Hydrolysis of Glucovanillin by ß-D-Glucosidase during Curing of Vanilla Bean (*Vanilla planifolia* Andrews)

E. ODOUX[1], J. ESCOUTE[2], J.-L. VERDEIL[2] and J.-M. BRILLOUET[1]
Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD)
[1]Département Flhor, TA 50/16, [2]Département Amis, TA 40/02, 34398 Montpellier Cedex 5, France

Hydrolysis of glucovanillin by β-glucosidase in vanilla beans is of major importance in flavor development. Nevertheless it seems to be incomplete during traditional curing (Fig. 1) (Odoux, 2000). The enzyme and substrate were thus localized at tissue and cell levels to gain further insight into this mechanism (Odoux et al., *2003).*

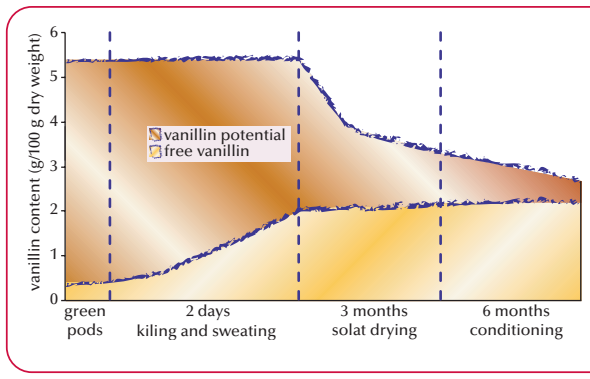

Figure 1. Free vanillin and vanillin potential content during the main stages of traditional curing of vanilla.

Materials and methods

Flesh samples were dissected from the epicarp to the placentae and papillae (Fig. 2) and tested for glucovanillin content and glucosidase activity. *In situ* localization of β-glucosidase activity was revealed by incubating sections with 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside followed by light microscopy examination.

Results and discussion

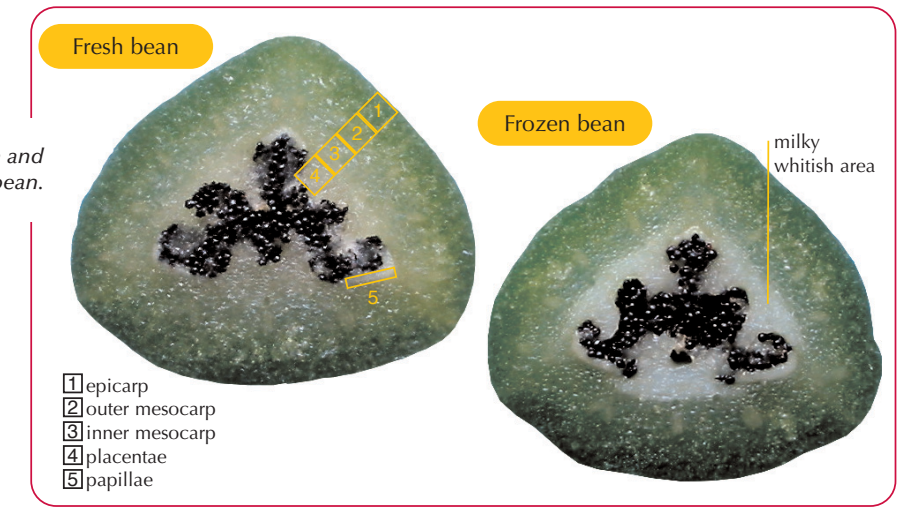

Figure 2. Cross-section of a fresh and frozen mature green vanilla bean.

Glucovanillin was absent from the epicarp and outer mesocarp area and mostly present in placentae and also in inner mesocarp and papillae (Fig. 3). Freezing beans leads to the appearance of a milky whitish area corresponding to the placentae (Fig. 2). By using this clear boundary for precise dissection, no glucovanillin was found in the non-whitish zone, thus in mesocarp.

β-glucosidase activity, measured with glucovanillin as substrate, was found in the whole pericarp (Fig. 3) but mostly in placentae. The high activity detected in so-called "inner mesocarp" tissues seemed to be due to the presence of placenta fragments, as the boundaries are unclear in fresh sections.

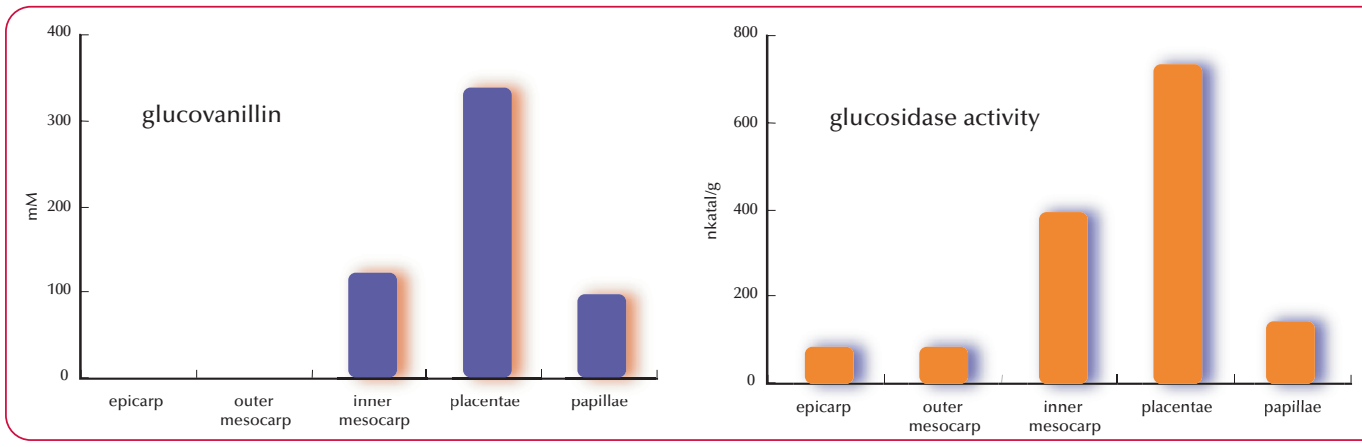

Figure 3. Distribution of glucovanillin and β-D-glucosidase activity in the different fresh vanilla bean tissues.

Staining revealed the presence of β-glucosidase activity at the periphery of cells (Fig. 4). As most of the intracellular volume is filled by the vacuole, β-glucosidase activity is probably cytoplasmic and/or apoplastic.

Although glucovanillin was not localized at the cellular level, it is probably present in the vacuole as this is the most common storage compartment for secondary metabolites, and since its high concentration in the placentae laminae (300 mM) would be incompatible with a cytoplasmic localization.

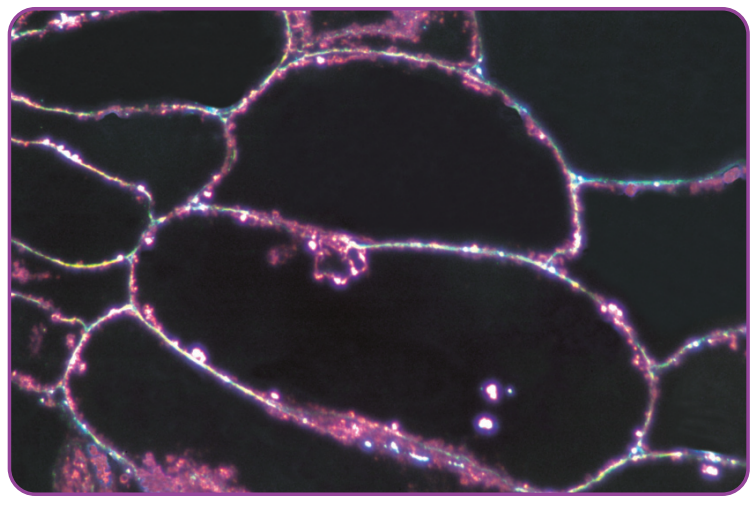

Figure 4. Thin cross-sections of the mesocarp of a mature vanilla bean after staining for β-glucosidase activity.

Conclusion

The tissue distribution of β-glucosidase activity is remarkably similar to that of glucovanillin but the enzyme activity and substrate seem to be located in two different cellular compartments (cytoplasmic/apoplastic and vacuolar, respectively). The incomplete hydrolysis of glucovanillin during traditional curing of vanilla beans could possibly be due to incomplete cellular decompartmentation between the enzyme and substrate.

References

Odoux E. 2000. Changes in vanillin and glucovanillin concentrations during the various stages of the process traditionally used for curing *Vanilla fragrans* beans in Réunion. *Fruits* 55: 119-125.

Odoux E., Escoute J., Verdeil J.L., Brillouet J.M. 2003. Localization of β-D-glucosidase activity and glucovanillin in vanilla bean (*Vanilla fragrans* Andrews). *Annals of Botany* 92: 437-444.

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