Interrelation of Curing and Botany in Vanilla (*Vanilla planifolia*) Bean

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Abstract

The fruit of the climbing orchid *Vanilla planifolia* (vanilla bean) is used for the commercial production of the prized vanilla flavor, consisting of vanillin and other numerous flavor compounds, with the use of a curing process. However, present curing methods yield only a fraction of the vanilla flavor from flavor precursors in green beans. Studies on the botany of vanilla beans revealed that flavor precursors are found in the bean interior, where they are secreted onto the placental region around the seeds, whereas hydrolytic or other degradative enzymes, which catalyze the release of the flavor precursors to flavor compounds, are localized mostly in the outer fruit wall region. This insight suggests that the objective of killing, the first curing stage carried out by hot water scalding, freezing or by other methods, is to disorganize the bean tissue, such that contact is created between substrates and their respective enzymes. Sweating, a subsequent step in curing, entailing high temperatures (usually around 45º to 65º C) and high humidity, provides conditions for enzyme-catalyzed production of flavor compounds and also for non-enzymatic reactions. The objective of the final curing steps, including drying and conditioning, is to dry the cured beans to preserve the formed flavor compounds. Further understanding on the botany and curing of the vanilla bean may render a full recovery of flavor from the flavor precursors in vanilla beans and, subsequently, significant economic gains.

HISTORY

Vanilla (*Vanilla planifolia* Andrews) is a climbing orchid indigenous to Mexico (figure 1A). Vanilla was introduced to Europe by the Spanish Conquistadores in 1520 but commercial production of vanilla started about 300 hundred years later with the discovery of hand pollination of the vanilla flower. In the wild, insects carry out the pollination of vanilla flowers (Childers et al., 1959). In commerce, vanilla is cultivated in tropical regions and is propagated by cuttings. The plant requires 3 to 4 years to set flower, and afterward flowers once a year. The pod-like fruit (vanilla bean) is allowed to develop for 8 to 10 months before harvesting. Worldwide production of vanilla beans is around 2,000 tons annually (US Department of Commerce).

Vanilla beans are harvested green, flavorless and are next subjected to a curing process for 3 to 6 months or longer, depending on various curing protocols in different production regions. The objective of the curing process is to develop the prized vanilla flavor and, in addition, to dry the cured beans for subsequent extraction that renders the familiar vanilla flavor. Vanilla cultivation, biosynthesis, and economic aspects are discussed extensively in other reviews (Ranadive, 1994; Dignum, 2001a; Havkin-Frenkel and Dorn, 1997; Rao and Ravishankar, 2000). We are providing, however, information on the botany of the vanilla bean and detailed descriptions of special papillary cells that are believed to be the site of vanillin biosynthesis. This information is vital to the understanding of the curing process and for further improvement of this process.

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BOTANY OF THE VANILLA BEAN POD

Two Fruit Regions

The syncarpous fruit of *Vanilla planifolia* develops from an inferior ovary that eventually splits open along three lines at maturity, and is thus a capsule. For the purposes of this study we have recognized two principal regions in the vanilla fruit: 1. The fruit wall, or "green" region including the epidermis, ground and vascular tissues of the fruit wall, and 2. The "white" region composed of the three parietal placentae (not including seeds), and the three bands of glandular hairs between them. (Figure 2). The glandular hairs play a role in the biosynthesis of vanillin. The “green”, “white” and seed components comprise about 60 % and 20 % of the fruit weight, respectively, with the seeds representing the balance.

Fruit Anatomy

The epidermis contains isodiametric ground epidermal cells, which lack prominent chloroplasts. Each epidermal cell contains a rhomboidal crystal of calcium oxalate and is bounded by thickened, pitted cell walls. Stomata are widely spaced. In some varieties dozens of extra floral nectaries occur on the fruit. In other varieties, extra floral nectaries are entirely absent. The fruit wall contains a ring of about 15 unbranched vascular bundles, each containing a strand of xylem and phloem with a sclerotic bundle sheath. Xylem consists of annular to helical and reticulate elements.

Tissue outside the ring of vascular bundles is composed of thin-walled parenchyma cells several times longer than wide. Each ground parenchyma cell in the outer fruit wall contains chloroplasts and occasional rhomboidal calcium oxalate crystals. Raphide "vessels" are abundant in the outer fruit wall and release mucilage-containing raphides when the fruit is cut, which is highly irritating if it contacts skin. No attempt was made to determine the development or structure of these large, complex cells, which are many times the length of ground epidermal cells, and contain tightly packed bundles of raphides if undisturbed. Compared with the outer fruit wall the wall tissue inside the ring of vascular bundles contains larger cells, with somewhat less abundant and smaller chloroplasts, so is much less green in freshly cut beans.

Pollination Initiates Fruit Development

The inferior ovary of the non-pollinated vanilla flower has three weakly developed parietal placentae separated from each other by the smooth inner epidermis of the ovary. Pollination triggers the placenta to begin extensive branching, followed by ovule development.

Vanillin Producing Cells

Following pollination large numbers of pollen tubes progress down the ovary. The pollen tubes move in three groups, each located in a narrow pocket at one side of each of the three placentae, flanked by the hairs. Unusual glandular hairs begin to develop quickly in the regions between the placentae. Each hair is unbranched, and then reaches a length of about 300 micrometers (figure 3). The hairs become cemented together during their development, and later break down, releasing their contents into the locule. The developing hairs have abundant endoplasmic reticulum, ribosomal structures, enlarged plastids containing lipid globules, and other features that are the hallmarks of metabolically active cells (data not shown). Swamy (1947) suggested that vanillin is produced in the glandular hairs, whose presence has been casually noted by previous investigators. This suggestion is confirmed by our work, showing that vanillin and related intermediates in the vanillin biosynthetic pathway accumulate in the inner white tissue of a developing vanilla pod, around the plancental hairs. The accumulation of vanillin as well as intermediates of vanillin biosynthesis is correlated to the growth of the special hair cells (figure 4). We also found that the placental tissue contains proposed intermediates of vanillin biosynthesis including 4-coumaric acid, 4-hydroxybenzaldehyde and 3,4-dihydroxybenzaldehyde (Havkin-Frenkel et al, 2003).

Mature Fruit

As the fruit develops the inter-placental hairs form thickened walls and a complex cytoplasm. Because of their size, number and thick walls the hairs are easily observed in transverse sections of vanilla fruits, as three lustrous white bands. Many seeds become appressed into the hairs in mature fruits. The three panels of hairs extend the full length of the fruit. The cells contain abundant lipids, which are released into the locule and coat the seeds when the hairs senesce later in ripening. The hairs develop complex cell walls, which cement the hairs together in mature beans. An understanding of the site of vanillin production in the vanilla pod is important for the control of the curing process.

THE CURING PROCESS OF VANILLA BEANS

Purpose of Curing

Vanilla beans are harvested green and are flavorless. During bean development on the vine, for 8 to 10 months, flavor precursors accumulate in the placental tissue surrounding the seeds in the inner core of the bean (figure 4 b, c). However, flavor precursors, glucovanillin for instance, and enzymes that catalyze conversion of these constituents to final products are apparently sequestered in different regions in the vanilla pod. It is estimated, for example, that activity of β-glucosidase, an enzyme that degrades glucovanillin to vanillin and glucose, was a several fold higher in the outer fruit wall than in the inner placental tissue and the glandular hair cells. This was confirmed also by activity staining of a cross section of vanilla pod for β-glucosidase (results not shown). These data, suggest that in intact tissues of green beans hydrolytic enzymes, including β-glucosidase and perhaps other glycosyl hydrolases, are spatially separated from glucovanillin or other flavor precursors, which are localized in the fruit interior. The purpose of the curing process, then, is to create contact between flavor precursors and the enzymes that catalyze the hydrolysis of precursor compounds to vanillin, a major flavor component, as well as other flavor compounds, amounting to around 250 identified constituents (Adedeji et al., 1993). An additional objective is the drying of cured beans for the preservation of the formed flavor compounds.

Traditional Methods of Curing

The curing process is comprised of four major stages including killing, sweating, drying and conditioning.

1. Killing. Modern methods of killing are based on the observation that, in the ancient Mexican method of curing, killing consisted of wilting the beans in the sun until beans became brown (Balls and Arana, 1941a). Contemporary methods for killing vanilla beans include: (1) sun killing, (2) oven killing, (3) hot water killing, (4) killing by scratching, and, (5) killing by freezing (Childers et al., 1959, Ranadive, 1994). The stated purpose of the various killing methods is to bring about the cessation of the vegetative life of the vanilla bean and allow contact between enzymes and substrates (Arana, 1943; Theodose, 1973). The most practical and most commonly used killing methods of green beans are exposure to the sun, killing by oven heat, or hot water killing (Ranadive, 1994), consisting of placing the green beans in wire baskets and submerging in hot water (60 º to 70 ºC) for 3 minutes. Freezing, by dipping in liquid nitrogen or by holding beans for a few hours in a freezer (0 º to –80 ºC), is yet another method of killing (Ansaldi et al., 1990). Our own experience and those of other workers (Dignum et al., 2001 ab) indicate, however, that storage of frozen beans must be carried out at -70 ºC or below to preserve the viability of enzymes that are involved in the curing process.

2. Sweating. After killing, beans are allowed to sweat. During this stage, killed beans develop the characteristic vanilla flavor, aroma and color. During the sweating stage, beans are held at high humidity and high temperature (45 º to 65 ºC) for 7 to 10 days (Balls and Arana, 1941 a, b). The purpose of sweating is to retain enough moisture to allow enzymes to catalyze various hydrolytic and oxidative processes. Apparently, non-enzymatic reactions might also occur during this stage. At the same time, some moisture

is permitted to escape to reduce the water content sufficiently to prevent spoilage by microorganisms.

3. Drying and Conditioning. At the end of a sweating period, beans are brown in color, and have developed most of the characteristic flavor and aroma of cured beans. However, at the end of this stage, beans contain about 60 to 70 % or higher moisture content and are traditionally dried for protection against microbial spoilage and to stop any further enzymatic activity. At the end of the drying process, the moisture content in the beans reaches 25 to 30 % of the bean weight (Ranadive, 1994). Drying is the most difficult stage in the curing process to control. Uneven drying may result from varying bean size, differences in bean moisture content, and from variable environmental conditions. The latter may include weather conditions during sun drying or variations in the relative humidity during sun or air-drying. The drying stage is apparently critical to the preservation of flavor quality, but prolonged drying may lead to loss in flavor and in vanillin content.

4. Vanillin and β-glucosidase. Vanillin was first isolated from vanilla beans in 1858 by Gobley, according to Arana (1944). Goris (1924) was the first to show that vanillin is formed from glucovanillin, during the curing process of vanilla beans. Other glycosyl conjugates of vanillin or other phenolic compounds conjugated to mannose, galactose and rhamnose are found in trace amounts in the developing vanilla pod (Leong et al., 1989 ab; Tokoro et al., 1990; Kanisawa et al., 1994; Pu et al., 1998; Dignum 2001 a). Accumulation of glucovanillin during vanilla pods development on the vine ensues during the fourth month after anthesis. It then rises sharply for the next 3 months and levels off during the last stages of pod development (Havkin-Frenkel et al., 1999). Glucovanillin may be sequestered mostly in the inner white placental tissue surrounding the seeds (Figure 4 c). The distribution of glucovanillin, along the longitudinal axis of green vanilla pods, was found to be as follows: 40% in the blossom end, 40 % in the central portion and 20 % in the stem end. This is in agreement with previous observation showing that vanillin crystals formed during curing appear mostly on the blossom end (Childers et al. 1959).

Because of the importance of vanillin to vanilla flavor, the enzymatic hydrolysis of glucovanillin to vanillin is one of the most studied processes in vanilla beans. To examine further the tissue distribution of β-glucosidase in green beans, we separated the green outer fruit region, the placental tissue (without the seeds) and the glandular hair cells. We found that, when protected against proteolysis, β-glucosidase activity expressed as μg product/ hr/ μg protein, as previously defined (Ranadive et al. 1983), was as follows: 75.2 in green outer fruit tissue, 32.3 in the placental tissue and 11.1 in the glandular hair cells. These results confirm that the enzyme is localized mostly in the outer fruit region and, furthermore, reinforce the need for proper killing in order to establish contact between enzymes and their corresponding substrates, which results from the bean tissue disorganization.

The conversion of glucovanillin to vanillin during the curing process is shown in figure 5. After 3 days of curing at 50 ºC, the glucovanillin content decreased from an initial level of 14 % to roughly 6 % on dry weight basis. At the same period, the vanillin content, liberated from glucovanillin, has risen to approximately 6 %. The content of the two compounds leveled off afterward (Figure 5a). This process was greatly accelerated in chopped beans where the glucovanillin disappeared in less than 24 hours (Figure 5b). This process was further enhanced in blender homogenized vanilla tissue (data not shown) suggesting that disruption of tissue organization by mechanical means simulates the effect of the commercial curing process. The release of vanillin appears to be accompanied by the accumulation of other flavor metabolites, for example, vanillic acid, p-hydroxybenzaldehyde and p-hydroxybenzoic acid (Figure 6).

CONCLUSIONS

The botany of the vanilla pod dictates the curing methods developed, throughout history, for generating the prized vanilla flavor. Because flavor precursors and enzymes,

which degrade them to flavor compounds, are sequestered in different fruit regions it is necessary to disorganize the bean tissue, in order to create contact between flavor substrates and enzymes, an objective carried out by killing. High temperature and high humidity provides conditions for enzyme-catalyzed production of flavor compounds in the subsequent sweating stage. In the final curing steps, including drying and conditioning, beans are dried to preserve the formed flavor compounds. An understanding of the botany of vanilla bean might be used for further improvement of the curing process.

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Figures

Fig. 1. Climbing vanilla plant bearing green pod-like fruit (vanilla bean).

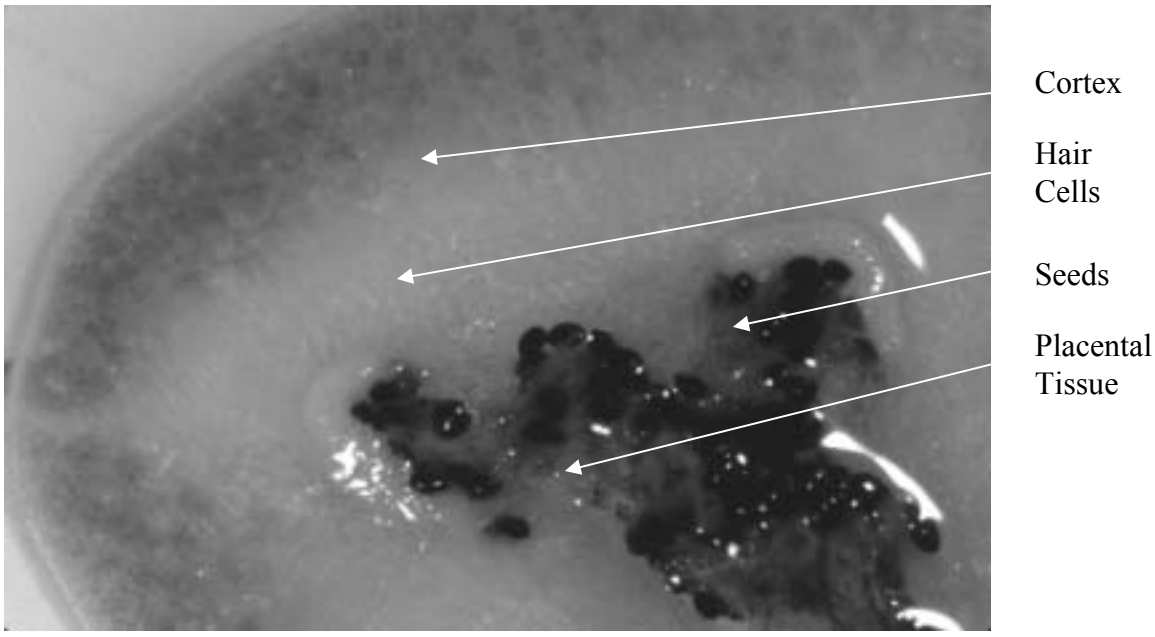

Fig. 2. Cross section (x 20) of freshly cut green vanilla bean. The figure shows an inner portion composed of seed (dark bodies). Arrows indicate a white placental tissue surrounding the seeds. Shown also are specialized hair cells as well as a green outer fruit region.

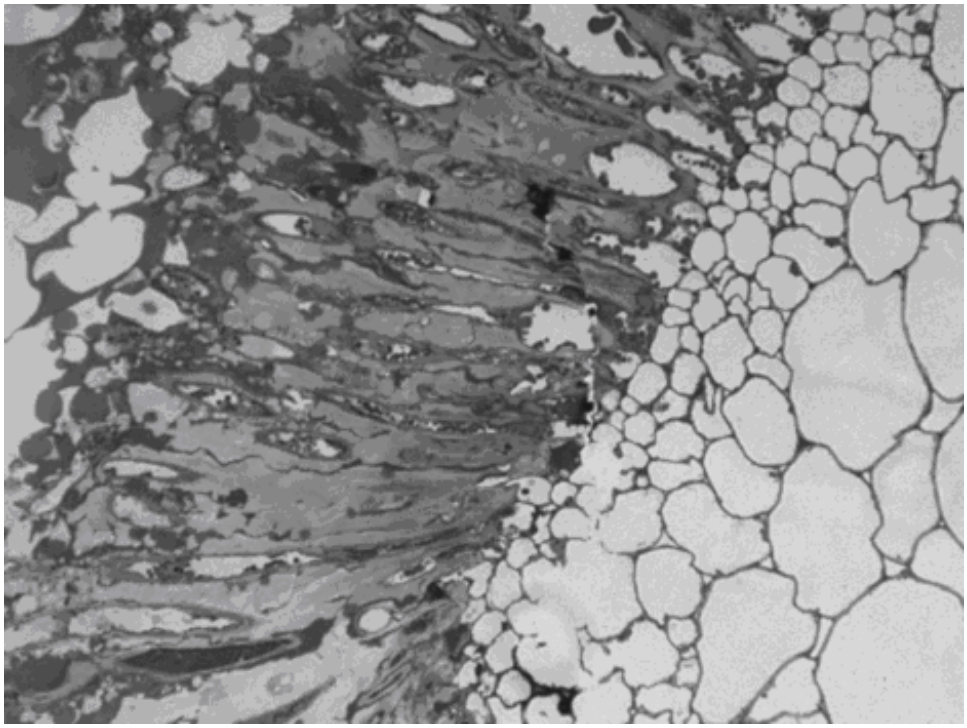

Fig. 3. A magnified view (x 400) of cross section of green vanilla bean.
The figure shows the senescing inter-placental hairs (left) and white parenchyma cells in the fruit wall (right). The hair-like cells contain enzymes in the vanillin biosynthetic pathway. These cells release abundant lipid seen as globular bodies (top left). The parenchyma cells comprise the white portion of the fruit wall. The outer fruit tissue contains hydrolytic and other degradative enzymes.

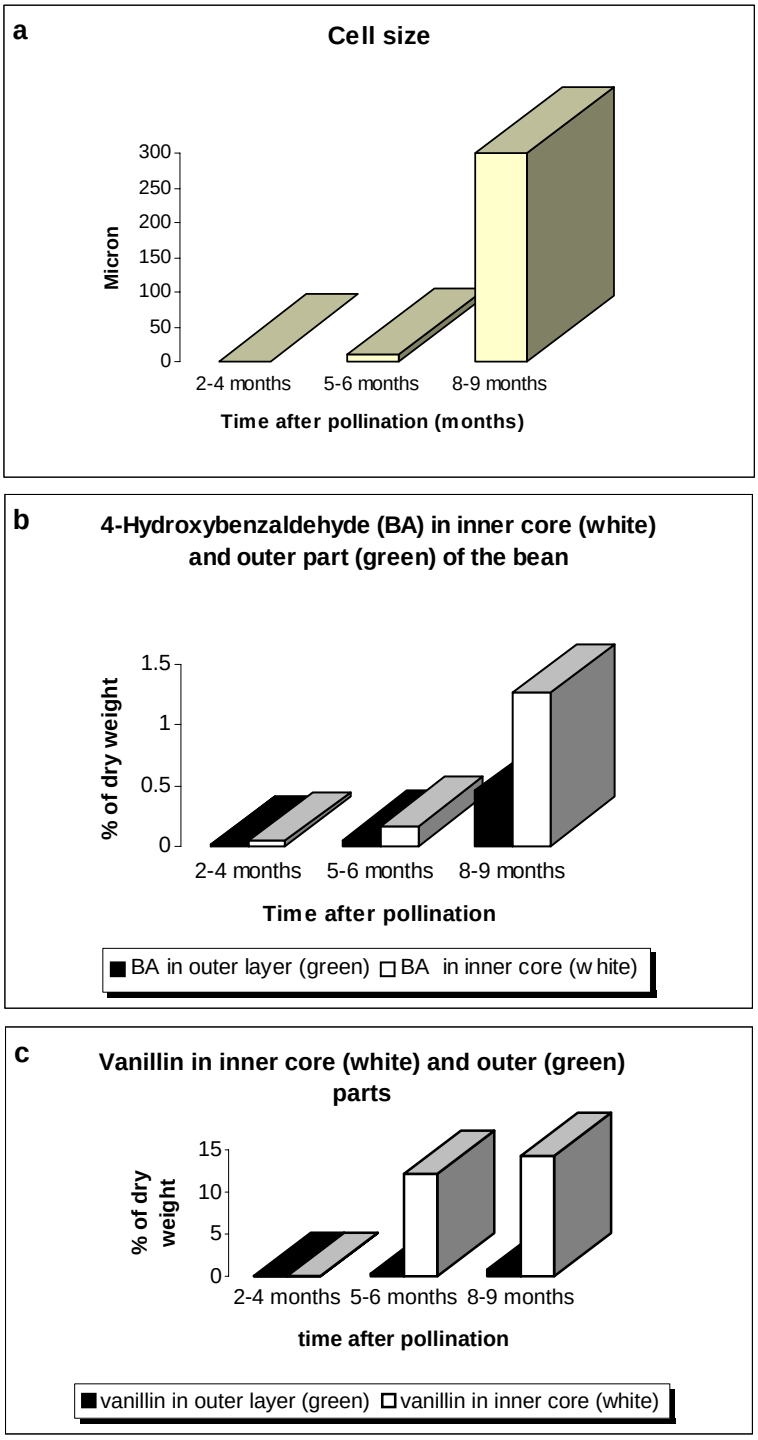

Fig. 4. Time course change in cell size (a) and the content of 4-hydroxybenzaldehyde (b) and vanillin (c) in the inner core and the outer green tissue of vanilla bean during pod development on the vine. Beans were harvested green at various stages of development. The various metabolites, present as glucosides, were hydrolyzed and the resulting aglycons determined as described previously (Podstolski et al., 2002).

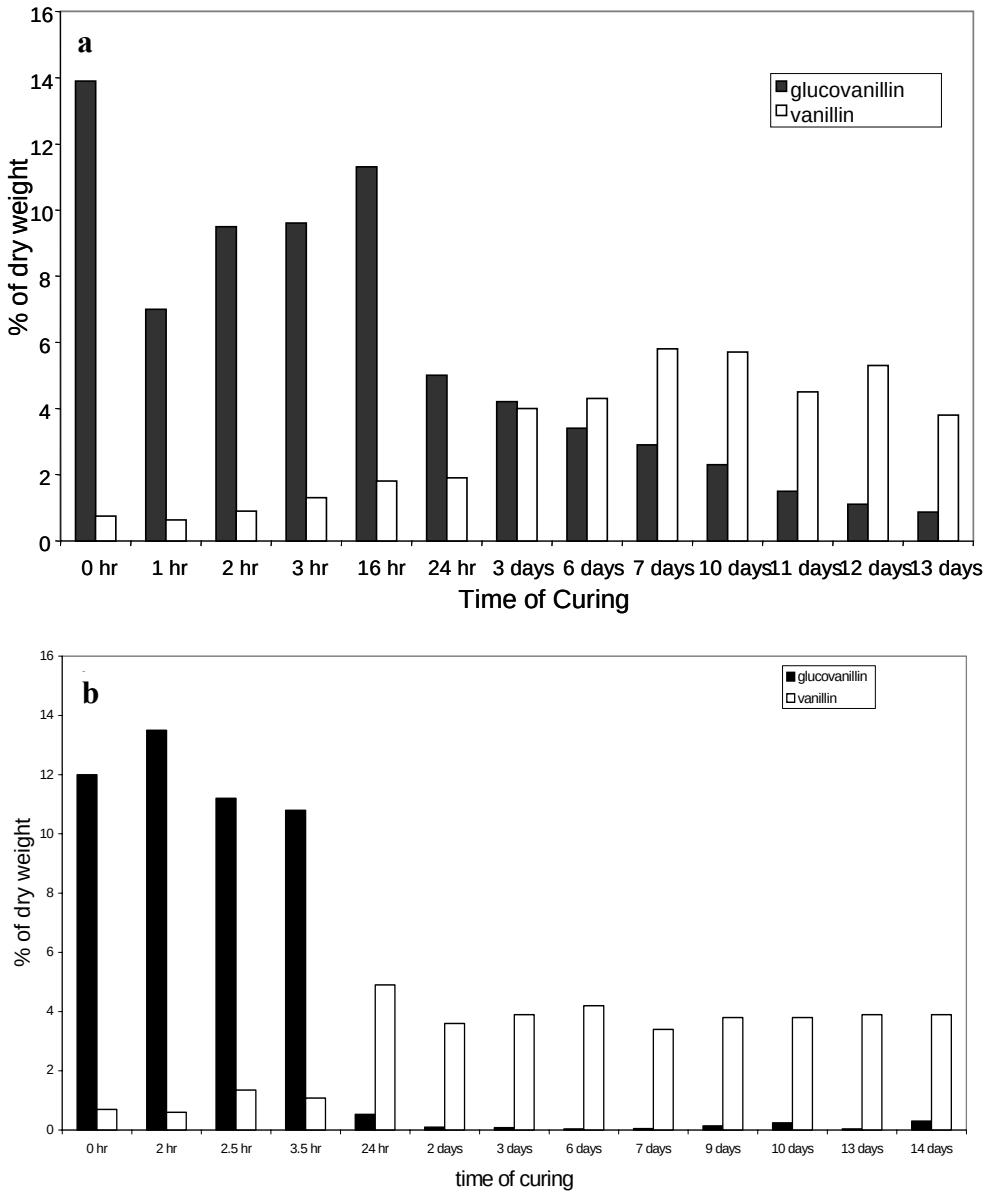

Fig. 5. Changes in the content of glucovanillin and in vanillin as function of Curing time in (a) whole vanilla bean and (b) chopped bean. Curing was carried out at 50 ^{O}C.

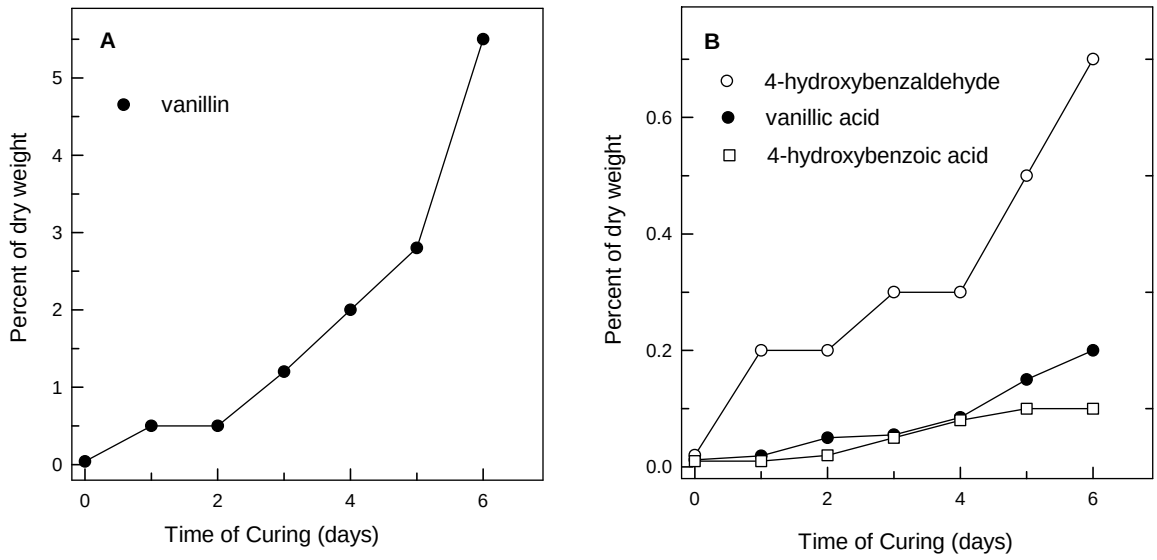

Fig. 6. Time course changes in the content of vanillin (A), vanillic acid, 4-hydroxybenzaldehyde and 4-hydroxybenzoic acid (B) in whole vanilla bean during curing at 50 ^{O}C.