P.O. Box 148, Meyrin 2 CH-1217, Switzerland

[c]Uvan Limited, P.O. Box 8813, Kampala, Uganda

Visual recognition of raw material vanilla beans; left to right are longitudinal sections of 1) unripe next season, 2) unripe this season, and 3) ripe this season; red arrow indicates placental region **F-3**

readily recognized by the flaccid nature of the pod after exposure.

In general, therefore, the pronounced yellowing at the distal end of the vanilla pod is a good indicator of fruit maturity. Supplementation of this state of ripening is enhanced by visual examination of longitudinal cut sections of the vanilla bean.

It is equally important during visual examination of the mature beans to remove any beans that are extensively split or show large black spots. Such beans are likely to be infected with mold and bacteria and may spread infection to healthy pods resulting in cured material with off flavors.

Why is it important to know when the vanilla pod is fully mature?

It is long established that the principal phenolics present in the cured bean, especially vanillin, are present in the mature pod as β-D-glucosides and as the result of the curing process they are liberated as free phenols by the action of an endogenous β-glucosidase.[8] On this basis, therefore, it is important to maximize the level of these glucosides ahead of the curing operation.

During the maturation process of the vanilla bean, the level of glucovanillin, as well as the glucosides of the other phenolics, accumulate in the placentae (92%) and marginally in the trichomes (7%) with traces in the mesocarp and intralocular interstitial cell free medium. The secretory tubular trichomes, on the other hand, are filled with oleoresin rich in γ-pyranone derivatives and a mucilaginous polysaccharide, along with lower amounts of glucovanillin.[7]

The levels of the phenolic glucosides build up over the nine months of bean maturation starting around week 15 after pollination and continuing until about week 30.[9] Reported levels of glucovanillin in ripe beans vary. Levels in the Madagascan crop, in 2000, averaged around 32 mMoles/100 g dry weight of tissue. Samples of beans from Papua New Guinea, in 2006, gave levels of glucovanillin in excess of 64 mMoles/100 g dry weight.[10] A glucovanillin level of 64 mMoles/100 g dry weight would yield a vanillin content, based on 100% conversion, of 9.72 g/100 g dry weight. High levels of vanillin in the final cured product are, as indicated above, dependent on the level of the precursor glucoside in the ripe bean, the efficiency of the enzymatic hydrolysis of the glucoside during the "sweating" and perhaps the sunning phases of curing. The survival of the phenols, once liberated from the glucoside, will depend on other transformations that occur to the free phenols during the sweating, sunning, drying and conditioning stages.

There is much speculation regarding the relative levels of glucovanillin and vanillin during maturation, and evidence exists for the early stages of maturation where it was shown that, for beans around three, five, seven and nine months after pollination, the percentage of free vanillin in relationship to the total (glucoside plus free form) was 33%, 6%, 1.5% and 0.2%, respectively.[11] The significance of this information is not clear and further work is required to clarify the interplay of vanillin and glucovanillin during maturation. What is clear is that at the mature stage >95% of the vanillin in the undamaged vanilla pod is present as the glucoside. Elevated levels of vanillin at the mature bean stage reflect the length of the comminution process prior to extraction, damage by insects or early stages of senescence. Such artifacts can be avoided in undamaged mature fruit by size reduction of pods in methanol, a solvent that inhibits hydrolytic enzyme activity.

Within the vanilla bean there has been much debate regarding the localization of glucovanillin and the β-glucosidase enzyme. The recent work of Odoux et al. demonstrated that the vanillin precursor was localized in the internal part of the fruit, predominantly in the placental region.[7,12] Based on the mass ratios of different tissues, 92% of glucovanillin was found in placental tissue compared to 7% in papillae, with only 1% in the mesocarp. The same workers found β-glucosidase distributed in the placenta (highest activity of 100%) compared to 20% in the papillae and 11% in the mesocarp. This glucosidase distribution pattern mirrored the situation for the glucoside. At the cellular level, β-glucosidase activity is located in the cytoplasm/apoplasm but absent from the vacuole. By analogy with other glycosides in plants and considering the very high levels of phenolic glucosides, it is very likely that these compounds are stored in vacuoles.[13] Thus it would appear that phenolic glucosides and β-glucosidase are present in the same cells within the vanilla pod and are compartmentalized at this level. This contrasts with an allium such as garlic where the compartmentation of the precursors, the S-alk(en)yl-L-cysteine sulfoxides, are present in mesophyll cells, while allinase, the enzyme responsible for the precursor transformation, is present in the vascular bundle sheath cells.[14] This situation in the vanilla bean has implications for the processing methods that facilitate contact between vanillin glucoside and β-glucosidase during curing. Such procedures need to facilitate decompartmentation at the intracellular rather than the intercellular level.[15]

Glucovanillin is the most important marker of vanillin potential of the vanilla bean. With knowledge of the level of this β-D-glucoside in the ripe bean and the yield and level of vanillin during each of the stages of the curing process, it is possible to measure both the vanillin potential related to state of maturity, and actual yield as an index of curing efficiency (**F-4**). Thus the assessment of glucovanillin content in the green vanilla beans is a key factor in determining the quality of the ripe vanilla bean.

Analytical methods are in place to determine both glucovanillin and vanillin from the same sample, using different extraction procedures in the work-up.[16] We, the authors of this paper, have refined this method to achieve a sound overall procedure.[6] At present there are few, if any, analytical laboratories carrying out routine simultaneous vanillin and glucovanillin determination from the same sample and extract.

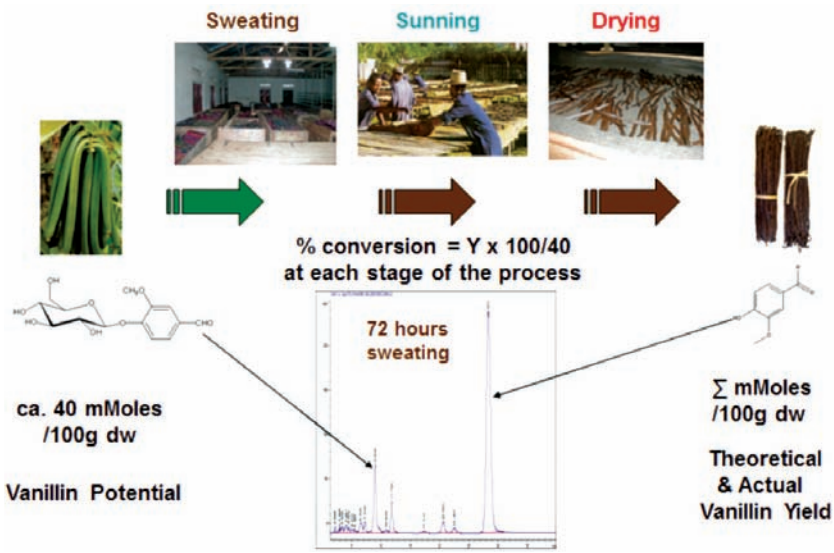
For the above reasons, determination of glucovanillin is not conducted at curing facilities. Additionally the lack of sophisticated and expensive equipment and training are a hindrance to instituting such methods in vanilla growing and curing areas. This deficiency should be addressed by analytical laboratories and major vanilla processors, since the benefits, in terms of knowledge of starting content of glucovanillin and its conversion during curing to vanillin, can outweigh the costs. In the absence of glucovanillin measurement, reliance will be on the visual methods described above, their inherent limitations being that they are only able to determine the maturity state without any indication of vanillin potential or efficiency of the curing operation. Any determination of both glucovanillin and vanillin levels should be quoted on a dry weight basis to eliminate the variable of moisture content.

Liberation of vanillin and the other phenolics from their β-D-glucosides is dependent on the presence of an endogenous β-glucosidase. The activity of this enzyme, though present at all stages of bean maturation, increases considerably during the third and fourth month post-pollination, reaching a maximum at month five.[17] Of greater significance, however, is the activity of β-glucosidase at the point of harvesting and during the early stages of curing.

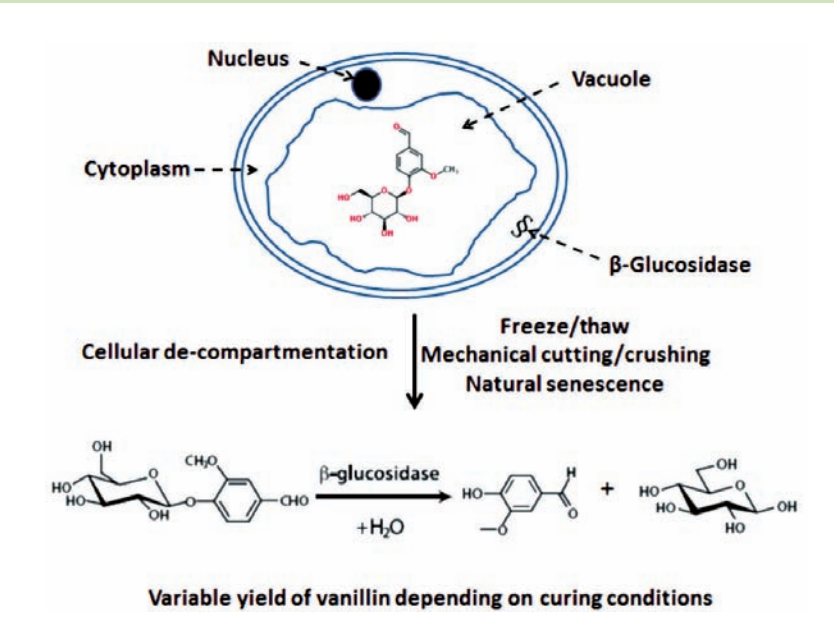
To improve the understanding of the traditional curing process, vanilla curing was conducted under laboratory conditions to mimic the commercial curing operation in Singaraja, Bali, Indonesia. Emphasis was placed on enzyme activity and aroma character.[18]

All of the enzymes monitored were most active in the green bean. β-Glucosidase found in the untreated bean could not be detected after 24 hours of autoclaving. Protease and peroxidase

Glucovanillin content in mature beans and the relationship to curing efficiency and vanillin content **F-4**



Cellular decompartmentation and glucovanillin hydrolysis **F-5**



activity decreased but were still active, at about 20% of initial level, after 29 days of curing. Blanching at 80°C for 20 min totally eliminated β-glucosidase activity.

From the standpoint of glucoside hydrolysis during curing, clearly the fragility of the glucosidase(s) is an issue. The efficiency of phenol liberation is additionally tied to the compartmentation/decompartmentation of this hydrolytic enzyme and the phenolic glucosides in the bean (**F-5**).

Any method facilitating rapid contact of substrate and enzyme would encourage extensive phenol glucoside hydrolysis. The processing options, particularly the pretreatments prior to the fermentation stages of curing that facilitate enzyme/substrate contact, are reported elsewhere.[7,19]

The measurement of moisture content in green vanilla beans and of vanilla beans during the fermentation stages and subsequent drying and conditioning stages is an important monitor. A number of methods are available, including the International Standards Method, which employs azeotropic distillation of water entrained in the water-immiscible organic solvent toluene.[20] This procedure is quite time-consuming and requires pure solvents and many pieces of apparatus. An alternative method relies on oven drying of the comminuted sample to constant weight. The procedure is simple but takes up to 48 hours to complete.[d] Water content ranging 15–80% can be determined using a moisture analyzer.[e] The moisture analyzer operates on thermo-gravimetric principles, i.e. the moisture is determined by the weight loss of a sample dried by heating. The instrument comprises a precision balance with an integrated halogen heating module, and final moisture content is automatically registered at the end of the heating cycle. We have employed for our curing studies this simple method of drying that is both accurate, easy to operate, requires one piece of equipment and is rapid in use, taking about 45–60 minutes/sample to complete. This facility is particularly valuable during the drying phase of curing, where the final moisture content influences the further extraction of the bean and probably the final flavor profile.

Besides the phenolic glucosides, which have been considered in detail, there are other macro components that may contribute to flavor generation during curing. There have been no significant studies directed to the role of the non-phenolics in the mature bean on the final flavor profile. The reasoning behind this is simple. Detailed knowledge on the key aroma and taste compounds contributing to vanilla flavor is not known. In addition and based on the limited knowledge that we do have, we cannot, except in a few cases, indicate for the identified flavor compounds their biogenetic and/or chemical origin. Significant reports on the flavor of cured vanilla beans are limited to a few important studies that identified a number of key aroma compounds and the newly described potential taste compounds; the latter were predominantly present in cured beans. These flavor compounds have been described elsewhere.[21–25] A listing of the key aroma compounds isolated from cured Mexican vanilla beans (**T-1)** shows that 13 of the 24 compounds listed are phenolic, with the rest distributed between short chain acids, alcohols ketones and aldehydes. The likely biogenetic origins of the listed compounds are known pathways of metabolism in plant tissues. Except for the phenolics, the rest of the pathways are mostly degradative and probably feed from substrate pools of monosaccharides, short and long chain fatty acids, and peptides and amino acids. These substrate pools would be dependent on the degradative transformation of complex lipids (galactolipids, phospholipids), polysaccharides and proteins. This catabolism of macromolecules probably occur by hydrolytic activity during the natural senescence process or during the induced senescence of curing.[26]

Some insight into the likely precursors to the characterized aroma compounds may be gleaned from examination of the composition of mature green beans, sampled at the normal harvest stage and recorded in **T-2**.[7]

The sugars, both reducing and non-reducing, as well as the unsaturated fatty acids and the proteins and their related amino acids, as indicated above, probably play a part as precursors in the formation of non-phenolic flavor compounds. Glucovanillin and the other glycosylated phenols, though particularly the former, are present at high levels and provide the free phenols after hydrolysis.

Of particular interest in the context of degradative processes, initiated with the onset of natural or artificial senescence, is the loss of vanillin potential. This is reflected in decreased yield of vanillin from the initial glucovanillin precursor, to the extent of ca. 50%, which normally occurs during the traditional curing operation.[15,27,28]

The fate of vanillin and the other phenols once liberated from the stable glucosides is not established. It is certainly the case that the free phenols are susceptible to oxidative processes, which probably account for some dimeric products reported by Schwarz and Hofmann.[22] Other transformations of these phenols may result in the formation of additional aroma compounds. This is an area for further study.

Summary and Conclusions

What can and cannot be concluded about the quality of mature vanilla beans?

- A good deal is known about the accumulation, content and activity of phenol glucosides and glycolytic enzymes, respectively, during vanilla bean maturation.
- Valuable information has been gathered on tissue sites within the vanilla beans and the cellular location of phenol glucosides and β-glucosidases.
- Methods are available to determine the content of glucovanillin in unripe or mature green beans. In addition, these methods permit the determination of the efficiency of each, or the overall curing operation, from both a theoretical and practical standpoint.

[d] *www.ehow.com/how_5961569_oven-method-measure-moisture-foods.html*

[e] Mettler Toledo Moisture Analyzer HB43, Mettler-Toledo GmbH 2003. 11780531. Printed in Switzerland 0311/2.12

Aroma active compounds in vanilla bean extracts; modified from reference 21 **T-1**

Compound	Bio-chemical origin
Phenolics	**Substituted phenols from shikimate/phenylalanine pathway**
Guaiacol	
4-Methyl guaiacol	
4-Vinyl guaiacol	
p-Cresol	
4-Vinyl phenol	
Vanillin	
Acetovanillone	
Vanillyl alcohol	
p-Hydroxybenzaldehyde	
p-Hydroxybenxyl alcohol	
Methyl salicylate	
Methyl cinnamate	
Anisyl alcohol	
Aliphatic acids	
Acetic acid	**Glycolysis/fatty acid and/or amino acid degradation**
Isobutyric acid	
Butyric acid	
Isovaleric acid	
Valeric acid	
C4 Alcohols and ketones	
2, 3-Butandiol	**Pyruvate/acetolactate pathway**
3-Hydroxy-2-butanone	
Aldehydes	
2-Heptenal	**Lipid oxidation pathway**
(E)-2-Decenal	
(E,Z)-2,4-Decadienal	
(E,E)-2,4-Decadienal	

- Researchers so far have limited information on the precursors of the non-phenolic aroma compounds and their pathways of formation.
- The precursors of the phenolic tastant and their formation route(s) have not been demonstrated.
- There is limited information on the structure of complex lipids in the mature vanilla bean, their potential breakdown during curing and their relationship to flavor formation.
- More information is required from the literature on the processes initiating and the sequence of the senescence process, naturally occurring or induced, in the vanilla bean.
- Additional indices of the quality of the mature vanilla bean are still required.

The answers to these and other questions will require further studies with ripe, green vanilla beans.

Typical composition of mature Madagascan vanilla beans[7] **T-2**

Fraction*	g/100 g/dw	Component and % of each		
Fiber	45	lignin 62	cellulose 27	hemicellulose 11
Sugars	10	sucrose 80	glucose 15	fructose 5
Lipids**	12	linoleic acid 54	oleic acid 10	palmitic acid 10
Protein	3			
Organic acids	5	citric 50	malic 30	
Minerals	10	potassium 28	calcium 10	magnesium 2
Glucovanillin	10			

*Moisture content 83% by weight
**Percentage of fatty acids is in relationship to saponifiable content

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