Changes in vanillin and glucovanillin concentrations during the various stages of the process traditionally used for curing *Vanilla fragrans* beans in Réunion

Éric Odoux

Laboratoire d'analyses chromatographiques du Cirad, Centre de la Réunion, Maison régionale des sciences et technologies, 100, route de la Rivière-des-Pluies, 97490 Sainte-Clotilde, Island of Reunion, France

Changes in vanillin and glucovanillin concentrations during the various stages of the process traditionally used for curing *Vanilla fragrans* beans in Réunion.

Abstract — Introduction. The study focused on the progression of vanillin and glucovanillin concentrations during any one specific stage of the traditional process used for curing vanilla. It aimed at identifying the most important stages of the hydrolysis of the glucovanillin into vanillin in order to improve the yield of vanillin. **Materials and methods**. A batch of 400 kg of green vanilla beans was cured using the traditional curing process consisting in killing, sweating, drying, washing, and conditioning. We sampled the vanilla beans after harvest (the green stage) and at each stage of the curing process. After extracting the aromatic compounds, the vanillin concentration was measured out by means of HPLC. The dry matter content of each sample was determined as well. **Results and discussion**. During the different stages of the curing process, the dry matter (dm) gradually progressed from 18 % on fresh vanilla beans to 56.6 % after conditioning. During the first phases of the curing process, the vanillin content rose in stages with each phase of the sweating process and remained steady (2.2 g·100 g^{-1} dm) when drying was initiated. The concentration in total vanillin (in its aglycone and glycosylated forms) was steady until the drying process (5.5 g·100 g^{-1} dm) after which it decreased through the conditioning stage (2.8 g·100 g^{-1} dm). The vanillin yield from the glucovanillin enzymatic hydrolysis amounted to about 40 %. **Conclusion**. The study revealed two crucial phases: the killing and sweating stages on the one hand and the drying stage on the other hand. The results of the study led to further research currently in progress. © Éditions scientifiques et médicales Elsevier SAS

France (Réunion) / *Vanilla flagrans* / processing / aroma recovery / optimization methods / vanillin / aroma precursors

Évolution des concentrations en vanilline et en glucovanilline au cours des différentes étapes de la préparation traditionnelle des gousses de *Vanilla fragrans* à la Réunion.

Résumé — Introduction. L'évolution des concentrations en vanilline et en glucovanilline pendant une phase de préparation traditionnelle de la vanille a été étudiée, afin d'identifier les étapes les plus importantes de l'hydrolyse de la glucovanilline en vanilline et, par la suite, de tenter d'en améliorer les rendements. **Matériel et méthodes**. Un lot de 400 kg de vanille verte a été traité par utilisation du procédé traditionnel, impliquant des étapes d'échaudage, d'étuvage, de séchage, de lavage et d'affinage. Des échantillonnages de gousses ont été réalisés lors de la réception des gousses vertes, puis à chaque étape de leur préparation. Après extraction des composés aromatiques, la concentration en vanilline a été dosée par HPLC. Par ailleurs, la teneur en matière sèche de chacun des échantillons a été déterminée. **Résultats et discussion**. Au cours des différentes étapes de la préparation de la vanille, la matière sèche (ms) évolue régulièrement de 18 % sur gousse fraîche jusqu'à 56,6 % après affinage. Pendant les premières phases de la préparation, la teneur en vanilline évolue par palier au cours de chaque étape d'étuvage, puis reste stable (2,2 g·100 g^{-1} de ms) à partir de la phase de séchage. La concentration en vanilline totale (formes aglycone et glycosylée) est stable jusqu'à l'étape de séchage (5,5 g·100 g^{-1} de ms), puis décroît jusqu'après l'affinage (2,8 g·100 g^{-1} de ms). Le rendement d'hydrolyse enzymatique de la glucovanilline en vanilline a été de l'ordre de 40 %. **Conclusion**. Deux phases critiques du procédé ont été mises en évidence : les étapes d'échaudage et d'étuvage et l'étape du séchage. Ces résultats ont justifié de nouvelles études en cours. © Éditions scientifiques et médicales Elsevier SAS

France (Réunion) / *Vanilla flagrans* / traitement / récupération d'arôme / méthode d'optimisation / vanilline / précurseur d'arôme

* *Correspondence and reprints*

Received 8 October 1999
Accepted 29 November 1999

Fruits, 2000, vol. 55, p. 119–125

Resumen Español, p. 125

1. introduction

Among all the different vanilla species known, *Vanilla fragrans* is the plant most commercialized with about 2 000 t of vanilla cured annually around the world [1]. The process for curing *Vanilla fragrans* used in Madagascar and Indonesia, the world's current principal vanilla producers, was developed on the island of Reunion at the end of the XIXth century and has fundamentally remained unchanged since then.

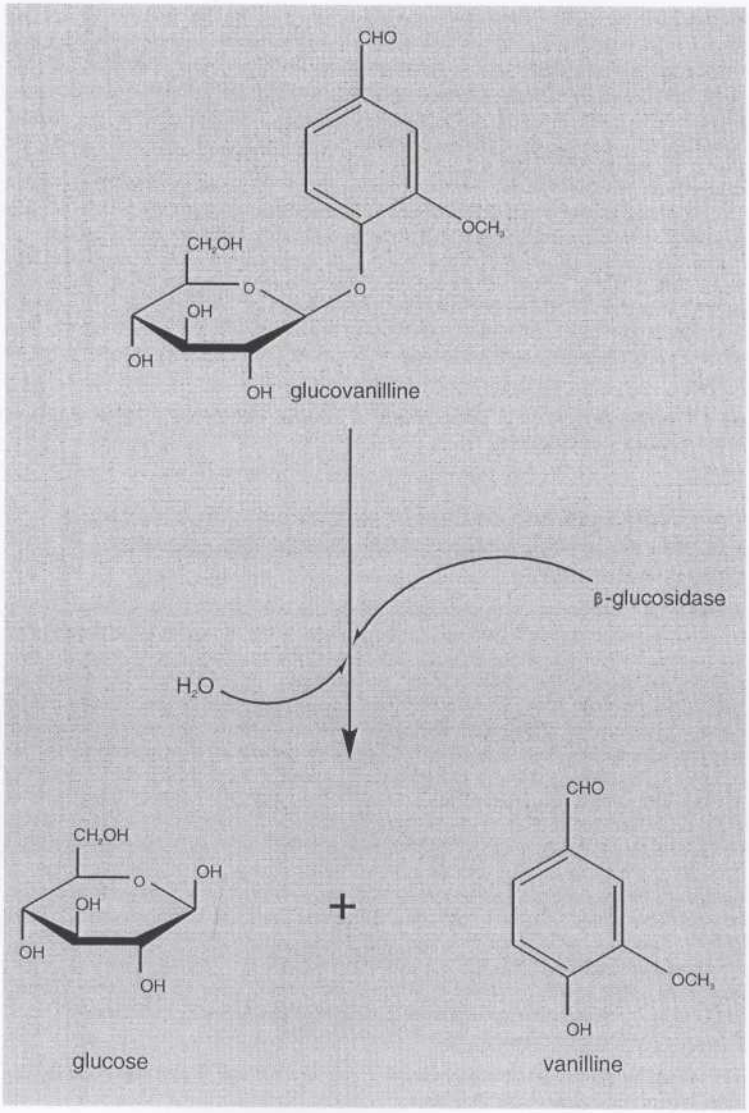

Figure 1.
Illustration of the hydrolysis of the glucovanillin by the β-glucosidase.

This curing process requires that the vanilla beans be harvested before complete ripening to avoid fruit dehiscence. The beans are thermally treated by immersing them in hot water and placing them in sweat boxes. They are then naturally or artificially dried by laying them in the sun or in dryers, and conditioned in curing trunks for several months to obtain the best aroma. Even though all processes for curing vanilla beans follow this basic method, there are some variations; these variations result in noticeable differences in the flavor of the vanilla bean.

The main biochemical reaction occurring during the curing process of the vanilla beans appears to be the hydrolysis of the vanillin's glycosylated precursor [2], the glucovanillin (vanillin-β-glucosid), by a β-glucosidase (*figure 1*). It has been demonstrated that, at the usual harvest time, 90 % of the vanillin potential in the vanilla beans are present in the form of glucovanillin [3]. The glucovanillin undergoes hydrolysis during the ripening of the fruit on the vine [2]. During the curing of the beans, other enzymatic reactions, which might be at the origin of the typical vanilla aroma, may be observed as well [4,5]. However, the literature does not mention any specific mechanism.

Also, the vanillin content in traditionally cured beans seems to represent about 2 % of the dry matter [6, 7], while its original potential in the green vanilla beans amounts to about 5 to 6 % [2, 8, 9]. Different patent applications for processes aimed at improving the yield of the hydrolysis have been filed [10–12]; however, the aroma obtained with these processes differs completely from the aroma produced by the traditional curing process.

Until now, no published study has shown with accuracy the development of vanillin and glucovanillin concentrations during any one specific phase of the traditional curing process. Nevertheless, the identification of the most important stages of the hydrolysis of glucovanillin would help to optimize the traditional process by retaining the typical vanilla aroma so popular among consumers while increasing the yield of vanillin. The

study here presented focuses on this development.

2. materials and methods

2.1. plant material

The vanilla beans used for the study were collected on the vines of the *Vanilla fragrans*, a plant commonly cultivated on the island of Reunion. They do not show any specific variety distinction. The vines are cultivated on a semi-intensive scale and grown between rows of sugar cane. The vanilla beans were harvested in August 1997, 9 to 10 months after pollination. At this stage of maturation, the vanilla pod turns a light yellow color at its floral extremity and the dehiscence process has not yet started.

2.2. curing vanilla

The vanilla beans were cured between August 1997 and May 1998 at *La Maison de la vanille*, a business located on the island of Reunion and specialized in the curing of vanilla. This company uses the traditional curing method to process 10 to 15 t of green vanilla a year.

For the study, a batch of 400 kg of green vanilla was immersed in water at 60 °C for 3 min (killing #1) and immediately placed in a cloth-lined wooden box for 24 h (sweating #1). After repeating the killing and sweating steps (killing #2 and sweating #2), the beans were spread out on racks where they dried in the morning sun; they were then moved to the shade in the afternoon. This long drying process lasted about 3 months. During these 3 months, the flexibility of the beans was checked on a regular basis. The beans that were considered dry enough, depending on their flexibility, were placed in a wooden trunk lined with wax paper to limit the risk of desiccation. After drying the whole batch, the beans were brushed by hand to remove any dirt and impurities with water and once again rapidly dried to remove any water excess off the surface. Then, the beans were conditioned by placing them for several months in a wooden trunk lined with wax paper at ambient temperature and with a humidity level similar to their moisture content. At this stage, the condition of the batch was inspected by opening the trunk on a regular basis. After 5 months of conditioning, the beans were sorted according to their length, tied in small bundles, and stored in trunks before marketing.

2.3. sampling

The beans were sampled at each stage of the curing process: at the green stage, after killing #1, at the end of sweating #1, after killing #2, at the end of sweating #2, at different stages of the drying process (beans "in an advanced state", "fine" and "dried" beans), after washing, and at different stages of the conditioning process performed in trunks (2, 4, and 6 months conditioning).

After 6 months of conditioning, we took 3 samplings among the beans sorted by length: in the bundles gathering the shortest and longest beans (13 cm and 21.5 cm) and in the bundles containing beans of average length (17.5 cm).

For each sampling, 60 beans [13] were cut into pieces, frozen, and stored at –18 °C up to their analysis.

2.4. extracting the aromatic compounds

To extract the aromatic compounds present in the samples, about 50 g of beans were cut into pieces, then mashed in a mixer. At about 3 g of dry matter, accurately weighed, extracted from the previous mashing, and diluted in about 20 mL of distilled water, we proceeded to a second fragmentation for about 1 min. We mixed the puree obtained with distilled water until we reached a total mass of about 80 g, accurately weighed. We centrifuged the mixture at 3,000 revs per minute for 5 min; 500 µL of the supernatant were diluted to a 1:50 ratio in the mobile phase and filtered at 0.45 µm before initiating the HPLC analysis.

We then mixed the residue and the supernatant together again. With the β-glucosidase of sweet almonds (Fluka, ref. 49290) and following the protocol derived from Brunerie [12] and Brodelius [3], we performed an enzymatic hydrolysis of the glycosylated precursors. The incubation was carried out at 35 °C with a pH of 5 for 1 h in a double boiler constantly agitated; the quantity of enzyme in the medium, which amounted to 50 mg or 100 $U \cdot g^{-1}$ of vanilla beans' dry matter, made possible a complete hydrolysis of the glycosylated precursors. After hydrolysis, we centrifuged the mixture and took a sample of the supernatant which we diluted and filtered as before. Each sample was treated so as to measure out both the free vanillin at the time the sampling was taken and the vanillin in its glycosylated form.

We carried out three extractions per sample.

2.5. measuring the vanillin via HPLC

We measured the vanillin with an instrument Thermo Separation Products (SpectraSERIES P100) equipped with a sample loop of 20 µL and a UV detector (λ = 254 nm). The separating column was a Licrospher® 100 (Merck) RP 18e (5 µm), 250 mm long and 4 mm in diameter. The column was thermostatted at 30 °C.

The mobile phase consisted of a combination of 35 % MeOH – 65 % H_3PO_4 10^{-2} M; the flow through the column amounted to 0.7 $mL \cdot min^{-1}$ which is equivalent to a pressure of about 135 bars.

The compounds were quantified using the external standard technique with a calibration curve. Each sample was injected three times. The reference vanillin was from Fluka (vanillin, ref. 94750).

2.6. measuring the dry matter

To determine the dry matter, we extracted 10 to 30 g of puree, depending on how dry the sample was, from the first mashing. We dried them for 24 h at 60 °C in an oven and weighed them before and after the drying process. We followed with three measurements of the dry matter on each sample.

3. results and discussion

During the different stages of the curing process, the dry matter (dm) increases steadily: from 18 % on fresh beans to 56.6 % after drying and 6 months of conditioning

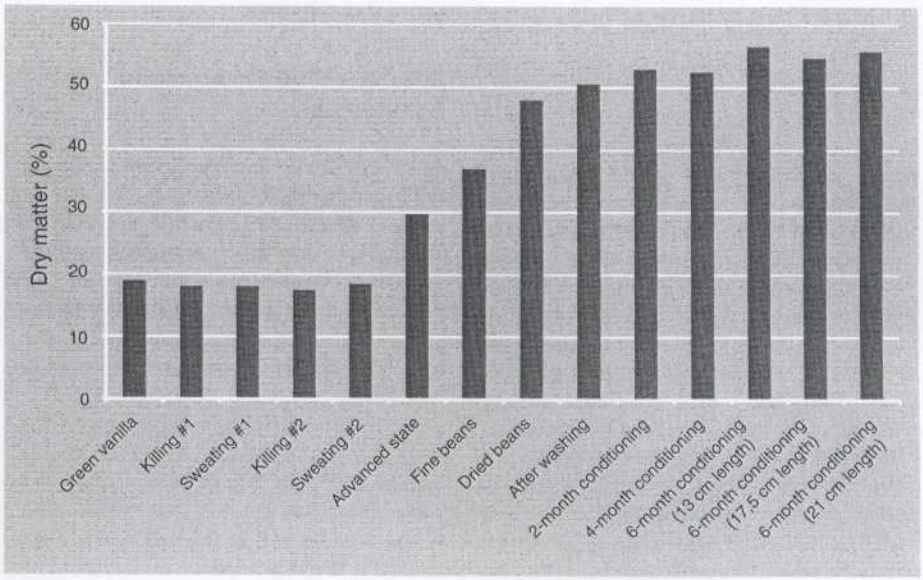

Figure 2.
Development of the dry matter of the vanilla beans during the different stages of the curing process; the curing is performed according to the traditional process used on the island of Reunion (averages of three determinations with variation coefficients between 0.9 and 6.2 %).

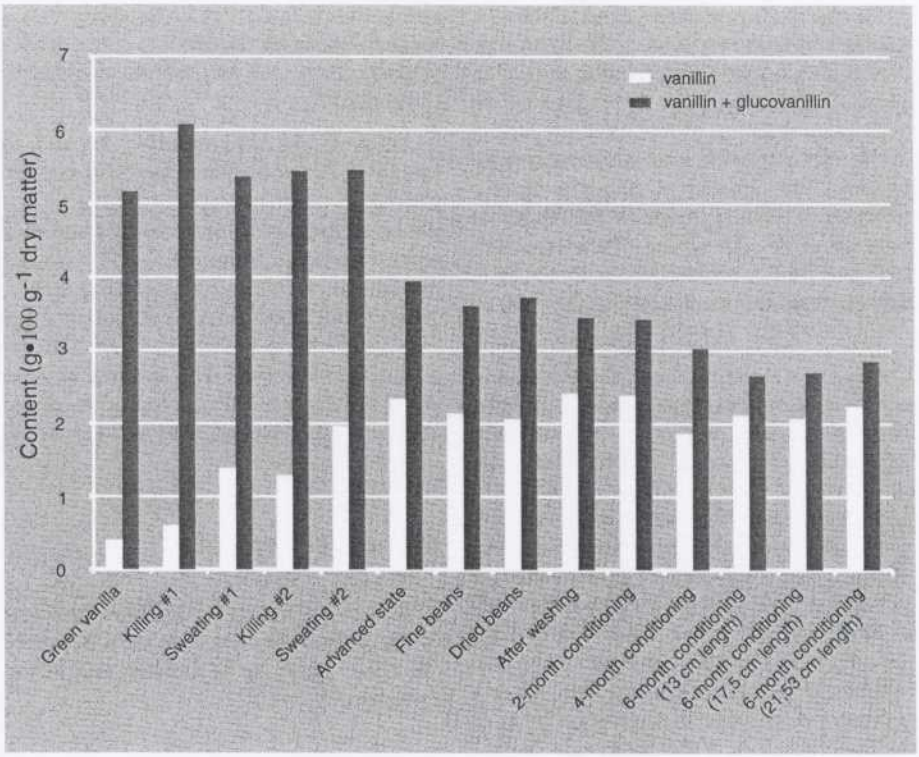

Figure 3.
Progression of the vanillin content and potential during the different stages of the traditional process for curing vanilla used on the island of Reunion. Averages of three extractions and three analyses per sample with variation coefficients between 1.8 and 9.9 %. The average variation coefficient is about 4.5 % for all the samples.

in trunks (*figure 2*). It is worth noting that the dry matter of the vanilla beans intended for industrial market ranges between 70 and 80 %.

During the first phases of the curing process, the vanillin content increases at intervals with each sweating stage; it runs successively from 0.6 g·100 g^{-1} dm in harvested green vanilla beans to 1.4 g·100 g^{-1} dm at the end of killing #1 and to 2.0 g·100 g^{-1} dm at the end of killing #2. When the drying phase is initiated, the progression of the vanillin content present in the beans is more subtle: it oscillates between 1.8 and 2.3 g·100 g^{-1} dm, with an average vanillin content in the 9 samples amounting to 2.2 g·100 g^{-1} dm (*figure 3*).

The vanillin contents (in its aglycone form), measured on the green beans gathered for the study at the usual harvest time, correspond with the figures obtained by other authors [2, 8, 14, 15]; the same applies to the vanillin contents measured on cured vanilla beans [6, 7] which are characteristic of high quality beans for *Vanilla fragrans*.

The total vanillin concentration (in its aglycone and glycosylated forms) averages 5.5 g·100 g^{-1} dm during the first five stages of the curing process. It remains steady until the drying stage (*figure 3*) and, thereafter, decreases considerably; from 3.9 g·100 g^{-1} dm in beans "in an advanced state" when the drying process is initiated, it then decreases steadily down to 2.8 g·100 g^{-1} dm after 6 months of conditioning in trunks (*figure 3*). The total vanillin contents (in its aglycone and glycosylated forms) measured for the study on the green vanilla beans also correspond with the figures obtained by other authors [2, 8–12].

The vanillin yield from the glucovanillin enzymatic hydrolysis amounted to about 40 %; it is generated when curing the vanilla following the traditional process and is calculated according to the ratio [final vanillin content of the cured bean / original vanillin potential of the green vanilla bean].

The process reveals two crucial phases.

During the first phase which includes the killing and sweating stages, the hydrolysis of the glucovanillin into vanillin is incomplete. Various hypotheses could explain such phenomenon: problems of accessibility between enzyme and substrate, inactivation of the enzyme during the thermal treatments, development of inhibitors of the enzymatic activity, etc. These hypotheses need further research so as to optimize this phase in the vanilla curing process.

During the second phase which mainly consists of the drying stage, the concentration of total vanillin (in its aglycone and glycosylated forms) decreases substantially while the concentration of free vanillin (in its aglycone form) remains steady. The measuring technique used for this assay cannot determine with certainty which form of the vanillin, aglycone or glycosylated, is lost during the drying process. Two hypotheses are proposed: either the loss occurs on the glycosylated form or the enzymatic hydrolysis continues through the drying process by releasing the aglycone form of the vanillin which is lost later on; in such a case, the drying process would have more than just a dehydration effect. The first theory suggests the optimization of the enzymatic hydrolysis before drying in order to improve the vanillin content of the vanilla beans; in the second hypothetical case, the losses in vanillin could well be limited by improving the drying process.

4. conclusion

The study focused on the progression of the vanillin and glucovanillin contents during the different stages of the traditional vanilla curing process. It proved that the vanillin potential of the green beans gathered at the usual harvest time amounts to 5.5 g·100 g^{-1} dry matter whereas the actual concentration of the cured beans averages 2.2 g·100 g^{-1} dm. Therefore, the yield of the enzymatic hydrolysis obtained after curing the vanilla does not exceed 40 %.

The analysis showed two critical phases: the killing and sweating stages during which the hydrolysis of the glucovanillin is incomplete, and the drying stage which leads to heavy losses in vanillin and/or glucovanillin.

These results require further research to identify the factors related to the enzymatic hydrolysis which takes place during the first phase of the curing process. At present, a work has been undertaken to go further into the first killing and sweating stages. Thus, it might be possible to identify the factors which are limiting the glucovanillin enzymatic hydrolysis. The aim would be to verify if there was a technological response improving the vanillin yield in the process.

acknowledgements

We are grateful to M. de Floris, manager of *La Maison de la Vanille* in Saint-André (Réunion), who let us use his premises to conduct this study.

references

[1] Todd H., The north American market for vanilla, Perfumer and Flavorist 23 (1998) 23–25.

[2] Arana F.E., Action of a β-glucosidase in the curing of vanilla, Food Res. 8 (1943) 343–351.

[3] Brodelius P.E., Phenylpropanoid metabolism in *Vanilla planifolia* Andr. (V) High performance liquid chromatographic analysis of phenolic glycosides and aglycones in developing fruits, Phytochem. Analysis 5 (1994) 27–31.

[4] Balls A.K., Arana F.E., The curing of vanilla, Ind. Eng. Chem. Res. 33 (1941) 1073–1075.

[5] Jones M.A., Vicente G.C., Inactivation and vacuum infiltration of vanilla enzyme systems, J. Agric. Res. 78 (1949) 435–443.

[6] Fayet B., Tisse C., Guerrere M., Estienne J., Nouveaux critères analytiques dans les gousses de vanille, Analusis 15 (1987) 217–226.

[7] Derbesy M., Uzio R., Falque V., Gousses de vanille Comores : Banque de données pour la récolte 1987, Parfums, cosmétiques, arômes 87 (1989) 77–90.

[8] Ranadive A.S., Szkutnica K., Guerrera J.G., Frenkel C., Vanillin biosynthesis in vanilla beans, In: Proc. 9th Int. Cong. Ess. Oils, Singapore, Indonesia, 1983, pp. 147–154.

[9] Leong G., Contribution à l'étude des hétérosides des gousses de vanille vertes, Thèse, Université d'Aix-Marseille III, Marseille, France, 1991, 152 p.

[10] Graves R.E., Hall R.L., Karas A.J., Method of producing cured vanilla extract from green vanilla beans, U.S. Patent, 1958, N 2 835 591.

[11] Ansaldi G., Gil G., Le Petit J., Procédé d'obtention d'arôme naturel de vanille par traitement des gousses de vanille verte et arôme obtenu, Institut National de la Propriété Industrielle, 1988, N FR 2 634 979.

[12] Brunerie P.M., Procédé d'obtention d'arôme naturel de vanille par traitement enzymatique des gousses de vanille verte et arôme obtenu, International Patent Application, 1991, N PCT/FR92/00837.

[13] Odoux E., Languille S., Leblé J.P., Lombard A., The effect of the nature of the soil amendments on the main aromatic compounds in *Vanilla fragrans* beans, Fruits 55 (1) (2000) 63–71.

[14] Shankaracharya N.B., Natarajan C.P., Vanilla – Chemistry, technology and uses, Indian food packer 27 (1973) 29–36.

[15] Sagrero-Nieves L., Schwartz S.J., Phenolic content of *Vanilla planifolia* as affected by harvested period, J. food comp. analysis 1 (1988) 362–365.

Evolución de las concentraciones de vainillina y glucovainillina durante las diferentes etapas de la preparación tradicional de vainas de *Vanilla fragans* en la Reunión.

Resumen — Introducción. Se ha estudiado la evolución de las concentraciones de vainillina y glucovainillina durante una fase de preparación tradicional de la vainilla, para identificar las etapas más importantes de la hidrólisis de la glucovainillina en vainillina y, seguidamente, intentar mejorar los rendimientos. **Material y métodos**. Un lote de 400 kg de vainilla verde fue tratado mediante utilización del procedimiento tradicional que comprende las etapas de escaldado, estufado, secamiento, lavado y maduración. Se tomaron muestras de vainas cuando se recibieron éstas y, posteriormente, en cada fase de la preparación. Tras extracción de los componentes aromáticos, se dosificó la concentración de la vainilla mediante HPLC. También se determinó el contenido de materia seca de cada una de las muestras. **Resultados y discusión**. Durante las diferentes etapas de la preparación de la vainilla, la materia seca (ms) evoluciona regularmente del 18 % en vaina fresca hasta el 56 % tras maduración. Durante las primeras fases de la preparación, el contenido de vainillina evoluciona gradualmente durante cada etapa de estufado para luego permanecer estable (2,2 g·100 g^{-1} ms) a partir de la fase de secado. La concentración de vainillina total (formas aglicona y glicosilada) es estable hasta el secado (5,5 g·100 g^{-1} ms), y luego decrece hasta después de la maduración (2,8 g·100 g^{-1} ms). El rendimiento de hidrólisis enzimática de la glucovainillina en vainillina ha sido de un 40 %. **Conclusión**. Se evidenciaron dos fases críticas del procedimiento: las etapas de escaldado y estufado y la etapa de secado. Estos resultados han justificado los nuevos estudios que están realizándose.

Francia (Réunion) / *Vanilla flagrans* / procesamiento / recuperación del aroma / métodos de optimización / vainillina / precursor de aroma