15 Vanilla

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15.1. Introduction

Vanilla, a vine known since the time of the Aztecs, is a genus of about 110 species in the orchid family. *Vanilla planifolia* yields the popular, commercial flavouring agent vanillin. Vanilla is the world's third most expensive spice, next to saffron and cardamom. *V.* Mill. is the only genus among orchids whose members produce a spice. The name originates from the Spanish word 'vainilla', the diminutive form of 'vaina' (meaning 'sheath'), which is in turn derived from Latin 'vagina'. It belongs to the family Orchidaceae, subfamily Epidendroideae, tribe Vanilleae and subtribe Vanillinae (Weiss, 2002.)

This evergreen genus occurs in tropical and subtropical regions, from tropical America to tropical Asia, New Guinea and West Africa. The main species harvested for vanillin is *V. planifolia*, a native of Mexico, though now grown widely throughout the tropics. Additional sources include *V. pompona* and *V. tahitiensis*, grown in Tahiti.

The total area of vanilla cultivation in the world during the year 2001 was 40,846 ha and production was 5583 t. There has been no appreciable increase in area under vanilla cultivation in the traditional vanilla-growing countries, according to the UN Food and Agriculture Organization (2005). The major vanilla-producing countries are Madagascar, The Comoro Islands, Indonesia, Mexico and Réunion. Among these countries Madagascar holds the prominent position, having a cultivated area of 25,550 ha under vanilla. Of late, Indonesia has started to produce more, with a production of 2102 t from 9700 ha. Others are Mexico, China, The Comoro Islands, Réunion, Tonga, French Guiana, Malawi, Uganda, Zimbabwe, Guadeloupe, Kenya, Fiji Islands, Cook Islands and Turkey. Madagascar stands as the world's largest producer of vanilla, e.g. 3.0 t, of a total world production of 7.3 t (Table 15.1).

The import of vanillin and ethyl vanillin together to India during 2000/01 was 404 t. Even if only 10% of import of these synthetic substitutes was replaced by natural product, the requirement of vanilla beans would be 2020 t at the rate of 2% vanillin content. This is almost one half of the entire global production of vanilla beans, indicating the great potential for vanilla development in India (http://www.hinduonnet.com/businessline/2003/03/03/).

Vanilla was highly regarded in pre-Columbian Mesoamerica and was brought back to Europe, and from there to the rest of the world, by the Spanish Conquistadors. In ancient Mexico, the Totonac people (present state of Veracruz (Papantla), Mexico) were regarded as the producers of the best vanilla.

 Chemistry of Spices
(eds V.A. Parthasarathy, B. Chempakam and T.J. Zachariah)

Table 15.1. Main vanilla-producing countries.

Country	Production, 2005 (t)
Madagascar	3.0
India	2.4
China	1.0
Mexico	0.2
Turkey	0.2
The Comoro Islands	0.1
World total	7.3

Source: UN Food and Agriculture Organization (2005).

They continued to be the world's chief producers through the mid-19th century. At that time, French vanilla growers in Mexico traded their knowledge of artificial pollination of flowers for the Totonac knowledge of preparing the beans (Correll, 1953; Purseglove *et al.*, 1981).

Vanillin, the crystalline component, was first isolated from vanilla pods by Gobley in 1858. By 1874, vanillin had been obtained from glycosides of pine tree sap, temporarily causing an economic depression in the natural vanilla industry. When vanillin, a cheaper synthetic substitute of vanilla, was used for New Coke in 1985 by the Coca-Cola Corporation, the world's largest customer of natural vanilla extract, the Madagascan economy crashed. It recovered only when New Coke flopped. The world market price of vanilla has been at the mercy of cartels, typhoons and political instability, apart from the ever-fluctuating demand and supply pressures. Production of secondary vanilla metabolites, particularly vanillin from *V. planifolia* cell suspension cultures from various plant parts, remains experimental (Havkin-Frenkel *et al.*, 1997.) This technology could reduce the cost of growing vanilla beans greatly, but could seriously affect the economy of vanilla-producing countries such as Madagascar, Java and Tahiti (Simpson and Ogarzaly, 1986).

15.2. Botany and Uses

The basic chromosome number of the genus is x = 16; *V. planifolia*, *V. fragrans*, *V. pompona*, *V. tahitensis* and other species are diploid, with 2n = 32. Vanilla is a tropical climbing orchid, with a long, green, fleshy stem that sprouts roots and clings to trees parasitically. Vanilla climbs over supports and can grow as high as possible. When cultivated, the vines are trained on to posts and support trees up to a height of about 130–135 cm, to facilitate trailing of the vines and artificial hand pollination. Suitable live supports are *Plumaria alba*, *Erythrina lithosperma*, *Jatropha carcas* and *Glyricidia maculata* (http://www.kissankerala.net), but *E. lithosperma* is highly susceptible to wasp attack and hence is not an ideal live support. Just before the plant flowers, the grower usually prunes 10–15 cm from the vine tip; this stops linear growth and seems to benefit flowering (Childers *et al.*, 1959). Its yellow or orange orchidaceous flowers grow in bunches, which bloom one flower each day, opening one by one, during the 2-month season.

Vanilla is a tropical crop and requires a warm climate with frequent rains (annual rainfall of 150–300 cm). Uncleared jungle areas with natural shade or filtered sunlight and soil or rich humus layer undisturbed on the top are ideal for vanilla plantations. The spice is cultivated on various types of soils from sandy loam to laterites and is propagated by planting shoot cuttings *in situ*. Rooted cuttings of 60 cm length (or even tissue culture-derived plants) are planted; longer cuttings bear earlier. The cutting is planted with the onset of the monsoon rains.

V. planifolia is the only orchid used for industrial purposes (in the food and cosmetic industries). *Vanilla* species are used as food plants by the larvae of some Lepidopteran species, including *Hypercompe eridanus* and *H. icasia*. The seeds will not germinate in normal soil; they need a certain symbiotic fungus.

The racemose inflorescences, short-lived flowers, arise successively on short peduncles from the leaf axils or scales (Weiss, 2002). The small lily-like, greenish-yellow vanilla flowers, 3.6 × 5.2 cm long, develop in axillary racemes (Woebse, 1963). There may be 20–100 flowers on a single raceme. Each flower opens up in the morning and closes late in the afternoon, never to re-open

(Childers *et al.*, 1959). If pollination has not occurred meanwhile, the flower will be shed. The flowers are self-fertile but need pollinators to perform this task. They are pollinated by stingless bees and certain hummingbirds, which visit the flowers primarily for its nectar (DeVarigny, 1894; Correll, 1953), but hand pollination is the best method in commercially grown vanilla as flowering is not synchronous. Practically all vanilla is produced now by hand pollination, a labour-intensive task, which accounts for 40% of the total labour cost in vanilla production (Gregory *et al.*, 1967). Approximately 6–8 months after pollination, the green vanilla beans are harvested.

The fruit ('vanilla bean') is an elongated, fleshy seed pod, 10–20 cm long. A single fruit is formed from the pollination of one flower and from this is derived the characteristic flavour compound. It ripens gradually (8–9 months after flowering), eventually turning black and giving off a strong aroma. Each pod contains thousands of minute seeds, but it is the pod that is used for vanilla flavouring. The vanillin in the green beans is present exclusively in conjugated form, principally as the β-D-glucoside and, at this stage, the beans display no trace of the characteristic vanilla flavour. This only develops during the fermentation or 'curing' process, which can take more than 6 months to occur. During curing, vanillin β-D-glucoside and related β-D-glucosides come into contact with β-D-glucosidases, resulting in the release of free vanillin and related substances (notably 4-hydroxybenzaldehyde) (Kanisawa *et al.*, 1994; Rao and Ravishankar, 2000b; Dignum *et al.*, 2001). The vanillin content of cured pods is usually *c.* 2–2.5% and, in addition, the number of minor components present is around 200.

Species with common names include:

- *V. aphylla*: leafless vanilla
- *V. barbellata*: small bearded vanilla, wormvine orchid, leafless vanilla, snake orchid
- *V. chamissonis*: Chamisso's vanilla
- *V. claviculata*: green withe
- *V. dilloniana*: leafless vanilla
- *V. edwallii*: Edwall's vanilla
- *V. mexicana*: Mexican vanilla
- *V. odorata*: inflated vanilla
- *V. phaeantha*: leafy vanilla
- *V. planifolia*: vanilla, flat plane-leaved vanilla, West Indian vanilla
- *V. poitaei*: Poit's vanilla
- *V. siamensis*: Thai vanilla.

Vanilla fruits are harvested when fully mature, but before they are too ripe. If harvested immature, the full-bodied aroma and requisite colour do not develop and the beans are more prone to fungal infection. The pods take about 9 months to mature. They are harvested when the tips (the thickest portion of the fruit, the blossom end) begin to turn pale yellow, overall pod colour changes from dark green to light green, the fruits lose their shine and two distinct lines appear from end to end (David, 1950; Purseglove, 1985). The flavouring comes from the vanilla bean. The prepared beans are very dark brown, slender, pleated and about 20 cm long, but tough and pliable. Quality vanilla has a frosting of crystal called *givre* (French for 'frost'), which contains the active ingredient 'vanillin' that produces the characteristic fragrance and is produced during the process of induced fermentation. These pods are called 'fine vanilla'. 'Woody vanilla' is shorter, lighter coloured, uncrystallized, stronger and slightly bitter.

Processing for vanillin

Unlike most spices, the processing or curing of vanilla is quite complicated, since fresh vanilla pods do not have any taste; this, and the need for manual pollination (outside Mexico), makes vanilla one of the most expensive spices. Vanillin is bound as a glycoside and must be set free by enzymatic reaction, normally induced by a sequence of blanching (Bourbon) or steaming (Mexico) operations. During the curing process, the flavour precursors, which are glucosides, are broken down by glucosidase into vanillin and glucose and some other minor aromatic substances. Odoux *et al.* (2003) have purified beta-D-glucosidase from beans of

V. planifolia and have found the enzyme to be a tetramer (201 kDa) made up of four identical subunits (50 kDa).

Weiss (2002) described in detail the stepwise procedure for curing vanilla by the traditional method. This includes killing the vegetative tissue of the vanilla pod to prevent it from growing further after harvest; sweating to allow enzymes to process the compounds in the beans into vanillin and other compounds important to the final vanilla flavour; and drying to prevent rotting and to lock in the aroma in the pods. After the final step of conditioning, the beans are graded for quality. At this point, the beans are dark, oily and pliable. One kg of cured beans is derived from about 6 kg of green pods. Theodose (1973) gives details of the two main traditional forms of curing employed in Mexico. The Bourbon curing technique developed on the island of Réunion is slightly different from the above and is described by Correll (1953). Steps for vanilla curing under laboratory conditions have been described by Dignum *et al.* (2002).

Uses

The predominant commercial use of vanilla is for its flavour compound, vanillin. It finds use not only as a flavouring agent in ice creams, bakery products and puddings, etc., but is also important in the perfumery and cosmetic industry. A few studies on its medicinal properties have also been reported, which are detailed in a subsequent section on culinary and medicinal uses.

15.3. General Composition

Cured vanilla beans contain vanillin, organic acids, fixed fatty oil, wax, gum, resins, tannins, pigments, sugars, cellulose and minerals. The relative amounts of these depend on the species, the environmental factors during growth, harvesting, processing and grading procedures (Purseglove *et al.*, 1981). Mature, fresh, green pods contain about 20% water and each 100 g of dried pod contains, on average, 3–5 g protein, 11 g fat, 7–9 g sugar, 15–20 g fibre, 5–10 g ash, 1.5–3.0 g vanillin, 2 g of a soft resin and an odourless vanillic acid (Weiss, 2002).

Vanilla contains 25% of sugars, 15% fat, 15–30% cellulose and 6% minerals (Uhl, 2000). Water content is unusually high (35%). The nutritional content of vanilla extract in 34.4% ethanol is given in Table 15.2 (USDA National Nutrient Database for Standard Reference, 2002).

Sagrero-Nieves and Schwartz (1988) studied the phenolic content of vanilla, vanillic acid and 4-hydroxybenzaldehyde (HBA) in *V. planifolia* with respect to the harvest period of beans (August–December). The moisture content of the beans decreased from 87.6 to 81.4%. Vanillic acid remained constant and both vanillin and HBA

Table 15.2. Nutrient content of vanilla extract in 34.4% ethanol.

Nutrient	Value per 100 g edible portion
Proximates	
Water (g)	52.58
Energy (kcal)	288
Protein (g)	0.06
Total lipid (g)	0.06
Ash (g)	0.26
Carbohydrate by difference (g)	12.65
Minerals	
Ca (mg)	11
Fe (mg)	0.12
Mg (mg)	12
P (mg)	6
K (mg)	148
Na (mg)	9
Zn (mg)	0.11
Cu (mg)	0.072
Mn (mg)	0.23
Vitamins	
Thiamin (mg)	0.011
Riboflavin (mg)	0.095
Niacin (mg)	0.425
Pantothenic acid (mg)	0.035
Vitamin B_6 (mg)	0.026
Lipids	
Total saturated fatty acids (g)	0.010
Monounsaturated fatty acids (g)	0.010
Polyunsaturated fatty acids (g)	0.004

Source: USDA National Nutrient Database for Standard Reference (2002), www.nal.usda.gov.

increased from 0.20 and 0.05 to 11.30 and 1.03 mg/g dry weight, respectively. The higher phenolic content could be attributed to fermentation on the vine. No correlation was observed between the extract colour (i.e. green versus brown) and the vanillin content. Funk and Brodelius (1990a) established a cell suspension culture of *V. planifolia* in MS-medium. 2,4-D suppressed, while NAA enhanced the formation of extractable phenolics and cytokinins appeared to favour lignin biosynthesis. Treatment of the culture with chitosan resulted in the induction of various enzymes of phenylpropanoid metabolism, while the amount of extractable phenolics decreased due to their rapid incorporation into polymeric ligneous material.

Bouquet: highly fragrant and aromatic.

Flavour: rich, full, aromatic and powerful. Madagascar and Mexico make the best quality. Indonesian and Tahitian vanilla is weaker and considered inferior.

Hotness scale: in the scale devised to relate the 'hotness' of spices, a value on the arbitrary scale ranging from 0 to 10, vanilla scores 1. Generally, most spices fall in the middle, with only the hottest of Mexican chillies scoring 9 or 10.

15.4. Chemistry

Volatiles

The main aroma compound in vanilla is vanillin. Several other volatile constituents are responsible for its characteristic aroma with sweet, balsamic, creamy, woody, spicy, fruity, herbaceous, phenolic and cinnamon-like notes. A compilation of volatile substances identified has been reported by Maarse *et al.* (1994) and Ranadive (1994).

Vanillin

The characteristic aroma of vanilla is obtained after a curing process of green fruits, which contain many different glucosidic compounds. The curing process is required to hydrolyse the glucosides and to release the aroma compounds. β-Glucosidases are believed to play an important role in this process (Arana, 1943). The β-glucosidase activity in vanilla beans is highest at 6–7 months after pollination (Arana, 1943; Wild-Altamirano, 1969) and the amount of glucosides is also at its highest level then (Kanisawa, 1993). The curing process kills the β-glucosidase activity (Ranadive *et al.*, 1983; Dignum *et al.*, 2002), indicating that the aroma formation might not be a completely enzymatic process.

Extraction

Aroma compounds from vanilla beans have been extracted using several extraction procedures, using alcohols and organic solvents (Galletto and Hoffman, 1978; Dignum *et al.*, 2002), direct thermal desorption (Hartman *et al.*, 1992; Adedeji *et al.*, 1993) and solid-phase microextraction (SPME) (Sostaric *et al.*, 2000), followed by identification of the compounds by gas chromatography-mass spectrometry (GC-MS).

Waliszewski *et al.* (2007a) found that a combination of hydration process in 5% ethanol for 48 h and enzymatic pretreatment with stable cellulolytic preparations up to 12 h could double vanillin content in the ethanolic extract and yield a product of excellent sensory properties. Extraction of glucovanillin from green pods of *V. fragans* (*V. planifolia*) and simultaneous conversion to vanillin by a combination of pectinase (polygalacturonase) and cellulase enzyme activities involving cell wall degradation and glucovanillin hydrolysis, in the presence of 47.5% ethanol for 8 h at 70°C, was found to be highly efficient (Ruiz *et al.*, 2001). Extracted vanillin was 3.13 times higher than the one obtained with the Soxhlet method. The classical curing/extraction process results in 1.1–1.8 g of vanillin/100 g of dry pods. Thus, it was concluded that the enzymatic reaction might substitute the microbial process involved in tissue fermentation previous to vanillin extraction with the simultaneous hydrolysis of glucovanillin.

Longares and Canizares (2006) devised a new method for the quick extraction of vanillin and *p*-hydroxybenzaldheyde (PHB) of vanilla beans from *V. fragans* by irradiating

with microwave energy to accelerate the extraction process. Combined with simultaneous determination (using the Vierordt's method) and photometric monitoring (at 348 and 329 nm), this resulted in a 62-fold decrease in the extraction time and a 40–50% increase in the vanillin and PHB concentrations compared with the official Mexican extraction method.

Nguyen *et al.* (1991) extracted oleoresin from vanilla beans with 2–62 g CO_2/g dried bean at 306–309 K and 10–13 MPa. Vanillin yields of up to 95% were attained. Vanillin purity was higher with supercritical CO_2 extraction than with conventional aqueous ethanol extraction, with vanillin representing 74–97% of the flavour and fragrance compounds, compared with 61% using alcohol extraction.

Other aroma compounds

Aroma compounds in cured vanilla beans from different countries, e.g. Madagascar, Tonga, Costa Rica, Java, Indonesia and Mexico, have been documented. Over 100 volatile compounds have been detected, including aromatic carbonyls, aromatic alcohols, aromatic acids, aromatic esters, phenols and phenol ethers, aliphatic alcohols, carbonyls, acids, esters and lactones, of which the aldehyde vanillin is the most abundant. The level of the aldehydes, e.g. vanillin and *p*-hydroxybenzaldehyde and their respective acids (vanillic acid and *p*-hydroxybenzoic acid), in cured vanilla beans is used as an indicator of cured vanilla bean quality for commercial purposes (Klimes and Lamparsky, 1976; Adedeji *et al.*, 1993; Ranadive, 1994).

Silva *et al.* (2006) showed from sensory analysis that aromatic extracts obtained with a pentane/ether (1/1 v/v) solvent mixture, from cured vanilla beans, provided the flavour most representative of vanilla bean. They found clear differences between the numbers of aroma compounds identified in different organic aroma extracts: 65 volatiles were identified in a pentane/diethyl ether extract by GC-MS analysis; ether extraction gave 54 volatiles; the pentane/dichloromethane solvent yielded only 41 volatiles. The volatile compounds identified included 25 acids, 15 phenolic compounds, ten alcohols, four aldehydes, four heterocyclic compounds, four esters, two hydrocarbons and one ketone (Table 15.3). The tentatively identified compounds 2-heptenal, (*E*)-2-decenal and 2-heptenoic acid, were reported for the first time. Aromatic acids, aliphatic acids and phenolic compounds were the major volatiles. Quantification of the aroma compounds revealed that vanillin, vanillic acid, *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid were the major compounds (Table 15.4). Vanillin is reported to represent 50% of the total quantified volatiles in Bourbon vanilla and 30% in Mexican vanilla whereas, in the study by Silva *et al.* (2006), the vanillin concentration (19,118 ppm) represented 85% of the volatile compounds.

When the pentane/diethyl ether extract was subjected to GC-O analysis, two of the 26 compounds detected were found at concentrations of less than 1 ppm, 13 at < 4 ppm, six at < 20 ppm, three at < 150 ppm and two at > 150 ppm. The compounds guaiacol, 4-methylguaiacol, acetovanillone and vanillyl alcohol, found at much lower concentrations in vanilla beans than vanillin, proved to be as intense as vanillin (Table 15.5). Ten phenolic compounds were detected in vanilla extracts as being aroma-active. Guaiacol, 4-methylguaiacol and acetovanillin, occurring at concentrations of 3.8–13.7 ppm, were similar in intensity to vanillin, which was detected at a concentration of more than 1000 times that of these compounds. Methyl salicylate, detected at a level of less than 1 ppm, was perceived as being as intense as vanillin. *p*-Cresol, methyl cinnamate and anisyl alcohol, occurring at concentrations of 1.1–2.6 ppm, were of medium intensity. Sweet, woody, balsamic, spicy, vanilla-like and toasted notes were attributed to phenolic compounds. Vanillic acid was not perceived by panellists because its elution required a high temperature, which caused a burnt odour in the sniffing port. The aldehydes 2-heptenal and (*E*)-2-decenal, identified here for the first time in vanilla beans, were perceived as being of medium intensity, with green, oily and herb-like floral notes. Aliphatic, acetic, isobutyric, isovaleric and valeric acids were

Table 15.3. Volatile compounds detected in aroma extracts from cured vanilla beans obtained using various organic solvents.

Compound	Ether	Pentane/ether	Pentane/dichloromethane
Phenols			
Guaiacol	*	*	*
4-Methylguaiacol		*	*
Phenol	*	*	
p-Cresol	*	*	*
4-Vinylguaiacol		*	
Vanillyl methyl ether		*	*
4-Vinylphenol	*	*	
Vanillin	*	*	*
Acetovanillone	*	*	*
Vanillyl alcohol	*	*	*
Vanilloylmethyl cetone	*	*	
p-Hydroxybenzaldehyde	*	*	*
p-Hydroxybenzyl alcohol	*	*	
Vanillic acid	*	*	*
p-Hydroxybenzoic acid	*	*	*
Aliphatic acids			
Acetic acid	*	*	*
Propanoic acid	*	*	*
Isobutyric acid	*	*	
Butyric acid	*	*	
Isovaleric acid	*	*	*
Valeric acid	*	*	*
Hexanoic acid	*	*	*
Heptanoic acid	*	*	*
Octanoic acid	*	*	*
2-Heptenoic acid	*	*	*
Nonanoic acid	*	*	*
Dodecanoic acid	*	*	
Myristic acid	*	*	
Pentadecanoic acid	*	*	
Hexadecanoic acid	*	*	*
9-Hexadecanoic acid		*	
Heptadecanoic acid	*	*	
Stearic acid	*	*	
Oleic acid	*	*	
Linoleic acid	*	*	
Aromatic acids			
Benzoic acid	*	*	
Benzene propanoic acid	*	*	*
Cinnamic acid (isomer 1)	*	*	*
Cinnamic acid (isomer 2)	*	*	*
Anisic acid	*	*	
Alcohols			
1-Octen-3ol	*	*	
2,3-Butanediol (isomer 1)	*	*	*
1-Octanol	*	*	
2,3-Butanediol (isomer 2)	*	*	*
1,2-Propanediol	*	*	
Benzyl alcohol	*	*	*
2-Phenylethanol	*	*	*
Benzene propanol	*	*	

Continued

Table 15.3. *Continued*

Compound	Ether	Pentane/ether	Pentane/dichloromethane
Anisyl alcohol	*	*	*
Cinnamyl alcohol	*	*	*
Aldehydes			
2-Heptenal	*	*	*
(*E*)-2-Decenal		*	*
(*E*,*Z*)-2,4-Decadienal	*	*	*
(*E*,*E*)-2,4-Decadienal	*	*	*
Esters			
Methyl salycilate		*	
Methyl cinnamate		*	
Anisyl formate		*	
Ethyl linolenate	*	*	*
Hydrocarbons			
Tricosane		*	*
Pentacosane		*	*
Heterocyclics			
Furfural	*	*	*
γ-Butyrolactone	*	*	*
Pantolactone		*	*
1H-pyrrole-2,5-dione, ethyl-4-methyl	*	*	*
Ketone			
3-Hydroxy-2-butanone	*	*	*

Souce: Silva *et al*. (2006).

perceived by the panellists as having sour, buttery and oily notes.

In the overall vanilla aroma, minor compounds like *p*-cresol, creosol, guaiacol and 2-phenylethanol have a high impact. This is shown by GC-olfactometry analysis of cured vanilla beans. Dignum *et al*. (2004) investigated the presence of β-D-glucosides of these compounds in order to determine whether these compounds were derived from glucosides or if they were formed during the curing process via different pathways. Glucosides of vanillin, vanillic acid, *p*-hydroxybenzaldehyde, vanillyl alcohol, *p*-cresol, creosol and bis[4-(β-D-glucopyranosyloxy)-benzyl]-2-isopropyltartrate and bis[4-(β-D-glucopyranosyloxy)-benzyl]-2-(2-butyl)tartrate have been identified in a green bean extract. Glucosides of 2-phenylethanol and *p*-cresol were not hydrolysed. β-Glucosidase does not have a high substrate specificity for the naturally occurring glucosides compared with the synthetic *p*-nitrophenol glucoside.

Werkhoff and Güntert (1997) reported for the first time the identification of some ester components in Bourbon vanilla beans – pentyl salicylate and citronellyl isobutyrate – as constituents of food aroma or essential oils (Table 15.6).

Adedeji *et al*. (1993) used a direct thermal desorption technique (220°C) to analyse the volatiles from beans that might cause the thermal degradation and transformation of sugar into common volatile compounds such as 3,5-dimethyl-2,4(3H,5H)-furandione and 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one. This last compound was detected at a high concentration (3880 ppm) in Mexican vanilla, being the third most abundant compound after vanillin and 2-furfural, and far more abundant than vanillic acid, *p*-hydroxybenzaldehyde or *p*-hydroxybenzoic acid.

Vanillin structure and properties

The compound predominantly responsible for the characteristic flavour and smell of vanilla is vanillin (4-hydroxy-3-methoxybenzaldehyde, MW 152.14), which is an aromatic aldehyde (Fig. 15.1). The fermented fruit

Table 15.4. Concentrations of volatile compounds in pentane/ether extract from cured vanilla beans.

Compound (quantified HPLC)	Concentration, ppm (mg/kg of cured vanilla)	Compound (quantified by HPLC)	Concentration, ppm (mg/kg of cured vanilla)
Guaiacol	9.3	Benzene propanoic acid	3.9
4-Methylguaiacol	3.8	Cinnamic acid (isomer 1)	3.4
Phenol	1.8	Cinnamic acid (isomer 2)	9.5
p-Cresol	2.6	Anisic acid	
4-Vinylguaiacol	1.2	*Alcohols*	
Vanillyl methyl ether	< 1	1-Octen-3ol	< 1
4-Vinyl phenol	1.8	2,3-Butanediol (isomer 1)	16.5
Vanillin	19,118	1-Octanol	1.1
Acetovanillone	13.7	2,3-Butanediol (isomer 2)	8.0
Vanillyl alcohol	83.8	1,2-Propanediol	< 1
Vanilloyl-methyl cetone	2.2	Benzyl alcohol	2.7
p-Hydroxybenzaldehyde	873.3	2-Phenylethanol	1.0
p-Hydroxybenzyl alcohol	65.1	Benzene propanol	< 1
Vanillic acid	1315	Anisyl alcohol	2.4
p-Hydroxybenzoic acid	255	Cinnamyl alcohol	< 1
Aliphatic acids		*Aldehydes*	
Acetic acid	124.3	2-Heptenal	2.1
Propanoic acid	1.7	(*E*)-2-Decenal	1.8
Isobutyric acid	1.7	(*E*,*Z*)-2,4-Decadienal	1.4
Butyric acid	< 1	(*E*,*E*)-2,4-Decadienal	1.2
Isovaleric acid	3.8	*Esters*	
Valeric acid	1.5	Methyl salycilate	< 1
Hexanoic acid	< 1	Methyl cinnamate	1.1
Heptanoic acid	1.9	Anisyl formate	2.3
Octanoic acid	5.5	Ethyl linolenate	13.5
2-Heptenoic acid	1.7	*Hydrocarbons*	
Nonanoic acid	15.7	Tricosane	15.9
Dodecanoic acid	2.2	Pentacosane	19.9
Myristic acid	12.4	*Heterocyclics*	
Pentadecanoic acid	13.4	Furfural	< 1
Hexadecanoic acid	126.6	γ-Butyrolactone	< 1
9-Hexadecanoic acid	5.7	Pantolactone	1.4
Heptadecanoic acid	5.7	1H-pyrrole-2,5-dione, ethyl-4-methyl	1.8
Stearic acid	13.9		
Oleic acid	16.3	*Ketone*	
Linoleic acid	225.6	3-Hydroxy-2-butanone	14.6
Aromatic acids			
Benzoic acid	2.6		

Source: Silva *et al.* (2006).

contains about 2% vanillin, depending on provenance (Mexico 1.75%, Sri Lanka 1.50% and Indonesia 2.75%). Vanillin is also found in gum benzoin, Peru balsam and clove oil. In clove bud oil, vanillin probably originates by air-oxidation of eugenol (Guenther, 1982).

According to Gildemeister and Hoffmann (1899), vanillin crystallizes from hot water in the form of colourless needles at 81–82°C. It possesses the strong and intensely sweet odour characteristic of vanilla. On careful heating, vanillin can be sublimated without decomposition; by prolonged heating at 105°C, vanillin decomposes with the formation of non-volatile products.

Vanillin is readily soluble in alcohol, ether, chloroform and hot water; relatively insoluble in cold water, for which reason vanillin can be recrystallized from water. At 75–80°C, one part of vanillin dissolves

Table 15.5. Aroma-active compounds detected by GC-O analysis of an aroma extract from cured vanilla beans.

Compound	Concentration, ppm	Odour quality	Intensity[a]
Phenols			
Guaiacol	9.3	Chemical, sweet spicy	+ + +
4-Methylguaiacol	3.8	Sweet, woody	+ + +
p-Cresol	2.6	Balsamic, woody, spicy	++
4-Vinylguaiacol	1.2	Chemical, phenolic	+
4-Vinylphenol	1.8	Sweet, woody	++
Vanillin	19,118	Vanilla, sweet	+ + +
Acetovanillone	13.7	Vanilla, sweet, honey	+ + +
Vanillyl alcohol	83.8	Vanilla-like	+ + +
p-Hydroxybenzaldehyde	873	Vanilla-like, biscuit	++
p-Hydroxybenzyl alcohol	65.1	Vanilla-like, sweet	++
Aliphatic acids			
Acetic acid	124	Sour, vinegar	++
Isobutyric acid	1.7	Buttery	++
Butyric acid	< 1	Buttery, oily	+
Isovaleric acid	3.8	Buttery, oily	++
Valeric acid	1.5	Cheese	+ + +
Alcohols			
2,3-Butanediol (isomer 2)	8.0	Floral, oily	+
Anisyl alcohol	2.4	Herbal	++
Aldehydes			
2-Heptenal	2.1	Green, oily	+
(*E*)-2-Decenal	1.8	Herb-like, floral	++
(*E*,*Z*)-2,4-Decadienal	1.4	Herb-like, fresh	++
(*E*,*E*)-2,4-Decadienal	1.2	Fatty, wood	++
Esters			
Methyl salicylate	< 1	Chalk	+ + +
Methyl cinnamate	1.1	Sweet	++
Ethyl linolenate	13.5	Sweet	++
Ketones			
3-Hydroxy-2-butanone	14.6	Buttery	+
Unknown[b]	6.2	Vanilla-like, chemical	+ + +

Note: [a](+) Weak, (++) medium, (+ + +) strong.
Source: Silva *et al.* (2006).

in 20 parts of water, at 14°C in 90–100 parts of water. At 7–8°C, most of the vanillin will crystallize from the solution gradually. Vanillin is soluble in sodium carbonate solution, but not in sodium bicarbonate solution (Guenther and Althausen, 1978).

About 170 other volatile constituents, mostly present at below 1 ppm levels, have been reported in vanilla by Klimes and Lamparsky (1976). Besides vanillin (85% of total volatiles), other important aroma components are glucovanillin, anisic acid, aldehyde, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde (up to 9%), vanillic acid, *p*-hydroxybenzyl alcohol, vanillyl alcohol and *p*-hydroxybenzyl methyl ether (1%), phenols, lactones, furans and esters (Uhl, 2000.)

The characteristic fragrance of Tahiti vanilla is due to the presence of piperonal (heliotropin, 3,4-dioxymethylenbenzaldehyde) and diacetyl (butandione). Piperonal is an aromatic aldehyde with a floral odour and is used in flavouring and perfumes. Separation of a fragrant 5-piperidone compound, containing three methyl groups, and also of methylbenzoate from Bourbon vanilla bean extract was achieved by liquid chromatography and gas-liquid partition

Table 15.6. Newly identified volatile ester components in Bourbon vanilla beans and their sensory impressions.

Component	Sensory impression
Hexyl butanoate	Fruity, green, sweet
Butyl hexanoate	Heavy-fruity, sweet
Fenchyl acetate	Fir needle oil, sweet, conifer-like
Menthyl acetate	Minty, woody-herbaceous, fruity, floral
α-Terpinyl acetate	Herbaceous, sweet, spicy, bergamot, lavender
Bornyl acetate	Herbaceous-piney, sweet, balsamic, camphory
Isobornyl acetate	Balsamic-camphoraceous, pine needle-like
Linalyl acetate	Sweet, floral-fruity, bergamot-like, lavender-like
Citronellyl isobutyrate	Fruity-rosy, sweet, bergamot-like
Phenethyl formate	Green-herbaceous, floral
Anisyl formate	Herbaceous-green, sweet, spicy, vanilla-like
Ethyl salicylate	Sweet, floral-fruity, heavy-fruity
Pentyl salicylate isoamyl	Floral, green, oil of winter-green, rose oxide-like
Salicylate hexyl	Sweet, clover-like
Salicylate	Sweet-herbaceous, floral, green, spicy

Source: Werkhoff and Güntert (1997).

chromatography, respectively (Feyertag and Hutchins, 1981).

The molecular structures of vanillin (4-hydroxy-3-methoxybenzaldehyde), isovanillin (3-hydroxy-4-methoxybenzaldehyde) and ethylvanillin(3-ethoxy-4-hydroxybenzaldehyde) were determined by Egawa *et al.* (2006) by means of gas electron diffraction. Among them, vanillin and ethylvanillin have a vanilla odour but isovanillin smells different. Vanillin and isovanillin have two stable conformers and ethylvanillin has four.

Non-volatiles

Oleoresin

Vanilla is available in three physical forms, whole beans, splits and cuts. Vanilla powder is a mixture of ground vanilla in a carrier such as 30% sugar (Purseglove *et al.*, 1981). Vanilla extract is made by cutting the cured beans into small pieces and percolating in successive quantities of hot 65–70% alcohol. The extract is very concentrated, a few drops sufficing for most uses. Vanilla oleoresin involves solvent extraction of chopped beans and later evaporation of the vanilla extract under vacuum, leaving a dark, viscous mass (Cowley, 1973). The oleoresin is diluted with solvents to give one- two- or 10-fold strengths (Uhl, 2000). Vanilla absolute and tincture are very concentrated ethanol or benzene extracts of vanilla aroma, used for perfumery purposes.

Vanilla essence comes in two forms: the actual extract of the seedpods and the far cheaper synthetic essence, basically consisting of a solution of synthetic vanillin in ethanol. Natural vanilla is an extremely complicated mixture of several hundred different compounds, versus synthetic vanillin which is derived from phenol and is of high purity. Many commercial vanilla extracts are now actually blends of natural and synthetic vanillin. The occurrence of several non-vanillin aroma and flavour components in minor or trace amounts in beans is the reason for their organoleptic superiority over synthetic vanilla and blends. Natural vanilla has a delicate, rich and mellow aroma and aftertaste, while the synthetic material is quite heavy, grassy and less pleasant.

Vanillin synthesis

Although more than 12,000t of vanillin is produced each year, less than 1% of this is natural vanillin from *Vanilla*; the remainder is synthesized much more cheaply via chemical processes.

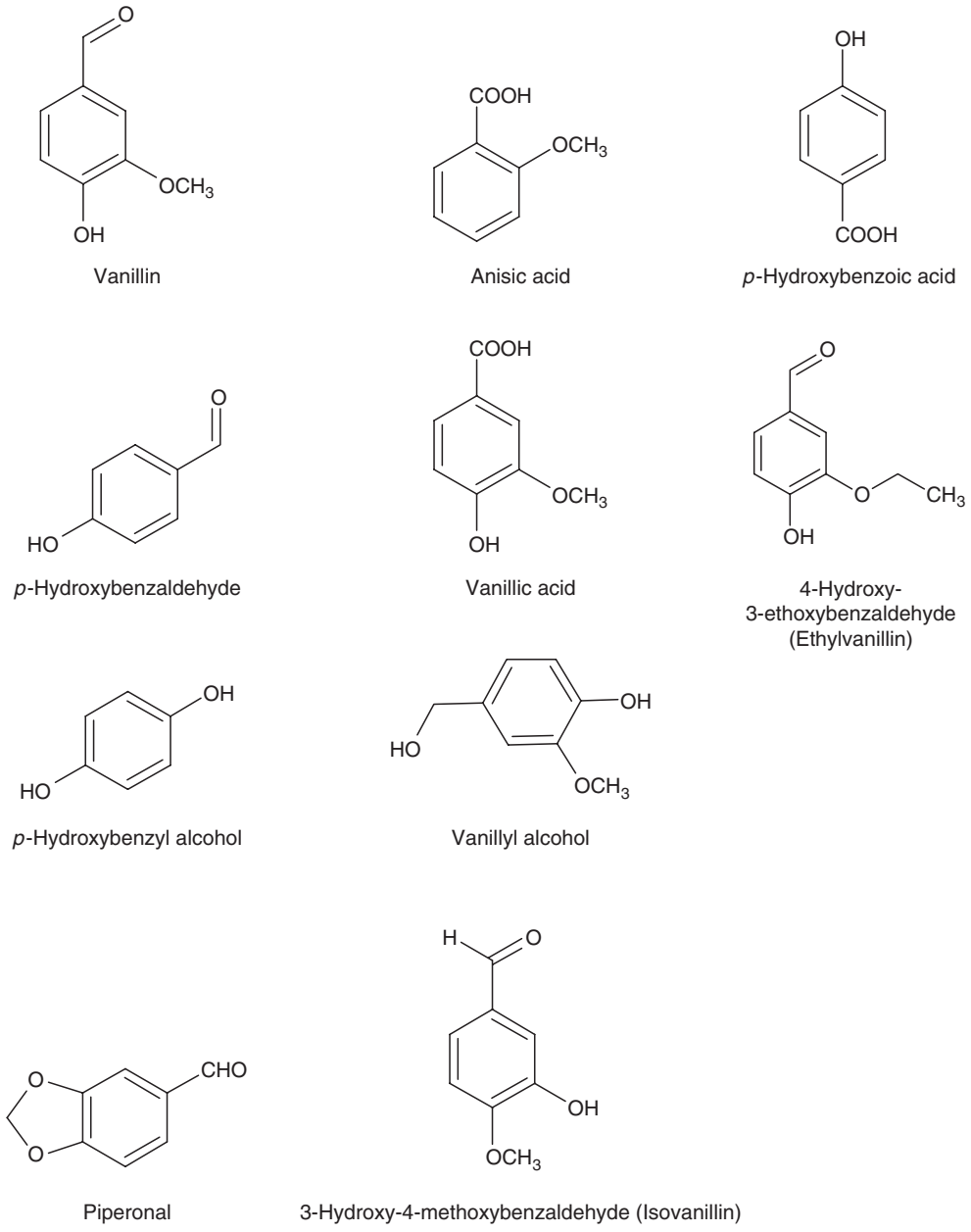

Fig. 15.1. Volatile compounds of vanilla.

The 'classical' synthesis of vanillin from eugenol or isoeugenol was developed in 1896 and it remained the preferred method for about 50 years. Vanillin is now prepared industrially in large amounts by the Reimer–Tiemann reaction, starting with guaiacol (catechol monomethylether), from which it is formed along with *o*-vanillin. Another source of vanillin is lignin, a by-product of paper pulp manufacture. Bioconversion of ferulic acid to vanillin is also possible by liquid cultures of various

fungi, which may be an economical route as well.

The value of vanillin extracted from *Vanilla* pods is calculated variously as being between US$1200/kg and US$4000/kg, in contrast to the price of synthetic vanillin, <US$15/kg (Muheim and Lerch, 1999). Synthetic vanillin is used in both food and non-food applications, in fragrances and as a flavouring in pharmaceutical preparations. Currently, approximately 50% of the worldwide production of synthetic vanillin is used as an intermediate in the chemical and pharmaceutical industries for the production of herbicides, antifoaming agents or drugs such as papaverine, L-dopa, L-methyldopa and the antimicrobial agent, trimethoprim (Hocking, 1997). Synthetic vanillin is also used in household products, such as air fresheners and floor polishes.

Biosynthesis

Biosynthesis of vanillin has been attempted by various workers, as reviewed by Walton *et al.* (2003).

In 1965, Zenk, using radioactively labelled ferulic and vanillic acids, proposed a route by which both vanillin and vanillic acid were derived from ferulic acid. A CoA-dependent β-oxidative cleavage of feruloyl-CoA led to the formation of vanilloyl-CoA, which was further reduced to vanillin, or alternatively deacylated to vanillic acid (Fig. 15.2). The addition of ferulic acid to callus cultures or tissue cultures resulted in increased levels of vanillin production, suggesting that ferulic acid might indeed be a precursor of vanillin (Romagnoli and Knorr, 1988; Labuda *et al.*, 1993).

A more complex pathway was proposed by Funk and Brodelius (1990a,b, 1992), where caffeic acid was first methylated at the 4-position, followed by the 3-position to produce 3,4-dimethoxycinnamic acid, which was then demethylated at the 4-position, prior to a glucosylation step. Side-chain cleavage was proposed to occur at a late stage to produce vanillic acid (or its β-D-glucoside), which was then reduced to vanillin (Fig. 15.3).

Enzyme preparations of β-glucosidase have been used to release vanillin from vanilla pods as an alternative to conventional curing (Dignum *et al.*, 2001; Ruiz *et al.*, 2001).

A new model (Fig. 15.4) for hydroxycinnamate chain-shortening and vanillin formation in plants was revealed with the isolation of 4-hydroxycinnamoyl-CoA hydratase/lyase (HCHL) and its gene from a soil bacterium, *Pseudomonas fluorescens* strain AN103, which had been isolated by growth on ferulic acid as a sole carbon source (Gasson *et al.*, 1998; Narbad and Gasson, 1998; Mitra *et al.*, 1999).

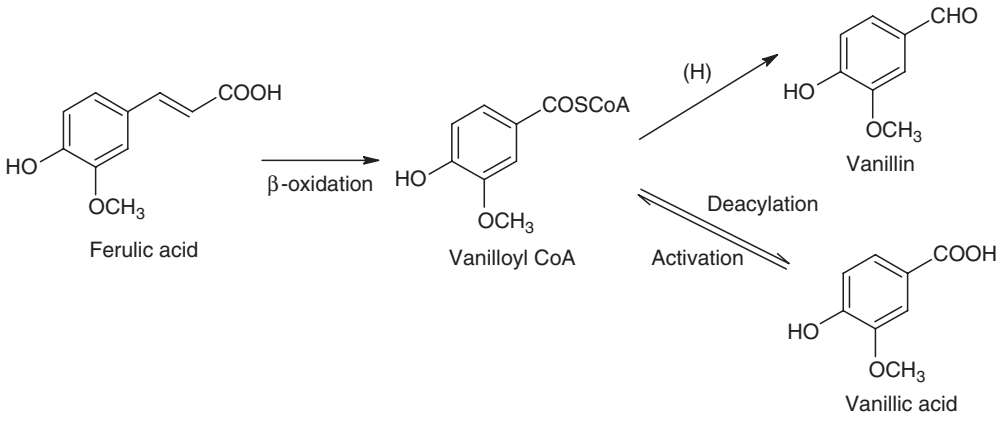

Fig. 15.2. Vanillin biosynthesis (Zenk, 1965).

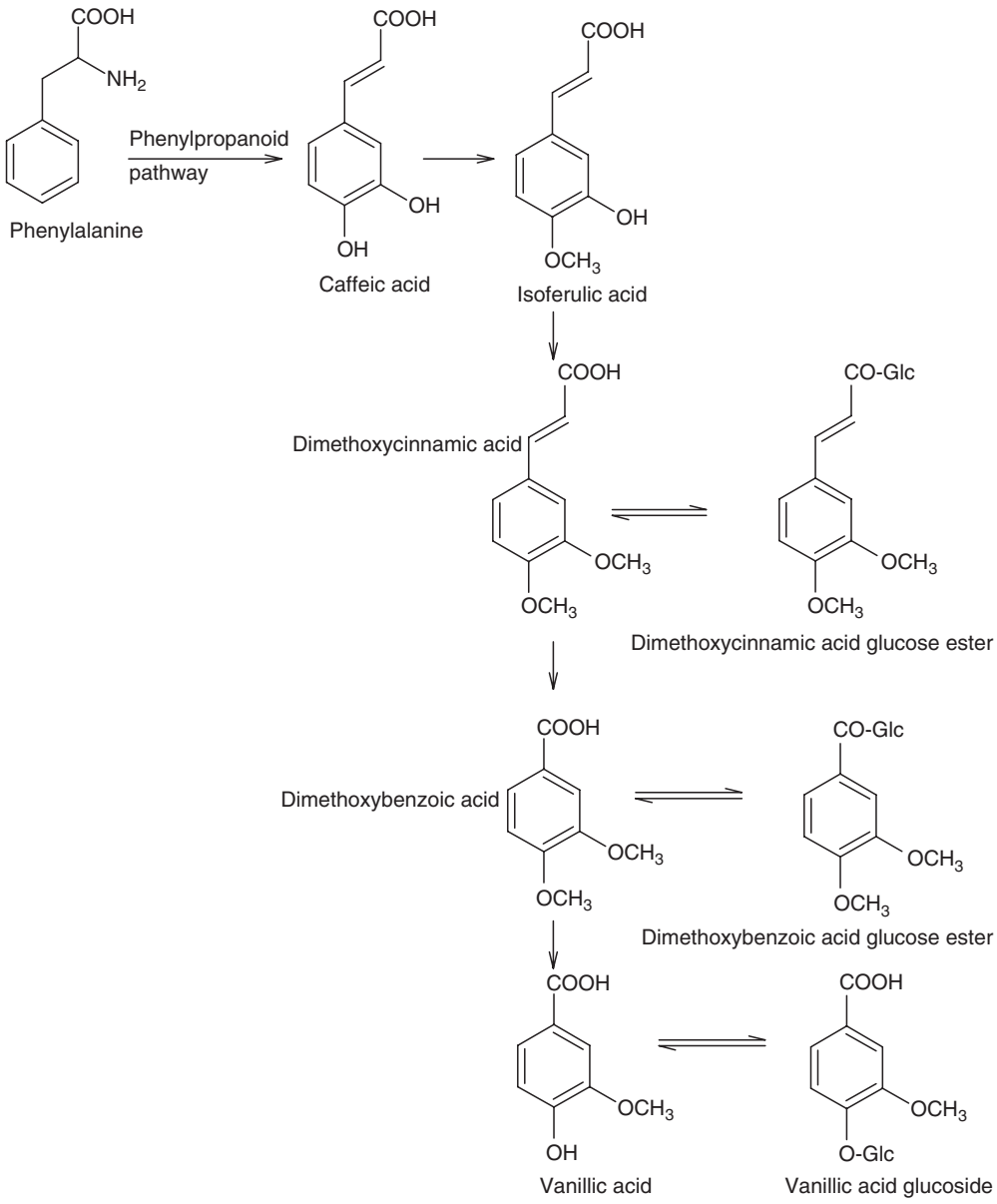

Fig. 15.3. A proposed route to vanillic acid via isoferulic acid (Funk and Brodelius, 1990a,b, 1992).

The biosynthetic origins of vanillin can be determined by the analysis of naturally occurring isotope ratios (in practice, chiefly $^{2}H/^{1}H$ and $^{13}C/^{12}C$), using isotope ratio-mass spectrometry (IR-MS) and nuclear magnetic resonance (site-specific natural isotope fractionation: SNIF-NMR®). Isotopic ratio characteristics are influenced by the biosynthetic route, as well as by the environmental (including climatic) conditions under which biosynthesis occurs (Martin *et al.*, 1992; Jamin *et al.*, 1997; Martin, 1998). The extent of the overall incorporation of naturally occurring ^{13}C, denoted by the $\delta_{PDB}{}^{13}C$

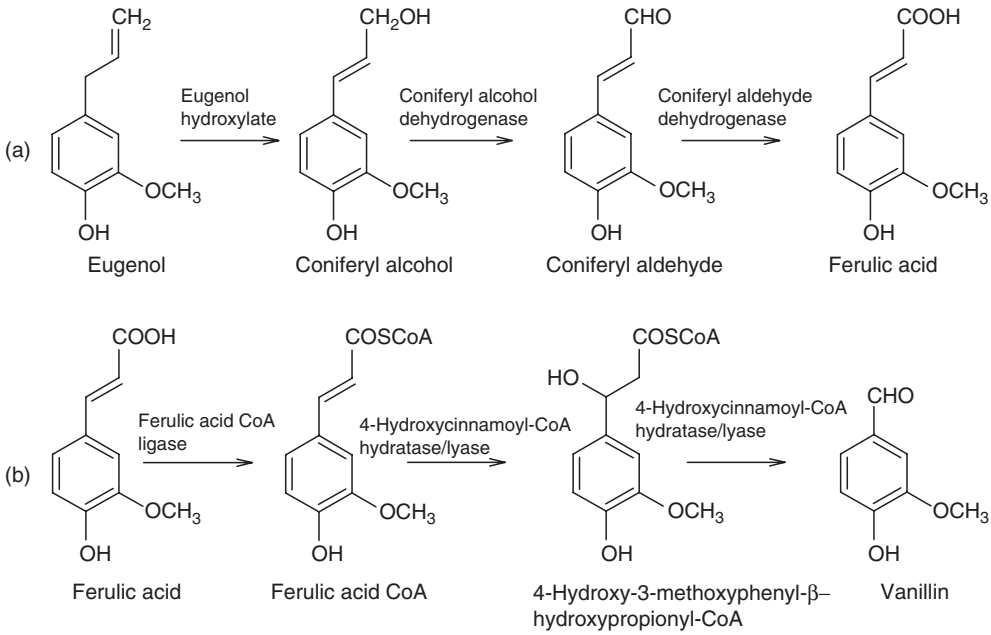

Fig. 15.4. The routes (a) from eugenol to ferulic acid and (b) from ferulic acid to vanillin in *Pseudomonas* strains (Gasson *et al.,* 1998; Mitra *et al.,* 1999).

(delta Pee Dee Belemnite) value, for vanillin and 4-hydroxybenzaldehyde samples isolated from *Vanilla* spp. falls within a characteristic range (around –21.0 for vanillin) that reflects the crassulacean acid metabolism (CAM) pathway of photosynthesis by which *Vanilla* fixes CO_2 (Lamprecht *et al.*, 1994; Remaud *et al.*, 1997). This is quite different from values determined for samples of vanillin that have been produced from the degradation of lignin, or chemically from fossil fuel sources, where CO_2 is not fixed originally by CAM metabolism (between *c.* –25 and –37). Vanillin samples produced by other means, for example by microbial fermentation or by metabolic engineering in plants (or microbes), or enzymatically, will display $\delta_{PDB}{}^{13}C$ values that will reflect the mechanism of the pathway involved.

Biotransformation

It is possible to generate vanillin from other plant-derived materials by biotransformation.

- Isorhapontin, a monoglucosylated stilbene constituent of spruce bark, can be cleaved by a dioxygenase isolated from *Pseudomonas* strain TMY1009 (Kamoda *et al.*, 1989).
- Soybean lipoxygenase can produce vanillin from esters of coniferyl alcohol (Markus *et al.*, 1992).
- van den Heuvel *et al.* (2001) used a broad-specificity *Penicillium* flavoenzyme, vanillyl alcohol oxidase, to produce vanillin by the biotransformation of vanillylamine (obtainable by the hydrolysis of capsaicin) and of creosol (a major component of creosote obtained from heating wood or coal tar).

Yoshida *et al.* (1997) achieved the production of vanillin by oxidation of vanillylamine using amine oxidase (AO) from *Aspergillus niger* and monoamine oxidase (MAO) from *Escherichia coli*. Enzyme kinetic studies have revealed that AO is

a more efficient producer of vanillin than MAO. Continuous production of vanillin with immobilized AO was also investigated, and industrial synthesis of vanillin through AO from *A. niger* was suggested as a possibility by the authors. Such approaches are, in principle, attractive since the technologies are reproducible, predictable and acceptable and, given adequate demand, scale-up and stability would also be cost effective.

Tripathi *et al.* (2002) studied the biotransformation of phenylpropanoid intermediates – ferulic acid, coniferyl aldehyde and *p*-coumaric acid in free and immobilized cell cultures of *Haematococcus pluvialis*, which accumulated vanilla flavour metabolites – vanillin, vanillic acid, vanillyl alcohol, protocatechuic acid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde and *p*-coumaric acid when treated with these precursors, to a range corresponding to vanilla flavour metabolites.

Tissue cultures

Tissue or organ cultures of *Vanilla* to produce vanillin and related flavour compounds have been explored by Knorr *et al.* (1993), Rao and Ravishankar (2000b), Dignum *et al.* (2001) and Priefert *et al.* (2001). Such cultures have the potential to produce *c.* 200 compounds that reportedly are present in (cured) vanilla pods; *Vanilla* cells and organs, and cells of *Capsicum frutescens* (Rao and Ravishankar, 2000a), have been cultured and successfully demonstrated to accumulate vanillin and associated metabolites (vanillic acid and ferulic acid), but production is low. Rao and Ravishankar (1999) reported that suspended and immobilized cell cultures of *C. frutescens* accumulated vanilla (and capsaicin) flavour metabolites when fed with isoeugenol. The addition of β-cyclodextrin and isoeugenol increased the accumulation of vanillin. Isoeugenol-treated immobilized cells, when challenged with aqueous mycelial extract of *A. niger*, yielded maximum vanillin concentrations, whereas the addition of a medium filtrate of *A. niger* led to a marginal increase in the vanillin.

A novel process for producing natural vanillin flavour from ferulic acid precursor has been developed by Westcott *et al.* (1993) using vanilla plant aerial roots as the biocatalyst. The charcoal used in the process acts as a product reservoir for the vanillin produced, thus relieving possible product inhibition and/or further metabolism. The vanillin is then removed from the charcoal by selective solvent extraction. The remaining unreacted ferulic acid remains adsorbed to the charcoal and can be recycled for further reaction. The aerial root tissue can be reused several times, but its activity gradually declines with reuse. Vanillin productivities of 400 mg/kg dry weight tissue/day and concentrations of 7 g/kg of root tissue can be obtained regularly. This concentration is *c.* 35-fold greater than the concentrations of vanillin originally present in the aerial root tissue and is about 40% of that present in matured vanilla beans. Using aerial roots supplied with ferulic acid, vanillin is produced five to ten times faster than its normal synthesis in vanilla beans, or in aerial roots not supplied with precursor. The composition of the vanilla flavour produced using the aerial root method is comparatively close to that of vanilla beans; in particular, it contains *p*-hydroxybenzaldehyde, at a vanillin:pHB ratio of 7.8:1, as compared with a ratio of 12.8:1 for bean-derived vanilla. This may impart a superior organoleptic value and make the product of this aerial root process more valuable.

Metabolic engineering

An innovative approach towards an enhanced capacity for vanillin formation would be to introduce an enzyme or pathway to generate vanillin from a mainstream intermediate of the plant phenylpropanoid pathway. The isolation of the gene encoding the bacterial vanillin-forming enzyme HCHL (detailed earlier) raised this possibility (Gasson *et al.*, 1998). In principle, feruloyl-CoA, an intermediate of the plant monolignol pathway (Whetten and Sederoff, 1995), could be converted

directly to vanillin and acetyl-CoA in a single step.

In summary, of the alternatives available for introducing a pathway of vanillin production *de novo,* or for enhancing vanillin production in *Vanilla*, HCHL presents the most attractive option of generating vanillin from a phenylpropanoid precursor (feruloyl-CoA) naturally present in plants (Whetten and Sederoff, 1995).

Qualitative and quantitative analysis of vanilla

Waliszewski *et al.* (2007b) described a simple and rapid HPLC technique for vanillin determination in alcohol vanilla extract, and the method has been applied successfully for the determination of vanillin in some commercial extracts for routine analysis. de Jager *et al.* (2007) developed a LC-MS method for the determination of vanillin, coumarin and ethyl vanillin in vanilla products using LC-electrospray ionization in the positive ionization mode. The limits of detection for the method ranged from 0.051 to 0.073 μg/ml.

Bettazzi *et al.* (2006) developed a disposable electrochemical sensor for the detection of vanillin in vanilla extracts and in commercial products. An analytical procedure based on square-wave voltammetry (SWV) was optimized and a detection limit of 0.4 μM for vanillin was found. The method was applied to the determination of vanillin in natural concentrated vanilla extracts and in final products such as yoghurt and compote. The results obtained with electrochemical quantification of vanillin in the extract samples correlated well with the HPLC results.

It has been shown by Tenailleau *et al.* (2004) that the $^{13}C/^{12}C$ ratio can be used to determine the origin of vanillin by quantitative measurements of the ^{13}C NMR signals for each of the eight C atoms. Thus, attempts to substitute cheaper synthetic vanillin fraudulently can be detected even when ^{13}C-substituted materials are used.

Boyce *et al.* (2003) reported the use of the mixed micellar electrokinetic capillary chromatography (MECC) method for the qualitative and quantitative determination of key components, including vanillin, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, vanillic acid and 3-methoxybenzaldehyde in natural vanilla extracts, nature identical extracts and synthetic flavourings. The limits of detection (LOD) ranged between 5–10 μg/ml. Bütehorn and Pyell (1996) had earlier demonstrated the potential use of micellar elektrokinetic chromatography (MEKC) in food analysis and a rapid method for the determination of vanillin and related compounds and possible synthetic additives to vanilla flavourings by MEKC as a screening method for quality control.

15.5. Culinary and Medicinal Uses

There are three main commercial forms of natural vanilla:

- Whole bean
- Powder
- Extract (alcoholic solution; as per Food and Drug Administration requirements, at least 35% vol. of alcohol).

Culinary uses

Vanilla has been coveted over the ages for culinary and medicinal reasons alike. While traditional medical uses of vanilla have faded away, its culinary traditions have changed little (Bythrow, 2005). Vanilla's high status in the culinary world comes from a long history of flavouring and its mellow fragrance enhances a variety of sweets and desserts, such as chocolates, custards, creams, soufflés, liqueurs, ice cream, sugar cookies, puff pastries and butter creams; its usage in salty foods is very uncommon.

Vanilla, being native to Central America and having a long record of pre-Columbian usage, was used by both the Mayas and, later, the Aztecs to flavour a special drink prepared from water, cocoa beans and spices: *chacau haa* (or *chocol haa*) in the Mayan and *cacahuatl* in the Aztec tongue (Náhuatl). Mayan chocolate, as still drunk

in southern Mexico (Yucatán), Guatemala and Belize, is often spicy, containing chillies and other spices, native (allspice, annatto) or imported (black pepper, cinnamon).

Vanilla was first used in Europe, mainly for the same purpose as earlier in America, to flavour drinking chocolate, a very popular drink among the 17th century European nobility. European drinking chocolate was almost exclusively sweet and might have used a lot of additional flavourings, e.g. anise, cinnamon, but also exotic animal products like musk and ambergris.

Vanilla flavour in foodstuffs may be achieved by adding vanilla essence or by cooking vanilla beans in the liquid preparation. A stronger aroma may be attained if the beans are split in two; in this case, the innards of the beans (the aroma-filled seeds – the tiny black grains) are mixed into the preparation. Natural vanilla gives a brownish to yellowish colour to preparations, depending on concentration. Good-quality vanilla has a strong aromatic flavour, but foodstuffs with small amounts of low-quality vanilla or artificial vanilla-like flavourings are far more common, since true vanilla is much more expensive. Methyl, as well as ethyl, vanillin is used by the food industry; the latter is more expensive and has a stronger note.

Medicinal properties

From the time of the Aztecs, vanilla was considered an aphrodisiac. This reputation was much enhanced in 1762 when a German study found that a medication based on vanilla extract cured impotence. It was also once believed that vanilla was a febrifuge, i.e. used to reduce fevers, though it is used rarely for any medicinal purposes other than as a pharmaceutical flavouring. Essential oil of vanilla and vanillin were and are sometimes used in aromatherapy.

Antimicrobial property

In common with many other low-molecular weight phenolic compounds, vanillin displays antioxidant and antimicrobial properties and hence has the potential for use as a food preservative (Burri *et al.*, 1989; Davidson and Naidu, 2000). It is active against both Gram-positive and Gram-negative food-spoilage bacteria and has been shown to be effective against both yeasts and moulds in fruit purées and laboratory growth media (Cerrutti *et al.*, 1997; López-Malo *et al.*, 1998; Fitzgerald *et al.*, 2003). When used at a concentration of ~ 13 mM, vanillin inhibited the growth of *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Debaryomyces hansenii* and *Z. rouxii* in culture medium and apple purée for 40 days. However, vanillin was less effective in banana purée, where ~ 20 mM was insufficient to inhibit the growth of *Z. bailii*; the authors concluded that the higher lipid/protein levels in bananas interfered with vanillin's antimicrobial activity (Cerrutti and Alzamora, 1996). The inhibition was biostatic in nature. During fermentation, the bioconversion of sub-MIC levels of vanillin to vanillyl alcohol and low levels of vanillic acid were demonstrated in the culture medium, presumably catalysed by constitutively expressed, non-specific dehydrogenases, neither of which was antagonistic to yeast cell growth (Fig. 15.5).

The results indicate the importance of the aldehyde moiety in the vanillin structure for its antimicrobial activity and that the bioconversion of vanillin could be advantageous for the yeasts, but only at levels below MIC. It was observed that increased vanillin concentrations inhibited its own bioconversion, suggesting that the activity required intact cells with metabolic capacity.

One limitation is the strong flavour of vanillin at the minimal inhibitory concentrations required, but this may be partially overcome by using it in combination with other, synergistic, antimicrobials, thus lowering the effective concentrations that are necessary (Gould, 1996).

Studies by López-Malo *et al.* (1995, 1997) showed the incorporation of vanillin (~ 3–7 mM) into fruit-based agars (apple, banana, mango, papaya and pineapple) inhibited the growth of *Aspergillus flavus*, *A. niger*, *A. ochraceus* and *A. parasiticus* for 2 months. Furthermore, synergistic effects

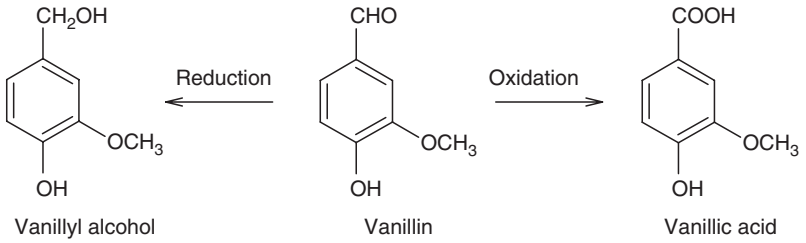

Fig. 15.5. The bioconversion pathway of vanillin in *Saccharomyces cerevisiae*.

were observed when vanillin and potassium sorbate were used in combination. Matamoros-León *et al*. (1999) established that, with a slight reduction in pH and water activity (a_w), ~ 3 mM vanillin in combination with ~ 2 mM potassium sorbate could inhibit the growth of *Penicillium digitatum*, *P. glabrum* and *P. italicum* for 1 month.

Antioxidant property

Vanillin has been reported to act as an antioxidant in complex foods containing polyunsaturated fatty acids (Burri *et al*., 1989).

Antigenotoxic effect

There is some evidence for the antimutagenic effects of vanillin; for example, in suppressing chromosomal damage induced by methotrexate in the Chinese hamster V79 cell line (Keshava *et al*., 1998). Inouye *et al*. (1988) reported the suppression of the induction of micronuclei by mitomycin C (MMC) in mouse bone marrow cells by post-treatment with vanillin. Post-treatment with vanillin at 500 mg/kg caused about 50% decrease in the frequency of micronucleated polychromatic erythrocytes (MN-PCEs). The suppressant effect was not due to a delay in the formation of MN-PCEs but to the cytotoxic action of vanillin. Vanillin acts as an anticlastogenic factor *in vivo*.

However, a study by Salih (2006) demonstrated that when a food additive was present in *E. coli* cell suspension during sunlight exposure, the number of induced mutations increased to varying extents over that seen with sunlight alone. Vanilla produced mutations in an additive fashion, while flavoured colourants like raspberry and peach increased the number of mutations in a dose-dependent manner. The impact of this investigation reflects the significance of the combination of sunlight and chemical food additives as a potential risk, which requires special attention and necessitates further investigations to evaluate this risk.

15.6. International Specifications for Quality and Desirable Limits

Everything expensive gets adulterated and faked – vanilla is no exception. Synthetic vanillin is an obvious choice to 'spice up' beans of low quality, or beans that have been extracted to yield the expensive vanilla extract (obtained by macerating vanilla pods in a mixture of water and alcohol). Synthetic vanillin could appear in the extract itself. Tonka bean extract features regularly in vanilla extract, especially in Mexico.

A high-performance liquid chromatographic procedure was developed for the isolation and quantitation of coumarin from vanilla-based liquid flavourings of Mexican origin by Thompson and Hoffmann (1988) in 40 products representing 14 different Mexican brands, which were assayed for coumarin, vanillin and ethyl vanillin. The procedure has been adapted to the analysis of other products including domestic vanilla extracts and imitation vanilla flavourings for 37 compounds, including vanillin, ethyl vanillin, 4-hydroxybenzaldehyde and piperonal.

The quality of the cured vanilla beans depends on a whole range of factors, starting

from the agroclimatic condition during cultivation, through raw material production to curing. According to the ISO 3493 International Standards for vanilla, four commercial forms have been established:

1. Vanilla pods, consisting of whole pods which may be split.
2. Cut vanilla, consisting of parts of pods, split or not, and deliberately cut or broken.
3. Vanilla in bulk, consisting of vanilla in pods and cut vanilla.
4. Vanilla powder; obtained by grinding vanilla pods with permitted additives after drying.

Whole vanilla

Vanilla pods

The general characteristics desired for vanilla pods are that they are whole, sound, supple and full, of typical flavour, of uniform chocolate brown to dark brown colour and without any other stain for the non-split and split pods. The pods must have been cured suitably to develop their flavour and contain optimal moisture content. The pods may be rimy, with a mark at the bottom one-third of their length.

Cut vanilla

Cut vanilla must be prepared from vanilla pods as specified above, be sound and of good flavour and be chocolate brown to dark brown in colour.

Vanilla in bulk

Vanilla in bulk is obtained from either the pods or cut vanilla; it must be sound and of good specific flavour, chocolate brown to dark brown in colour, generally wooded and have several large stains.

Vanilla powder

Vanilla powder is obtained from either of the above three forms and must be able to pass through a mesh of 1.25 mm, be brown to dark brown in colour and have the characteristic flavour of vanilla.

Non-split and split vanilla

Non-split and split vanilla may fall into any of the four categories, depending on their quality. Their moisture contents may be 38% for categories 1 and 2, 30% for category 3 and cut and bulk vanilla pods, 25% for category 4 and 20% for vanilla powder (ISO 5565–2).

Vanilla extract

The quality of vanilla extract is defined by Winton's analytical values (Merory, 1960) (Table 15.7). The concentration of vanillin is a major criterion, although organoleptic quality does not depend on it entirely. The various characteristic flavour notes that define vanilla are woody, pruney, resinous, leathery, floral and fruity aromatics (Gillette and Hoffman, 1992). Bourbon vanilla serves as the standard by which the chemical and sensory qualities

Table 15.7. Quality parameters for vanilla extract.

Quality factor	Minimum	Maximum	Average
Vanillin (g/100 ml extract)	0.11	0.35	0.19
Ash (g/100 ml extract)	0.220	0.432	0.319
Soluble ash (g/100 ml extract)	0.179	0.357	0.265
Lead number (Winton)	0.40	0.74	0.54
Alkalinity of total ash (N/10 acid/100 ml extract)	30.00	54.00	30.00
Alkalinity of soluble ash (N/10 acid/100 ml extract)	22.00	40.00	42.00
Total acidity (N/10 alkali/100 ml extract)	30.00	52.00	30.00
Acidity other than vanillin (N/10 alkali/100 ml extract)	14.00	42.00	

Source: Merory (1960).

of other types of vanilla can be assessed. Imitation vanilla, when spiked with vanillin, is inferior in quality compared with the natural extract, as the characteristic flavour components are missing. No modern processing technology can improve the quality of a poor bean; deterioration in quality can also result from improper curing and handling.

Specifications

The standards defined by the Food and Drug Administration of the USA for vanilla products are given below.

21CFR 169.3
Sec. 169.3 Definitions

a) The term vanilla beans mean the properly cured and dried pods of *Vanilla planifolia* Andrews and of *Vanilla tahitensis* Moore.
b) The term unit weight of beans means, in the case of vanilla beans, containing not more than 25% moisture; it means the weight of such beans equivalent in content of moisture-free vanilla-bean solids to 13.35 ounces of vanilla beans containing 25% moisture.
c) The term unit of vanilla constituent means the total sapid and odorous principles extractable from one unit weight of vanilla beans, as defined in paragraph (b) of this section, by an aqueous alcohol solution in which the content of ethyl alcohol by volume amounts to not less than 35%.

21CFR 169.175
Sec. 169.175 Vanilla extract

a) Vanilla extract is the solution in aqueous ethyl alcohol of the sapid and odorous principles extractable from vanilla beans. In vanilla extract, the content of ethyl alcohol is not less than 35% by volume and the content of vanilla constituent, as defined in Sec. 169.3 (c), is not less than one unit per gallon. The vanilla constituent may be extracted directly from vanilla beans or it may be added in the form of concentrated extract or concentrated vanilla flavouring or vanilla flavouring concentrated to the semi-solid form called vanilla oleoresin. Vanilla extract may contain one or more of the following optional ingredients:
   (1) Glycerin
   (2) Propylene glycol
   (3) Sugar (including invert sugar)
   (4) Dextrose
   (5) Maize syrup (including dried maize syrup).

Source: US Food and Drug Administration (2002), Code of Federal Regulations, 21CFR Part 169.

Packing and marking

Vanilla pods are to be put in packets of pods of the same length and then packaged in clean, sound, watertight containers of a material that will not affect the product in any way (e.g. tin-plate boxes). The containers must have vanilla of the same category and homogeneous. The same specifications hold good for different forms of vanilla. The packets of the above products of vanilla must contain the following information: name of the product (botanical species), commercial form, producing country, code, batch or test certificate number, or similar means of identification, any other information required by the customer, and reference to the international standard (ISO, 1997.)

15.7. Conclusion

To conclude, it is worth reiterating that the only orchid used as a spice, the vanilla pod, has been recognized for its culinary and medicinal uses since the time of the Aztecs. Vanilla is the world's third most expensive spice, from which is obtained the popular commercial flavouring agent, vanillin. The characteristic aroma of vanilla is obtained only after a time-consuming and labour-intensive curing process. The main aroma compound in vanilla is vanillin; over 100 volatile compounds have been detected, including aromatic carbonyls, aromatic

alcohols, aromatic acids, aromatic esters, phenols and phenol ethers, aliphatic alcohols, carbonyls, acids, esters and lactones. The level of the aldehydes, vanillin and *p*-hydroxybenzaldehyde and their respective acids (vanillic acid and *p*-hydroxybenzoic acid) in cured vanilla beans is used as an indicator of bean quality for commercial purposes. Vanilla's high status in the culinary world comes from a long history of flavouring chocolates, sweets and desserts. Its medicinal uses are demonstrated in its antimicrobial, antioxidant and antigenotoxic effects. The ISO 3493 International Standards for vanilla dictates the quality standards for the different forms of vanilla: vanilla pods, cut vanilla, vanilla in bulk and vanilla powder.

References

Adedeji, J., Hartman, T.G. and Ho, C. (1993) Flavor characterization of different varieties of vanilla beans. *Perfumer and Flavors* 18, 115–133.

Arana, F.E. (1943) Action of β-glucosidase in the curing of vanilla. *Food Research* 8, 343–351.

Bettazzi, F., Palchetti, I., Sisalli, S. and Mascini, M. (2006) A disposable electrochemical sensor for vanillin detection. *Analytica Chimica Acta* 555(1), 134–138.

Boyce, M.C., Haddad, P.R. and Sostaric, T. (2003) Determination of flavour components in natural vanilla extracts and synthetic flavourings by mixed micellar electrokinetic capillary chromatography. *Analytica Chimica Acta* 485(2), 179–186.

Burri, J., Graf, M. Lambelet, P. and Löliger, J. (1989) Vanillin: more than a flavouring agent – a potent antioxidant. *Journal of the Science of Food and Agriculture* 48, 49–56.

Bütehorn, U. and Pyell, U. (1996) Micellar electrokinetic chromatography as a screening method for the analysis of vanilla flavourings and vanilla extracts. *Journal of Chromatography A* 736(1–2), 321–332.

Bythrow, J.D. (2005) Historical perspective: vanilla as a medicinal plant. *Seminars in Integrative Medicine* 3(4), 129–131.

Cerrutti, P. and Alzamora, S.M. (1996) Inhibitory effects of vanillin on some food spoilage yeasts in laboratory media and fruit purées. *International Journal of Food Microbiology* 29, 379–386.

Cerrutti, P., Alzamora, S.M. and Vidales, S.L. (1997) Vanillin as an antimicrobial for producing shelf-stable strawberry purée. *Journal of Food Science* 62, 608–610.

Childers, N.E., Cibes, H.R. and Hernandez-Medina, E. (1959) Vanilla – the orchid of commerce. In: Whithner, C.L. (ed.) *The Orchids – A Scientific Survey*. Ronald Press Co., New York, pp. 477–508.

Correll, D.S. (1953) Vanilla. Its botany, history, cultivation and economic importance. *Economic Botany* 7(4), 291–358.

Cowley, E. (1973) Vanilla and its uses. In: *Proceedings of the Conference on Spices*, Tropical Products Institute, London, p. 79.

David, P.A. (1950) Vanilla culture in the College of Agriculture at Los Banos. *Phillippines Agriculture* 33, 239–349.

Davidson, P.M. and Naidu, A.S. (2000) Phyto-phenols. In: Naidu, A.S. (ed.) *Natural Food Antimicrobial Systems*. CRC Press LLC, Boca Raton, Florida, pp. 265–294.

de Jager, L.S., Perfetti, G.A. and Diachenko, G.W. (2007) Determination of coumarin, vanillin, and ethyl vanillin in vanilla extract products: liquid chromatography mass spectrometry method development and validation studies. *Journal of Chromatography A* 1145(1–2), 83–88.

DeVarigny. C. (1894) Fertilization of the vanilla flower by bees. *Bombay Natural History Society* 4, 555–556.

Dignum, M.J.W., Kerler, J. and Verpoorte, R. (2001) Vanilla production: technological, chemical, and biosynthetic aspects. *Food Review International* 17, 199–219.

Dignum, M.J.W., Kerler, J. and Verpoorte, R. (2002) Vanilla curing under laboratory conditions. *Food Chemistry* 79(2), 165–171.

Dignum, M.J.W., van der Heijden, R., Kerler, J., Winkel, C. and Verpoorte, R. (2004) Identification of glucosides in green beans of *Vanilla planifolia* Andrews and kinetics of vanilla β-glucosidase. *Food Chemistry* 85(2), 199–205.

Egawa, T., Kameyama, A. and Takeuchi, H. (2006) Structural determination of vanillin, isovanillin and ethylvanillin by means of gas electron diffraction and theoretical calculations. *Journal of Molecular Structure* 794(1–3), 92–102.

Feyertag, E. and Hutchins, R. (1981) Isolation and tentative identification of a new constituent of bourbon vanilla bean extract. *Food Chemistry* 7(4), 311–315.

Fitzgerald, D.J., Stratford, M. and Narbad, A. (2003) Analysis of the inhibition of food spoilage yeasts by vanillin. *International Journal of Food Microbiology* 23rd International Specialized Symposium on Yeasts (ISSY-23) 86(1–2), 113–122.

Funk, C. and Brodelius, P.E. (1990a) Influence of growth regulators and an elicitor on phenylpropanoid metabolism in suspension cultures of *Vanilla planifolia*. *Phytochemistry* 29(3), 845–848.

Funk, C. and Brodelius, P.E. (1990b) Phenylpropanoid metabolism in suspension cultures of *Vanilla planifolia* Andr. II. Effects of precursor feeding and metabolic inhibitors. *Plant Physiology* 94, 95–101.

Funk, C. and Brodelius, P.E. (1992) Phenylpropanoid metabolism in suspension cultures of *Vanilla planifolia* Andr. IV. Induction of vanillic acid formation. *Plant Physiology* 99, 256–262.

Galletto, W.G. and Hoffman, P.G. (1978) Some benzyl ethers present in the extract of vanilla (*Vanilla planifolia*). *Journal of Agricultural and Food Chemistry* 26, 195–197.

Gasson, M.J., Kitamura, Y., McLauchlan, W.R., Narbad, A., Parr, A.J., Parsons, E.L.H., Payne, J., Rhodes, M.J.C. and Walton, N.J. (1998) Metabolism of ferulic acid to vanillin. *Journal of Biological Chemistry* 237, 4163–4170.

Gildemeister, E. Von and Hoffmann, Fr. (1899) *Die Ätherischen Öle*, 3rd edn., Volume III. Julius Springer, Schimmel & Co., Leipzeig, Berlin, pp. 288.

Gillette, M.H. and Hoffman, P.G. (1992) Vanilla extract. In: Hui, Y.H. (ed.) *Encyclopedia of Food Science and Technology*, Volume 4. Wiley, New York, pp. 2641–2657.

Gould, G.W. (1996) Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. *Journal of Food Protection* (suppl.), 82–86.

Gregory, L.E., Gaskins, M.H. and Colberg, C. (1967) Parthenocarpic pod development by *Vanilla planifolia* Andrews induced with growth-regulating chemicals. *Economic Botany* 21, 351–357.

Guenther, E. (1982) Oil of clove. In: *The Essential Oils*, Volume 4. Robert E. Krieger Publishing Co., Florida, pp. 435.

Guenther, E. and Althausen, D. (1978) Aromatic aldehydes. In: *The Essential Oils*, Volume 2. Robert E. Krieger Publishing Co., Huntington, New York, pp. 361–362.

Hartman, T.G., Karmas, K., Chen, J., Shevade, A., Deagro, M. and Hwang, H. (1992) Determination of vanillin, other phenolic compounds, and flavours in vanilla beans. In: Ho, C.-T., Lee, C.Y. and Huang, M.T. (eds) *Phenolic Compounds in Food and Their Effects on Health I*, *ACS Symposium Series No. 506*. American Chemical Society, Washington, DC, pp. 60–76.

Havkin-Frenkel, D., Dorn, R. and Leuster, T. (1997) Plant tissue culture for production of secondary metabolites. *Food Technology (Chicago)* 51(11), 56–58.

Hocking, M.B. (1997) Vanillin: synthetic flavoring from spent sulfite liquor. *Journal of Chemical Education* 74, 1055–1059.

http://www.hinduonnet.com/businessline/2003/03/03/.

http://www.kissankerala.net.

Inouye, T., Sasaki, Y.F., Imanishi, H., Watanabe, M., Ohta, T. and Shirasu, Y. (1988) Suppression of mitomycin C-induced micronuclei in mouse bone marrow cells by post-treatment with vanillin. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 202(1), 93–95.

Jamin, E., Naulet, N. and Martin, G.J. (1997) Multi-element and multi-site isotopic analysis of nicotine from tobacco leaves. *Plant, Cell and Environment* 20, 589–599.

Kamoda, S., Habu, N., Samejima, M. and Yoshimoto, T. (1989) Purification and some properties of lignostilbene-α,β-dioxygenase responsible for the C_α–C_β cleavage of a diarylpropane type lignin model compound from *Pseudomonas* sp. TMY1009. *Agricultural and Biological Chemistry* 53, 2757–2761.

Kanisawa, T. (1993) Flavor development in vanilla beans. *Kouryou* 180, 113–123.

Kanisawa, T., Tokoro, K. and Kawahara, S. (1994) Flavor development in the beans of *Vanilla planifolia*. In: Kurihara, K., Suzuki, N. and Ogawa, H. (eds) *Olfaction Taste XI, Proceedings of the International Symposium*. Springer, Tokyo, pp. 268–270.

Keshava, C., Keshava, N., Whong, W.-Z., Nath, J. and Ong, T.-M. (1998) Inhibition of methotrexate-induced chromosomal damage by vanillin and chlorophyllin in V79 cells. *Teratogenesis, Carcinogenesis, and Mutagenesis* 17, 313–326.

Klimes, I. and Lamparsky, D. (1976) Vanilla volatiles – a comprehensive analysis. *International Flavours and Food Additives* 7, 272–291.

Knorr, D., Caster, C., Dörneburg, H., Dorn, R., Gräf, S., Havin-Frenkel, D., Podstolski, A. and Werrman, U. (1993) Biosynthesis and yield improvement of food ingredients from plant cell and tissue cultures. *Food Technology* 47, 57–63.

Labuda, I.M., Keon, K.A. and Goers, S.K. (1993) Progress in flavour precursor studies: analysis, generation, biotechnology. In: Schreier, P. and Winterhalter, P. (eds) *Proceedings of the International Conference, Würzburg, Germany*. Allured Publishing Corp., Carol Stream, Illinois, pp. 477.

Lamprecht, G., Pichlmayer, F. and Schmid, E.R. (1994) Determination of the authenticity of vanilla extracts by stable isotope ratio analysis and component analysis by HPLC. *Journal of Agricultural and Food Chemistry* 42, 1722–1727.

Longares, P.A. and Canizares, M.M.P. (2006) Focused microwave-assisted extraction and simultaneous spectrophotometric determination of vanillin and *p*-hydroxybenzaldehyde from vanilla fragans. *Talanta* 69(4), 882–887.

López-Malo, A., Alzamora, S.M. and Argaiz, A. (1995) Effect of natural vanillin on germination time and radial growth of moulds in fruit-based agar systems. *Food Microbiology* 12, 213–219.

López-Malo, A., Alzamora, S.M. and Argaiz, A. (1997) Effect of vanillin concentration, pH and incubation temperature on *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus* and *Aspergillus parasiticus* growth. *Food Microbiology* 14, 117–124.

López-Malo, A., Alzamora, S.M. and Argaiz, A. (1998) Vanillin and pH synergistic effects on mould growth. *Journal of Food Science* 63, 143–146.

Maarse, H., Visscher, C.A., Willlemsen, L.C. and Boelens, M.H. (1994) *Volatile Compounds in Food. Qualitative and Quantitative Data*, Supplement 4. TNO, Zeist, Netherlands, pp. 219–227.

Markus, P.H, Peters, A.L.J. and Roos, R. (1992) Process for the preparation of phenylaldehydes. European Patent Application, EP 0 542 348 A2.

Martin, G.J. (1998) Recent advances in site-specific natural isotope fractionation studied by nuclear magnetic resonance. *Isotopes in Environmental and Health Studies* 34, 233–243.

Martin, G.J., Martin, M.L. and Zhang, B.-L. (1992) Site-specific natural isotope fractionation of hydrogen in plant products studied by nuclear magnetic resonance. *Plant, Cell and Environment* 15, 1037–1050.

Matamoros-León, B., Argaiz, A. and López-Malo, A. (1999) Individual and combined effects of vanillin and potassium sorbate on *Penicillium digitatum*, *Penicillium glabrum*, and *Penicillium italicum* growth. *Journal of Food Protection* 62, 540–542.

Merory, J. (1960) *Food Flavorings: Composition, Manufacture and Use*. AVI Publ. Co. Inc., Westport, Connecticut.

Mitra, A., Kitamura, Y., Gasson, M.J., Narbad, A., Parr, A.J., Payne, J., Rhodes, M.J.C., Sewter, C. and Walton, N.J. (1999) 4-hydroxycinnamoyl-CoA hydratase/lyase (HCHL) – an enzyme of phenylpropanoid chain cleavage from *Pseudomonas*. *Archives of Biochemistry and Biophysics* 365, 10–16.

Muheim, A. and Lerch, K. (1999) Towards a high-yield conversion of ferulic acid to vanillin. *Applied Microbiology and Biotechnology* 51, 456–461.

Narbad, A. and Gasson, M.J. (1998) Metabolism of ferulic acid via vanillin using a novel CoA-dependent pathway in a newly-isolated strain of *Pseudomonas fluorescens*. *Microbiology* 144, 1397–1404.

Nguyen, K., Barton, P. and Spencer, J.S. (1991) Supercritical carbon dioxide extraction of vanilla. *The Journal of Supercritical Fluids* (Proceedings of the Symposium on Supercritical Fluids) 4(1), 40–46.

Odoux, E., Chauwin, A. and Brillouet, J.M. (2003) Purification and characterization of vanilla bean (*Vanilla planifolia* Andrews) beta-D-glucosidase. *Journal of Agricultural and Food Chemistry* 51(10), 3168–3173.

Priefert, H., Rabenhorst, J. and Steinbüchel, A. (2001) Biotechnological production of vanillin. *Applied Microbiology and Biotechnology* 56, 296–314.

Purseglove, J.W. (1985) *Tropical Crops: Monocotyledons*. Longman Group Ltd., Harlow, UK.

Purseglove, J.W., Brown, E.G., Green, C.L. and Robbins, S.R.J. (1981) *Spices*, Volume 2. Longman, Inc., New York, pp. 736–813.

Ranadive, A.S. (1994) Vanilla cultivation, curing, chemistry, technology and commercial products. In: Charalambous, G. (ed.) *Spices, Herbs and Edible Fungi*. Elsevier, Amsterdam, pp. 517–577.

Ranadive, A.S., Szkutnica, K., Guerrera, J.G. and Frenkel, C. (1983) Vanillin biosynthesis in vanilla beans. In: *Proceedings of the IX International Congress of Essential Oils*, Singapore, pp. 147–154.

Rao, S.R. and Ravishankar, G.A. (1999) Biotransformation of isoeugenol to vanilla flavour metabolites and capsaicin in suspended and immobilized cell cultures of *Capsicum frutescens*: study of the influence of β-cyclodextrin and fungal elicitor. *Process Biochemistry* 35(3–4), 341–348.

Rao, S.R. and Ravishankar, G.A. (2000a) (Review) Vanilla flavour: production by conventional and biotechnological routes. *Journal of the Science of Food and Agriculture* 80, 289–304.

Rao, S.R. and Ravishankar, G.A. (2000b) Biotransformation of protocatechuic aldehyde and caffeic acid to vanillin and capsaicin in freely suspended and immobilized cell cultures of *Capsicum frutescens*. *Journal of Biotechnology* 76, 137–146.

Remaud, G.S., Martin, Y.-L., Martin, G.G. and Martin, G.J. (1997) Detection of sophisticated adulterations of natural vanilla flavors and extracts: application of the SNIF-NMR method to vanillin and *p*-hydroxybenzaldehyde. *Journal of Agricultural and Food Chemistry* 45, 859–866.

Romagnoli, L.G. and Knorr, D. (1988) Effects of ferulic acid treatment on growth and flavour development of cultured *Vanilla planifola* cells. *Food Biotechnology* 2, 83–104.

Ruiz, T.F., Perez, A.I. and Lopez, M. (2001) An enzymatic extraction and transformation of glucovanillin to vanillin from vanilla green pods. *Journal of Agricultural and Food Chemistry* 49(11), 5207–5209.

Sagrero-Nieves, L. and Schwartz, S.J. (1988) Phenolic content of *Vanilla planifolia* as affected by harvest period. *Journal of Food Composition and Analysis* 1(4), 362–365.

Salih, F.M. (2006) Risk assessment of combined photogenotoxic effects of sunlight and food additives. *Science of the Total Environment* 362(1–3), 68–73.

Silva, A.P., Odoux, E., Brat, P., Ribeyre, F., Rodriguez-Jimenes, G., Robles-Olvera, V., García-Alvarado, M.A. and Günata, Z. (2006) GC-MS and GC-olfactometry analysis of aroma compounds in a representative organic aroma extract from cured vanilla (*Vanilla planifolia* G. Jackson) beans. *Food Chemistry* 99(4), 728–735.

Simpson, B.B. and Ogarzaly, M.C. (1986) *Economic Botany: Plants in Our World*. McGraw-Hill, New York.

Sostaric, T., Boyce, M.C. and Spickett, E.E. (2000) Analysis of the volatile compounds in vanilla extracts and flavorings by solid-phase microextraction and gas chromatography. *Journal of Agricultural and Food Chemistry* 48, 5802–5807.

Tenailleau, E.J., Lancelin, P., Robins, R.J. and Akoka, S. (2004) Authentication of the origin of vanillin using quantitative natural abundance [13]C NMR. *Journal of Agricultural and Food Chemistry* 52, 7782–7787.

Theodose, R. (1973) Traditional methods of vanilla preparation and their improvement. *Tropical Science* 15, 47–57.

Thompson, R.D. and Hoffmann, T.J. (1988) Determination of coumarin as an adulterant in vanilla flavoring products by high-performance liquid chromatography. *Journal of Chromatography A* 438, 369–382.

Tripathi, U., Rao, S.R. and Ravishankar, G.A. (2002) Biotransformation of phenylpropanoid compounds to vanilla flavor metabolites in cultures of *Haematococcus pluvialis*. *Process Biochemistry* 38(3), 419–426.

Uhl, S.R. (2000) Emerging flavor contributors. In: *Handbook of Spices, Seasonings and Flavorings*. Technomic Publishing Co., Inc., Pennsylvania, pp. 217–219.

UN Food and Agriculture Organization (2005) http://www.hinduonnet.com/businessline/2003/03/03/.

US Food and Drug Administration (2002) Code of Federal Regulations, 21CFR Part 169.

USDA National Nutrient Database for Standard Reference (2002) www.nal.usda.gov.

van den Heuvel, R.R.H., Fraaije, M.W., Laane, C. and van Berkel, W.J.H. (2001) Enzymatic synthesis of vanillin. *Journal of Agriculture and Food Chemistry* 49, 2954–2958.

Waliszewski, K.N., Ovando, S.L. and Pardio, V.T. (2007a) Effect of hydration and enzymatic pretreatment of vanilla beans on the kinetics of vanillin extraction. *Journal of Food Engineering* 78(4), 1267–1273.

Waliszewski, K.N., Pardio, V.T. and Ovando, S.L. (2007b) A simple and rapid HPLC technique for vanillin determination in alcohol extract. *Food Chemistry* 101(3), 1059–1062.

Walton, N.J., Mayer, M.J. and Narbad, A. (2003) Molecules of interest – vanillin. *Phytochemistry* 63(5), 505–515.

Weiss, E.A. (2002) Orchidaceae. In: *Spice Crops*. CAB International, Wallingford, UK, pp. 136–154.

Werkhoff, P. and Güntert, M. (1997) Identification of some ester compounds in Bourbon vanilla beans. *Lebenomittel-Wissenschaft und Technology* 30, 429–431.

Westcott, R.J., Cheetham, P.S.J. and Barraclough, A.J. (1993) Use of organized viable vanilla plant aerial roots for the production of natural vanillin. *Phytochemistry* 35(1), 135–138.

Whetten, R. and Sederoff, R. (1995) Lignin biosynthesis. *Plant Cell* 7, 1001–1013.

Wild-Altamirano, C. (1969) Enzymic activity during growth of vanilla fruit. 1. Proteinase, glucosidase, peroxidase and polyphenoloxidase. *Journal of Food Science* 34, 235–238.

Woebse, A. (1963) Pollinating the vanilla flower. *American Orchid Society Bulletin* 32(12), 1009–1010.

Yoshida, A., Takenaka, Y., Tamaki, H., Frebort, I., Adachi, O. and Kumagai, H. (1997) Vanillin formation by microbial amine oxidases from vanillylamine. *Journal of Fermentation and Bioengineering* 84(6), 603–605.

Zenk, M.H. (1965) Biosynthese von vanillin in *Vanilla planifolia* Andr. *Z. Pflanzenphysiology* 53, 404.