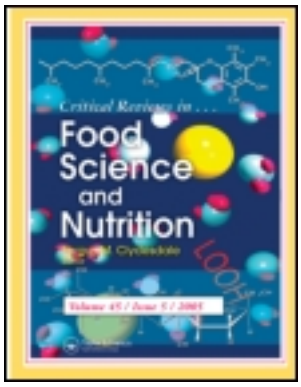


Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Vanilla- Its Science of Cultivation, Curing, Chemistry, and Nutraceutical Properties

Krushnamurthy Anuradha [a] , Bellur Nanjundaiah Shyamala [b] & Madeneni Madhava Naidu [c]
[a] Plantation Products, Spices & Flavour Technology , Mysore , India
[b] Central Food Technological Research Institute , Mysore , India
[c] Constituent Laboratory of Council of Scientific and Industrial Research , Mysore , India
Accepted author version posted online: 01 Aug 2012.Published online: 03 Oct 2013.

To cite this article: Krushnamurthy Anuradha , Bellur Nanjundaiah Shyamala & Madeneni Madhava Naidu (2013) Vanilla- Its Science of Cultivation, Curing, Chemistry, and Nutraceutical Properties, Critical Reviews in Food Science and Nutrition, 53:12, 1250-1276, DOI: 10.1080/10408398.2011.563879

To link to this article: http://dx.doi.org/10.1080/10408398.2011.563879

Critical Reviews in Food Science and Nutrition, 53:1250–1276 (2013)

ISSN: 1040-8398 / 1549-7852 online
DOI: 10.1080/10408398.2011.563879

Taylor & Francis
Taylor & Francis Group

Vanilla- Its Science of Cultivation, Curing, Chemistry, and Nutraceutical Properties

KRUSHNAMURTHY ANURADHA,[1] BELLUR NANJUNDAIAH SHYAMALA,[2] and MADENENI MADHAVA NAIDU[3]

[1]Plantation Products, Spices & Flavour Technology, Mysore, India
[2]Central Food Technological Research Institute, Mysore, India
[3]Constituent Laboratory of Council of Scientific and Industrial Research, Mysore, India

Vanilla is a tropical orchid belonging to the family Orchidaceae and it is mainly used in food, perfumery, and pharmaceutical preparations. The quality of the bean depends on the volatile constituent's, viz., the vanillin content, the species of the vine used, and the processing conditions adopted. Hence, proper pollination during flowering and curing by exercising utmost care are the important aspects of vanilla cultivation. There are different methods of curing, and each one is unique and named after the places of its origin like Mexican process and Bourbon process. Recently, Central Food Technological Research Institute, Mysore has developed know-how of improved curing process, where the green vanilla beans are cured immediately after harvest and this process takes only 32 days, which otherwise requires minimum of 150–180 days as reported in traditional curing methods. Vanillin is the most essential component of the 200 and odd such compounds present in vanilla beans. Vanillin as such has not shown any antioxidant properties, it is along with other compounds has got nutraceutical properties and therefore its wide usage. The medicinal future of vanilla may definitely lie in further research on basic science and clinical studies on the constituents and their mechanism of action.

Keywords Vanillin, flavor, antioxidant properties, food application, vanilla pods, glucovanillin

INTRODUCTION

Vanilla is a costly spice next to saffron. The vanilla originated in Mexico, is a tropical orchid belonging to the family Orchidaceae (Childers et al., 1959). The family comprising 788 genera with 18,500 species is one of the largest families of flowering plants in the world. Vanilla is mainly used in food, perfumery, and pharmaceutical industries. It is the hot topic of 21st century. About 110 species have been identified, but only 3 have been reported to be important in terms of commerce and cultivation: *Vanilla fragrans* (Salisbury) Ames; also known as *Vanilla planifolia* Andrews; *V pompona* Schiede; and *Vanilla tahitensis* J. W. Moore (Ranadive 1994; Reineccius 1994; Webster 1995). Among these, *V. planifolia* is the most valued for its flavor qualities and is, therefore, widely cultivated and used for the production of food additives (Purseglove et al., 1988). The characteristic flavor of vanilla is due to the presence of large number of aromatic compounds, which are produced during various stages of curing/processing. Principal component of vanilla is "Vanillin" which contributes to 1/3rd of total flavor whereas other volatile and nonvolatile compounds contribute to the remaining 2/3rd flavor components in vanilla (Ranadive, 1994). Vanilla and its extracts are very important and popular so far as its natural flavorings used in food, beverages, and confectionery preparations. Vanilla is also used as a fragrance ingredient (Purseglove, 1972; Funk and Brodelius, 1994).

Vanilla is extensively cultivated in Madagascar (80%), Comoro, Réunion and Indonesian Islands of Jawa, Bali, Sulawesi, Lombox and Papua, and the South Pacific islands of Tonga. Small amounts of vanilla beans are also produced in Costa Rica, Jamaica, Bolivia, Tahiti, Uganda, and Hawaii Islands. Since recently it has taken roots in India. The species V. *planifolia* has its origin in Mexico where it was already in use by the Aztecs even before the Spaniards arrival. The Spanish brought vanilla to Europe in 1520; it became very popular. Since then vanilla has spread and cultivated throughout the tropics between 25° above and below the equator. At present, India produces about 100 tons of vanilla beans from 2000 hectares as against the world

Address correspondence to M. Madhava Naidu, Constituent Laboratory of Council of Scientific and Industrial Research, Mysore, India. E-mail: madhavanaidu45@yahoo.com

production of 5600 tons (Ayyappan, 1990). Even if 10% of synthetic substitute is replaced by natural products, then the requirement of vanilla beans would be around 2020 tons at the rate of 2% vanillin content in cured beans. This amounts to almost half the entire global production of vanilla beans. Therefore, there is a vast potential for development of vanilla cultivation in India.

Green vanilla beans are almost odorless and aroma develops in the pods or beans through the labor-intensive process called curing. This process of aroma development is carried out through drying vanilla beans and allowing the chemical and enzymatic reactions (Dignum et al., 2001). Every vanilla growing country has developed its own curing process, but all processes generally involve 4 common steps: scalding, sunning/sweating, drying, and conditioning. The whole process takes 6–8 months (Rao and Ravishankar, 2000). During the important process of curing, the beans undergo enzymatic reactions which are responsible for the development of characteristic vanilla aroma and flavor (Dignum et al., 2001). The extract of *Vanilla planifolia* contains not only vanillin but also a range of related phenylpropanoid (C6–C3) compounds, which combine together to impart the unique subtlety to natural vanilla flavor (Clark, 1990). Green vanilla beans (pods) although contain a little free vanillin but aroma precursors such as β-D-glucosides come into contact with β-D-glucosidases only during the curing process (Walton et al., 2003).

The anticlastogenic properties of vanillin have been studied over the last 2 decades by many researchers (Odoux et al., 2003). A study even explored that vanillin has the ability to reduce chromosomal damage caused by X-ray and ultraviolet (UV) light (Keshava et al., 1998). The antimutagenic property of vanillin was further evident when it significantly reduced the number of micronuclei, and at all dose levels, vanillin reduced the frequency of chromosomal aberrations (Bythrow, 2005). Vanillin also showed anticarcinogenic effects in a family of DNA-PK inhibitors (Duran and Karran, 2003). Vanillin's antimicrobial properties against yeasts have been studied, and it was found that the structure of vanillin played an important role (Fitzgerald et al., 2003).

Between 1700 and 1800 AD vanilla appeared in the European pharmacopoeia and was indicated for "fevers, melancholy, and hysteria probably because of its supposed diuretic, sedative, and purifying qualities." Because of advances made in chemistry and pharmacology, most of the earlier medicinal uses of vanilla have given way to functional uses of vanillin, vanilla's main constituent. Currently basic science research is exploring vanillin's properties as an anticarcinogen to have ability to inhibit tumor formation and as an anticlastogen to have the ability to inhibit chromosome breakage. Biomedical research has also discovered that vanillin is an effective inhibitor of red blood cell sickling in patients with sickle cell disease. Vanillin has also found to possess powerful antimicrobial property. This has possible implications in using it as a natural food preservative.

World over, there is an increasing trend toward using natural products and vanilla being an important food flavoring agent, the demand for vanilla extracted from vanilla beans is bound to grow. Encouraging price offers from the western countries has really enthused the farmers. But the farmers are unable to produce quality beans due to lack of adequate knowledge on the scientific way of cultivation and technology of processing, depriving them of the desired benefits. Hence, the purpose of this study is to review and interpret the relatively recent research and developments on the cultivation, curing, chemistry, and nutraceutical properties of natural vanilla and its products. This ensemble would help easy access to the art and science of vanilla.

SCIENCE OF CULTIVATION

History of Vanilla

Vanilla has a long and interesting history. In Mexico, Aztecs used vanilla to flavor a drink called "Tlilxochitl," which is considered as ancestor of chocolate. The drink was prepared using pulverized pods of cocoa and sweetened with honey. In 1520, Spanish conquistadors led by Hernan Cortes entered Mexico, Bernal Diza, a Spanish officer under Cortes observed Montezumma, the Azetec Emperor drinking this flavored drink—chocolate. This drink was served to Cortes in golden goblets. Spaniards were impressed by the flavor of vanilla, as they took the beans to Spain in 1520, this was followed by the demand for vanilla beans in France and other European countries that led to spread of the vanilla plant. Though vanilla was introduced to Spain during 1520 and subsequently, to other European countries in 1733, Mexico remained the sole producer of vanilla for the next 350 years. The plants were again reintroduced by Marquis of Blandford for cultivation successfully by Charles Greville at Paddington in England in 1807. Greville supplied the cuttings to Paris and Antwerp. In 1819, plants were sent to Bogor, Java from Antwerp and plants flowered in 1825. Plants were taken to Réunion and from there to Mauritius in 1827. Vanilla was introduced to Malagasy Republic in 1840. Although flowering occurred, fruiting did not take place due to the absence of natural pollinators. Edmond Albius (1841) developed a practical method of artificial pollination using bamboo sticks to pollinate vanilla flowers. Even before Albius, Professor Charles Morren of Liege (1836) had discovered artificial method of pollination for production of capsules. By 1890, vanilla cultivation was prevalent in Mauritius, Réunion, Seychelles, Madagascar, Tahiti, Jamaica, and other several tropical countries (Cunningham, 1920; Ranadive, 1994; Reineccius, 1994; Webster, 1995).

Vanilla cultivation was introduced in Java in 1846 by Teysmann, 1866 in Seychelles, in Tahiti in 1848 by Hamelin, in Comoro islands in 1893, in West Indies and Guadelope in 1839, and in Uganda in 1912 (Ramarosom-Raonizafinimanana et al., 1998 and 1999; Hanum, 1997). Though no authenticated information is available regarding the entry of vanilla to India, it is believed that it was introduced in 1835 for "Spice Garden" in Kurtallam, owned by East India Company. At present,

Table 1 Area of vanilla harvested in hectares

Country	2003	2004	2005	2006	2007	2008
Madagascar	25,750.00	25,880.00	26,050.00	26,220.00	63,764.00	63,764.00
Indonesia	8539.00	8903.00	9216.00	9216.00	9216.00	9216.00
China	1200.00	1200.00	1200.00	1200.00	1300.00	1400.00
Mexico	1046.00	6567.00	826.00	575.00	681.00	582.00
Turkey				NA		
Tonga	290.00	290.00	290.00	268.00	255.00	NA
Uganda	100.00	140.00	140.00	136.00	138.00	138.00
Comoros	700.00	700.00	650.00	700.00	700.00	700.00
French Polynesia	400.00	450.00	450.00	460.00	470.00	NA
Réunion	350.00	351.00	290.00	290.00	290.00	290.00
Malawi	80.00	80.00	80.00	80.00	80.00	80.00
Portugal				NA		
Kenya	40.00	40.00	40.00	40.00	40.00	40.00
Guadeloupe	40.00	40.00	40.00	40.00	40.00	40.00
Zimbabwe	45.00	45.00	45.00	30.00	23.00	23.00

the major vanilla growing countries are Madagascar, Indonesia, India, Mexico, the Comoros, and Réunion (Tables 1 and 2). Other countries where vanilla is cultivated to a limited extent are French Polynesia, Tonga, Guadeloupe, and Zimbabwe (Potty, 2004).

Nomenclature of Vanilla and Important Varieties

Vanilla is a climbing orchid belonging to the family Orchidaceae, which has about 788 genera and 18,500 species, is one of the largest families of flowering plants in the world. The genus itself consists of over 100 species (Mabberley, 1997) although this is under revision (Bory et al., 2007). Other species of the genus represent valuable sources of useful traits for the improvement of *V. planifolia* with regards to disease resistance, self-pollination, or aromatic quality (Soto Arenas, 2003; Minoo et al., 2006). Generally most of the Orchidaceae species are well known for their ornamental flowers. Surprisingly, vanilla belonging to this particular family is the only orchid bearing flavoring material of commercial importance. Three species, viz., *V. fragrans* or *V. planifolia*, *V. tahitensis,* and *Vanilla pompana* are of commercial value.

Detection of genetic variability in vanilla through Polyacrylamide Gel Electrophoresis (PAGE) studies has been reported (Rao et al., 1992). V. *pompana* is cultivated widely in Mexico, Central America, Trinidad and South America, Guadelope, and Marinique. Leaves of this species are about 15–30 cm long and 5–12 cm broad. The pods are thick and short. It is 2.5–3.5 cm in diameter and 10–12.5 cm long (Childers and Cibes, 1948). The bean quality of this species is low. Vanilla of this species can grow under favorable moisture conditions and soils with lower nutritive value. Though the quality of beans is low, the species is resistant to root rot disease caused by *Fusarium batatis* and thus takes an important place for cross-breeding of disease resistant variety of *V. fragrans*. The *V. pompana* is widely used in manufacture of perfumes and fragrance items (Land, 1986).

V. tahitensis is grown widely in Tahiti and Hawaii. The species has narrow leaves of 2.5–3.0 cm wide and 12–14 cm long. The pods are about 9–10 mm broad and 12–14 cm long.

Table 2 World vanilla production in tones

Country	2003	2004	2005	2006	2007	2008
Madagascar	920.00	880.00	525.00	510.00	1240.00	1240.00
Indonesia	2198.00	2731.00	2375.00	2387.00	2399.00	2399.00
China	4100.00	4150.00	4252.00	4354.00	5089.00	1200.00
Mexico	299.00	189.00	240.00	177.00	251.00	306.00
Turkey				NA		
Tonga	130.00	130.00	130.00	139.00	144.00	NA
Uganda	50.00	70.00	70.00	72.00	75.00	75.00
Comoros	140.00	140.00	110.00	60.00	65.00	60.00
French Polynesia	30.00	35.00	31.00	43.00	50.00	NA
Réunion	34.00	32.00	31.00	31.00	23.00	23.00
Malawi	20.00	20.00	20.00	20.00	20.00	20.00
Portugal	10.00	10.00	10.00	10.00	10.00	10.00
Kenya	8.00	8.00	8.00	8.00	8.00	8.00
Guadeloupe	8.00	8.00	8.00	8.00	8.00	8.00
Zimbabwe	10.00	10.00	10.00	10.00	10.00	10.00

Source: FAO statistics division 2009.

Beans of V. *tahitensis* have pods with a very strong aroma on processing. Important aromatic constituents of this bean include anisic acid, anisyl alcohol, anisyl aldehyde, *p*-hydroxybenzoic acid, and heliotrophin. To Surprise, these aromatic compounds are actually not present in beans of *V. fragrans* though it is extensively sold in the market (Krishnamurthy and Melanta, 2002).

Vanilla Plant

V. planifolia, formerly known rather by an appropriate name *V. fragrans* (Salisb) is known for its quality vanillin. It is a large green-stemmed creeping or climbing perennial plant. The vine has a fleshy succulent stem, smooth, thick, oblong-lanceolate bright green leaves, and numerous twining adventitious aerial roots arising opposite each leaf, by which it clings to trees or supports. It is the fruit which is a commercially important part of the plant. The fruit/pod of vanilla is known as vanilla bean (Krishnamurthy, 2004). *V. planifolia* climbs by means of adventitious roots up the trees or other given supports to a height of 10–15 m. It is usually up to height of 5 feet (150 cm) which facilitates hand pollination and harvesting. Long whitish aerial adventitious roots of 2 mm in diameter are produced singly opposite the leaves and remain firmly oppressed to the support of which the plant climbs. The roots at the base ramify in to humus of the mulch layer. An endotrophic mycorrhiza is present along the roots. The long, cylindrical, monopodial stems are simple or branched, and are succulent, flexuose, and brittle. They are 1–2 cm in diameter and are dark green with stomata and are photosynthetic. The internodes are 5–15 cm long (Knudson, 1946). The large flat, fleshy subsessile leaves are alternate, oblong-elliptic to lanceolate and are 8–25 cm long and 2–8 cm broad. The tip is acute to acuminate and the base is somewhat rounded. The petiole is short, thick, and canalized above.

The stout inflorescence are auxiliary, usually simple, and only rarely branched. They are usually borne toward the top of the vine and are 5–8 cm long, comprising of 29–30 flowers, but more usually 8–15, opening from the base upward, generally with only 1–3 flowers open at one time and each lasting single day. The large waxy fragrant, pale greenish yellow flowers are about 10 cm in diameter and are fugacious. The pedicel is very short. There are three oblong-lanceolate sepals, 4–7 cm long and 1–1.5 cm wide. The 2 upper petals resemble the sepals in shape but are slightly smaller. The lower petal is modified as a trumpet shaped labellum or lip which is shorter than the other perianth lobes and is 4–5 cm long and 1.5–3 cm broad at its widest point. It is attached to the column which it envelops. The tip of lip is obscurely 3-lobed and is irregularly toothed on its revolute margin. The gynostemium is 3–5 cm long and is attached to the labellum for most of its length. It is hairy on the inner surface, bearing at its tip the single stamen containing the 2 pollen masses or pollinia covered by a cop and below is the concave sticky stigma, which is separated from the stamen by the thin flap-like rostellium. The fruit is a capsule known in the trade as a bean, 10–25 cm long and 5–15 mm in diameter. The other species are occasionally cultivated besides the yield is an inferior quality product.

Climate

Vanilla grows well in presence of a support and shade. It also grows well in hot, moist climate with infrequent rainfall. The optimum temperature for its growth is 21–32°C with an average of about 27°C. High lands up to about 600 m above mean sea level (msl) found to be ideal for vanilla cultivation. The best-suited land for vanilla growth is a gently sloping land with checkering light, thick surface layer, and adequate drainage facility. Partial shade required which can be made possible by shrubs or trees that provide support to the vine (Childers et al., 1959). Vanilla requires tropical climate for its growth. It grows well in soils rich in humus and nutrients like N, P, K, and with a soil pH of 6–7. Vanilla should be partially protected from light because it cannot tolerate direct strong sunlight and thick shading produces vines with smaller leaves, thin stems, and reduced flowering and fruiting. Excessive rainfall may cause mildew and root diseases.

Propagation

Vanilla is the most labor-intensive agricultural product cultivated in the world. It takes 3 years from planting a cutting of the vine to produce vanilla pods. The orchids bloom and die within a few hours and they are to be pollinated within that period by hand. The beans (which are actually seed pods) must stay in the vine for 9 months before being harvested in order to completely develop their signature aroma. However, when the beans are harvested, they have neither flavor nor fragrance. They develop only when the beans go through curing, drying, and resting process lasting for several months. Each vanilla bean is handled hundreds of times before it is ready to use They develop flavor, and fragrance develops when the beans go through curing, drying, and resting processes for several months. Each vanilla bean is handled hundreds of times before it is ready to use (http://www.vanilla.com).

Vanilla propagation can be carried out with using seeds or by stem cuttings. Scientific information on seed propagation methods for vanilla is limited to the reports (Knudson, 1946, 1950; Withner, 1955). Vanilla seeds can also be germinated under laboratory conditions for cross-breeding purpose to improve varieties.

Raised Rooted Cuttings

Vine cuttings with 4 nodes and pretreated with plant growth regulators are taken for planting in the polythene bags (15 × 25 cm size) provided with 4–5 drainage holes at the bottom. The bags are filled with potting mixture prepared with jungle top soil, dry powdered farm yard manure, and sand in the ratio of

3:1:1 (Siddagangaiah et al., 1996). The soil mixture in the poly bags is drenched with water containing 1% copper oxy-chloride as disinfectant. The vine cuttings are to be planted in these poly bags in such a way that the bottom 2 nodes excised of leaves are buried inside the soil mixture while the remaining 2 top nodes with the leaves intact remain above the soil mixture level. Then the soil mixture in the poly bags is compacted and watered daily. These planted poly bags have to be kept in shade. When the cuttings strike roots the signs of fresh growth can be seen. At this stage, to provide nutrition, well-decanted oil cake slurry may be poured into poly bags. Care should be taken that there is no cake formation of the slurry on the soil surface. If it forms, then the top soil in the poly bags may be stirred to provide proper aeration. Application of fertilizers to the bags may be avoided as they damage the young roots. The best way would be to give a spray with multiplex nutrient solution (containing all macro and micro nutrients). To protect the nursery plants in the poly bags from pests, 0.2% methyl parathion has to be sprayed and for protecting them from diseases, drench the soil mixture with 0.2% Captan or Kavach (2 g/l of water) as well as drenching the plants with Bavistin (1 g/l of water). When these nursery plants attain 8–12 months age, they can be planted in the main field (Rao et al., 1992).

In Situ Planting

In the main field, pits of the size 40 × 40 × 40 cm to be dug and are opened close to the standard plants (pillars or posts). Planting of the rooted vine should be done in the wet weather. The pit may be filled at the bottom with pebbles, gravel, and sand and then with loamy soil. Pretreated cuttings of the vines with 6–8 nodes are used for planting. From the rooted planting vines, the bottom 3–4 leaves are removed and buried into the pit. The other top 3–4 nodes of the growing end with the leaves intact are left above the soil and tied to the supporting plants. Then the base is compacted and watered regularly. In about 4–8 weeks the planted cuttings show the sign of growth. Here, also the planted vines in the pits have to be attended with plant protection schedules (Krishnakumar, 1995).

Planting

Preliminary studies on trailing of vanilla are reported (Muralidharan et al., 1974). Since vanilla is a climbing vine, it has to be raised on a support. These support trees have to be planted 1 year in advance. Any tree like Sesbania, Glyricidia, Silver oak, Bauhinia, Plumaria, Jatropa, Jack, Indian almond, and Cashew will meet the purpose. Even stone pillars, trellis, or wooden poles of 2 m height could be fixed for support along with providing shade nets to meet this purpose but it would be expensive. Standard supporting trees have to be raised at a spacing of 2.5–3 m between rows and 2 m between trees within a row (Correll, 1953; Ridley, 1992). A plant population of 1600–2000 plants per hectare may be obtained. From one support tree or pillar to another, at a height of about 1.5 m, polyvinyl chloride (PVC) pipes or bamboos are to be tied horizontally, so that the vines train on them. This method provides better conditions for the training of vines, proper inspection, easy pollination, better training, and coiling of vines to the pipes or bamboos. Vines are to be inspected regularly (Fig. 1).

Aftercare

The base of the vines has to be worked and mulched with dry leaves, straw, coconut husk, or any other organic material to

Figure 1 Vanilla planting. (Color figure available online.)

conserve moisture and suppress the weeds (Mc Clelland, 1919; Lionnett, 1958, 1959). As the vines grow, they have to be trained on the pipes or poles or bamboo's and later coiled around them. The vines should not be allowed to proceed high up on the trees. In that condition, they seldom flower and further inspection, pollination, and harvest become difficult. The shade from the support trees has to be regulated periodically by pruning to form an umbrella shape. If the plants are made to trail horizontally on wires tied on to trellis the height should be 1.5–2 m. If height is increased then it will be inconvenient for pollination (Medina, 1943).

Manuring

Vanilla prefers manure from the plant sources. Manure is supplied twice or thrice in a year. This is done in the month of May-June, June-July, and August-September. Organically grown vanilla gets a premium price in the market. Organic manures like the compost prepared from the dung of various kinds of animals, the bio-compost prepared from the sugar factory wastes (such as press mud, fly ash, and cane yard manure, spent wash), vermin compost, bio-fertilizers, various cakes of oil seeds (like Neem, Honge, and Hippe), wood ash, dry, or green leaves are all useful for vanilla cultivation. But there should be good composting. This compost can also be enriched by adding bio-organisms like *Plurotus, Azatobacter, Azospirillium,* (Phospho bacteria), Rhizobium, *Aspergillus avamori*, peat mass, *Azolla*, green algae, etc. Fertilizers like Nitrogen, Phosphorus, and Potash (NPK) can be applied to vanilla through the soil only prior flowering stage when the vines are 1–3 year old. These make the crop grow well. Based on the soil test report, each vine has to be supplied with (g/plant/year) 40–60 N, 20–30 P, and 60–100K, in 2–3 split doses, mixed with organic manure supplied 3 times a year.

Flowering

Flowering of vanilla vines depends on the size of the cuttings placed during planting. Generally, flowering in vanilla vines occur in the third year of planting (Ranadive, 1994). The inflorescence having 20–25 flowers emerge as light green protrubences from the leaf axils. One to two flowers open in a day. In some cases, there is a gap of about 5 days in flower openings in the same inflorescence. In Indian conditions, flowering period extends for 2–3 months and generally spread over from March to May. Much of the flower formation is recorded between March and April. Flowers formed are pale green in color, large, bisexual, and zygomorphic. Sepals and petals of flower look alike/in color and are called as perianth. The lower part of the petal is broad, short and is modified into labellum. The lower part of labellum has a structure called column/gynostemium. The tip of column possesses stamen with 2 pollinia covered by stigma (cap-like structure). Rostellum covers stigma. Slender stalk portion is ovary which is about 4–5 cm in long. The flowers open during morning and have to be pollinated on the same day

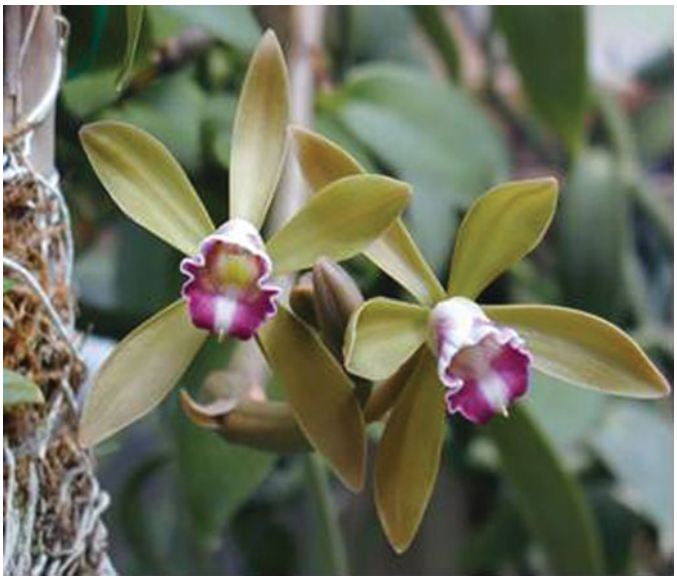


Figure 2 *V. planifolia*. (Color figure available online.)

(Figs. 2 and 3). The detailed study on floral biology of vanilla is reported (Kuruvilla et al., 1996).

Pollination

Large number of factors such as climatic conditions, temperature, ages of the vine, etc., influences the flowering process of vanilla. It takes 2–3 years for vanilla vines to blossom after cuttings are planted. Vanilla vines promote flowering 6–8 weeks before they blossom and this flowering is due to the ability of the cuttings to store carbohydrate and other substances. Once it blossoms, pollination has to be completed within half a day because each flower blossoms only for 1 day as it opens in the early morning and closes in the afternoon. Raceme of each cutting has 15–20 flowers and 6–8 flowers can be pollinated everyday. Humming birds and bees of genus *Melipona* are effecting natural pollination in Mexico and Central America. It is observed that $>1\%$ of flowers are pollinated naturally in Puerto Rica (Ranadive, 1994). Since this bee is indigenous to Mexico, the process of hand pollination, a substitute for natural pollination was developed by Prof. Charles Morren and Edmund Albius, a farmer of Réunion. Pollination is carried out using bamboo stick or with a material having the size of a toothpick (Ranadive, 1994). Flowers are held in one hand and pollens are rubbed against stigma. This process stimulates the ovary. A skilled worker on an average can pollinate about 1000 flowers a day (Fig. 4). If fertilization had taken place, pollens stick to raceme and enlarge in size. Unfertilized flowers wilt within a day. In the next 6–8 weeks, pods develop from fertilized ovary and attain complete maturity in the next 6–7 months (Bhat and Sudharshan, 1998). However, attempts were made to use growth regulators to avoid pollination (Leopold, 1958; Crane, 1964; Nickell, 1982). Studies showed that application of 2, 4-dichlorophenoxy acetic acid, 2-methoxy-6-dichloro benzoic acid and indolacetic

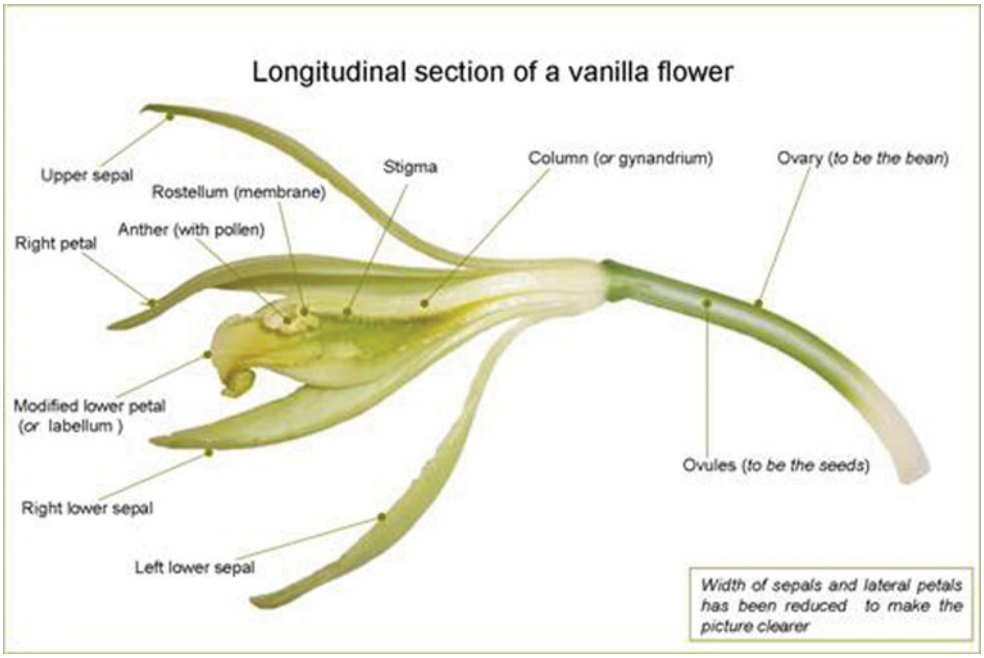


Figure 3 Typical structure of vanilla flower. (Color figure available online.)

acid-indolbutyric acid resulted in parthenocarpic pods that were lower in weight and smaller in length and diameter compared to pods that were pollinated by hand (Gregory et al., 1967). In *V. wightiana*, a wild species from Andhra Pradesh (India), natural fruit production is reported (Rao et al., 1994).

Harvesting of Vanilla Beans

Flowering of vanilla beans occurs usually after third year of planting, and it takes about 8–9 months from flowering to harvesting (Lionnett 1959). Detailed study on anatomy and biochemistry of vanilla beans are described by Fabienne Lapeyre-Montes et al. (2010). Fully mature pods (indicated by tips turning yellow) are harvested at right stage and are carried out rotationally. Harvesting is done using thumb or knife. Around 75–80 beans of 18–20 cm long are needed to get 1 kilogram of fresh beans (Fig. 5). Variation in quality of cured beans occurs depending on the time of harvest (Jones and Vincente 1949). The effect of harvesting date on vanillin content of pods of V. *tahitensis* in French Polynesia was studied (Lancher, 1989). Curing of harvested beans should start within a week. One kilogram of cured beans requires 6 kilograms of green beans (6 kg green beans: 1 kg cured beans).

CURING AND PROCESSING TECHNOLOGY

Green vanilla beans (pods) contain small amount of vanillin and is odorless and flavorless. They develop a faint phenolic odor, unlike the cured beans, as beans reach harvest maturity. In the commercial practice, mature green beans are subjected to curing process, which allows the characteristic flavor, aroma, and color to develop; excess moisture is also removed to prevent spoilage of the beans during storage. Curing brings about physical, biochemical, and chemical changes necessary for imparting the desired attributes (Ranaddive, 1994). The curing of vanilla pods has been defined as their controlled ripening (Lionnett 1959). It is a process involving alternative sweating and drying of pods until they lose most of their moisture as much as 80% curing in Mexican beans (Correll, 1953). The process of curing is an important stage in its production as it is during curing that the beans undergo the enzymatic reaction responsible for the development of characteristic aroma and flavor of vanilla (Ranaddive, 1994). Each vanilla-growing region has devised its own method suitable for that region based on available resources and experience gained through trial and error. The primary quality requirement for cured vanilla beans is the typical aroma/flavor character, which is essential (Muralidharan and Balagopal, 1978). Other factors of significance in quality assessment are the general appearance, flexibility, size of the bean, and vanillin content.

The 4 steps involving in proper curing are:

- Killing
- Sweating
- Drying and
- Conditioning

Killing

In this stage, further vegetative development of the fresh bean is stopped/disrupted and the enzymatic reaction

Figure 4 Different stages of pollination. (Color figure available online.)

responsible for the production of aroma and flavor is initiated (Ranaddive, 1994). The disruption of cell structure can be carried out by any 1 of the following methods; hot water scalding, sun and oven wilting, scarification, treatment with ethylene gas, and freezing. The process is called killing because it disrupts the respiratory function by destructing cell membranes and cell walls of the bean tissue and thus bringing about their physiological death.

Figure 5 Freshly harvested vanilla pods. (Color figure available online.)

Of the various killing methods mentioned, only the most practical methods such as sun, oven and hot water killing are commonly practiced (Fig. 6).

Sweating

In this step, which follows the killing process, moisture is initially allowed to escape rapidly to attain a level which will reduce the risk of microbial spoilage during the subsequent operation but will be sufficient for further enzyme activity (Ranaddive, 1994). Improper handling of beans at this stage will produce markedly inferior beans. In general, in this step vanilla beans develop their characteristic color, aroma, and flavoring properties. The process is carried out in sweat boxes, enclosed rooms, and rarely in ovens. The process lasts for 7–10 days (Fig. 7).

Figure 6 Hot water treatment of vanilla beans. (Color figure available online.)

Drying

At the end of sweating period, the cured beans, which are now brown in color and aromatic, still have about 60–70% moisture. The beans need further drying to reduce their moisture content in order to protect them from microbial spoilage and to allow for further beneficial chemical changes to take place. The lower moisture in the beans after drying also significantly reduces undesirable enzyme activities and biochemical changes. At the end of drying the beans have about 25–32% moisture.

Conditioning

For conditioning, the beans are stored in closed boxes. This step lasts from 1 month to several months. Various chemical and biochemical reactions such as esterification, etherification, oxidative degradation, etc. take place during this step, which produce various aroma constituents and further enhance overall flavor quality of cured beans (Fig. 8).

Curing Methods

The methods of curing followed are named after the places of its origin like Mexican process, Bourbon process, Peruvian process, and Guyana process. Under Indian conditions, either Mexican or Bourbon process is followed. The desired method of curing process may be slightly modified based on the experience gained in order to obtain best quality cured beans. At any stage of processing, beans should not come in contact with any external odor. The blankets, boxes, bins, planks, and drying halls should be perfectly clean so that best quality cured beans could be obtained.

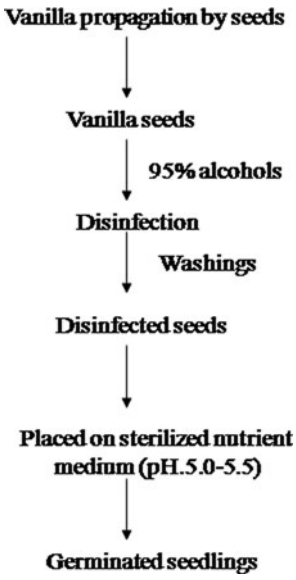
Figure 7 Sweating of vanilla beans. (Color figure available online.)

Mexican Curing Process

The 2 main traditional forms of curing employed in Mexico are sun wilting and oven wilting procedures (Chalot and Bernard, 1920; Bouriquet, 1954; Theodose, 1972). Both methods are still widely applied by the specialist curing firms in Mexico who processes the major bulk of the vanilla crop (Mallory and Walter, 1942; Correll, 1953; Merory, 1968).

Harvested green beans are made to shrink and loose some moisture by spreading them in shade for a week. Then the shrunken beans are spread in open, hot sun, on a thick dry woolen blanket and allowed for sweating. During the mid day, when the beans have gained heat, the woolen blanket is folded, and covered over the beans and left as such in open for the rest of the day. Then by evening, the beans are tied into small bundles and stored overnight in boxes (called sweating boxes) that are provided with a lining of woolen blanket. This is the beginning of fermentation stage. The beans while in these boxes undergo fermentation and sweating. This process (Flow chart 2) of spreading the beans in hot sun up to mid day, later covering them with blanket for rest of the day followed by keeping overnight in bundles in boxes has to be continued for 2–3 days, when this, the beans turn into chocolate-brown color and become soft and flexible. After this, the drying process starts and the beans are spread over the grass mats and dried in sun for 6–7 days. Each day after the sun drying, the beans should be

Figure 8 Conditioning of vanilla. (Color figure available online.)



Flow chart 1

kept packed overnight in sweating boxes. Later, the beans are completely dried in shade and are sorted for conditioning. The beans are sorted out into groups of 2 or 3 sizes, tied as per size into bundles of 50. Later these are stored in closed boxes for 3 months or more so as to allow them to develop aroma and flavor. Longer the time allowed (more than 3 months) for aging of beans, the better it is and these beans are preferred to freshly cured ones. It is estimated that the Mexican curing process allows the beans to have about 4.15–4.40 per cent vanillin content.

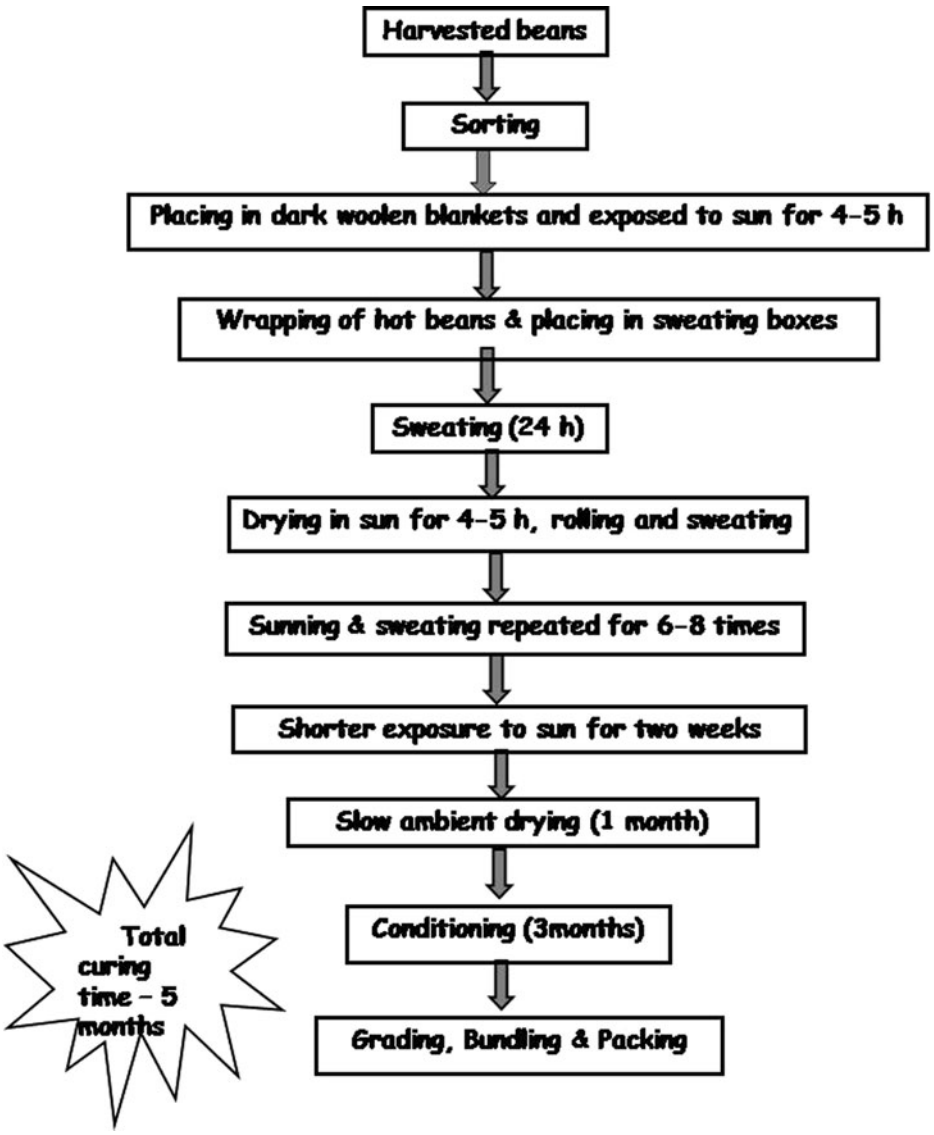
Bourbon Curing Process

The Bourbon curing technique is distinguished from that of Mexican in that the killing is achieved by scalding the beans in hot water and sweating are undertaken. The Bourbon product usually has higher moisture content than the corresponding Mexican grade and is frequently frosted. Slight variations in the curing technique are practiced in various producing islands (Lionnett, 1959; Chalot and Bernard, 1920; Bouriquet, 1954; Hibon, 1966). The island of Réunion was formerly known as Bourbon and this method was since developed there, it derives this name. This process is followed in Madagascar, the world's largest vanilla producing country. In the Bourbon process (Flow chart 3), there are 2 types: (1) hot water and (2) hot water vapor precuring. Any one of these could be followed (Krishnamurthy and Melanta, 2002; Jones and Vincente 1949).

Considerable research and development have gone into speed up the curing process (Ranaddive, 1994). As already mentioned, a number of early improvements have been patented (Towt, 1952; Graves et al., 1958; Karas et al., 1972) in which the beans were cut and cured completely within a few days resulting in a product having high uniformity and consistency, independent of the weather conditions. Another US Patent (Kaul, 1967) describes a process where green beans are covered with plastic sheets. The beans are then heated to 60°C at high humidity. This process lasts only for 1 day. The following drying step is carried out at room temperature at low humidity prior to shipping. Beans frozen immediately after harvest and thawed, storage at −18°C for 5 days, contained up to 4.7% vanillin. Conventionally cured beans contained between 2% and 2.5% vanillin. Vanilla bean with 6% vanillin was obtained after successive treatment of green beans with pectinase and β-glucosidase (Mane and Zucca, 1993). The same beans cured in traditional way contained only 1.75% vanillin. The influence of enzymatic processing of green vanilla pods is described in detail (Brunerie, 1993). The beans were crushed and enzymes were added. The best vanilla was obtained when pectinase and hemicellulase were added and the beans were treated at 37°C for 7 hours. Although the processes seem successful in shortening the curing process but they are not widely practiced in industry.

Variations effected to traditional curing methods that make the process independent of weather conditions are probably more effective (Broderick, 1956). The most important steps in vanilla curing are: first of all, the beans should be harvested when their apical ends turn yellow. This was confirmed by the fact that the highest glucovanillin concentration is reached at full maturity (Ranadive, 1994). Secondly, scratching the surface of the bean or cutting the bean as pieces is desirable, because this results in increased contact between enzymes and substrates. Furthermore, scalding of the green beans should be done by immersion in hot water (65°C) for 2–3 minutes. Subsequently, the beans are sweated after cutting them as pieces of 0.5–1 cm and wrapping them in wax paper in a closed container for 48 hours at 38°C. Drying lasts for 48 hours at 38°C on open trays, and the final conditioning step is also done at 38°C for 2–3 months in a closed container. The beans obtained through exhibited an odor comparable to that of traditionally cured beans (Broderick, 1956). Madhava Naidu et al. (2009) was reported that, preparation of vanilla extract from green beans without going through the elaborate and time-consuming conventional curing process. Vanilla beans after size reduction were mixed in a suitable proportion with tea leaf enzyme extract (TLEE) and incubated to facilitate action of enzymes on vanilla flavor precursors. The beans mix was squeezed, and the filtrate was treated with ethanol to extract the vanilla flavor. TLEE-treated extracts had higher vanillin content (4.2%) compared to Viscozyme extract (2.4%). Also, it had higher intensity of vanilla flavor, sweet, and floral notes. Further, electronic nose analysis confirmed the discrimination between extracts. It was concluded that the use of TLEE is very much useful to obtain higher yield of vanilla extract and superior quality vanilla flavor, which avoids the traditional laborious and time-consuming curing process.

Jones and Vincente (1949) suggested almost the same process, except that they used 45°C instead of 38°C. The simple mathematical procedure was developed for use in curing of vanilla to safe moisture content (Cernuda, 1949). The quality of the beans was tested by a trained panel and they equated the flavor to that of traditional beans. A later study investigated was on the quality of cured vanilla in relation to natural flavours (Bouriquet, 1954). The conclusion of their work was that the best quality beans were obtained if the beans were harvested

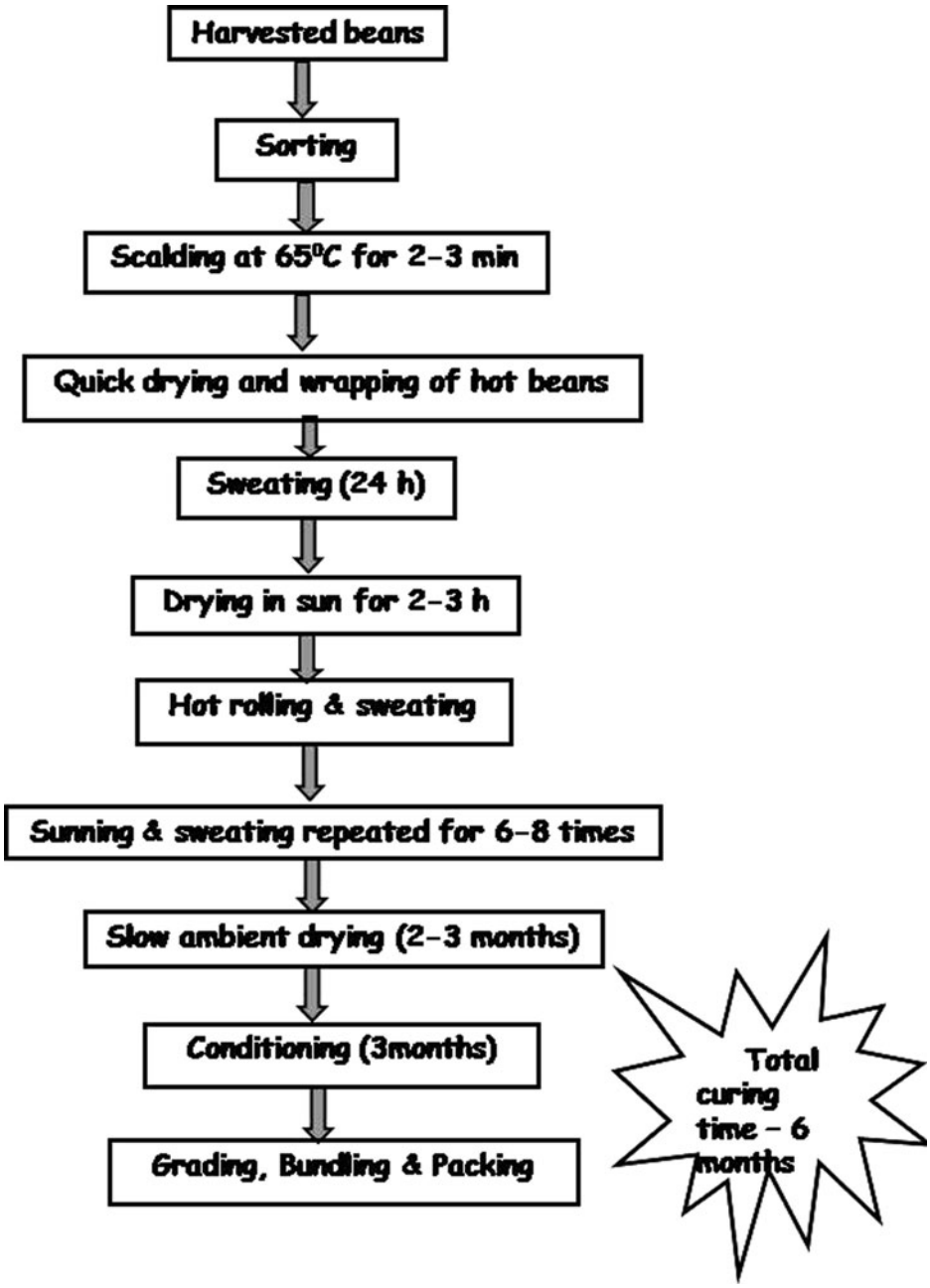


Flow chart 2 Mexican curing method of vanilla.

when the blossom ends had turned yellow (Karas et al., 1972). Beans that were ripe early in the growth season resulted in higher vanillin content than those ripened late in the season. A simple mathematical procedure was developed for use in curing of vanilla to safe moisture content (Cernuda, 1949).

Traditional Curing Process and Improvements

The vanilla aroma develops in the pods or beans through a quite labor-intensive process called curing. This process of aroma development is allowed to occur first by drying the vanilla beans and to allow chemical and enzymatic reactions to occur. Every vanilla-cultivating country has developed its own curing process, but all processes generally consist of the following mentioned common 4 steps: scalding, sweating, drying, and conditioning. In the course of the scalding procedure, also called killing, the vegetative phase is stopped. Most scalding methods involve submersion of the beans in hot water for 1 or 2 minutes. By disruption of cells, enzymes can get into contact with their substrates. During sunning/sweating, enzymes like glucosidase and oxidase are very active and the beans get their characteristic brown color. Sunning in this case means that the beans are spread out in the sun until they are hot. Then they are wrapped up in sheets and put into an airtight container overnight (sweating). This process is repeated for 1–2 weeks. The drying of the beans prevents them from being spoiled by microorganisms and allows other reactions to take place which are beneficial for the aroma to develop. The drying step usually lasts for 2–4 weeks and is performed indoors avoiding direct sunlight, sometimes with the use of a fan to create an air circulation. The most time consuming step in vanilla curing process is, conditioning will take several months. During conditioning, the beans are stored in a conditioned room. Several (bio) chemical reactions such as

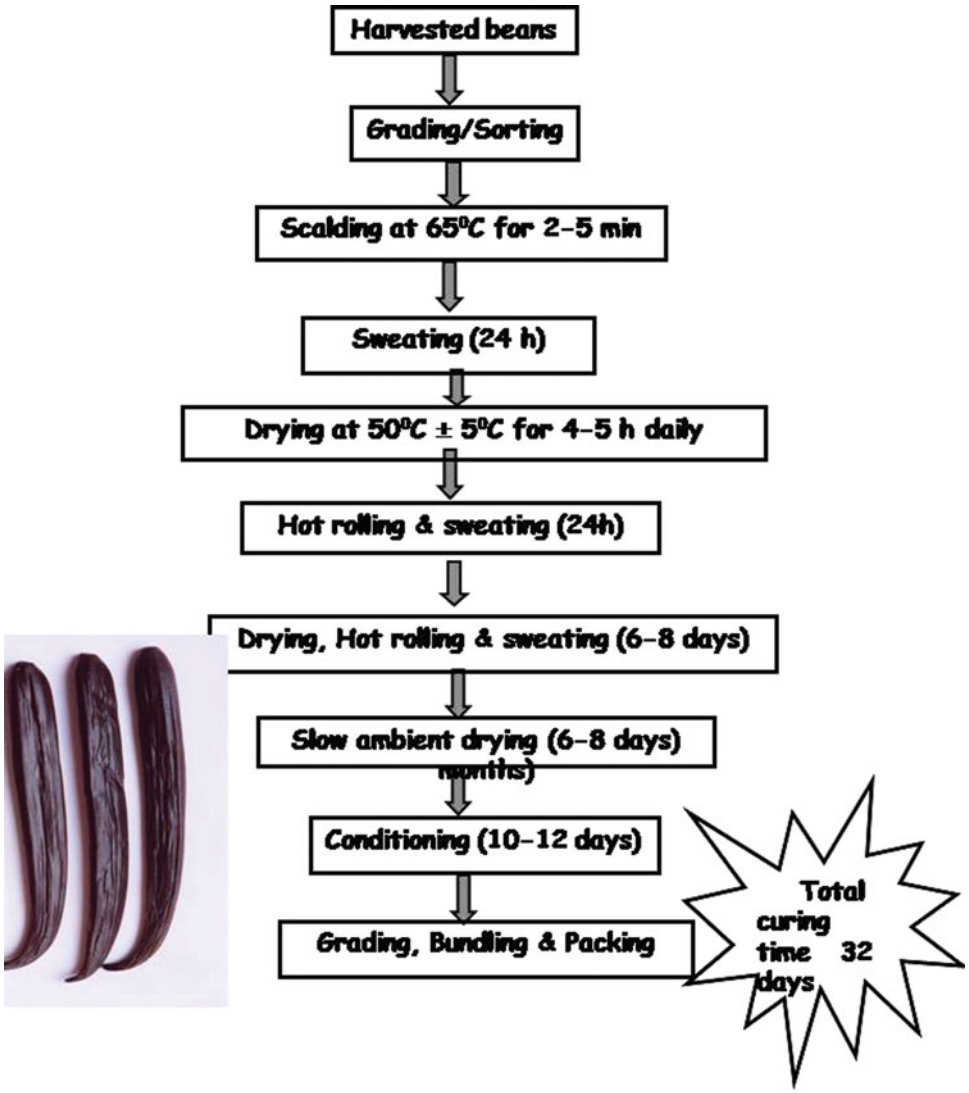


Flow chart 3 Bourbon curing method for vanilla.

esterification or oxidative degradation occur (Ranadive, 1994). The process can last up to a total of 6 months.

Conventional curing of vanilla supplemented with hot-air drying was reported (Theodose, 1973). Other groups (Abdulla, 1997; Kamaruddin and Mursalim, 1997; Ratobison et al., 1998) have investigated the possibility of using drying equipment based on solar energy. The best quality beans were obtained with a vanillin content of 2.36% by a greenhouse effect solar dryer at 45°C (Kamaruddin and Mursalim, 1997).

Recently, Central Food Technological Research Institute (Madhava Naidu et al., 2008; Sreedhar et al., 2007) has developed a process for faster curing of vanilla, which is considered to be a new approach is based on conventional curing supplemented with hot-air drying in well-organized curing facilities. This would assure a constant quality and lower labor costs and the process know-how is already available for commercialization. The process involves freshly harvested mature green vanilla beans are procured from vanilla gardens, are subjected to size grading manually and processed immediately. Graded beans are transferred to a bamboo basket and immersed in hot water at a suitable temperature for a few minutes. The scalded beans are subjected to sweating followed by mechanical drying to reduce the moisture and improve the vanilla flavor. The beans lose moisture quite rapidly and become very flexible. At the



Flow chart 4 Faster curing method for vanilla.

end of this period, the beans reduce half of their initial weight, turn to a shining dark brown color, develop wrinkles and also, improve their aroma. In the next phase, which lasts for a period of 6–8 days, the beans are allowed to dry slowly in properly ventilated rooms. For conditioning, the beans are placed in air-tight chests or waxed paper lined metal containers or polythene covers for about 10–15 days to develop desired vanilla flavor (Flow chart 4). The findings of studies that to date have not revealed the exact nature of reaction process involved in the highly complex phenomena that take place during vanilla curing (Erix Odoux, 2010).

Sensory Studies

The method of processing or curing influences the aroma quality of vanilla pods. Odor and flavor profiling of vanilla by sensory analysis is a convenient method of determining the quality of cured vanilla pods. The medium used for dispersing the vanilla extract exerted some influence on flavor perception (Hariom et al., 2006). Vanilla flavored milk was prepared incorporating vanilla extracts/concentrates (0.1% of sample1 and 0.2% of samples 2 and 3). The flavored milk was evaluated using quantitative descriptive analysis among the trained panelists.

As shown in Fig. 9, the sample 1 is having more of vanilla concentration which is reflected in having high score for vanilla flavor, pleasantness, and typical floral notes compared to milk prepared with vanilla extract samples 2 and 3. Milk prepared with samples 2 and 3 are having almost same sensory profile.

An attempt has been made to evaluate the utilization of vanilla extract as a source of flavorant as well as an antioxidant in biscuits (Anuradha et al., 2010). Biscuit doughs were incorporated with 0.4% vanilla extract and synthetic vanillin 0.2%. The biscuits were powdered, extracted with aqueous ethyl alcohol and screened for radical scavenging activity. Natural vanilla and synthetic vanillin biscuit extracts at a concentration of 200 ppm showed 70% and 43% of radical scavenging activity,

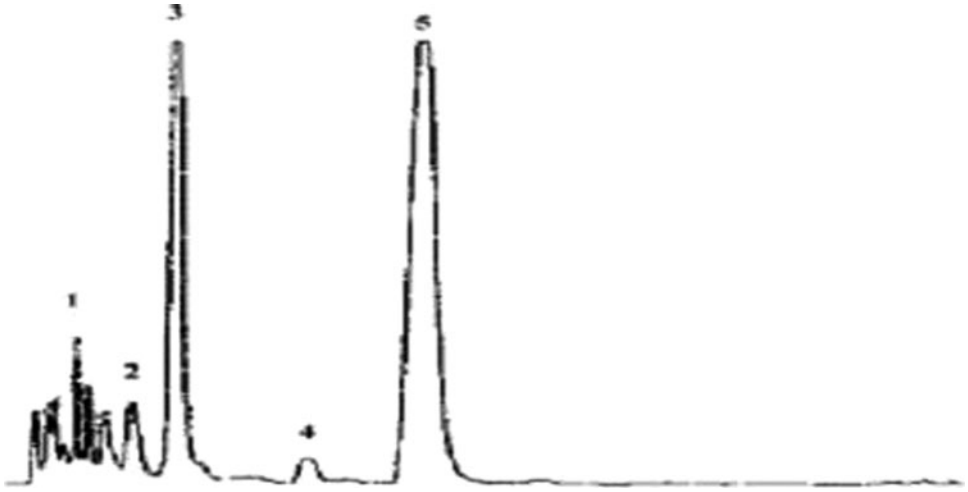


Figure 9 HPLC pattern of vanilla extract. (1) vanillic acid. (2) 4-hydroxy-benzyl alcohol. (3) 4-hydroxy-3-methoxybenzyl alcohol. (4) 4-hydroxybenzaldehyde. (5) vanillin (Shyamala et al., 2007).

respectively, by the 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) method in comparison to a corresponding value of 93% for Butylated hydroxyanisole (BHA). The extract was profiled by high-performance liquid chromatography (HPLC). The effect of moisture content on retention of flavor in biscuits was studied. The loss of flavor in packed and stored biscuits with time was also studied. Addition of vanilla extract showed higher antioxidant activity and lowered the peroxide value in biscuits. The results revealed that the natural vanilla extract 0.4% level could be used in glucose biscuit preparation, since this concentration imparted considerable antioxidant properties to the biscuits.

Sorting and Grading

After completing the curing process, the next operation to be followed is grading. Each country which produces vanilla follows its own system of grading. Three grades of vanilla exist in Seychelles (Lionnett, 1959) generally, the first grade (best) beans consist of those with lengths of 13–23 cm, which are flexible with good aroma, luster, appearance, and flavor besides having no infection by pests or diseases and possess dark brown color with oily look. The second grade comprise consists of harvested immature and having inferior color with reddish streaks. All the remaining beans are graded as third-grade beans. Further, the surface of the beans show crystals of vanillin during storage and such beans fetch premium price and are called frosted vanilla. According to the grades, beans are separately bundled into known weight (Rao et al., 1992).

In Mexico, 5 classes of vanilla are marketed. The best ones are called primeria, the pods of which are 24 cm long and proportionately thick; the second ones are called chica prima, the pods being shorter and 2 of them being counted as 1; the third is sacate; the fourth, vesacate and still smaller and are gathered before they are ripe; the fifth quality is basura with small, spotted, and much broken pods (Ridley, 1992). In India, the beans are graded into 4 groups. The first (best) grade beans consist of those above 16 cm in length, soft to touch, without any blemishes and having 2.5% vanillin. The second-grade beans consist of those having 12–15 cm in length, without blemishes and having more than 1.8% vanillin. The third-grade beans are shorter having a length of 8–12 cm and with some blemishes. The rest of the beans are taken as fourth (last) grade and are of low quality (Kuruvialla et al., 2000; Krishnakumar et al., 2003).

Quality of Beans

The volatile constituents and among them mainly the vanillin content of beans primarily decides the quality of cured vanilla beans. Even there are numerous nonvanillin aroma components present in beans which contribute for taste and flavor. Vanillin has its characteristic aroma and flavor. These differ according to the species of vanilla, agro-climatic and ecological conditions of place of cultivation, cultivation system, method of curing, pest and mould attack, and moisture content. The percentage of vanillin in beans varies from 1.5% to 2.6%. Among the different species, *Vanilla fragrance* is the most popular one valued for its flavor and aroma (Rao et al., 1992).

CHEMISTRY

Biosynthesis of Aroma Compounds

Glucovanillin is present as gradient increasing from the basal to the apical end of the bean. The central part of the bean, containing seeds and placental tissue is having maximum concentration of glucovanillin. In contrast to this β-glucosidase is present in the outer part rather than in the central part. Therefore, in order to hydrolyze the entire amount of glucovanillin in the central part of the bean containing the glucovanillin portion must be able to diffuse to the outer region during the initial day of curing, in order to get in contact with the hydrolyzing enzyme (Arana, 1943).

The formation of glucovanillin starts from the third month after pollination, when the bean is almost fully grown. Kanisawa, (1993) succeeded in identifying glucovanillin directly in a growth experiment with beans on the vine. Others also confirmed this observation after hydrolysis of the glucosides by *β-glucosidase* (Zenk, 1965; Brodelius, 1994). The activities of polyphenoloxidase, glucosidase, and peroxidase enzymes also increased from the third month after pollination. In contrast, proteinase loses its activity and probably does not have an effect on curing (Wild-Altamirano, 1969). When beans are injured during the scalding, either by dipping in hot water or by scratching the outer surface, an increase in the carbon dioxide release from the beans can be observed. This is probably due to an initial acceleration of the rate of oxidation in the tissues. Apparently, peroxidases play an important role (Balls and Arana, 1941).

Other investigators (Jones and Vimcente 1949; Arana 1944) reported that oxidases also play an important role in curing by producing quinines and condensed stable pigments. On heating (120°C) fresh beans, all enzyme activities are stopped. However, some peroxidase activity can be observed at a later stage of curing. The beans remain green and develop no vanilla aroma. Similarly, when the beans are heated after 2 days of sweating, no vanilla aroma is formed, but peroxidase activity recovers after a few weeks. These results indicate that the principal changes involving enzymes take place in the first week of curing. Since a large part of the aroma is formed during conditioning of the beans, it was thought that a considerable part of aroma formation must be nonenzymatic (Jones and Vimcente 1949). Another possibility is that the peroxidase system is responsible for further oxidation of aroma compounds to other structures with presumably different aroma. This would explain the fact that the quality of vanilla bean does not depend only on vanillin content (Kanisawa et al., 1994). The enzymes during curing apparently glucosidase activity is high in the first week of curing and then decreases rapidly (Hanum, 1997). At the same time, the enzyme activities of the oxides decrease more slowly. These results agree with the earlier reports (Balls and Arana, 1941; Jones and Vimcente, 1949).

The effect of scalding on enzyme activities in green beans was investigated (Krishnamurthy and Melanta, 2002). According to them, the relationship between vanillin biosynthesis and maturation is independent of the geographic origin of the beans. Apparently known differences between cured beans must occur during curing (Balls and Arana, 1941). Furthermore, the soluble protein content has been reported to decrease after scalding at 65°C (Balls and Arana, 1941; Arana, 1944; Ranadive, 1994). The β-glucosidase activities as well as glucovanillin concentration are very high at the time of harvest after 7 months. On the other hand, the glycosidases are not very resistant to heat. After scalding, most of their activity is lost and decreases even more, a day after scalding (Ranadive, 1994). If β-glucosidase is really inhibited by the scalding procedure, a significant part of vanilla aroma formation must be due to nonenzymatic reactions. One of the most obvious aspects of curing is that vanillin β-D-glucoside and related β-D-glucosides come into contact with β-D-glucosidases, with the result that free vanillin and related substances (notably 4-hydroxy-benzaldehyde) are released (Kanisawa et al., 1994; Ramachandra Rao and Ravishankar, 2000; Dignum et al., 2001). The vanillin content of cured pods is usually 2-2.5%, and in addition more than 200 minor components are reported.

The appearance of vanillin during curing is very simple, unlike the mechanism by which vanillin β-D-glucoside is initially synthesized. Several biosynthetic routes have been proposed, but much uncertainty still remains concerning the chain shortening and other reactions leading from the putative hydroxycinnamic acid precursor to vanillin β-D-glucoside. An earlier study reported the results of feeding radioactively-labeled ferulic and vanillic acids and proposed a route by which both vanillin and vanillic acid were derived from ferulic acid (Zenk, 1965). By analogy with fatty acid β-oxidation, a CoA-dependent β-oxidative cleavage of feruloyl-CoA was suggested, leading to the formation of vanilloyl-CoA; this compound would then be reduced to vanillin or alternatively deacylated to vanillic acid (Fig. 10).

It was considered that glucovanillin or vanillin was derived from conifer in either via glucovanillin or via coniferyl alcohol. The conclusion was reached by identifying the compounds present in an extract by Thin Layer Chromatography (TLC) and infrared (IR) spectroscopy, but it was also thought that ferulic acid is the precursor of vanillin rather than coniferin. Zenk used (O-$^{14}CH_3$)-labeled ferulic acid and found 100% incorporation in the vanillin formed after administering ferulic acid to 2-mm-thick bean parts (Zenk, 1965). Labeled ferulic acid was found in vanillic acid, but this vanillic acid could not be derived from vanillin. It is still unknown when glucosylation of the aroma compounds takes place.

Several groups tried to isolate the glycosidic fraction from green beans and to identify the glycosides present and 30 glycosides were identified either directly by means of HPLC and Nuclear Magnetic Resonance (NMR) or after β-glucosidase treatment (Kanisawa, 1993). They proposed a pathway for the formation of vanillin and other phenolic compounds in the beans of *V. planifolia* (Kanisawa et al., 1994). This pathway leading from 4-coumaric acid via *p*-hydroxybenzaldehyde glucoside to glucovanillin is based on the glycosides identified in the extract of green beans (Fig. 11). It does not agree with the earlier observations about conifer in, but it matches Zenk's findings using labeled ferulic acid. Ferulic acid can contribute to vanillin formation, but it might not be the main precursor. Studies on application of metabolic engineering to vanillin biosynthetic pathways in *V. Planifolia* were reported (Havkin-Frenkel and Belanger, 2007). Biotechnological Applications in Vanilla was described by Divakaran, et al. (2010).

Identification of Compounds in Green Beans

Many aroma compounds, including vanillin, are present in green beans as glycosides. Glucosides isolated from green beans using methanol was reported (Kanisawa, 1993). The extract was purified by selective elution on an Amberlite XAD-2 column, as well as by silica gel chromatography and HPLC.

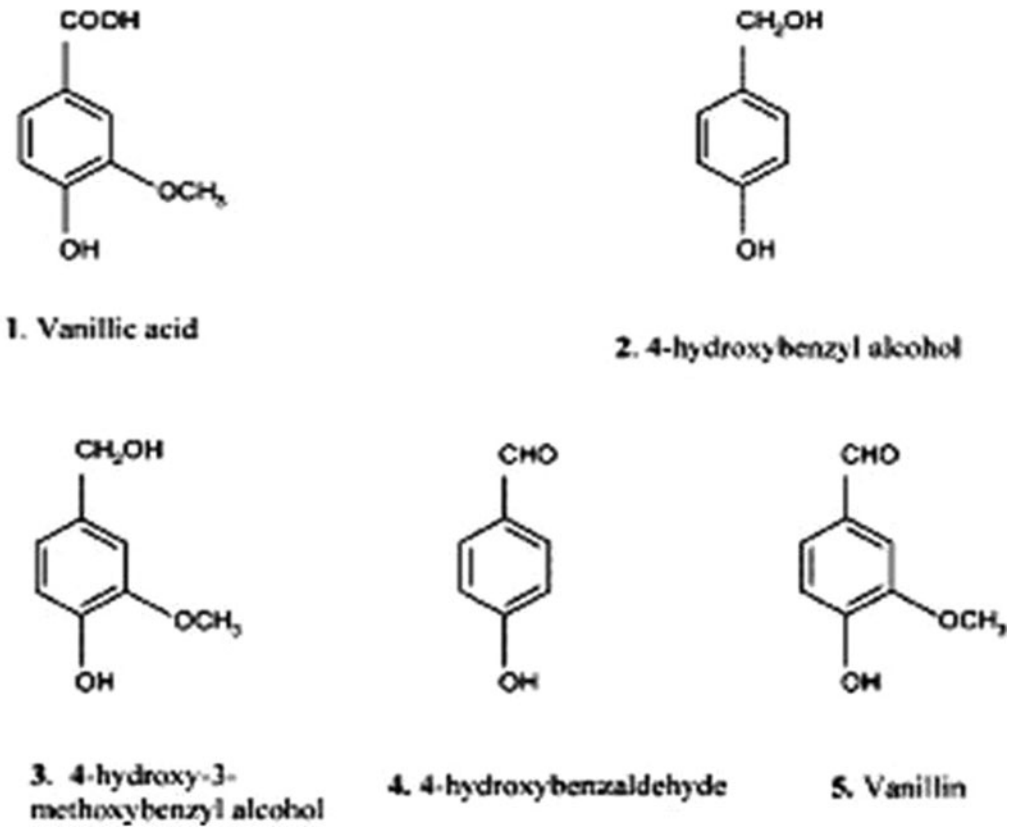


Figure 10 Chemical structures of the major flavor compounds found in cured beans of *V. planifolia*.

The major glucosides were identified using NMR and mass spectrometry (MS) (Tables 3 and 4), of which glucovanillin, bis {4-(β-glucopyranosyloxy)-benzyl}-2-isopropyltartarate (glucoside A) and bis {4-(β-glucopyranosyloxy)-benzyl}-2-(2-butyl)-tartarate (glucoside B) were the most abundant. Glucosides A and B belong to the loroglossins that have been reported in other Orchidaceae plants (Kanisawa et al., 1994). The minor glucosides were identified after hydrolysis with β-glucosidase by gaschromatography-MS (GC-MS).

Glucovanillin from an aqueous extract of green beans using a silica column was separated (Leong et al., 1989). The compound was identified by means of HPLC, NMR, Fast atom bombardment mass spectra (FAB-MS) and UV, and IR spectrometry. The purified glucovanillin was identified by means of NMR, UV spectroscopy, and static probe liquid secondary ion MS. Later, the same group investigated whether sugars other than glucose were bound to the aroma compounds (Leong et al., 1991). After hydrolysis of glycosidic extract, 20% of the dry weight was glucose, 1% mannose, and only a trace was rhamnose.

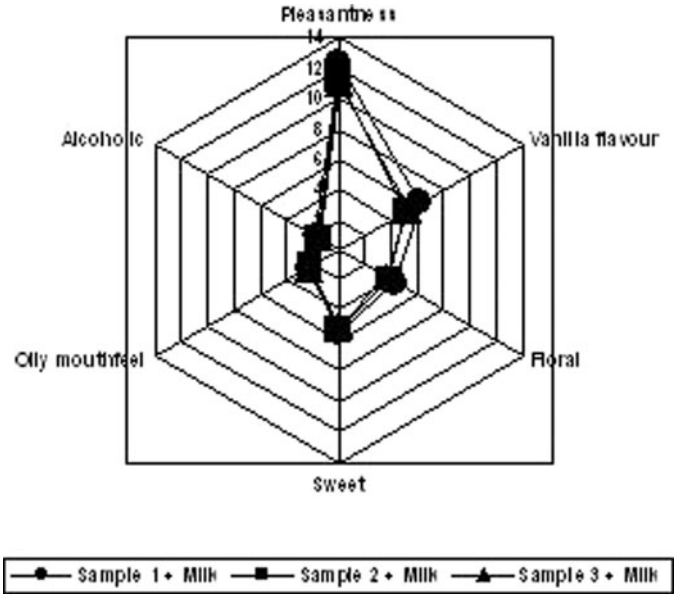


Figure 11 Sensory profile of vanilla extracts.

Table 3 Glucosides identified directly in green beans extract using NMR and MS

Glucovanillin
Methyl-4-β-glucopyranosyloxy ferulate
4-methylphenyl-β-D-glucopyranoside
4-formylphenyl-K-D-glucopyranoside
4-hydroxymethylphenyl-β-D-glucopyranoside
Methyl-2-β-D-glucopyranosyloxybenzoate
Methyl-4-β-D-glucopyranosyloxybenzoate
β-Phenylehtyl-β-D-glucopyranosid
Bis{4-(β-D-Glucopyranosyloxy)-benzyl}-2-isopropyltartarate (glucoside A)
Bis{4-(β-D-Glucopyranosyloxy)-benzyl}-2-(2-butyl)tartarate (glucoside B)

NOTE: Kanisawa et al. 1994.

Table 4 Glycosides identified after β-glucosidase treatment of the extract using GC-MS

Mono substitutes	
Cinnamic acid	Benzyl alcohol
Cinnamic alcohol	3-phenylprapanol
Phenethyl alcohol	
Di substitutes	
p-cresol	*p*-hydroxy benylalcohol
p-hydroxybenzaldehyde	*p*-hydroxy benzoic acid
p-hydroxycinnamic acid	*p*-hydroxy benzylmethyl ether
Methyl-*p*-hydroxybenzoate	Methyl-*p*-hydroxy cinnamate
4-vinylphenol	Methyl salicylate
Tri substitutes	
Methyl ferulate	3,4-dihydroxybenzoic acid
4-vinylguaiacol	Homovanillyl alcohol
Vanillin caffeic acid	Methyl vanillate
Ferulic acid acetovanillon	Vanillyl alcohol
2-methoxy-4-cresol	Methyl-3,4-dihydroxy cinnamate
Ethyl-4-hydroxy-3- methoxyphenylacetate	Vanillic acid

NOTE: Kanisawa et al. 1994.

Glucosides were synthesized and used for HPLC analysis (Negishi and Otawa, 1996). They based their research on glucosides identified earlier (Kanisawa, 1993). However, only glucovanillin and *p*-hydroxylbenzaldehyde glucoside could be identified as beans were immature. Glucosides of vanillin, *p*-hydroxybenzaldehyde, vanillic acid, and *p*-hydroxy benzoic acid were synthesized using a 2-step process (Leong et al., 1989). First, acetylated glucosides were prepared with acetobromoglucose, and deacetylated to form the glucosylated compounds. The yield of this process was not very high; also, in the case of vanillic acid and *p*-hydroxybenzoic acid, as some de-esterification took place. All 4 glucosides were detected in a green bean extract by HPLC. Recently, 5 glucosides were identified in green vanilla beans and also kinetics of vanilla β-glucosidase was reported (Dignum et al., 2004).

A completely different approach to obtaining glycosides is the use of plant cell cultures. Glycosylation is part of the detoxification process in plant cells. *Catharanthus roseus* cell suspension culture was able to produce glucovanillin on feeding of 1.24 g/L vanillin (Sommer et al., 1997). Glucovanillin reached a concentration of 1.54 g/L; this might be a good way to make more glycosides available for vanilla glycoside identification. Recently, 5 glucosides were identified in green vanilla beans and also kinetics of vanilla β-glucosidase was reported (Dignum et al., 2004).

Identification of Compounds in Cured Beans

The compounds identified in cured *V. planifolia* beans (Dignum et al., 2001) are listed in Table 5. Vanillin is the most

Table 5 Compounds identified in cured vanilla beans of *V. planifolia*

Reference	Compound
Werkoff et al. 1997	Hexyl salicilate, trans-α-ionone, menthyl acetate, isobornyl acetate, phenethyl formate, pentyl salicilate, and hexyl butanoate, butyl hexanoate, α-terpinyl acetate, linalyl acetae and anisyl formate, fenchyl acetate, bornyl acetate, citronellyl isobutyrate and ethyl salicilate
Galetto and Hofman, 1978	Vanillyl ethyl ether, *p*-hydroxybenzylmethyl ether, vanillyl methyl ether
Klimes and Lamparsky, 1976	Isobutyric acid, formic acid, propionic acid, glycolic acid, methoxy acetic acid, myristic acid and benzoic acid, nonane, ethyl benzene, propylbenzene, *p*-ethyltoluene, myrcene, *p*-cymene and α-curcumene, butane-2,3-diol, pentanol, 2-methyllbutanol, 3-methylbutanol, phenyethyl alcohol, myrtenol, α-terpineol, cresol, *P*-iinylphenol and *p*-hydroxybenzyl alcohol, protocatechualdehyde, pentanal and *p*-methoxy benzaldehyde, acetophenone, hexan-2-one, heptan-2-one, octan-2-one, 7,10,14-trimethylpentadecan-2-one, furfural hydroxyl-methylketone, 5-piperidone and dihydroactinidiolid, prenol, pentan-2-ol, hexanol, 3-methylpentanol, heptanol, terpinen-4s-ol, linalool, nerol, *p*-ethylguaiacol, *p*-vinylguaiacol, *p*-methoxybenzyl alcohol, benzaldehyde, furfural, β-cyclocitral and *p*-methoxy benzaldehyde, diacetyl, nonan-2-one, decan-2-one, 3-penten-2-one, 5-hydroxy heptan-2-one, and 1-hydrxy hexan-2-one, 2-acetylfuran, and vanillin2,3-butylene-glycolacetal, *p*-cresol, guaiacol, octanol, nonan-2-ol, decnol, benzyl alcohol, geraniol, *B*-bisabolol, phenol, furfurylalcohol and *p*-methoxybenzylalcohol, anisol and 2-acetylpyrrole, decenoic acid, protocatechuic acid, butyric acid, valeric acid, salicylic acid, isovaleric acid, methyl butyrolactone, naphthalate, benzene, dimethyl benzene, trimethyl benzene, 4-hydroxy-3-methoxy benzyl methyl ether, *p*-hydroxybenzyl ethylether, octanoic acid, decanoic acid, caproic acid, lauric acid, and lactic acid, anisic acid, α-muurolene toluene, 1,2-dimethoxy benzene, styrene, methyl cyclohexane carboxylate, diphenylether, and *p*-hydroxybenzyl methyl ether, salicylic aldehyde, 5-methylfurfural, heliotropin, acetaldehyde, 1-hydroxy pentan-2-one, acrolein, 3-octen-2-one, octa-4,7-dien-3-one, 3-hydroxybutan-2-one, and 2-hydroxyethyl-5-methylfuran, benzyl acetate, benzyl benzoate, benzyl butyrate, propyl valerate, isopropyl valerate, ethyl decanoate, isobutyl valerate, methyl caproate, methyl heptanoate, methyl nonanoate, methyl *cis*-cinnamate, di-*n*-propyl phthalate, di-*n*-butyl phtalate, methyl pentadecanoate, ethyl 2-methyl butyrate, 4-hydroxy-3-methoxy benzylethyl ether and *p*-cresyl isopropyl ether
Morison Smith 1964	Vanillin, vanillic acid, *p*-hydroxybenzaldehyde, acetic acid
Ramaroson-Raonizafinimanana et al. 1997	*n*-1-alkenes, 5-ethylalkanes, brassicasterol, β-Sitosterol, Δ^5-avenasterol, and vanillic alcohol
Ramaroson-Raonizafinimanana et al. 1998	Stigmasta-5, 22, 25-trien-3β-ol, Δ^7-avenasterol, ergosta-7,24(28)-dien-3β-ol
	Cis/*trans*-vitispirane
Ramaroson-Raonizafinimanana et al. 1999	Dihydromethylpyranones

NOTE: Dignum et al., 2001.

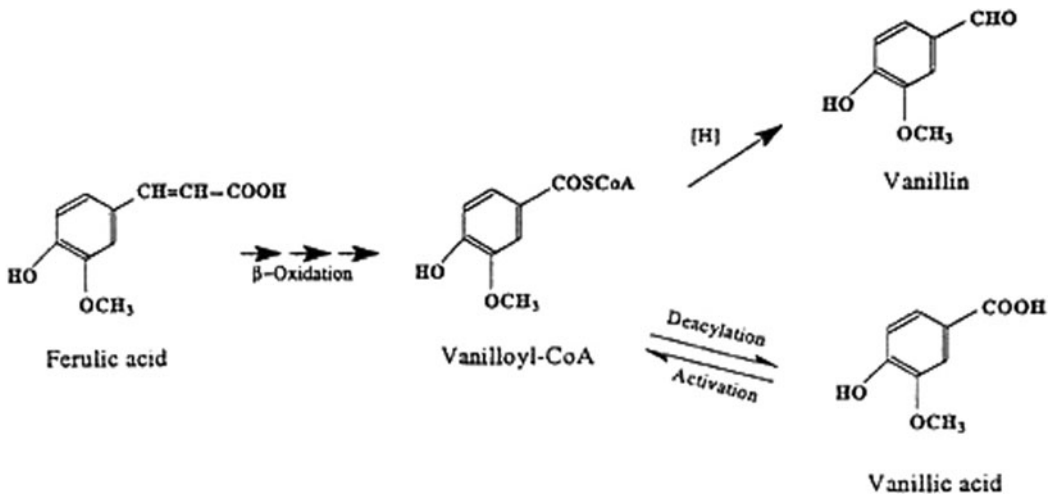


Figure 12 An outline suggested route of vanillin formation (Kanisawa et al., 1994).

abundant aroma compound in cured beans (∼2%), followed by *p*-hydroxybenzyl methyl ether (0.02%), and acetic acid (0.02%). Others are present in amounts less than 10 ppm (Klimes and Lamparsky 1976). The aroma compounds of 10 different cured bean extracts of various origins, including Tahiti beans were compared (Adedeji et al., 1993). A large range of compounds was found and some compounds seemed to be characteristic of beans from certain origins. The vanillin content in the beans varied from 0.34% for Java to 2.0% for Bali beans. Determination of glucovanillin and vanillin in cured beans was reported (Voisine et al., 1995).

Piperonal as an important characteristic compound in both vanillion and Tahiti beans has been reported (Lhuguenot et al., 1971). Its concentration should be higher than the vanillin concentration as detected by gas chromatography. Anisic acid and anisaldehyde are also characteristic of Vanillin and Tahiti beans. However, no piperonal was found in Tahiti beans (Fayet et al., 1987; Ehlers and Bartholomae, 1993; Lechat-Vahirua and Bessiere, 1998). It is reported that the presence of piperonal in Tahiti beans was the result of adulteration (Ehlers et al., 1994). Identification of ester compounds and hydrocarbons in Bourbon vanilla beans was reported (Werkoff and Guntert, 1997; Ramaroson-Raonizafinimanana et al., 1997). Also some benzyl esters and vanillin related compounds in the vanilla extract were reported (Morison Smith 1964; Galetto and Hoffman, 1978). Vanilla extract prepared from cured vanilla beans characterized by HPLC (Shymala et al., 2007) and 5 major compounds were identified (Figs. 12 and 13).

Detection of Vanilla Constituents

A "VANILLA EXTRACT" as defined by a standard of the Food and Drug Administration is the solution, containing not less than 35% alcohol, of the sapid and odorous principles extracted from one or more than 25% moisture in 1 gal of finished product. No addition of artificial vanillin is permitted in products designated as "VANILLA EXTRACT" (Martin et al., 1977).

Adulteration of vanilla extracts with synthetic, lignin-derived vanillin is a major problem in the commercial market. Low vanillin containing Indonesian vanilla extracts can be made to premium Bourbon extracts by addition of synthetic vanillin. Adulteration of this type can often frustrate the conventional quality assurance tests of lead number, organic acid profile, and vanillin/potassium ratio (Hoffman and Salb, 1979). This type of adulteration may be readily detected by carbon stable isotope ratio analysis (SIRA). It was found that the $^{13}C/^{12}C$ ratio of the carbon in vanillin from vanilla differed from that in synthetic vanillin derived from lignin and other sources. Vanillin is oxidized with sodium chlorite to vanillic acid, which is then decarboxylated with bromine. The CO_2 derived from the carbonyl carbon, is analyzed mass spectrometrically to determine $^{13}C/^{12}C$. Vanillin from *V. planifolia* gives carbonyl values near to −25%; vanillin from lignin yields carbonyl values near to −35%. Lignin vanillin, labeled with ^{13}C from (carbonyl-13C)-vanillin to resemble that from authentic vanilla, gives carbonyl values near +20% (Dana and Harold, 1985). Vanillin and related flavor compounds in vanilla extracts from various global origin was reported (Ranadive, 1992). Enzymatic extraction and transformation of glucovanillin to vanillin from vanilla green pods was reported (Ruiz-Teran et al., 2001). Recently, a process toward preparation of vanilla extract from green vanilla beans was reported (Madhava Naidu et al., 2009). Vanilla extracts examined by SIRA and chemical component analysis are described (Lamprecht et al., 1994). Vanillin was extracted and purified by semipreparative HPLC, and the $^{13}C/^{12}C$ isotope ratio was determined by MS. The concentrations of the main flavoring components relative to vanillin were determined by HPLC. Authentic extracts, prepared in the laboratory from vanilla pods, as well as commercially available extracts were examined. The authenticity and the degree of adulteration in blended samples were determined by both the methods. The analytical data demonstrated that SIRA and component

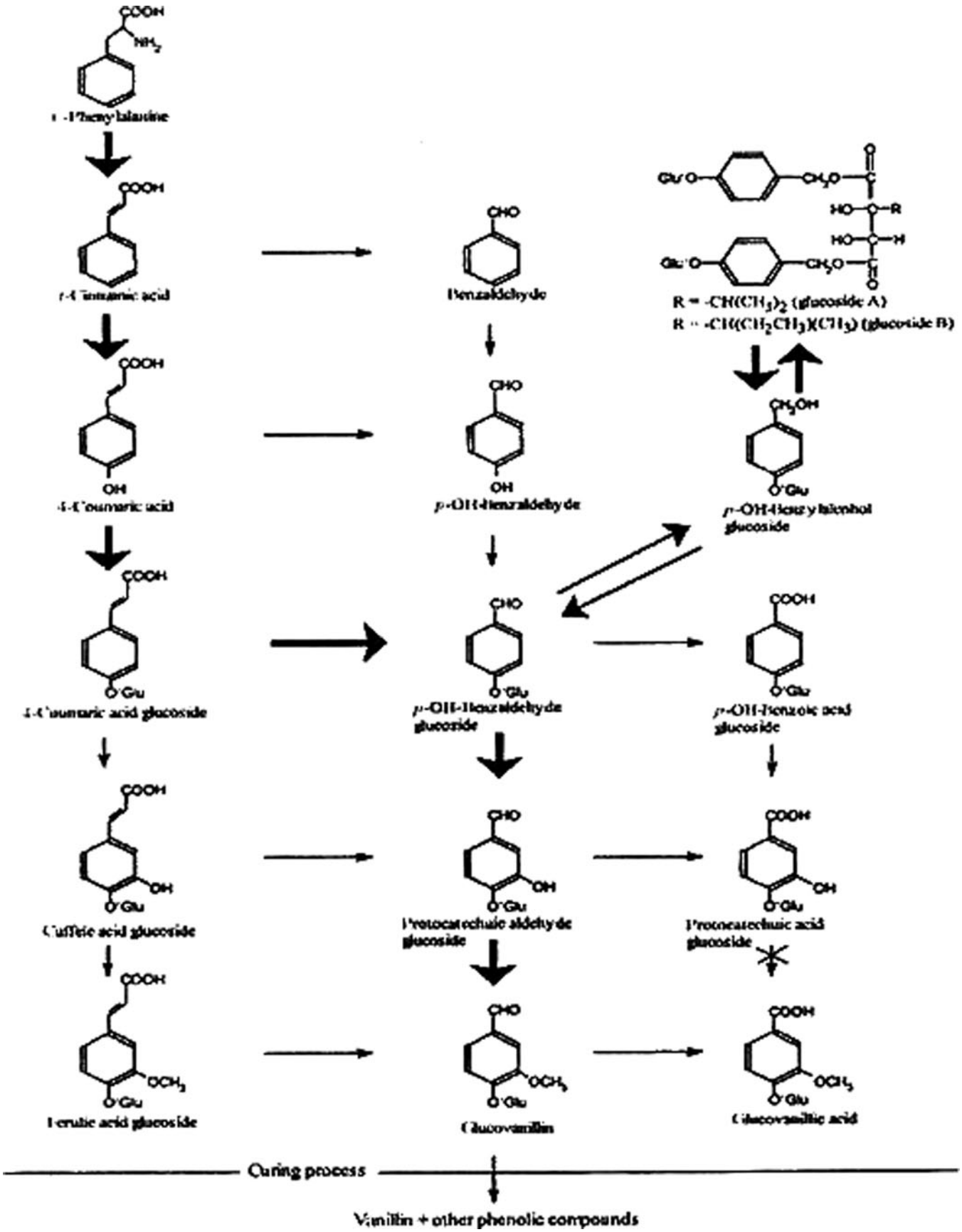


Figure 13 Proposed pathway for the formation of vanillin and other phenolic compounds in the beans of *vanilla planifolia* (Dignum et al., 2001).

analysis show comparable results; SIRA detects the amount of added synthetic vanillin much more precisely and is, therefore, taken for quantification of adulterations.

On the other hand, component analysis of vanilla extracts has the advantage that component concentrations and added substances, which did not occur in authentic vanilla extracts, can be determined. Simple adulterations can easily be recognized by component analysis, whereas the addition of flavoring compounds, which occur in vanilla extracts (mostly vanillin), is much more difficult to detect. Because of the relatively wide concentration range in which the components occur, the estimation of the degree of adulteration is hardly possible. Nevertheless, estimation by component analysis is feasible, if analytical data of vanilla pods of the same origin are available. Their chromatograms, recorded under the same conditions, show almost identical elution patterns. The application of pattern recognition is, therefore, an appropriate method for the determination of the origin and crop of vanilla pods, reducing further the range of variability of the components in vanilla pods and allowing statements of their authenticity by component analysis.

Using 2H NMR Solid Phase Micro Extraction (SNIF-NMR), it is possible to measure the 2H content even at individual atomic sites of molecules (Kauzinger et al., 1997). Regarding the molecule vanillin, 5 different monodeuterated structures are used for comparing samples. The values of these monodeuterated compounds are known, a unique isotopic fingerprint closely related to the origin of the molecule and inimitable by synthetic copies (Martin and Remud, 1993). Very recently, cGC coupled online via combustion interface with isotope MS (IRMS) has been reported successfully in the case of $^{13}C/^{12}C$ ratios. The substances eluting from the cGC column are converted into carbon dioxide in a combustion oven and then directly analyzed in the isotope mass spectrometer adjusted for the simultaneous recording of masses 44, 45, and, 46 in the nanomole range with high precision. The instrumental configuration of cGC-IRMS combines the precision of IRMS with the high purification effect of cGC separation, with large savings on laborious sample cleanup procedures. Stable carbon and hydrogen isotopes have been used in the deciphering of source materials for flavors such as vanilla, linalioil, and citral (Bricout and Koziet, 1978). Isolation and tentative identification of new constituent of bourbon vanilla bean extract was reported (Feyertag and Hutchins, 1981).

The major flavor constituent of vanilla extract is vanillin (4-hydroxy-3-methoxybenzaldehyde) besides many other volatile compounds such as guaiacol, *p*-anisaldehyde and methyl cinnamate have been reported to contribute to its flavor (Lamprecht et al., 1994; Perez-Silva et al., 2006). Artificial vanilla extracts are generally less complex and contain vanillin, ethyl vanillin, and other related compounds produced using inexpensive starting materials. Coumarin (2H-1-benzopyran-2-one) has a sweet herbaceous odor and has been detected in some imported vanilla products (Thompson and Hoffmann, 1988). Several methods for the detection of vanillin, ethyl vanillin, and/or coumarin in vanilla extract have been published including TLC (Beley and Poole, 1993; Mckone Puerto and Chambers, 1988) HPLC-UV (Martin et al., 1973; Kahan and Kruegar 1997; Jagerdeo et al., 2000a; Jagerdeo et al., 2000b; Waliszewski et al., 2007) and GC-MS (Sostaric et al., 2000). A method for on-line dilution and detection of vanillin in vanilla extracts was obtained by ultrasound (Flores and Canizares-Macias, 2007). Each of these methods has 1 or more drawbacks and no single method has been reported for the quantitative determination of vanillin, ethyl vanillin and coumarin in vanilla extract that provides qualitative mass spectral confirmation. Recently a Solid Phase Micro Extraction (SPME)-GC-MS and LC-MS method for the quantification of coumarin, vanillin, and ethyl vanillin in vanilla products was reported (De Jager et al. 2007, 2008). RP-HPLC method was developed for quantitative determination of vanillin and related phenolic compounds in vanilla (Sinha et al., 2007, 2008).

NUTRACEUTICAL PROPERTIES OF VANILLA

Vanilla has been coveted over the ages for culinary and medicinal reasons alike. Vanilla's high status in the culinary world comes from a long history of flavoring sweet, sensual desserts such as ice-cream, sugar cookies, puff pastries, and butter creams (Jenna, 2005). While history of vanilla is steeped in culinary traditions, its lesser-known uses as an aphrodisiac and a medico-botanical uses stretch back to its discovery in Mesoamerica by ancient cultures that cultivated and honored the sweet orchid. European nations also historically valued vanilla for its flavor, its lore as a love potion, and its medicinal uses. Present day advances in basic science research have shed light on the medicinal benefits of vanillin, the most active constituent of vanilla.

Vanilla was used medicinally for many women's concerns, including hysteria and depression. During 1700s and 1800s, vanilla appeared in the European pharmacopoeia and was indicated for "fevers, melancholy, and hysteria probably because of its supposed diuretic, sedative, and purifying qualities." Vanilla was brought to the United States by Thomas Jefferson in 1789 upon returning from a tour of France. In 1898, King's American Dispensary cited vanilla as an: "Aromatic stimulant. Vanilla is said to exhilarate the brain, prevent sleep, increase muscular energy, and stimulate the sexual properties. It is useful in infusion, hysteria, rheumatism, and low forms of fever. It is also considered as an aphrodisiac, powerfully exciting the generative system. Much used in perfumery, and to flavor tinctures, syrups, ointments, and confectionary."

Because of advances in chemistry and pharmacology, most of the earlier medicinal uses of vanilla have given way to functional uses of vanillin. Current basic research is exploring vanillin's properties as an anticarcinogen that has the ability to inhibit tumor formation and as an anticlastogen that has the ability to inhibit chromosome breakage. Biomedical research has also discovered that vanillin is an effective inhibitor of red blood cell sickling in patients with sickle cell disease. Vanillin is also found to be a powerful antimicrobial. This has possible implications in

creating a natural food preservative. Vanilla remains one of the most widely used culinary flavorings in the world. If research can make vanillin orally available for medicinal uses, the results could create a new legacy for the aromatic orchid.

The anticlastogenic properties of vanillin have been studied over the last 2 decades by many researchers (Odoux et al., 2003). Study explored vanillin's ability to reduce chromosomal damage caused by X-ray and UV light (Keshava et al., 1998). This study clearly indicated that vanillin has antimutagenic properties. Further studies *in vivo* would be useful to determine whether vanillin could be an effective part of a prevention diet. Vanillin has also been found to have anticarcinogenic effects (Bythrow, 2005). Vanillin has been characterized as a family of DNA dependent protein kinase (DNA-PK) inhibitors. This finding has powerful implications in the process of DNA strand repair. Generally, when there is a DNA strand break, the body mediates the joining of the ends in an orderly fashion. However, when something goes wrong, the DNA strands are joined in no specific order, this is called nonhomologous end-joining (NHEJ). This can result in deletions and mutations in the DNA strand that may lead to cancer. Since DNA-PK also inhibits NHEJ and they by reduces the chances of deleterious effects in DNA strand breaks (Duran and Karan, 2003).

Vanillin pro-drug is made for alleviating red blood cell sickling in rats with the sickle cell mutation. Vanillin is shown to covalently bond with sickle hemoglobin, inhibit cell sickling, and shift the oxygen equilibrium to the left based on *in vitro* studies. Orally, vanillin's effectiveness *in vivo* is problematic because it is degraded in the digestive tract before it can become functional in the body. To over come this, researchers developed a vanillin pro-drug, MX-1520, which turns into vanillin when broken down in the body. A baseline was established in healthy rats by determining the rate at which vanillin and the vanillin pro-drug were eliminated (Zhang, 2004). The bioavailability of orally administered MX-1520 was found to be 30 times higher than that of vanilla.

Vanillin (vanilla) is indicated against caries, cramp, dysmenorrheal, fever, heptoses, hysteria, immunodepression, inflammation, nervousness, pain, polyp, rhinosis, sickle cell anemia, tumor, virus, and water retention. Vanillin is also reported as, antipolio, antiviral, cholertic, immunosuppressive, and irritant. Vanillic acid is antibacterial, antifatigue, anti-inflammatory, antioxidant, antitumor, ascaricide, choleretic, immunosuppressive, laxative, and vermifuge. O-Vanillin proved to be very active in forming adducts with amino groups, there by inhibiting the gelation of the hemoglobin. This activity is dose dependent (Adesanya and Wora, 1995; Ravindran, 2006). Vanillic acid is found to have suppressive effect on sickle cell production in case of sickle cell anemia. Different components present in vanilla are responsible for the above medicinal properties (Table 6).

Table 6 Vanilla components and their properties

Property	Components responsible
Antiaggregant	Annisyl alcohol, catechin, ferulic acid
Anticancer	Benzyldehyde, catechol, salicylic acid, syringaldehyde, vanillic acid, vanillin, catechin, ferulic acid, cinnamic acid, ethyl ester, *p*-cresol
Antiedemic	Catechin, coumarin, syringaldehyde
Antiestrogenic	Ferulic acid
Antihaepatotoxic	Catechin, ferulic acid, protoatechic acid
Anti-inflammatory	Catechin, ferulic acid, n-hentriacontane,syringaldehyde. umbelliferone, vanillic acid
Antilipoperoxidant	Catechin
Antimutagenic	Anisaldehyde, ferulic acid, vanillin
Antineoplastic	Ferulic acid
Antinitrosaminic	Ferulic acid
Antioxidant	Catechin, catechol, ferulic acid, protocatechuic acid, salicylic acid, syringaldehyde, vanillic acid, vanillin
Antiperoxidant	Protocatechuic acid
Antitumour	Benzaldehyde ferulic acid, vanillic acid, vanillin
Anesthetic	Benzaldehyde, benzyl-alcohol
Immunostimulant	Belzaldehyde, catechin, ferulic acid
Hepatoprotective	Catechin, ferulic acid
Ornithine decarboxylase inhibitor	Ferulic acid

NOTE: Ravindran P. N., 2006.

Antimicrobial Activity

The use of chemicals to preserve food is a well-established practice in controlling microbial spoilage by their use. One normally employs chemical additives classified as preservatives. The examination of food use chemical other than direct preservative for their antimicrobial activity was stimulated by the finding that some antioxidants possess significant antimicrobial properties. Vanillin has been found to be a powerful antimicrobial. This has possible implications for creating a natural food preservative.

Antimicrobial activity was tested at pH 6 at either 1,000 ppm or 10 mM level and vanillin was found to be inhibitory than ethyl vanillin (Jay and Rivers, 1982). The antimicrobial activity of essential oils of herbs, spices, and vanillin could be the result of damage to enzymatic cell system, including those associated with energy production and synthesis of structural compounds (Conner and Beuchat, 1984). Phenolics could denature the enzymes responsible for spore germination, or interfere with amino acids involved in germination (Nychas, 1995). The mode of action of phenolic compound as antimicrobial agent is concentration dependent (Prindel and Wright, 1997). At low concentration, phenolic affects enzyme activity related to energy production, but at high concentration they cause protein precipitation. The exact cause–effect relation for mode of action of phenolic compound had not been determined, but they may inactivate essential enzymes, reacting with the cell membrane or disturb the genetic material functionality (Davidson, 1993).

Vanillin (4-hydroxyl-3-methoxy-benzaldehyde) was used as an antimicrobial agent in fruit purées, where it inhibited inoculated yeast, molds and bacteria at 2000–3000 ppm (Cerrutti and Alzamora, 1996; Cerrutti et al., 1997). However, to establish the usefulness of natural antimicrobials, they must be evaluated

either alone or in combination with other preservative factor to determine whether they have synergistic effects. Additionally, there is no model to predict performance when natural preservatives are used in combination with other factors. (Board and Gould, 1991). The effect of pH and vanillin concentration on the growth of *Aspergillus flavus, A. niger, A. ochraceus,* and *A. parasiticus* in potato-dextrose agar adjusted to a_w 0.98 was evaluated.

Molds can grow in a wide range of pH. However, pH considerably affects growth rate and growth limit is dependent on other extrinsic and intrinsic factors (Holmquist et al., 1983). The germination time dependent on the vanillin concentration and pH; the generation time increased when vanillin concentration increased and pH decreased. An increase in lag time of *A. flavus, A. niger, A. ochraceus,* and *A. parasiticus* with increasing concentration of vanillin (1500 ppm) in laboratory media and fruit based agar at pH 3.5 was reported (Lopez-Malo et al., 1995).

Results demonstrated that a combination of vanillin with pH reduction had an additive or synergistic effect on mold growth depending on the *Aspergillus* species. *A. ochraceus* was inhibited at pH 3.0 with 1000 ppm vanillin. Higher concentrations could inhibit other molds. Greater antimicrobial effectiveness of phenolic compound with pH reduction also has been reported (Sikes and Hooper, 1954). The effect has been attributed to increased solubility and stability. If the pH is reduced the effect of other inhibitory microbial hurdles could be enhanced (Skirdal and Eklund, 1993). The pH and citric acid concentration effects on lag time of the molds were not sufficient to delay germination after more than 46 hours, but the addition of vanillin increased lag time by 48% to >1000%. The study of vanillin's antimicrobial properties against the growth of 3 different yeasts associated with food spoilage, found that the structure of vanillin may play an important role in its antimicrobial properties (Fitzgerald et al., 2003). An analysis of the inhibition of food spoilage yeasts by vanillin was reported (Daniel et al., 2003). Microbial contamination was investigated using ice-cream with vanilla, chocolate, and strawberry flavor commercially available in Korea. Radiation sensitivity of food-borne pathogens such as *Listeria ivanovii, Escherichia coli,* and *Salmonella typhimurium* was determined by an inoculation test. Results suggested that a low-dose irradiation can improve the microbial quality and reduce the risk by food-borne pathogens of ice-cream, which have limited alternative sterilization methods due to the temperature sensitivity of the products (Cheorun et al., 2007).

Vanillin has been used in the past exclusively for flavoring purposes. It will continue to be used for this, but by adding it to foods susceptible to lipid oxidation thus the additional benefits of its antioxidant properties can be exploited (Burri et al., 1989). Recently, Shyamala et al. (2007) reported that vanilla extracts showed antioxidant activity and interestingly, 4-hydroxy-3-methoxybenzyl alcohol and 4-hydroxybenzyl alcohol exhibited higher antioxidant activity than vanillin. Also addition of vanilla extracts to biscuits increased the antioxidant activity and lowered the peroxide value compared to synthetic vanillin-incorporated biscuits (Anuradha et al., 2010). These studies points toward the potential benefit of vanilla extracts as antioxidants for food preservation and in health supplements as nutraceuticals.

CONCLUSIONS

Vanilla is the principal source of the natural vanilla of commerce. Vanilla is coveted over the ages for culinary reasons. The largest single use of vanilla is in ice-creams. It is also used to flavor beverages, cakes, chocolates, confectionaries, custards, liquors, puddings, soft drinks, syrups, yogurts, and so on. Besides, it is used in medicine, perfumes, sachet powders, soaps, and others. It is also used in medicine, perfumes, sachet powders, soaps, and other toilet preparations. Vanilla has so deeply steeped as a food flavor that it is an ingredient that cannot be forgone with. Being a new crop, vanilla poses specific bottlenecks and also scanty research work has been done. Vanilla is a labor intensive crop as it takes 3–4 years after the vines are planted before the first flower appears and it has to be hand pollinated by trained skilled worker. The fruits, which resemble big green beans, must remain on the vine for 9 months in order to turn to slight yellow in color. Currently, a very limited number of personnel who have some knowledge on the cultivation and processing of vanilla are available. Hence, natural vanilla is so expensive.

Recently, Central Food Technological Research Institute has developed a process for faster curing of vanilla is a new approach, based on conventional curing supplemented with hot-air drying in well-organized curing facilities. The mature green vanilla beans, immediately after harvest, when cured by this process will take only 32 days which otherwise requires 120–140 days in traditional curing methods. Vanilla beans of good flavor quality with typical floral aroma are obtained by this process and the finished products such as vanilla powder, extract/concentrate are hygienic compared to traditional curing. Further, the use of plant based enzymes is very much useful to obtain higher yield of vanilla extract and superior quality vanilla flavor, which avoids the traditional laborious and time-consuming curing process. This external treatment of plant based enzymes can be used for commercial production of vanilla extract with high-quality natural products. Also, addition of natural vanilla extract to biscuits gave higher antioxidant activity and lowered the peroxide value compared to synthetic vanillin-incorporated biscuits. In addition, vanilla extract is safe, imparts health benefits to the consumer and also provides economic returns to vanilla growers.

Vanillin has posed a fascinating biosynthetic problem for many years, closely associated with the more general issue of phenylpropanoid cleavage and the generation of benzaldehyde and benzoates. During the last few years encouraging progress has been made in elucidating this process, although a complete biochemical and molecular genetic characterization in a plant level has yet to be achieved. In vanilla, formation of vanillin β-D-glucoside appears to be a much more complex

process than originally envisaged. Vanilla beans contain more than 200 volatile compounds, the most important one being vanillin. Other major compounds present in cured beans are vanillic acid, 4-hydroxybenzyl alcohol, 4-hydroxy-3-methoxybenzyl alcohol, and 4-hydroxybenzaldehyde.

Though vanillin has nutraceutical properties, vanilla extract contains vanillin and other compounds that have shown antioxidant properties. The medicinal future of vanilla lies in pursuing further basic and clinical research on the constituents and their mechanism of action. They act against such varied illnesses as cancer, chromosomal breakage, polio, nervousness, hysteria, anemia, sickle celled anemia, immunodepression, dysmenorrheal, fever, hepatosis, caries, cramp, fatigue, water retention, rhinosis, polyp, and much more. This would avail vanilla to the consumers to get at an affordable price without compromising on the sweet scent, heartful aroma, and pleasant flavor of the sacred orchid.

ACKNOWLEDGMENTS

We thank Dr. V. Prakash, Director, CFTRI, Mysore, for his keen interest in this work. The financial support from CSIR, New Delhi (COR-0010) is gratefully acknowledged.

REFERENCES

Abdulla, K. M. (1997). Drying of vanilla pods using a green house effect solar dryer. *Drying Tech* **15**:685–698.

Adedeji, J., Hartman, T. G. and Ho, C. T. (1993). Flavour characterization of different varieties of vanilla beans. *Perf Flav* **18**:25–29.

Adesanya, S. A. and Wora, A. (1995). Phytochemical investigations of candidate plants for the management of sickle cell anemia. Phytochemistry of Plants Used in Traditional Medicine. Oxford Science, New York.

Anuradha, K., Madhava Naidu, M., Sai Manohar, R., and Indiramma, A. R. (2010). Effect of vanilla extract on radical scavenging activity in biscuits. *Flavours Frag. J.* **25**:488–492. DOI 10.1002/ffj.2009

Arana, F.E. (1943). Action of β-glucosidase in the curing of vanilla (*Vanilla planifolia*). *Food Res* **8**:343.

Arana, F. E. (1944). Vanilla Curing and Its Chemistry, Federal Experiment Station of the USDA, Mayaquez, Puerto Rico., Bulletin No. 42, Washington, DC.

Ayyappan, P. (1990). Vanilla a money making venture for ambitious farmers. *Kisan World* **7**:24–26.

Balls, A. K. and Arana, F. E. (1941). The curing of vanilla. *Ind Eng Chem* **33**:1073–1075.

Beley, M. T. and Poole, C. F. (1993). Determination of vanillin and related flavor compounds in natural vanilla extracts and vanilla-flavored foods by thin-layer chromatography and automated multiple development. *Chromatographia* **37**:365–373.

Bhat, S. K. and Sudharshan, M. R. (1998). Floral biology and fruit growth in vanilla (*V. planifolia* Andr.). **In**: Recent advances in Plantation Crops Research, pp. 110–112. Muraleedharan, M. and Rajkumar, R., Eds., Allied Publishers, Coimbatore.

Board, R. G. and Gould, G. W. (1991). Future prospects. **In**: Food Preservatives, pp. , 267–284. Russell, N. J. and Gould, G. W., Eds., Chapman & Hall, London.

Bory, S., Grisoni, M., Duval, M. F. and Besse, P. (2008). Biodiversity and preservation of vanilla: Present state of knowledge. *Genet Resour Crop Evol* **55**(4):551–571.

Bouriquet, G. (Ed.). (1954). Le vanillier et la vanilla dans le Monde. Paris Editions. Paul Lechavalier, Paris.

Bricout, J. and Koziet, J. (1978). Characterization of synthetic substances in food and flavours by isotopic analysis. **In**: Flavours of Foods and Beverages, Chemistry and Tehnology, pp. 199–208. Charalambous, G., and Inglett, F. E., Eds., Academic Press, New York.

Brodelius, P. E. (1994). The phenylalanine ammonia-lyase gene family in raspberry. *Phytochem Anal* **5**:27–29.

Broderick, J. J. (1956). The science of curing vanilla. *Food Tech* **10**:184–187.

Brunerie, P. (1993). Process of the production of natural vanilla extracts by enzymatic processing of vanilla beans. Patent PN FR 2691880A1

Burri, J., Graf, M., Lambelet, P. and Loliger, J. (1989). Vanillin: More than a flavouring agent–a potent antioxidant. *J Sci Food Agric* **48**:49–56.

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

Cernuda, F. C. (1949). A simple mathematical procedure for use in curing vanilla to desired moisture content. *Tropical Agric* **26**:7–12, 124–125.

Cerrutti, P., Alzamora, S. M. and Vidales, S. L. (1997). Vanillin as an antimicrobial for producing shelf-stable strawberry puree. *J Food Sci* **62**:608–610.

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

Chalot, C. and Bernard, U. (1920). Culture et preparation de la vanilla, Paris. E. Larose.

Cheorun, J. O., Hyun-Joo, Kim, Dong–Ho, Kim, Wan-Kyu, Lee, Jun-Sang, Ham, and Myung-Woo, Byun. (2007). Radiation sensitivity of selected pathogens in ice- cream. *Food Control* **18**:859–865.

Childers, N. F. and Cibes, H. A. (1948). Vanilla culture in Puerto Rico. USDA Fed. Expt. Station. Mayaguez. Puerto Rico Circular No. 28. Washington, DC, USA, Federal Experiment Station of the US Department of Agriculture.

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

Clark, G. S. (1990). Vanillin. *Perf Flav* **15**:45–54.

Conner, D. E. and Beuchat, L. R. (1984). Effect of essential oils from plants on growth of food spoilage yeast. *J Food Sci* **49**:429–434.

Correll, D. S. (1953). Vanilla - its botany, history, cultivation and economic import. *Eco Bot* **7**:291–358.

Crane, J. C. (1964). Growth substances in fruit setting and development. *Ann Rev Plant Physiol* **15**:303–308.

Cunningham, C. H. (1920). Vanilla production in Mexico. *Us Bur Foreign Dom Com* **274**:826–827.

Dana, A. K., and Harold, W. K. (1985). Detection of fraudulent vanillin labeled with carbon-13 in the carbonyl carbon. *J Agric Food Chem* **33**:323–325.

Daniel, J. F., Malcolm, S., and Arjan, N. (2003). Analysis of the inhibition of food spoilage yeasts by vanillin. **In**: Edible fungi. pp. 113–122. Charalambous, G., Ed., Elsevier Publishers, Charalambous.

Davidson, P. M. (1993). Parabens and phenolic compounds. **In**: Antimicrobial in Foods. 2nd ed., pp. 263–305. Davidson, P. M., and Branen, A. L., Eds., Marcel Dekker, Inc., New York.

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. *J Chromatography A* **1145**:83–88.

De Jager, L. S., Perfetti, G. A., and Diachenko, G. W. (2008). Comparison of headspace-SPME-GC-MS and LC-MS for the detection and quantification of coumarin, vanillin and ethyl vanillin in vanilla extract products. *Food Chem* **107**:1701–1709.

Divakaran, M., Nirmal Babu, K., and Grisoni, M. (2010). Biotechnological applications in vanilla. **In**: Vanilla, Odoux, E., and Grisoni, M., Eds., CRC Press, Taylor and Francis Group, Boca Raton, USA.

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

Dignum, M. J. W., Rob, Van der Heijden, Kerler, J., Chris, W. and Verpoorte, R. (2004). Identification of glucosides in green beans of V*anilla planifolia* Andrews and kinetics of vanilla β-glucosidase. *Food Chem* **85**:199–205.

Duran, S. and Karran, P. (2003). Vanillins: A novel family of DNA-PK inhibitors. *Nucleic Acids Res* **31**:5501–5512.

Ehlers, D. and Bartholomae, S. (1993). Analysis of Co2 vanilla extracts by high-performance liquid-chromatography-Comparison with the usual alcoholic vanilla extracts. *Z Lebensm Unters-Forsch* **197**:550–557.

Ehlers, D., Pfister, M., and Bartholomae, S. Z. (1994). Analysis of Tahiti Vanilla by high-performance liquid chromatography. *Z Lebensm Unters-Forsch* **199**:38–42.

Fayet, B., Tisse, C. and Guérère, M. (1987). *J Estienne Analusis* **15**:217–226.

Feyertag, E. and Hutchins, R. (1981). Technical note: 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. *Int J Food Microbiol* **86**:113–122.

Flores, C. V. and Canizares-Macias, M. P. (2007). On-line dilution and detection of vanillin in vanilla extracts obtained by ultrasound. *Food Chem* **29**:613–619.

Funk, C. and Brodelius, P. (1994). *Vanilla planifolia* Andrews: In vitro biosynthesis of vanillin and other phenylpropanoid derivatives. **In**: Biotechnology in Agriculture and Forestry. Medicinal and Aromatic Plants VI, 26, pp. 377–402. Bajaj, Y. P. S., Ed., Springer, Berlin.

Galetto, W. G. and Hoffman, P. G. (1978). Some benzyl ethers present in the extract of vanilla (*Vanilla planifolia*). *J Agric Food Chem* **26**:195–197.

Graves, R. E., Hall, R. L. and Karas, A. J. (1958). Method of producing cured vanilla extract from green vanilla beans. US Patent 2835591

Gregory, L. E., Murray, H. G. and Colberg, C. (1967). Parthenocarpic pod development by *vanilla planifolia* Andrews induced with growth-regulating chemicals. *Econ. Bot.* **21**:351–357.

Hanum, T. (1997). Changes in vaillin and activity of β-glucosidase and oxidases during post harvest processing of vanilla beans (*Vanilla planifolia*). *Bull Teknologia dan Industri Pangan*. **8**:46–52.

Hariom, B. N. Shyamala, Prakash, M., and Bhat, K. K. (2006). Vanilla Flavor Evaluation by sensory and electronic nose techniques. *J. Sens. Stud.* **21**:228–239.

Havkin-Frenkel, D., and Belanger, F. C. (2007). Application of metabolic engineering to vanillin biosynthetic pathways in *Vanilla Planifolia*. **In**: Applications of Plant Metabolic Engineering, Verpoorte, R., Alfermann, A. W., and Johnson, T. S., Eds., pp. 175–196. The Netherlands. DOI: 10.1007/978-1-4020-6031-1_7. Springer, UK.

Hibon, Th. E. (1966). Connerissance de la vanilla. *J Agric Trop* **13**:353–384.

Hoffman, P. G. and Salb, M. (1979). Isolation and stable isotope ratio analysis of vanillin. *J. Agric Food Chem* **27**:352–355.

Holmquist, G. U., Walker, H. W. and Stahr, H. M. (1983). Influence of temperature, pH, water activity and antifungal agent on growth of *Aspergillus flavus* and A. *parasiticus*. *J. Food Sci.* **48**:778–782.

http://www.vanilla.com/vanilla-beans/

Jagerdeo, E., Passetti, E. and Dugar, S. M. (2000a). Liquid chromatographic determination of vanillin and related aromatic compounds. *J AOAC Int* **80**:564–570.

Jagerdeo, E., Passetti, E. and Dugar, S. M. (2000b). Liquid chromatographic determination of vanillin and related aromatic compounds. *J AOAC* Int **83**:237–240.

Jay, J. M. and Rivers, M. G. (1982). Antimicrobial activity of some food flavoring compound. *J Food Safety* **6**:129–139.

Jenna, D. B. (2005). Vanilla as a medicinal plant. *Semin Integr Med Elsevier Inc* **3**:129.

Jones, M. A. and Vincente, G. C. (1949a). Criteria for testing vanilla in relation to killing and curing methods. *J Agric Res* **78**:425–434.

Jones, M. A. and Vincente, G. C. (1949b). Quality of cured vanilla in relation to some natural factors. *J Agric Res* **78**:445–450.

Jones, M. A. and Vincente, G. C. (1949c). Vanilla production: Technological, chemical and biosynthetic aspects. *J Agric Res* **78**:435–441.

Kahan, S. and Kruegar, D. A. (1997). Liquid chromatographic method for determination of vanillin and ethylvanillin in imitation vanilla extract (modification of AOAC Official Method 99025): Collaborative study. *J AOAC* Int **80**:564–570.

Kamaruddin, A. and Mursalim. (1997). Drying of vanilla pods using a green house effect solar dryer. *Drying Tech* **15**:685–698.

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

Kanisawa, T., Tokoro, K. and Kawahara, S. (1994). **In**: Olfaction Taste IX, Proceeding of the International Symposium, Kurihara, K., Suzuki, N., Ogawa, H., Eds., Springer, Tokyo.

Karas, A. J., Hall, R. L., and Stahl, W. H. (1972). Vanilla bean drying and curing. US Patent 3,663, 238

Kaul, R. J. (1967). Curing of vanilla beans. US patent 3352690

Kauzinger, A., Juchelka, D. and Mosandl, A. (1997). Progress in the authenticity assessment of vanilla. Initiation of authenticity profiles. *J Agric Food Chem* **45**:1752–1757.

Keshava, C., Keshava, N. and Ong, T. M. (1998). Protective effect of vanillin on radiation-induced micronuclei and chromosomal aberrations in V 79 cells. *Mutat Res* **397**:149–159.

Klimes, I. and Lamparsky, D. (1976). Vanilla volatiles a comprehensive analysis. *Int Flavours* 272–278.

Knudson, L. (1946). A new method for the germination of orchid seeds. *Am Orchid Soc Bull* **15**:214–217.

Knudson, L. (1950). Germination of seeds of vanilla. *Am J Bot* **37**:241–247.

Krishnakumar V., Potty, S. N. and Sudharshan, M. R. (2003). Harvesting and processing. **In:** Vanilla, The Prince of Spices, pp. 55–69. Thomas, J., and Rao, Y. S., Eds., Spices Board, Cochin, Kerala, India.

Krishnakumar, V. (1995). Production of planting materials in vanilla. *Spice India (Malayalam)*, May, 5-6.

Krishnamurthy, K. and Melanta, K. R. (2002). Vanilla–A fragrant crop for export. 15–16. Sapna Online.com Publishers, New Delhi.

Krishnamurthy, K. (2004). Advances in cultivation of exportable vanilla for promoting value addition. Spice India **10**:3–4.

Kuruvialla, K. M., Pradipkumar, K. and Madhusoodanan, K. J. (2000). A descriptor for vanilla. *Indian Spices* **37**:10–11.

Kuruvilla, K. M., Radhakrishnan, V. V., Madhusoodanan, K. J. and Potty, S. N. (1996). Floral biology of vanilla. *Spice India* **9**:20–22.

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

Lancher, J. (1989). Effect of harvesting date on vanillin content of pods of V. tahitensis J. W. Moore in French Polynesia. *Agron Trop* **41**:143–146.

Land, L. (1986). The new cullure of Tahitian vanilla. *The New York Times Magazine*. September, 28.

Lapeyre-Montes, F., Conéjéro, G., Verdeil, J.-L., and Odoux, E. (2010). In: Anatomy and Biochemistry of Vanilla Bean Development (Vanilla planifolia G. Jackson), Odoux, E. and Grisoni, M., Eds., CRC Press, Taylor and Francis Group, Boca Raton, USA.

Lechat-Vahirua, I. and Bessiére, J. M. (1998). Common fragrance and flavor materials. *Riv Ital Eppos* **9**:569–578.

Leong, G. (1991). Contribution à l'étude des hétérosides des gousses de vanilla verts. Ph. D. dissertation. University of Marseille, Marseille.

Leong, G., Uzio, R. and Drbesy, M. (1989). Synthesis, identification, and determination of glucoside present in green vanilla beans (*Vanilla Fragrans* Andrews). *Flav Fragr J* **4**:163–167.

Leopold, A. C. (1958). Auxin uses in the control of flowering and fruiting. *Ann Rev Plant Physiol* **9**:281–286.

Lhuguenot, J. C., Maume, B. F. and Baron, C. (1971). Gas chromatographic & mass spectrometric study of vanillin related compounds. *Chromatographia* **4**:204–208.

Lionnett, J. F. G. (1958). Seychelles Vanilla I. A valuable orchid crop. *World Crops* **10**:441–444.

Lionnett, J. F. G. (1959). Seychelles vanilla–II. Curing and marketing of the crop. *World Crops* **11**:15–17.

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

Mabberley, D. J. 1997. The Plant-Book. 2nd ed., University Press, Cambridge, UK.

Madhava Naidu, M., Sampath, S. R., Raghavan, B., Thakur, M. S., Sujith Kumar, P. V., Prakash, V., Srinivas, P., Prema Vishvanath and Maya Prakash. (2008). A process for faster curing of vanilla beans and products thereby. Indian patent 0811/DEL/2008.

Madhava Naidu, M., Sujith Kumar, P. V., Shymala, B. N., Sulochanamma, G., Prakash, M. and Thakur, M. S. (2009). Enzyme-assisted process for production of superior quality vanilla extracts from green vanilla pods using tea leaf enzymes. *Food Bioprocess Technol* doi: 10.1007/s11947-009-0291-y

Mallory, L. D. and Walter, K. (1942). Mexicos' vanilla production. US Dept. of Commerce. *Foreign Commerce Weekly* **7**:8–10.

Mane, J. and Zucca, J. (1993). Method for obtaining a natural vanilla aroma by treatment of vanilla beans and and aroma thus obtained. French patent, 2691880

Martin, G. E., Guinand, G. G. and Figert, D. M. (1973). Comparison of gas-liquid, gas-solid, liquid-liquid, and liquid-solid chromatographic techniques in analysis of vanillin and ethyl vanillin in alcoholic solutions. *J Agric Food Chem* **21**:544–547.

Martin, G. and Remud, G. (1993). Isotopic methods for control of natural flavor's authenticity. *Flav Fragr J* **8**:97–107.

Martin, G. E., Ethridge M. W., and Kaiser, F. E. (1977). Determining the authenticity of vanilla Extracts. *J Food Sci* **42**:6.

Mc Clelland, T. B. (1919). Vanilla–a promising new crop for Puerto Rico. *Puerto Rico Agr Expt Sta Bull Mayaguez* **26**:32P.

Mckone Puerto, H. T. and Chambers, T. E. (1988). Identification of coumarin in vanilla extracts by Tlc and Hplc. *J Chem Edu* **65**:628.

Medina, H. E. (1943). The value of utilizing the existing shade in the growing of vanilla. *J Agric Univ Puerto Rico* **27**:117–124.

Merory, J. (1968). Food Flavourings, Consumption, Manufacture and Use. Avi Pub. Co., Westfort, Conn.

Minoo, D., Nirmal Babu, K., Ravindran, P. N. and Peter, K. V. (2006). Interspecific hybridization in vanilla and molecular characterization of hybrids and selfed progenies using RAPD and AFLP markers. *Sci Hortic* **108**: 414–422.

Morison Smith, D. (1964). Flavors and non-alcoholic beverages: Determination of compounds related to vanillin in vanilla extract. *J AOAC* **47**:808.

Muralidharan, A. and Balagopal, C. (1978). Studies on curing of vanilla. *Indian Spices* **10**:3–4.

Muralidharan, A., Nair, E. V. G. and Balakrishnan, S. (1974). Preliminary studies on trailing of vanilla (*Vanilla planifolia L*). *Agric Res J Kerala* **12**:174–177.

Negishi, O., and Otawa, T. (1996). Determination of hydroxycinnamic acids, hydroxybenzoic acids, hydroxybenzaldehydes, hydroxybenzyl alcohols and their glucosides by HPLC. *J Chromatogra A* **756**:129–136.

Nickell, L. G. (1982). Plant growth Regulators, Chapter 4 & 7, Springer-Verlag, Berlin Heidelberg, New York.

Nychas, G. J. E. (1995). Natural antimicrobials from plants. In: New Methods of Food Preservation, pp. 58–89. Gould, G. W. Ed., Blackie Academic and Professional, Glasgow.

Odoux, E., Escoute, J. and Verdeil, J. L. (2003). Localization of β-D-glucosidase activity and glucovanillin in vanilla bean. *Ann. Bot*. **92**:437–444.

Odoux, E. (2010). Vanilla curing. **In**: Vanilla, Odoux, E., and Grisoni, M., Eds., CRC Press, Taylor and Francis Group, Boca Raton, USA.

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

Potty, S. N. (2004). Vanilla - The Prince of Spices. *Spices Board India* **3**: 517–577.

Prindel, R. F. and Wright, E. S. (1997). Phenolic compounds. **In**: Disinfection, Sterilization and Preservation, Block, S. S., Ed., Lea and Febiger, Philadelphia.

Purseglove, J. W. (1972). Tropical Crops: Monocotyledons, Longamn, London.

Purseglove, J. W., Brown, E. G., Green, C. L. and Robbins, S. R. J. (1988). **In**: Spices, Vol. 2, Scientific and Technical, Longman.

Ramachandra Rao, S. and Ravishankar, G. A. (2000). Vanilla flavor: Production by conventional and biotechnological routes. *J Sci Food Agri* **80**:289–304.

Ramaroson-Raonizafinimanana, B., Gaydou, E. M. and Bombarda, I. (1997). Hydrocarbons from three vanilla beans species: *V. fragrans, V. madagascariensis, and V. tahitensis*. *J Agric Food Chem* **45**:2542–2545.

Ramaroson-Raonizafinimanana, B., Gaydou, E. M. and Bombarda, I. (1998). 4-Demethylsterols and triterpene alcohols from two vanilla bean species: *V. fragrans and V. tahitensis*. *Am Oil Chem Soc* **75**: 51–55.

Ramaroson-Raonizafinimanana, B., Gaydou, E. M. and Bombarda, I. (1999). Long–chain γ-pyrons in epicuticular wax of two vanilla bean species: *V. fragrans* and *V. tahitensis*. *J Agric Food Chem* **47**:3202–3205.

Ranadive, A. S. (1992). Vanillin and related flavor compounds in vanilla extracts made from beans of various global origins. *J Agric Food Chem* **40**: 1922–1924.

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

Rao, R. S., and Ravishankar, G. A. (2000). Vanilla flavor: Production by conventional and biotechnological routes. *J Sci Food Agric* **80**:289–304.

Rao, Y. S., Madhusoodanan, K. J. and Naidu, R. (1992). Detection of genetic variability in vanilla through PAGE studies. *J Plant Crops* **21**: 363–365.

Rao, Y. S., Kuruvilla, K. M. and Madhusoodanan, K. J. (1994). Collection of vanilla wightiana Lindl- an endangered wild species from Eastern Ghats of Andhra Pradesh. *Indian J Pl Genet Resources* **7**:257–259.

Ratobison, R., Zeghmati, B., Reddy, T. A. and Daguenet, M. (1998). *Sol. Energy* **62**:131–138

Ravindran, P. N. (2006). Medicinal properties of vanilla. *Spice India* **19**:8–11.

Reineccius, G. 1994. **In**: Source Book of Flavors, 2nd edn., pp. 351–361. Chapman and Hall, New York.

Ridley, H. N. (1992). Spices Book on Medicinal Plants, Chapter II. Vanilla. Mac Millan, London.

Ruiz-Teran, F., Perez-Amador, I. and Lopez-Mungiia, A. (2001). Enzymatic extraction and transformation of glucovanillin to vanillin from vanilla green pods. *J Agric Food Chem* **49**:5207–5209.

Shyamala, B. N., Madhava Naidu, M., Sulochanamma, G. and Srinivas, P. (2007). Studies on the antioxidant activities of natural vanilla extract and its constituents compounds through in vitro models. *J Agric Food Chem* **55**:7738–7743.

Siddagangaiah, Vadiraj, B. A., Sudarshan, M. R., and Krishnakumar, V. (1996). Standardization of rooting media for propagation of vanilla. (*Vanilla planifolia* Andr.). *J Spices Aromatic Crops* **5**:131–133.

Sikes, G. and Hooper, M. C. (1954). Phenol as the preservative in insulin injections. *J Pharm Pharmac* **6**:552–557.

Sinha, A. K., Verma, S. C. and Sharma, U. K. (2007). Development and validation of an RP-HPLC method for quantitative determination of vanillin and related phenolic compounds in *Vanilla planifolia*. *J Sep Sci* **30**:15–20.

Sinha, A. K., Sharma, U. K. and Sharma, N. (2008). A comprehensive review on vanilla flavor: Extraction, isolation and quantification of vanillin and others constituents. *Int J Food Sci Nutr* **59**:299–326. doi:10.1080/09687630701539350

Skirdal, I. M. and Eklund, T. (1993). Microculture model studies on the effect of sorbic acid on *Penicillium chrysogenum, Cladosporium cladosporioides* and *Ulocladium atrum* at different pH levels. *J App Bacteriol* **74**: 191–195.

Sommer, J., Schroeder, C. and Stockigt, J. (1997). *In vivo* formation of vanillin glucoside. *Plant Cell Tiss Org Cult* **50**:119–123.

Sostaric, T., Boyce, M. C. and Spickett, E. E. (2000). Analysis of the volatile components in vanilla extracts and flavorings by solid-phase microextraction and gas chromatography. *J Agric Food Chem* **48**:5802–5807.

Soto Arenas, M. A. (2003). Vanilla. **In**: Genera Orchidacearum: Orchidoideae, pp. 321–334. Pridgeon, A. M., Cribb, P. J., Chase, M. W., and Rasmussen, F. N. Eds., Oxford University Press, USA.

Sreedhar, R. V., Roohie, K., Venkatachalam, L., Narayan, M. S. and Bhagyalakshmi, N. (2007). Specific pretreatments reduce curing period of vanilla beans. *J Agric Food Chem* **55**:2947–2955.

Theodose, R. (1972). Traditional methods of vanilla preparation, improvement of these techniques at the Antalaha station. Proceedings of the International Conference on Spices *Trop. Prod. Inst*. London, pp. 71–77.

Theodose, R. (1973). Traditional methods of vanilla preparation and their improvement. *Trop Sci* **15**:45–47.

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

Towt, L. V. (1952). Methods of dehydrating and curing of vanilla fruit. US Patent 2, 621, 127

Voisine R., Carmichel L., Chalier P., Cormier, F. and Morin, A. (1995). Determination of glucovanillin and vanillin in cured vanilla pods. *J Agric Food Cnem* **43**:2658–2661.

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

Walton, J., Mayuer, M. J. and Narbad, A. (2003). Molecules of interest: Vanillin. *Phytochem* **63**:505–515.

Webster, T. M. 1995. New perspectives on vanilla. *Cereal Foods World* **40**:198–200.

Werkoff, P. and Guntert, M. (1997). Identification of some ester compounds in Bourbon vanilla beans. *Lebensm-Wiss u-Technol* **30**: 429–431.

Wild-Altamirano, C. J. (1969). Enzymic activity during growth of vanilla fruit. *J Food Sci* **34**:235.

Withner, C. L. (1955). Ovule culture and growth of vanilla seedlings. *Amer Orchid Soc Bull* **24**:381–392.

Zenk, M. H. Z. (1965). Vanilla production: Technological, chemical, and biosynthetic aspects. *Z Pflanzenphysiol* **53**:404–408

Zhang, C. (2004). Anti-sickling effect of MX-1520, a pro drug of vanillin: An *in vitro* study using rodents. *Br J Haematol* **125**:788–795