

# A Review of *Vanilla planifolia* Andrews Horticulture and Curing, Phytochemistry and Quality Evaluation

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## ABSTRACT

**Introduction:** Vanilla is popular for its flavour and aroma characteristics, although its long and labour-intensive curing period impede its commercial production and result in high commercial prices. This manuscript focuses on the cultivation and processing (curing) of *Vanilla planifolia* beans, their phytochemistry, and vanillin biosynthetic pathways. We also discuss quality parameters and their analytical determination, with the aim of focussing future studies in this field. **Materials and Methods:** An extensive literature review was undertaken on the cultivation and curing of vanilla, its phytochemistry, vanillin biosynthetic pathways and quality evaluation. Several search engines including Science direct, Google Scholar and Scopus search engines were used as sources of information. **Results:** *Vanilla planifolia* cultivation and processing are lengthy and labour-intensive, resulting in high production costs. This results in high costs to the consumer and relatively low availability. Improvements in these processes may substantially increase the production and quality of the vanilla produced. Several methods have already been incorporated to optimise production. Hand pollination of *V. planifolia* flowers increases fruiting, and better horticultural practices improve the yield of beans. However, the beans are generally still processed by traditional curing methods. This review highlights processes that may be optimised and discusses the use of biotechnological advances to further enhance the production and quality of vanilla. **Conclusion:** Commercial vanilla production is growing, with quality vanilla being the second most expensive spice globally. In particular, improvements in *Vanilla planifolia* horticultural methods and growth conditions, as well as optimising the processing methods, may increase the production and quality of vanilla. Better analytical methods are also required to monitor these improvements.

**Keywords:** Orchidoideae, *Vanilla planifolia*, Vanilla, Glucovanillin, Biosynthetic pathway, Biotechnology, Traditional curing, Analytical methods.

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## INTRODUCTION

Vanilla, a luscious and unique fruit product of the Orchidoideae family, is the second most expensive spice globally (after saffron).<sup>1</sup> Intensive labour, restrictive growing conditions and lengthy cultivation/processing timelines are largely responsible for the high costs of vanilla. Generally, it takes a total of 5-6 years from initial cultivation for the cured product to be ready for export and eventual sale.<sup>2</sup> Despite these factors, vanilla is highly sought after globally for its flavouring and aromatic properties, and is commonly incorporated into foodstuffs, cosmetics, pharmaceuticals and medicine.<sup>2</sup>

## History and origins of vanilla use and cultivation

Vanilla use and cultivation originates from pre-Columbian Mesoamerica, where the indigenous Totonac people of North-central Veracruz, Mexico, pioneered its cultivation.<sup>3</sup> Following the 15<sup>th</sup>-century Aztec conquest of the Totonac people, vanilla became a necessary ingredient in a cultural specialty called Chocolate.<sup>4</sup> Hernán Cortés, a Spanish conquistador, later overthrew the Aztec empire and returned to Europe with the spice, thereby spreading the use and popularity of vanilla throughout Europe, and subsequently across the globe.<sup>4</sup> More recently, modifications to vanilla cultivation, notably the method of manual pollination and engineering of specialised greenhouse structures, have allowed non-native locations including Indonesia, Réunion and the Dominican Republic to participate in the cultivation and trade of the spice.<sup>5</sup>

## Botany and taxonomy

*Vanilla planifolia* Andrews is a species of vanilla orchid belonging to the *Vanilla* Plumier ex. Mill genus of the Orchidaceae



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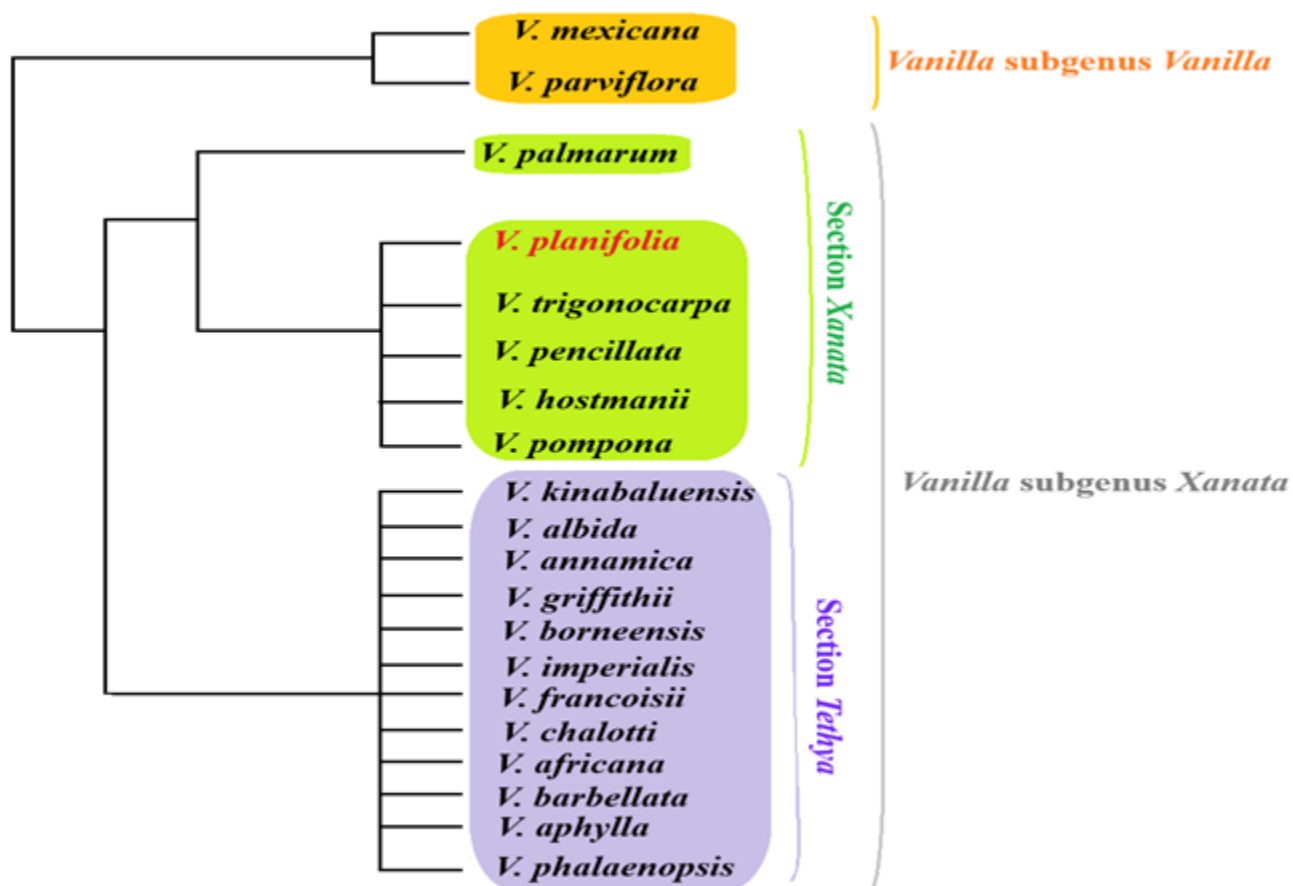
family), which consists of approximately 140 other species.<sup>6-10</sup> A simplified phylogenetic tree, showing the relationship between the most commercially important species is depicted in Figure 1. Interestingly, of all the species in the Vanilla family, only 2-3 species (*V. planifolia* Andrews, *Vanilla tahitensis* J.W. Moore and the *Vanilla pompona* Schiede) are cultivated at a commercial scale for their fruit products.<sup>6</sup>

The *V. planifolia* orchid is a stout climbing vine (Figure 2a), with large, flat, oblong-shaped leaves, which produces yellowish-green or white hermaphroditic flowers (Figure 2b).<sup>12,13</sup> Following pollination, *V. planifolia* produces a vivid-green fruit (Figure 2c), commonly referred to as pods or beans because their interior flesh contains small, black seeds.<sup>13</sup> These pods are cylindrical, odourless and vary in length and diameter (between 10-25 cm and 5-15 mm respectively).<sup>14</sup> When the mature beans are cured, they become black in colour (Figure 2d) and take on the characteristic aroma and flavour properties of vanilla.

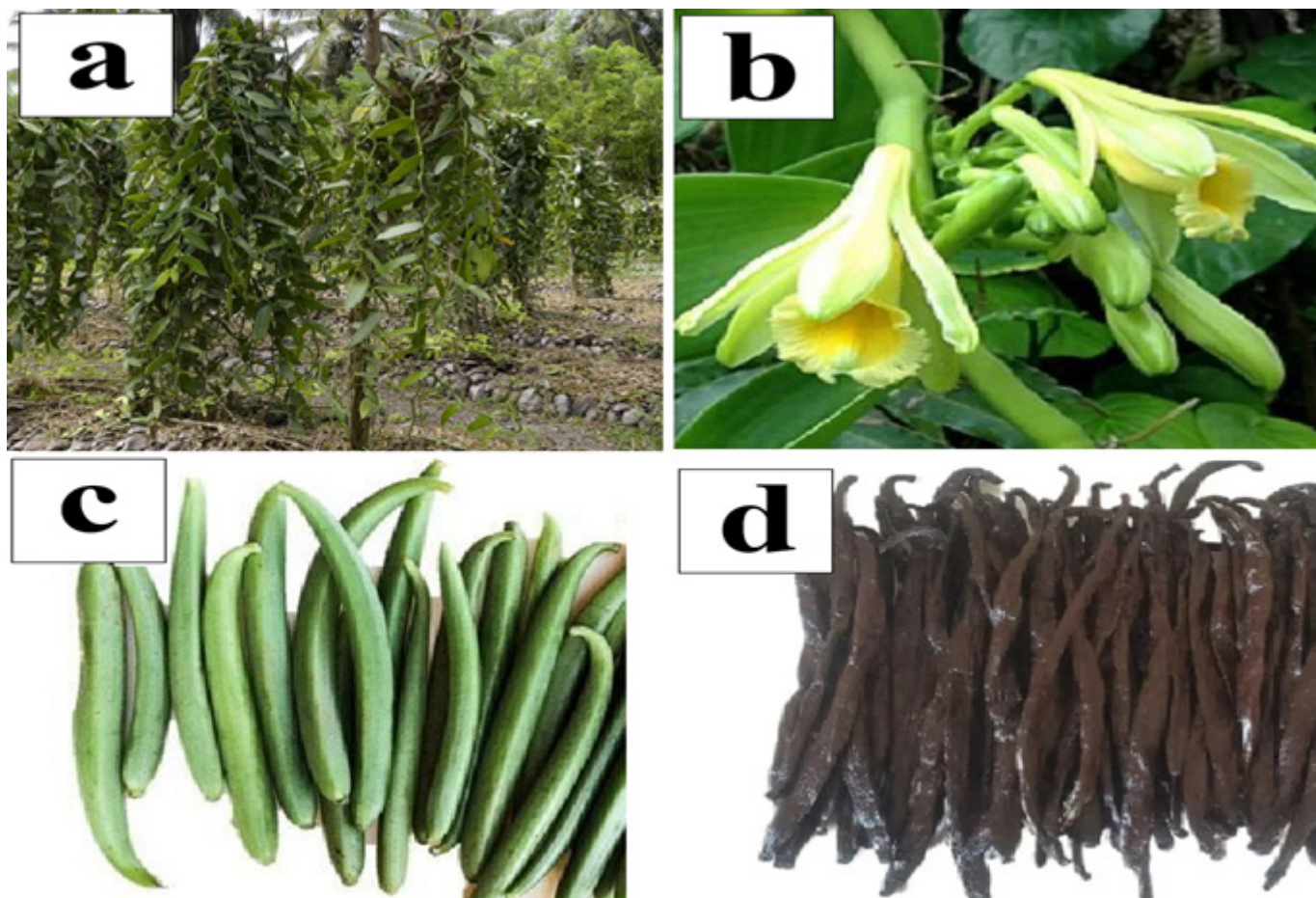
Due to the increasing demand for high quality vanilla products, evaluation methods need to be able to rapidly and accurately evaluate the quality of vanilla beans. Notably, the origin of cultivation, the curing and storage methods, as well as the age of the plant substantially affect the quality of the vanilla end product.

Whilst more than 200 compounds have been reported in vanilla, most are present at trace levels (as reviewed in.<sup>15</sup> Indeed, only 26 compounds occur in levels  $\geq 1$  mg/kg of beans. Vanillin is the considered to be the most important constituent for the aroma, flavour and therapeutic qualities of vanilla. It is also relatively abundant in *V. planifolia*'s beans, occurring at 1-2% of the mass of the cured beans.<sup>15</sup> Therefore, most laboratory-based methods to evaluate the quality of vanilla beans are based on the relative abundances of vanillin.

Several methods that utilise gas chromatography (GC) and high-performance liquid chromatography (HPLC), often coupled to mass spectrometers (MS), have been developed. However, many of these methods are sub-optimal and may not provide an accurate picture of the quality of the vanilla. Some methods quantify the total vanillin quantity (free and glycosylated forms). Notably, free vanillin is responsible for the aroma and flavour characteristics of vanillin. Therefore, quantifying the total vanillin concentration may provide a misleading evaluation of quality. Instead, most methods focus on the levels of free vanillin as a measure of quality. Whilst this may provide a more direct measure of vanilla quality, it fails to recognise the potential of modified post-harvest curing procedures (or even post curing treatments) to increase the quality of the vanilla by releasing vanillin from the relatively



**Figure 1:** Simplified representation of the taxonomic relationship between members of the genus *Vanilla*. This figure was adapted from data presented in.<sup>11</sup>



**Figure 2:** *Vanilla planifolia* (a) whole plant showing growth habit, (b) flower, (c) green beans, and (d) cured beans.

abundant glycosylated form, glucovanillin. An understanding of *V. planifolia* chemistry and biosynthetic pathways is necessary to develop better quality evaluation methods, and to develop optimised growth and curing conditions. This review examines *V. planifolia* cultivation and curing methods, as well as vanilla phytochemistry and vanillin biosynthetic processes, with the aim of highlighting future areas of research in this field

## MATERIALS AND METHODS

### Search strategy

This study aimed to document the phytochemical and quality information related to *Vanilla planifolia* beans. A thorough literature survey was undertaken using published reviews and surveys, which were identified by searching the following electronic databases: ScienceDirect, Google Scholar, PubMed, and Scopus. The filters that were used (either alone or in combination) to identify the relevant literature were: “Orchidoideae”, “Vanilla”, “*Vanilla planifolia*”, “vanillin”, “glucovanillin”, “biosynthesis pathway”, “cultivation”, “curing process”, “phytochemistry”, “secondary metabolites”, and “quality evaluation”.

### Eligibility criteria

Publications retrieved from the various electronic database searches were evaluated for eligibility based on their titles. The abstracts of all retrieved publications were read to confirm their eligibility to this study. For studies that met the initial study criteria, full-text manuscripts were then obtained, and the full texts were read to verify suitability.

### Inclusion criteria

To be deemed eligible for this study, the following inclusion criteria were evaluated:

Only English language publications were used to avoid misinterpretation.

All studies that reported on botanical aspects, cultivation methods, phytochemistry and quality assurance methods were deemed to be eligible for inclusion in this study.

### Exclusion criteria

Publications were deemed ineligible and were not included herein if they met the following exclusion criteria:

- Publications that was not available in English.



- Incomplete publications, where only the abstract and references were readily available.
- Studies that reported on *Vanilla* spp. other than *Vanilla planifolia*, or studies where the identity of the species studied was in doubt.

## RESULTS

### Traditional cultivation of *Vanilla planifolia*

Traditionally, vanilla is propagated as a cutting and is grown supported by an "Acahual" (secondary forest), which acts as support for vine growth and mimics vanilla's preferred natural territory (Figure 3a).<sup>16</sup> Optimal conditions for the growth of *V. planifolia* include temperatures between 20-30°C, an annual precipitation of 2000-3000 mm and approximately 50 % shade from sunlight.<sup>16</sup> Due to the environmental conditions that are required for optimal *Vanilla* bean production, *V. planifolia* is primarily grown in regions between 10-20° north and south of the equator.<sup>17</sup>

### Pollination and Harvest

Vanilla orchids reach flower-bearing maturity after 3-5 years of growth on the vine.<sup>18</sup> Flowering (Figure 3b) occurs over a 1-2 month period and continues for 12-14 years, at varying rates.<sup>19</sup> Previous studies have reported a positive correlation between the percentage of vines that bloom and the year of flowering for the first 3 years.<sup>16</sup> Generally, vanilla flowers open in the cooler temperatures of the morning and close the same day as temperatures rise in the afternoon, allowing approximately 6-8 hr for pollination to be completed.<sup>16-18</sup> Pollination can be completed naturally (e.g. insect), although in commercial cultivation, pollination is generally assisted by manual pollination (Figure 3b).<sup>16</sup> Knowledge about insectile pollination (or other natural processes) and the insect species that are responsible is significantly limited, although it is estimated that only 1-5% of total fruit pollinations occur through natural intervention.<sup>16</sup> Instead, manual pollination is most frequently used in commercial settings to enhance the fruiting rate and yield. Manual pollination generally uses a toothpick-like tool to manually move pollen from the anther sac (male) to the stigma (female), thereby bypassing the rostellum (Figure 3b).<sup>1</sup>

Once flowers are successfully pollinated, growth of the fruit commences and proceeds for approximately 7-10 months, depending on the environmental growth conditions.<sup>18</sup> Generally, beans reach their maximal length and diameter approximately 45 days after pollination, with the remaining time of growth regarded as a "maturation" stage.<sup>16</sup> Following maturation, the crops are rapidly harvested and the curing process begins immediately.

### Curing and its effects on vanilla bean quality

The processing of the green fruit pods is called 'curing'. Curing is completed post-harvest and allows the mature green pods

to develop a complex flavour and aroma profile. Traditionally, curing operations involve four critical steps (Figure 3d-g): known as "killing", "sweating" or "insolation", "drying" and finally, "conditioning".<sup>19</sup>

Variations to the curing process are generally based on the origin of curing. For example, the Mexican curing process deviates from the Bourbon method (practised in Madagascar, Réunion and the Comoro Islands) in the killing stage. The Mexican curing method relies on exposure to the sun, termed "sun killing", or the use of uniquely designed rooms called a "calorifico", which can reach high temperatures and humidity.<sup>20,21</sup> In contrast, the Bourbon method 'kills' the beans by immersion in hot water for 2-3 min (Figure 3d), depending on the quality of the beans (higher quality pods will be immersed for the longer period).<sup>20</sup> Ultimately, the techniques used to complete curing vary considerably and are unique to the location where the curing process occurs. Despite the regional differences, the purpose of each stage is shared and is summarised and explained in the following sections:

### Killing

The killing step of curing (depicted in Figure 3d) is generally believed to be the most crucial step in vanilla bean curing.<sup>22</sup> This step is required to halt vegetative growth in green pods and disrupt internal structures, thereby releasing the plants enzymes, allowing them to interact with the target substrates.<sup>23</sup> In most curing processes, killing involves immersion of the beans in hot water, although as noted above, some regional curing variations use other methods of heating the beans.

### Sweating

Sweating (depicted in Figure 3e) follows the killing process and involves storing the beans in enclosed containers or bags. This stage allows the *V. planifolia* bean enzymes to catalyse various hydrolytic and oxidative processes, thereby yielding vanillin and (to a lesser extent) several other phytochemicals that provide the favourable aroma and flavour qualities of vanilla.<sup>24</sup>

### Drying and Conditioning

The drying and conditioning phase of curing (Figure 3f) is used for quality-control. They ensure the aromatic compounds produced by preceding stages are preserved, to protect cured pods from microbial activity (particularly mould), and to ensure vanilla is in a suitable condition to market.<sup>24</sup> The quality of the vanilla beans is assessed through grading and largely determines the cost of product. The grading system criteria and nomenclature also varies with origin, although generally includes categories "whole" and "splits", and is followed by a series of subcategories, depending on deficits or damage to pods.<sup>25</sup> Overall, grading evaluates appearance (fullness, thickness, and colour), vanillin content (taste and smell) and moisture content.<sup>26</sup> Beans classified as gourmet or top-grade quality possess a rich brown colouring, have an oily lustre, are more than 12 cm in length, and have a



**Figure 3:** The stages of *Vanilla planifolia* cultivation and processing. (a) *Vanilla planifolia* plants are grown commercially in controlled environments; (b) hand pollination is used to ensure maximum bean production; (c) the beans are harvested when mature and then cured by (d) killing in scalding water, (e) sweating the beans in hot boxes or bags; (f) drying in sunlight; (g) bundling the beans for transporting and (h) marketing the beans.

maximum moisture content (MC) of 30%.<sup>25</sup> In comparison, green mature fruits pre-processing will have an average MC within the range 75-82% of wet weight (WW).<sup>27-29</sup>

### Novel methods of cultivating and curing *V. planifolia* beans

Despite the vanilla orchid being one of the more challenging crops to cultivate, efforts to revolutionise traditional methods are emerging. These include artificial growing environments, modern drying technology, and enzyme-assisted curing. The growth of vanilla in greenhouse structures has been investigated in the Netherlands, United Arab Emirates, and more recently in Australia.<sup>30-32</sup> Greenhouses and shade-houses are designed to imitate traditional environments and allow farmers to have control of conditions including temperature, humidity, carbon dioxide (CO<sub>2</sub>), and water.<sup>16</sup> By overcoming the limitations of traditional cultivation, these structures have the potential to expand vanilla cultivation, maximise crop yield and elevate the quality of product.<sup>32</sup> However, research regarding production capacity and product quality is limited.

Similarly, recent studies have also investigated modifications to the curing process, with the aim of increasing the levels of the major

aroma and flavour components, and thereby the quality of the beans. A modified drying method that reportedly supplements the traditional sun/air drying methods was reported to substantially enhance the quality of *V. planifolia* beans.<sup>33</sup> Indeed, beans dried using the modified protocol dried the beans significantly more rapidly than sun drying, and the content of vanillin (the main aroma and flavour component) was also slightly higher.<sup>30</sup>

The implementation of high hydrostatic pressure (HHP) to the curing stages of beans has been reported to increase  $\beta$ -D-glucovanillin and vanillin content, and reduce the overall time taken to process pods.<sup>34</sup> Similarly, the application of microwave and ultrasound technology during the killing stage of curing reduces the overall time required without negatively impact the quantity of vanillin in final product.<sup>35</sup> Additionally, the addition of specific microorganisms and/or exogenous enzymes (cellulase, pectinase,  $\beta$ -glucosidase) enhances the traditional curing process, or may replace the traditional curing process.<sup>36-40</sup> Indeed, notable increases have been reported for  $\beta$ -D-glucosidase content and vanillin production in *Bacillus* spp. assisted curing of *V. planifolia* beans.<sup>41</sup> Microbial assisted vanillin synthesis is promising for improving vanilla quality and substantially more work is required to optimise vanillin content using these methods.



## Threats to *V. planifolia* commercialisation

Several factors negatively impact the growth and production of *V. planifolia*, which has led to vanilla's listing as an endangered species under section B2 ab (iii, v) of the International Union for Conservation of Nature Red List.<sup>42</sup> The growth conditions required for *V. planifolia* growth and fruiting (warm, humid climates) also create an ideal environment for the growth of fungal pathogens. The most significant fungal threat to *V. planifolia* is *Fusarium oxysporum* f. Sp. *Vanillae* strain, which causes root and stem rot (RSR) disease and has significant impacts on the plant's growth.<sup>43,44</sup> Similarly, insect pests including the *Conchaspis angraeci* Cockerell, are also significant threats to vanilla cultivation, and cause substantial annual crop damage.<sup>45</sup> Unfavourable conditions or extreme weather events, particularly weather changes related to climate change, also pose threats to vanilla production globally.<sup>46,47</sup> The Vanilla Growers Association (VGA) of Vava'u has highlighted droughts and cyclones as the main environmental factors impacting their vanilla production.<sup>48</sup> That report focussed particularly on the 2016-2019 El Niño climatic event, and the 2016 cyclone season, which reduced vanilla production in Tonga to 10% of its usual capacity.

The high value of *V. planifolia* beans has made them a target for theft and other illegal activities. *Vanilla planifolia* plantations in Madagascar and Mexico have been the focus of theft and money laundering schemes connected to organised crime networks.<sup>49</sup> Indeed, the Global Initiative against Transnational Organised Crime (GI-TOC) reported over 5000 kilograms of vanilla, included green and cured product, was stolen from farmers in Madagascar's Sava region in 2020.<sup>49</sup> Additionally, vanilla adulteration has become a substantial issue in recent years. Adulteration is the process of modifying an original (often low-quality) vanilla product, typically by adding cheaper extracts and/or synthetic vanilla flavourings to increase the (perceived) quality and quantity of product, at reduced costs.<sup>50</sup> The availability of adulterated vanilla products on the market has threatened vanilla's reputation, and therefore has negatively impacted vanilla prices. This has induced the development of rapid and reliable analytical methods to evaluate vanilla extract authenticity. As a result, more stringent criteria are enforced regarding the labelling of vanilla extracts and products.<sup>25</sup> The Association of Official Agricultural Chemists (AOAC International) classifies nine compounds as economically motivated adulterants (EMAs) of vanilla, with *p*-coumaric acid, piperonal and ethyl vanillin being the most commonly reported.<sup>51</sup> Under this standard, the presence and concentration of these compounds is used to confirm the authenticity and/or severity of adulteration for vanilla extracts.

## Vanilla uses and applications

Vanilla is well established for its flavour and aroma applications, including use in foodstuffs, pharmaceuticals, and traditional/experimental therapeutic treatments.<sup>52,53</sup> More recently, vanilla's

principal compound (vanillin) has been studied to better understand the molecule's bioactivity and potential therapeutic uses, including anti-inflammatory, neuroprotective and anti-cancer effects.<sup>54-57</sup> The therapeutic properties of vanilla are not a focus of this review, and the reader is directed to those reviews for a comprehensive discussion of the pharmacological properties of *V. planifolia*.

## Phytochemistry and aromatic profile of *V. planifolia*

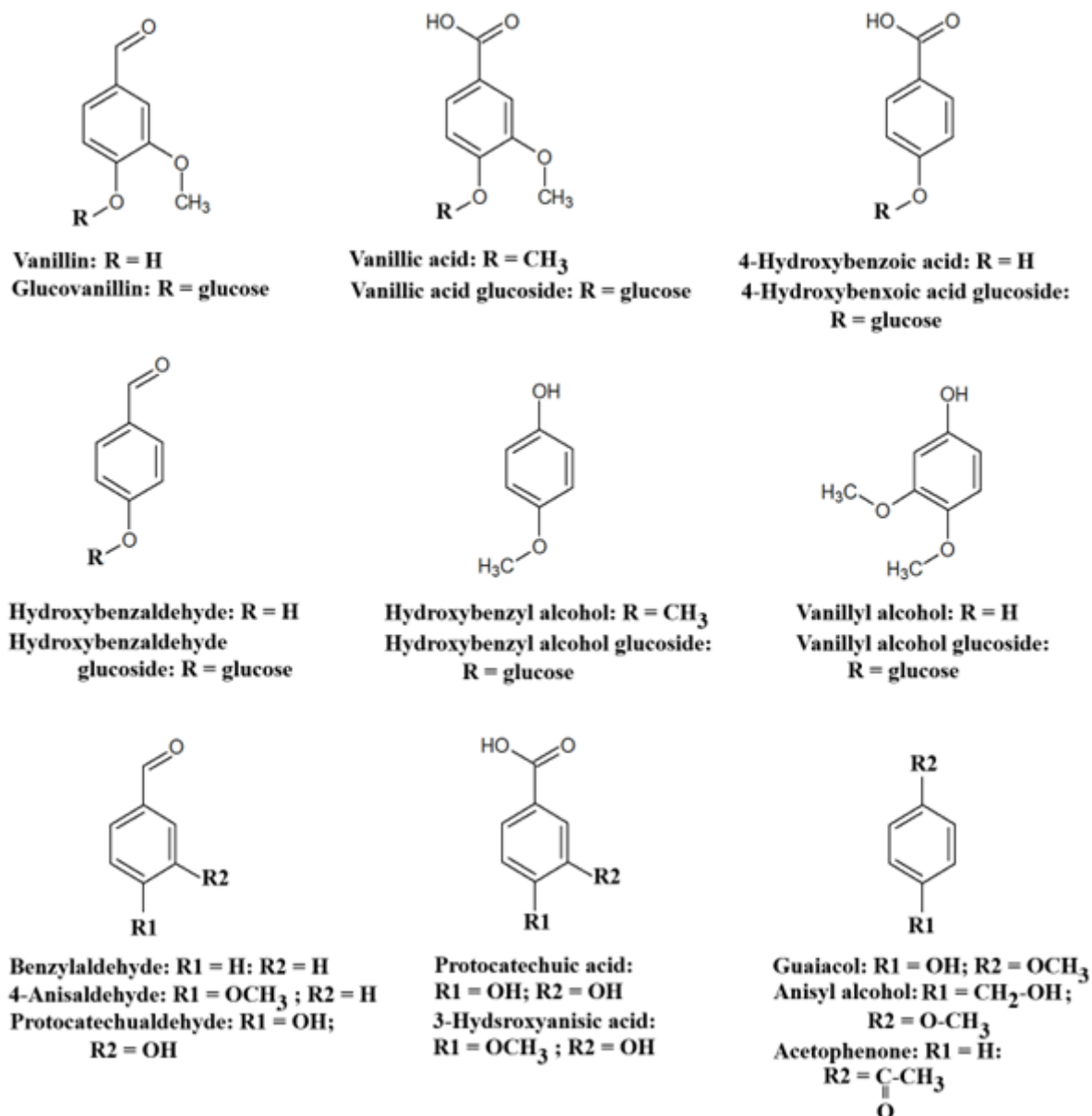
Cured vanilla has an intricate chemical profile, characterised by high vanillin content, complimented with a series of additional constituents. The most abundant and commonly reported compounds found in cured vanilla pods (in addition to vanillin), include vanillic acid, vanillyl alcohol, *p*-hydroxybenzyl alcohol, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid (Figure 4).<sup>58-62</sup> It is generally agreed that these compounds are all derived from their glycosylated form(s), which is/are present in green vanilla beans, and are also depicted in Figure 4.<sup>58</sup> The phytochemical complexity of *V. planifolia* beans, as well as the abundance of each compound, is dependent on multiple factors including the length of maturation, the curing practises used, and the method of analysis.<sup>62-66</sup> Furthermore, the ratios between compounds provides a method of predicting authenticity, origin, and the species of vanilla/vanilla products.<sup>60</sup> Notably, the vanillin content in top-grade *V. planifolia* beans can reach 2-2.5% dry weight.<sup>25,60</sup> In contrast, *p*-hydroxybenzaldehyde and vanillic acid are generally present at concentrations of approximately 1 g/kg (in dried and cured pods), whilst *p*-hydroxybenzoic acid is detected at lower concentrations (~0.1 g/kg).<sup>60</sup>

The average total sugar content of mature green *V. planifolia* beans generally accounts for <4% total wet weight.<sup>29</sup> Sucrose is found in the highest abundance of the sugars, followed by glucose and fructose.<sup>27,28</sup> Notably, the maturity of a green bean substantially influences the sugar content and profile of the bean. Beans aged between 3 to 5 months generally contain a higher abundance of glucose, compared to beans of 6 to 8 months maturity that contain higher levels of sucrose.<sup>63</sup>

## Biosynthesis of vanillin

### *Vanilla planifolia* de novo vanillin synthesis

Vanillin (3-methoxy-4-hydroxybenzaldehyde) is considered the principal aromatic compound in vanilla due to its significant contribution to flavour and aroma.<sup>66</sup> The *de novo* pathways for vanillin biosynthesis are not fully understood, although the currently accepted mechanism is summarised in Figure 5. However, this mechanism remains controversial, with each new advance in research providing an alternative model of vanillin biosynthesis, without effectively disproving existing models.<sup>67</sup> Natural vanillin synthesis uses phenylalanine (which is produced by the Shikimic acid pathway) as a precursor. Biosynthesis can occur by several routes, although in all cases *p*-coumaric acid is



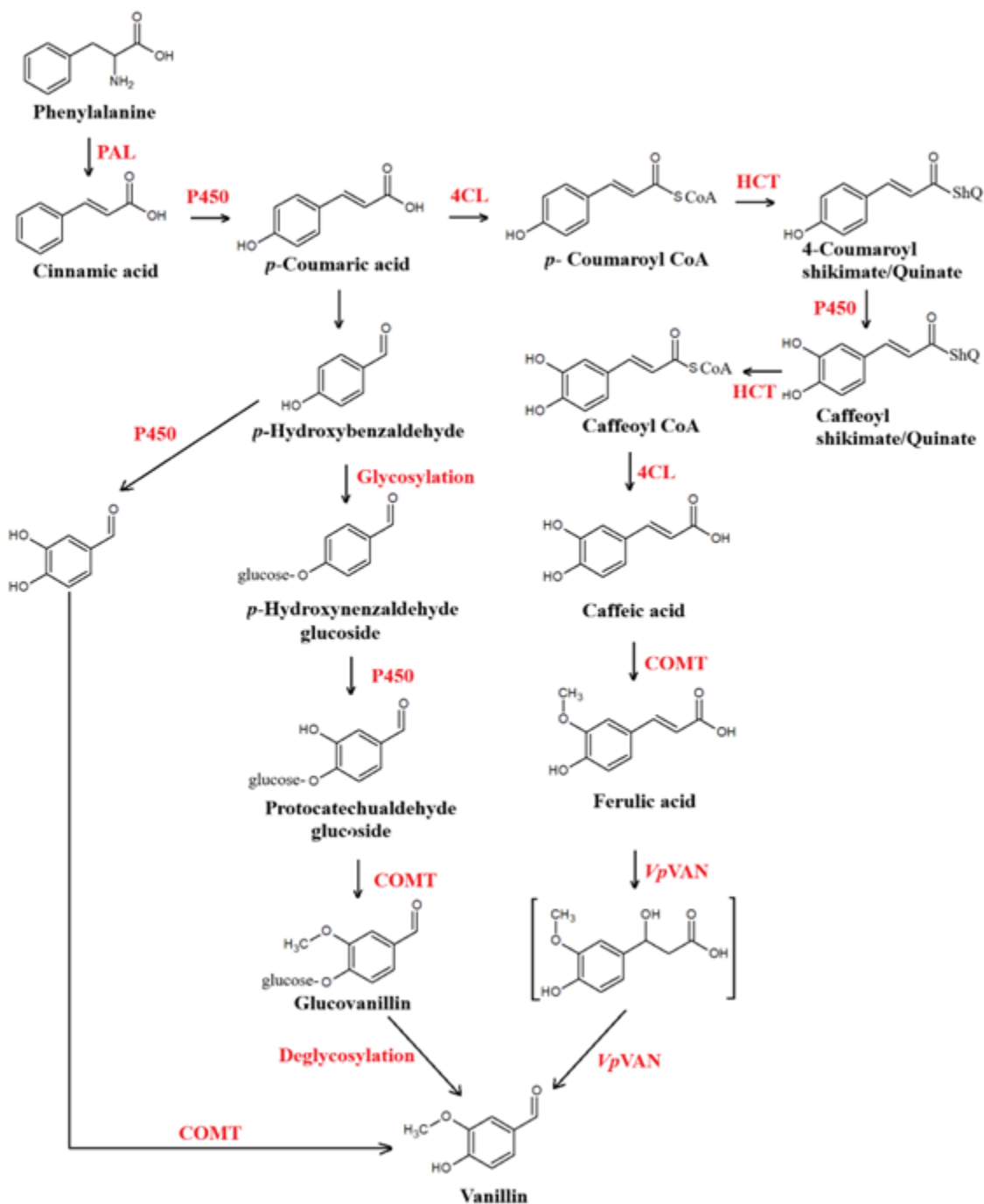
**Figure 4:** Chemical structures of natural flavour components and their glycosylated derivatives reported in *V. planifolia*, as reported in.<sup>59</sup> Structural figures were created using ChemDraw (V.23.1.1).

produced. This can then be converted (via several intermediates) to caffeic acid, ferulic acid and ultimately to vanillin (depicted as the right-hand branch in Figure 5). Alternatively, *p*-coumaric acid may be converted directly to *p*-hydroxyaldehyde, which serves as an intermediate in two vanillin synthetic pathways. The *p*-hydroxyaldehyde can be directly methylated in a caffeic acid 5-hydroxyferulic acid *O*-methyltransferase catalysed reaction to produce vanillin (depicted as the left-side branch in Figure 5). However, the majority of the *p*-hydroxyaldehyde is believed to be converted to glucovanillin, which is then hydrolysed to vanillin (depicted as the middle pathway in Figure 5).

It is generally agreed that a unique enzyme ( $\beta$ -D-glucosidase), is responsible for hydrolysing the glucovanillin precursor to liberate a free vanillin structure (Figure 5).<sup>18</sup> The temperatures

that *V. planifolia* beans are subjected to during the curing process modulates the activity of this enzyme, and thus affects vanillin synthesis.<sup>68-71</sup> A substantial volume of research has focussed on the isolation and characterisation of this enzyme, as well as evaluating the effects of temperature and pH on enzyme activity.<sup>72-74</sup> Evidence suggests that there are two separate glucosidase enzymes: one enzyme that targets the vanillin precursor glucovanillin as well as the *p*-hydroxybenzaldehyde glucoside; and a secondary glucosidase whose role is not yet understood.<sup>69-75</sup>

The primary location for storage of glucovanillin (and thus, the primary location of vanillin biosynthesis) has also been disputed through the literature, and our knowledge has evolved with the emergence of new technologies. It has been reported that glucovanillin is primarily stored in the central placental region of



**Figure 5:** *Vanilla planifolia de novo* pathway for the synthesis of vanillin from phenylalanine. PAL=phenylalanine ammonia lyase; P450=cytochrome P450 system; 4CL=4-hydroxycinnamoyl-CoA ligase; HCT=hydroxycinnamoyltransferase; COMT=caffeic acid 5-hydroxyferulic acid *O*-methyltransferase; VpVAN=vanillin synthase. The structures were prepared using ChemDraw (V.23.1.1) and the figure was subsequently assembled using Microsoft Paint (11.2410.39.0).

green pods, although it has also been observed to disperse to the surrounding papillae.<sup>76</sup> In contrast, other studies have reported that the highest content of glucovanillin is localised in the outer portions of the bean.<sup>23</sup> Brillouet *et al.*, 2010 proposed that these disparities may exist simply due to variations in the glucovanillin and  $\beta$ -glucosidase contents of the beans in the individual studies.<sup>77</sup> Nevertheless, it is understood glucovanillin content in

mature green beans ranges from 10-15% on a dry weight basis, or approximately 1.7% in wet beans.<sup>29,61,63</sup>

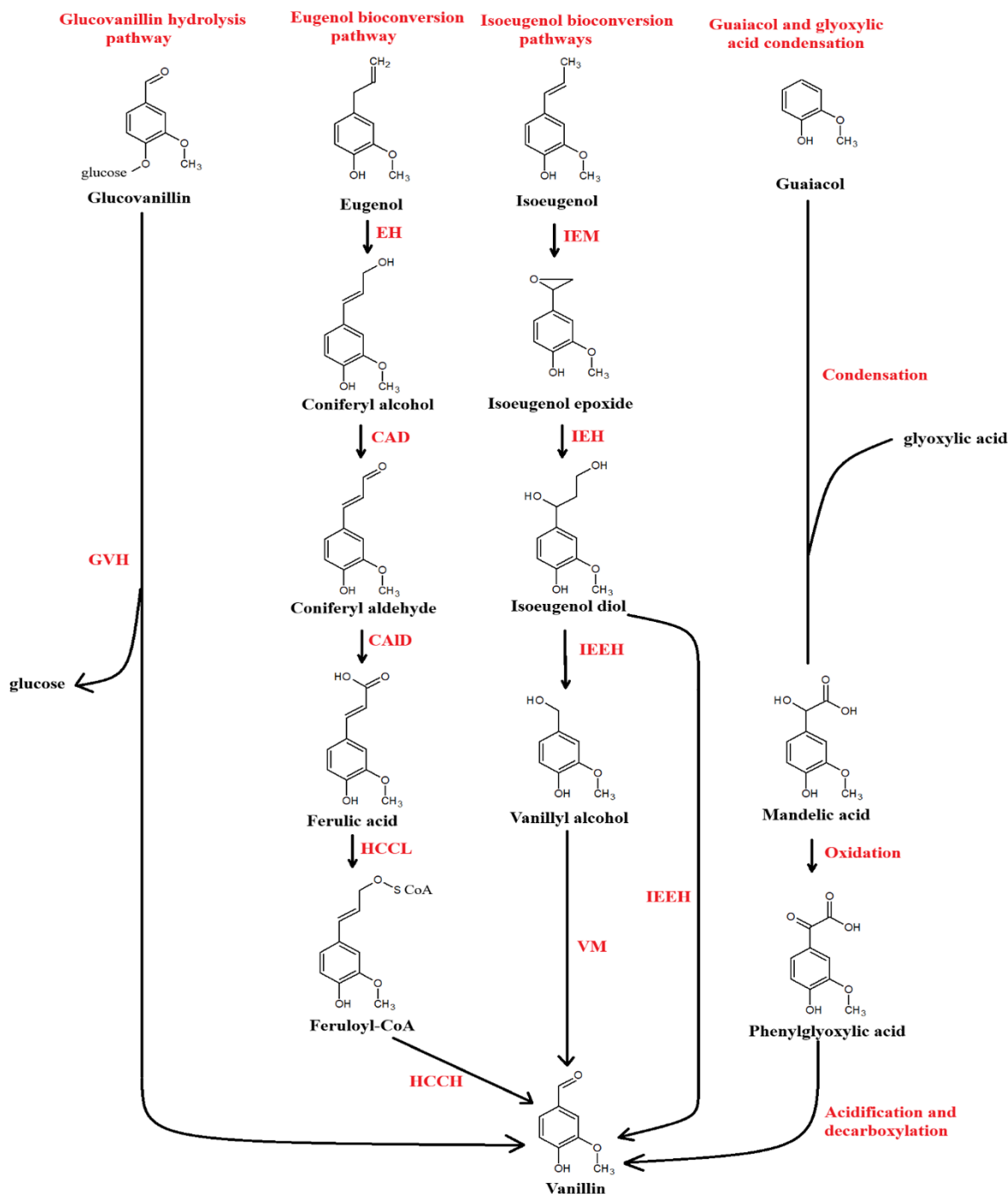
Notably, glucovanillin content accounts for 90% of total vanillin content in the beans at harvest.<sup>78</sup> In contrast, free vanillin is only present in low levels at harvest, and the majority of free vanillin is released from glucovanillin during curing. Interestingly,  $\beta$ -D-glucosidase activity decreases as curing stages progress,



whilst vanillin content continues to rise throughout the curing process.<sup>20</sup> It is believed that several microorganisms associated with the *V. planifolia* beans provide vanillin biosynthetic enzymes and enhance synthesis of vanillin, particularly in the later phases of curing.<sup>79</sup>

### Enzymatic and microbial vanillin synthesis

Due to the high costs and lengthy production times, natural plant-based synthesis of vanillin currently accounts for <1% of the global market.<sup>79</sup> Instead, the majority of vanillin production arises from precursors other than phenylalanine, and may involve both microbe-assisted synthesis, or chemical synthesis. Most directly,



**Figure 6:** Pathways for microbial synthesis of vanillin using eugenol, isoeugenol, guaiacol as precursors. GVH=glucovanillin hydrolase; EH=eugenol hydroxylase; CAD=coniferyl alcohol dehydrogenase; CAID=coniferyl aldehyde dehydrogenase; HCCL=ferulic acid CoA ligase; HCCH=hydroxycinnamoyl-CoA hydratase; IEM=isoeugenol monooxygenase; IEH=isoeugenol hydratase; IEEH=isoeugenol epoxide hydratase; VM=vanillin monooxygenase. The structures were prepared using ChemDraw (V.23.1.1) and the figure was subsequently assembled using Microsoft Paint (11.2410.39.0).



**Figure 7:** Novel methods for commercial cultivation of *Vanilla planifolia*: (a) growing in greenhouses and shade houses and; (b) the use of biodomes, which allows for regulation of multiple environment conditions, including, temperature, humidity and gas content. The photographs were provided by David Soo of OrbiGo Pty Ltd, and are reproduced here with permission of the photographer.

exogenous  $\beta$ -D-glucosidase enzyme can be added to the beans to induce substantially higher levels of glucovanillin hydrolysis (left-hand pathway in Figure 6), and therefore increased free vanillin production.<sup>80</sup> However, the use of exogenous enzymes in large scale vanillin production is expensive and time consuming. Instead, microbial assisted vanillin synthesis using eugenol and isoeugenol (middle pathways in Figure 6) or chemical synthesis using guaiacol (or isoeugenol) as a precursor are preferred.<sup>79</sup>

Microbial biosynthesis of vanillin can use several natural substrates as precursors, including eugenol, isoeugenol, ferulic acid, lignan and glucose, although, eugenol and isoeugenol are most frequently used and therefore are summarised herein. For a more complete review of vanillin synthesis from the other precursor compounds the reader is referred to.<sup>79</sup> Microbial vanillin synthesis uses relatively cheap growth media, and requires standard culturing equipment, making it relatively cheap and easy to establish. Despite this, microbial-assisted vanillin production is substantially more expensive than natural *V. planifolia* curing and can increase the costs of vanillin to US\$1200-1500/kg and substantially more work is required to make these processes more financially viable for large-scale production.

Eugenol can be converted by several microorganisms, including several *Pseudomonas* and *Corynebacterium* strains.<sup>81</sup> The eugenol is converted via coniferyl intermediates to ferulic acid, which is then ligated to a CoA moiety to produce feruloyl-CoA (Figure 6). The enzyme 4-hydroxycinnamate CoA hydratase then catalyses the conversion the release of the CoA moiety and the conversion to vanillin. Similarly, isoeugenol may either be converted to eugenol via isomerisation, or may function as a precursor of vanillin in its own right. The bioconversion proceeds via several intermediates, including isoeugenol epoxide, eugenol diol and vanillyl alcohol (Figure 6). Several studies have reported that *Bacillus* spp. cultures are effective for the conversion of isoeugenol to vanillin. *Bacillus subtilis* B2 strain converts isoeugenol to vanillin with >12% efficiency,<sup>79</sup> whilst *Bacillus fusiformis* assisted isoeugenol

conversion resulted in substantially higher vanillin yields.<sup>82</sup> Similarly, some fungi (particularly *Aspergillus niger*) have also been reported to efficiently catalyse the conversion of isoeugenol to vanillin.<sup>85</sup> Despite these promising results, the use of isoeugenol as a precursor for microbial vanillin synthesis is limited due to its water insolubility (700-810 mg/mL),<sup>80</sup> significantly limiting the amount of vanillin that can be synthesised from isoeugenol.

Alternatively, chemical synthesis of vanillin can produce substantial yields.<sup>79</sup> Guaiacol can be condensed with glyoxylic acid to produce mandelic acid.<sup>84</sup> Following an oxidation reaction, this is converted to phenylglyoxilic acid, which is subsequently decarboxylated to produce vanillic acid. The conversion of guaiacol to vanillin can be accomplished via microbe assisted methods,<sup>79</sup> or via synthetic pathways,<sup>84</sup> although the yields are substantially higher via synthetic methods. However, the use of synthetic vanillin (particularly as a food additive and flavouring) is restricted by many countries regulatory authorities,<sup>83</sup> thereby limiting its production to aroma and industrial uses only.

## CONCLUSION

Vanilla is a high-priced product that is sought after for its aroma and flavour characteristics. The high price of vanilla products is due to its high cost of production. Natural vanilla production is a lengthy and labour-intensive process that uses traditional methods to cure the beans. The purpose of the curing is to release free vanillin from the conjugated form glucovanillin, and to produce further free vanillin from other sources. As free vanillin is largely responsible for the aroma and flavour characteristics of vanilla, quality evaluations of the vanillin produced are based on the levels of free vanillin. Therefore, recent research has focussed on the development of methods to increase the yields of free vanillin in *V. planifolia* beans.

This may be achieved in several ways. Firstly, the development of more efficient horticultural methods to increase the production

biomass of *V. planifolia* beans would maximise the overall yield of total vanillin (glucovanillin and free vanillin) in the beans.<sup>85</sup> Alterations in environmental conditions during plant growth, as well as flowering and fruiting phases, are useful in maximising yields, and substantial work is underway to develop environments (e.g. biodomes Figure 7) with readily controlled moisture and humidity levels, temperatures and gas levels. The ability to control and monitor the growth conditions may substantially improve yield, bean quality and consistency. However, substantially more work is required to understand the optimal cultivation conditions, as well as timing for changes those conditions. Such studies may greatly enhance vanilla quality, production and profitability in the future.

Additionally, optimisation of the curing methods may also have substantial effects on the quality of vanilla produced. Whilst the traditional methods are effective, a greater understanding of the biochemical processes occurring during curing may allow for more efficient and time-effective processes. Additionally, the use of biotechnological advancements may have profound effects on vanilla quality and vanillin yield. Indeed, as discussed earlier in this review, microbial assisted curing not only greatly reduces curing time, but also increases free vanillin yield, and therefore the vanilla quality. Further studies focussed on the identification of specific microbial genes required for production vanillin biosynthetic enzymes, and the use of biotechnology to enhance the expression of those genes may have profound effects on the production of quality vanilla. Furthermore, more rapid production via microbial-assisted curing would allow for greater production at substantially reduced costs, which would also benefit the end-user.

Finally, assessments of vanilla quality generally focus on the amounts of free vanillin present, as it is the main aroma and flavour compound. However, substantial amounts of vanillin is stored in beans as the glycosylated form, glucovanillin. Thus, beans that show lower levels of free vanillin may also have substantial potential to increase their free vanillin content through modifications of the curing process. Thus, reliable and accurate methods to rapidly quantify the levels of these compounds would provide a more complete understanding of the quality and potential of the *V. planifolia* beans. However, quantification of both compounds using a single method is difficult because of the different physiochemical properties of these compounds. With improvements in analytical technologies (e.g. UPLC), methods that quantify both compounds at once may be developed, and substantially more work is needed in this field. Ideally, the new method should not only be able to identify and quantify these compounds simultaneously, but it should also be rapid and reproducible, as well as being relatively inexpensive.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**GC:** Gas Chromatography; **HHP:** High Hydrostatic Pressure; **HPLC:** High Performance Liquid Chromatography; **MC:** Moisture Content; **MS:** Mass Spectrometry; **WW:** Wet Weight; **UPLC:** Ultra Performance Liquid Chromatography.

## SUMMARY

- High quality *Vanilla planifolia* beans (with high vanillin contents) are the second most expensive spice globally.
- The expense of vanilla is related to its relatively slow cultivation, as well as its long and labour-intensive traditional curing process.
- Several steps in vanilla horticulture and processing are highlighted as targets for optimisation
- The potential for incorporation of biotechnological advancements in curing and quality assessment methods are also highlighted.

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