Vanilla Around the World:
New Challenges, Old Opportunities

SFC Symposium

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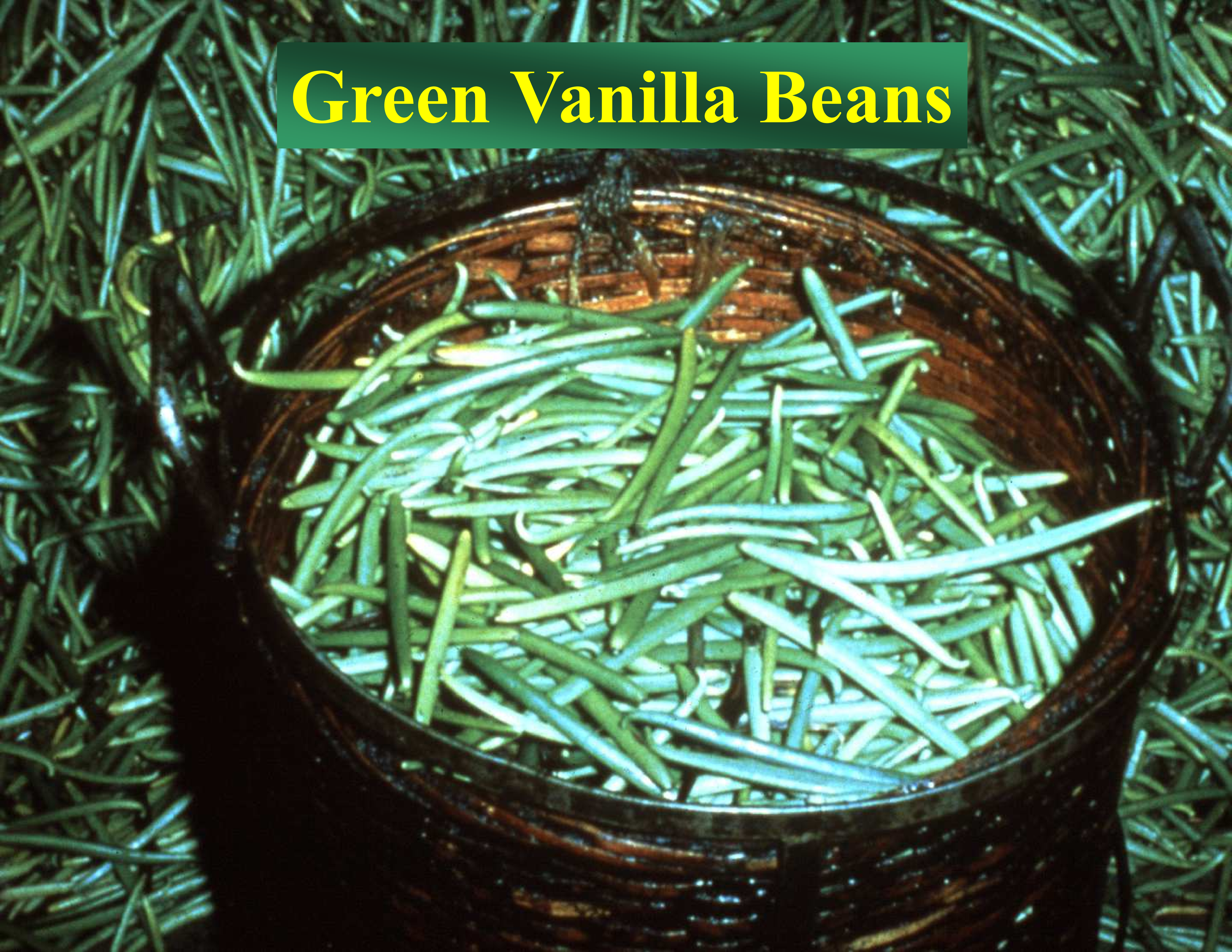
Edison NJ

Vanilla Plantation In Mexico

Green Vanilla Beans



Introduction

- Vanilla is the world's most popular and used flavor by both dollar and tonnage basis.
- Vanilla, a tropical climbing orchid, originated in Mexico.
- Beans are harvested green and cured at high-heat high-humidity conditions to release vanillin and other flavor compounds from their glucoside precursors. Vanilla extract contains more than 600 chemicals.
- Vanilla extract is obtained by ethanolic-water extraction of cured vanilla beans.
- The US annual consumption of vanilla beans in 2013 was around 2000 tons with a market value of approximately $100 million.
- The vanilla market has been volatile and the rate of consumption has been fluctuating.
- Most of the vanilla beans are used in the US market, where around 40% are used in ice cream.
- Vanilla beans are imported from tropical production regions but supply, cost and quality are subjected to fluctuations.

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Introduction

- Vanillin is one of the most important aromatic flavor compounds used in foods, beverages, perfumes, and pharmaceuticals.
- More than 15 thousand tons of synthetic vanillin per year are used by the industry globally. Around 4000 tons in the US alone.
- The demand for vanilla flavor cannot be met by vanilla extract, not only because of the vanilla bean price.
- There are not enough vanilla beans to satisfy the demand for natural vanillin.
- There is a demand for natural vanillin.
- Vanilla is the only flavor with a standard of identity regulated by FDA.

How Can We Maintain A Steady Supply Of Vanilla Beans?

- Consistent Quality
- Accessibility
- Stable Price

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Sustainability

- Sustainability
- Biodiversity

Which Are The Producing Countries?

- Madagascar
- Indonesia
- India
- Uganda
- Comoros
- Tanzania
- Mexico
- Tonga
- French Polynesia
- Papua New Guinea
- Costa Rica
- Hawaii

World Vanilla Production

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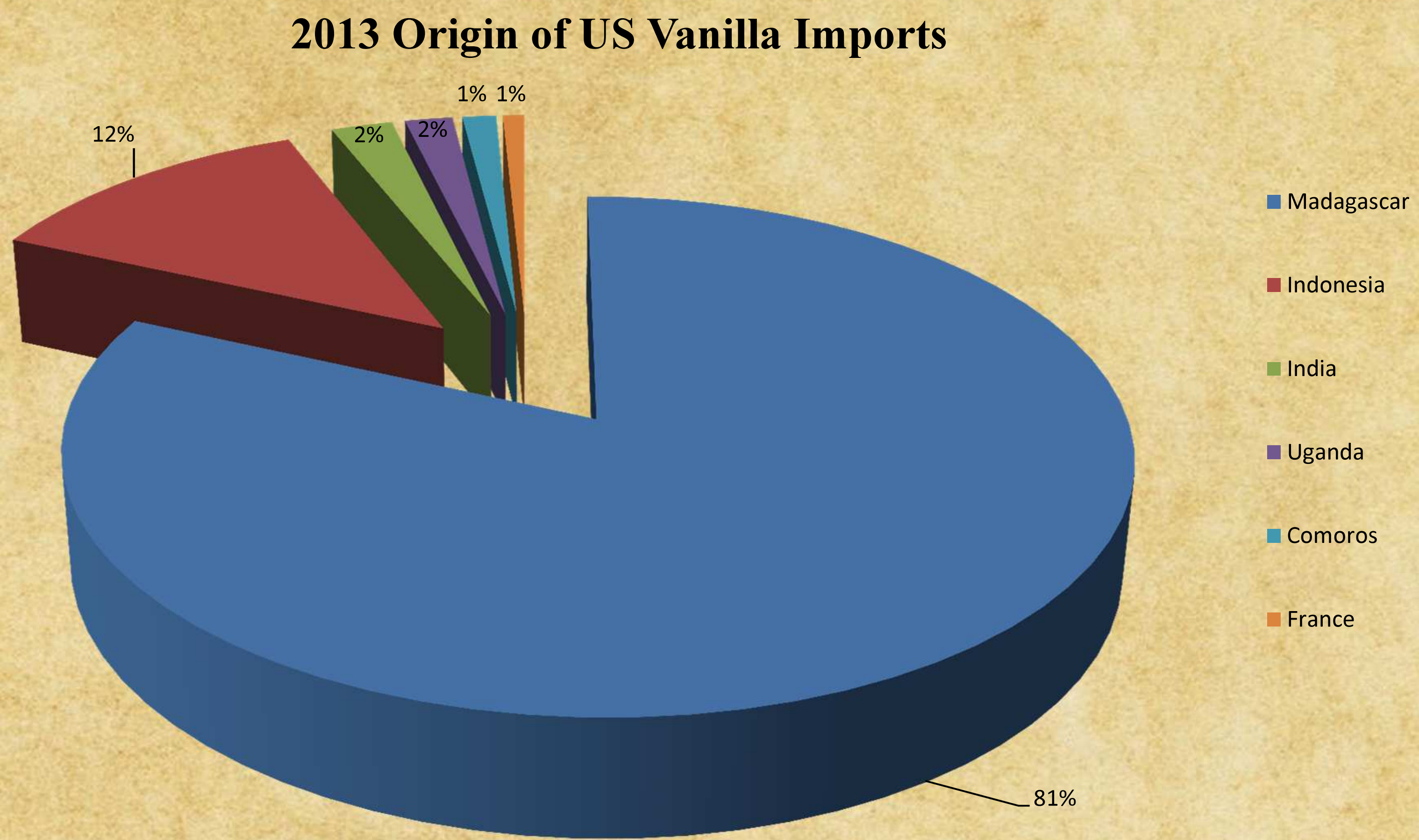
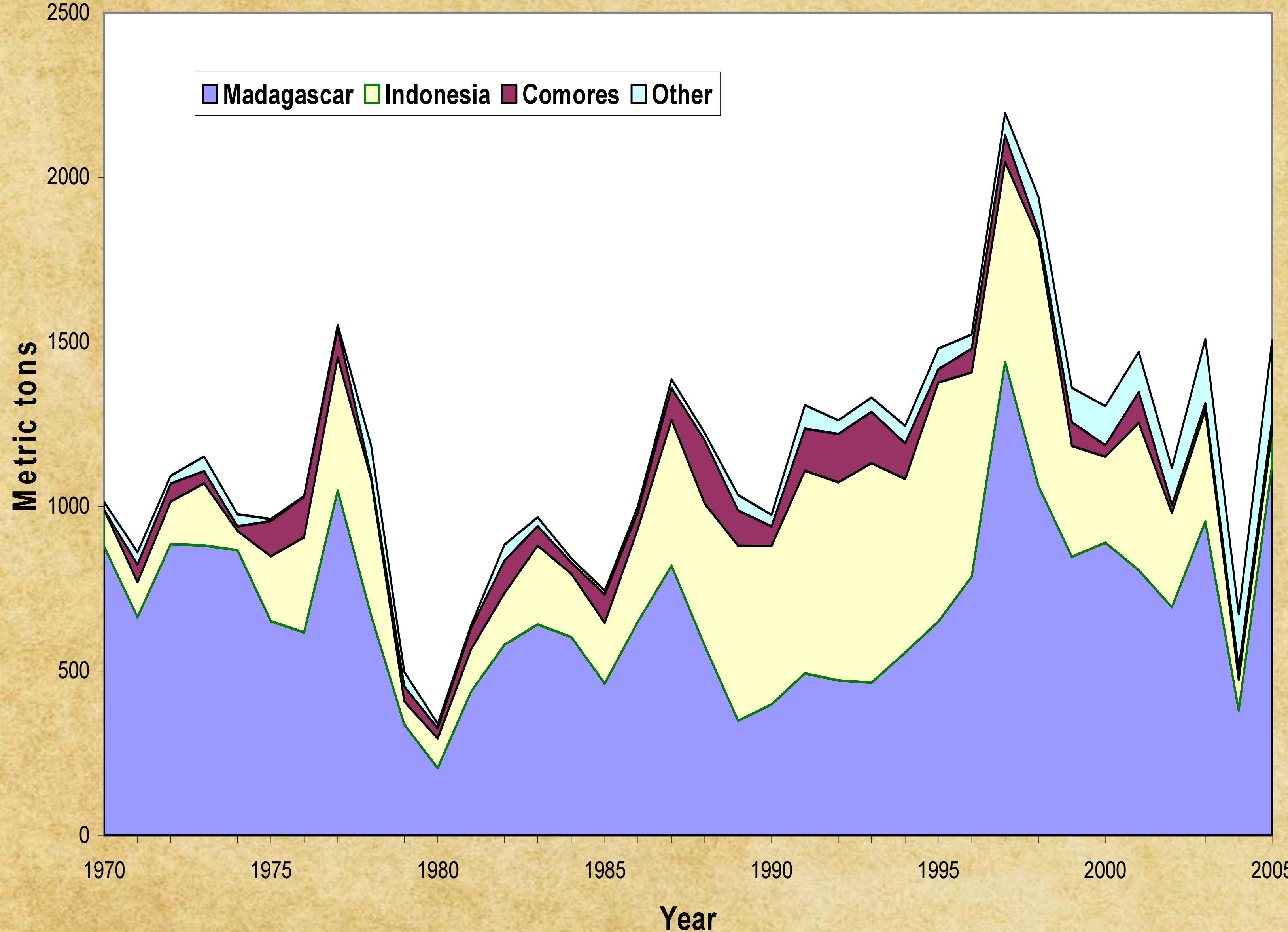
Import statistics



USA Vanilla Imports from 1970 to 2005



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Parameters Affecting Vanilla Production

- Political: When a political body intervenes with a professional judgment
- Weather: When harsh climate conditions interfere with agricultural production
- Growing conditions: Long time before harvest, 9 months to maturity
- Pollination and flowering: 2 months long, over pollination
- Nutrition: Not very well established
- Age variability: Long pollination period
- Harvest time: One time fits all ages

Parameters Affecting Vanilla Production

- Curing methods: Still an art and not science
- Disease/insects: Monoculture, globalization spared disease
- Only 2 Species permitted to be used in food
- Commitment of the industry/GOV: Long-term commitment needed from producing country
- Working with Farmers: Government, academia, private sectors, NGO
- Need Control of production: Agricultural production in Porto Rico.

Vanilla planifolia

Vanilla tahitensis

Species of Vanilla Beans

Vanilla planifolia Andrews

Madagascar

Comoros

Réunion

Indonesia

Puerto Rico

Mexico

Tonga

Uganda

India

Tanzania

Hawaii

Species of Vanilla Beans

Vanilla tahitensis Moore

- French Polynesia (Tahiti)
- Papua New Guinea

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Vanilla Flavor Quality

- Vanilla Species
- Vanilla Maturation/age when picked
- Agricultural Practices
- Geographic Factors
- Curing Method
- Post Harvest Handling
- Results in many different flavor profiles!!!

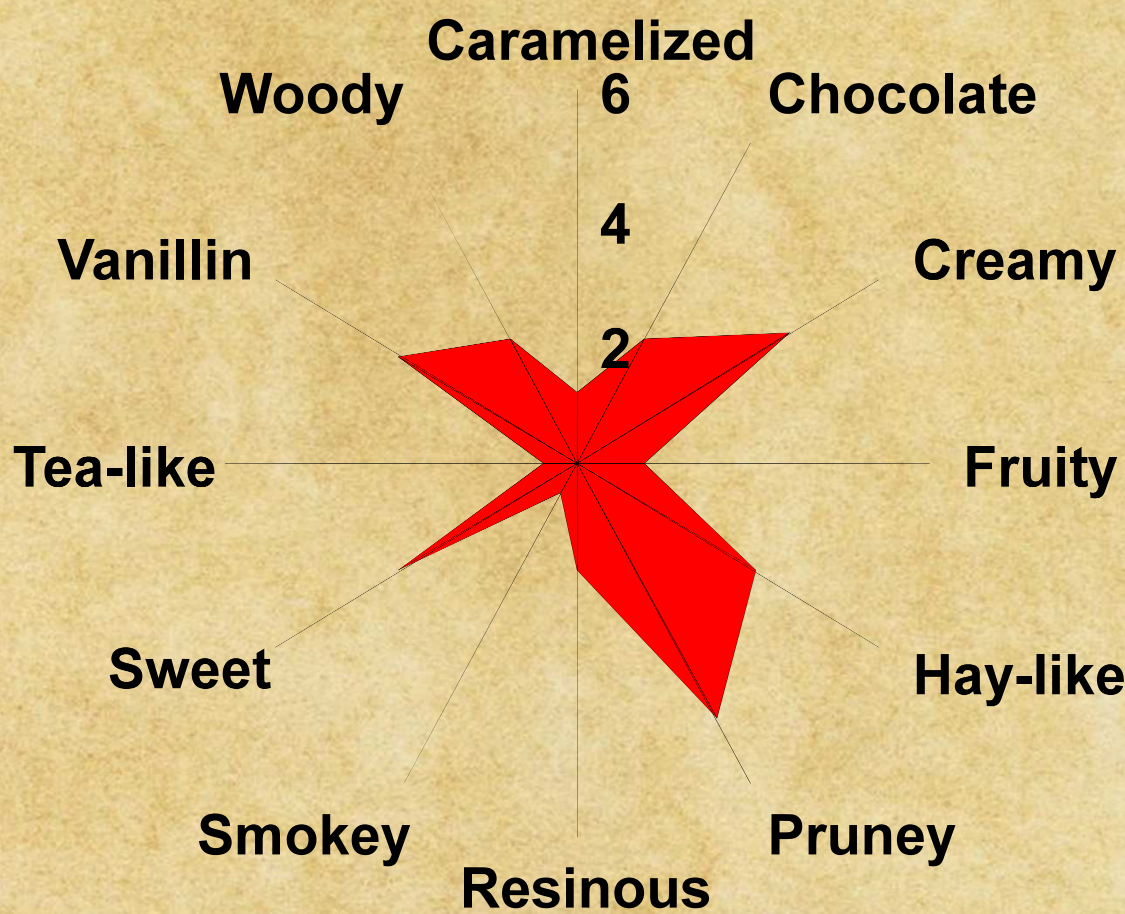
Vanilla Extract Flavor Descriptors

- **Anisic** → Sweet, Flowery, Anise mint like
- **Caramelized** → Brown Sugar, Butter
- **Chocolate** → Chocolate Syrup
- **Creamy** → Sweet Cream
- **Floral** → Sweet, Flowery
- **Fruity** → Cherry, Coconut
- **Hay** → Dry Grass, Straw
- **Prune/Raisin** → Prune Juice, Raisins
- **Resinous** → Wood Sap, Camphorous
- **Smokey** → Burning Wood, Charcoal
- **Sweet** → Sugar Syrup
- **Tea** → Astringent, Tea Extract
- **Vanillin** → Artificial Vanillin
- **Woody** → Wood Chips

Pure Bourbon Vanilla Extract

Species: *V. planifolia*

Profile: Pruney. Creamy, Hay-like, Vanillin



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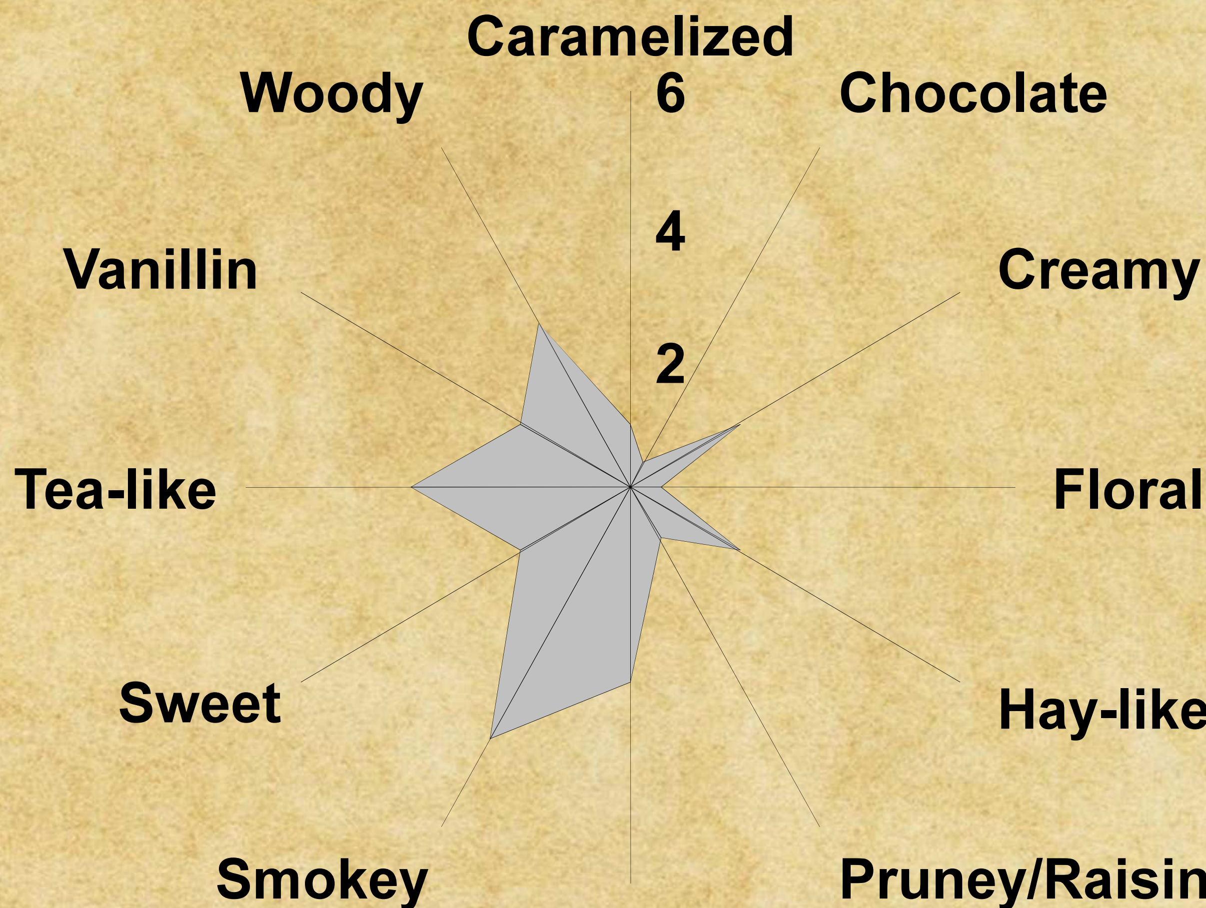
Pure Indonesian Vanilla Extract

Species: *V. planifolia*

Profile: Smokey, Tea-like, Resinous



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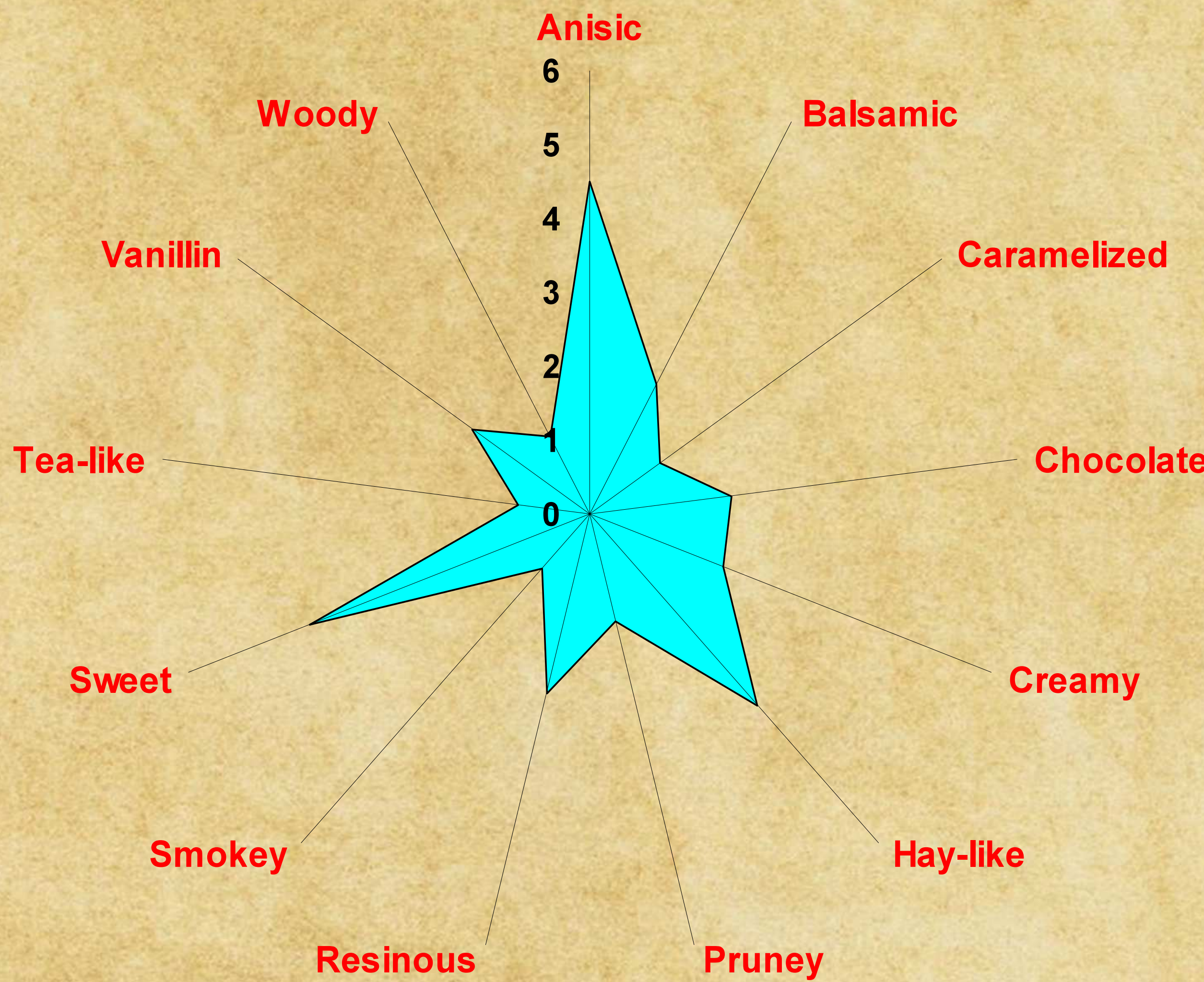
Pure Tahitian Vanilla Extract

Species: *V. tahitensis*

Profile: Anisic, Sweet,(floral perfumy)



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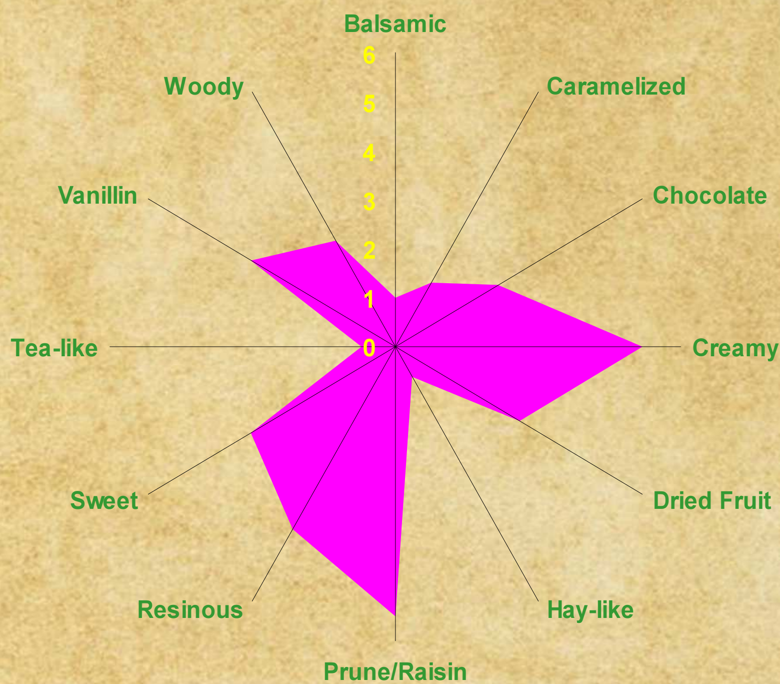
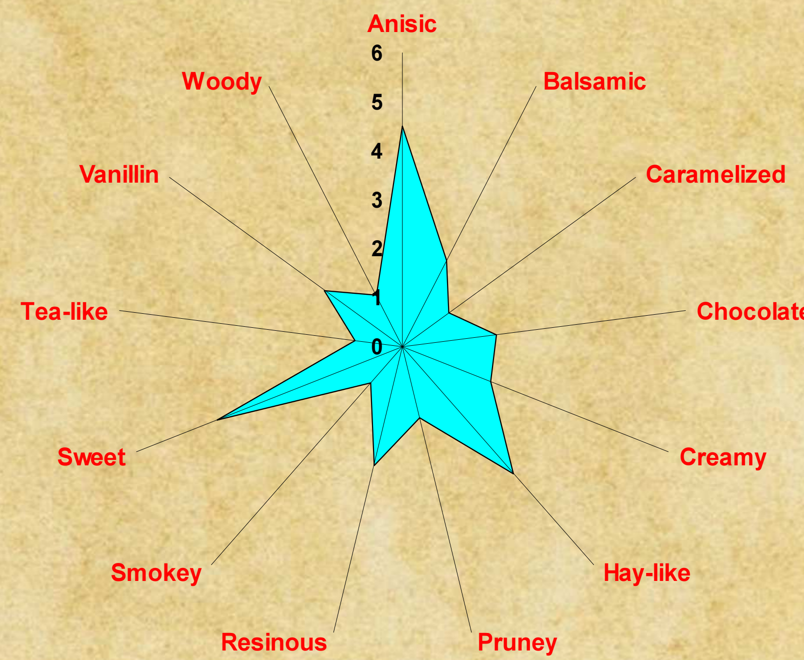
Flavor Quality

Effect of Vanilla Species

Bourbon - Black



Tahitian



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Lack of Sustainability

- Low price to farmers
- High price to the industry
- Only 2 species permitted to be used in food
- Lack of biodiversity
- Vanilla is a clone (has identical genetic makeup)
- Susceptible to disease (*Fusarium* wilt)
- Bad practices by producers: EP, vacuum packed
- High moisture content
- Lack of long term commitment. It is a Journey not Destiny

Alternatives to Vanilla/Vanillin

- Plant tissue culture, vanilla and other
- Lignins and ligninase
- Enzymes: Beta glycosidase, cellulases, pectinases…
- Microorganisms producing vanillin-FA to vanillin
- Physical conditions, high heat/high pressure-eugenol
- Regulation: strict standard of identity.

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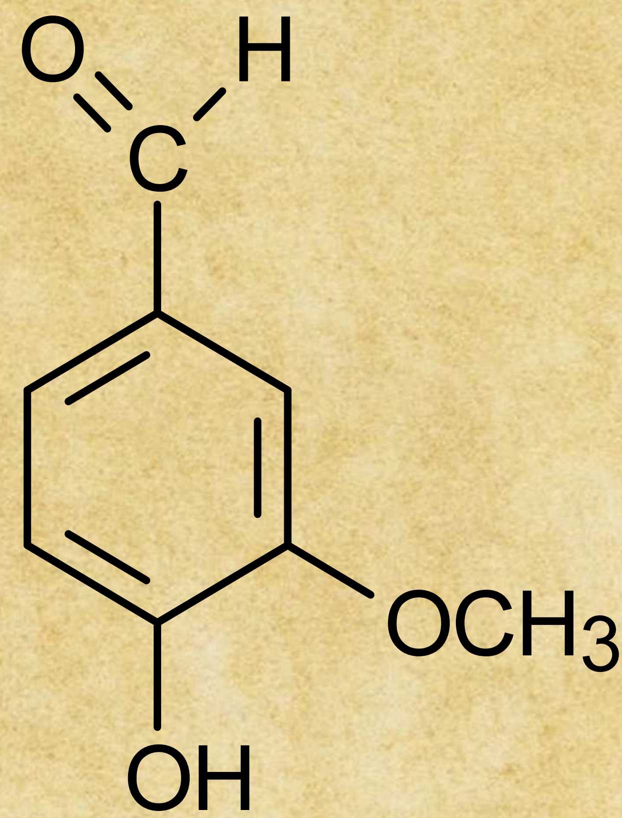
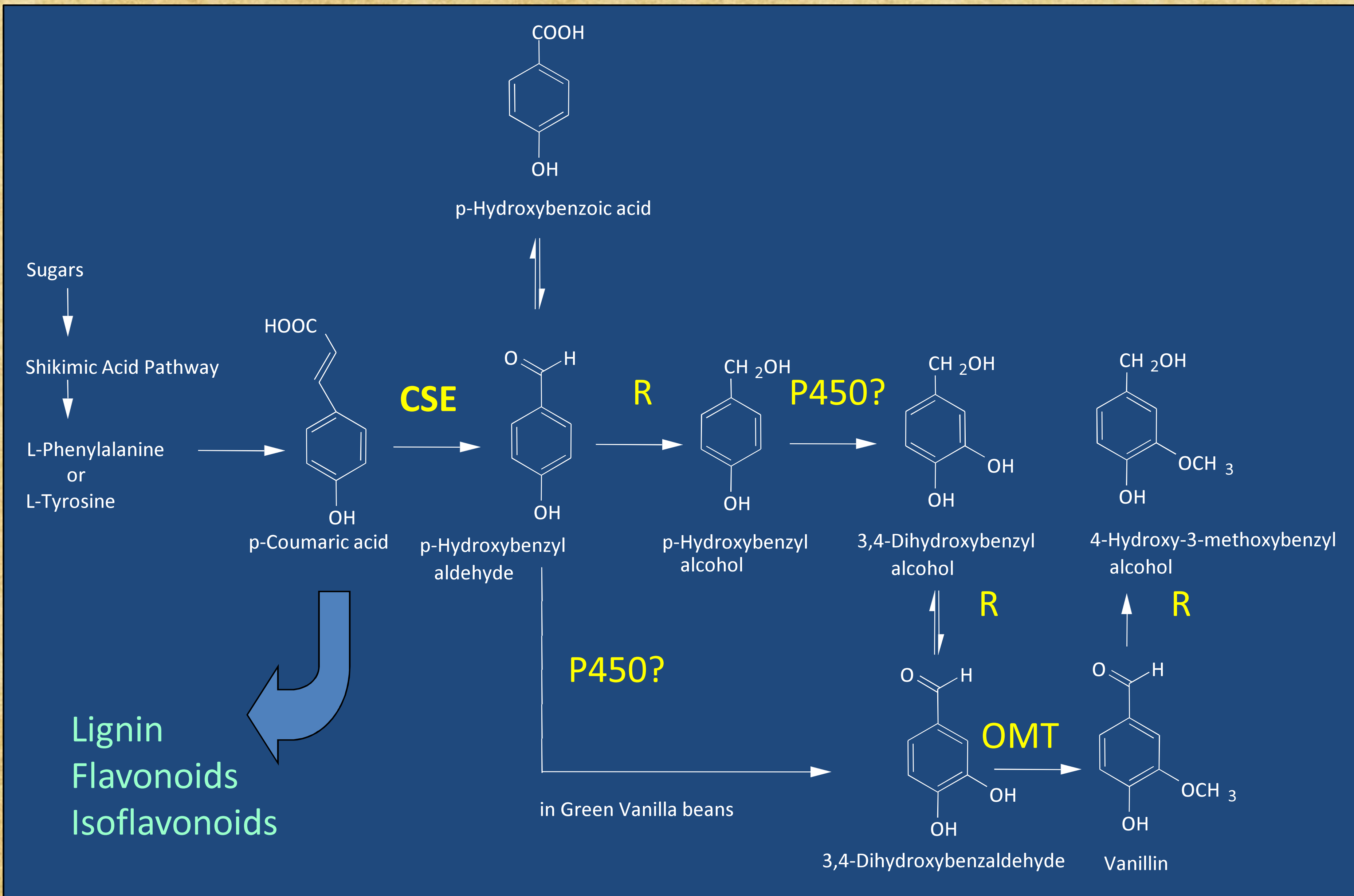
Vanillin

- Molecular Weight 152
- CAS 121-33-5
- FEMA-GRAS 3107
- Classification: A monocyclic aromatic aldehyde-phenol containing a methoxy group, found in a number of natural products in low levels.

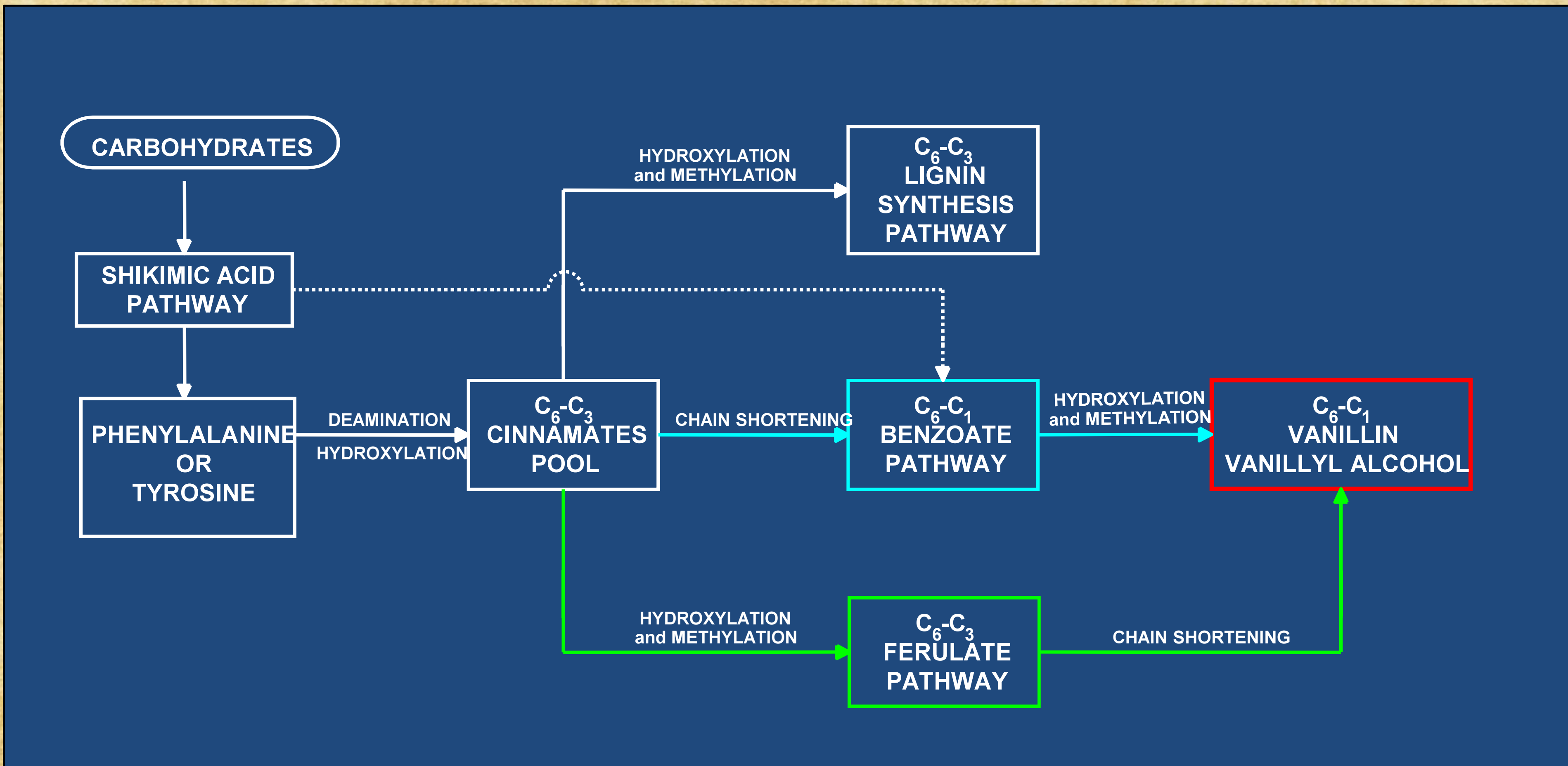


Proposed pathway for vanillin biosynthesis in *Vanilla* embryo culture

[Daphna Havkin-Frenkel and Andrzej Podstolski, 1999]



Possible Routes of Vanillin Biosynthesis



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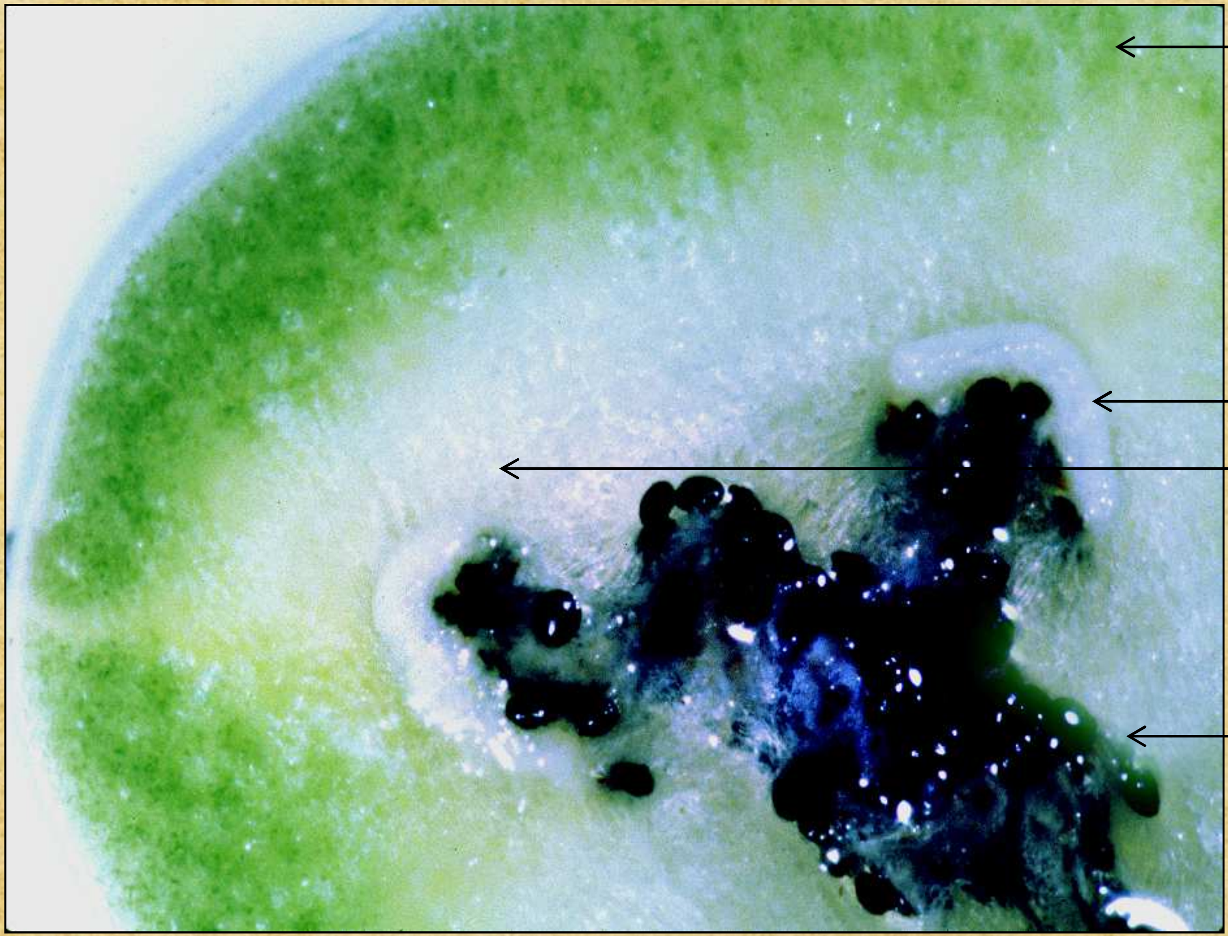

Cross section (x 20) of freshly cut green vanilla bean

The figure shows an inner portion of a vanilla pod composed of seed (dark bodies) and a white placental tissue surrounding the seeds.

Shown also are specialized hairy tissue cells, where vanillin is apparently synthesized as well as a green outer fruit region, which contain degradative enzymes.

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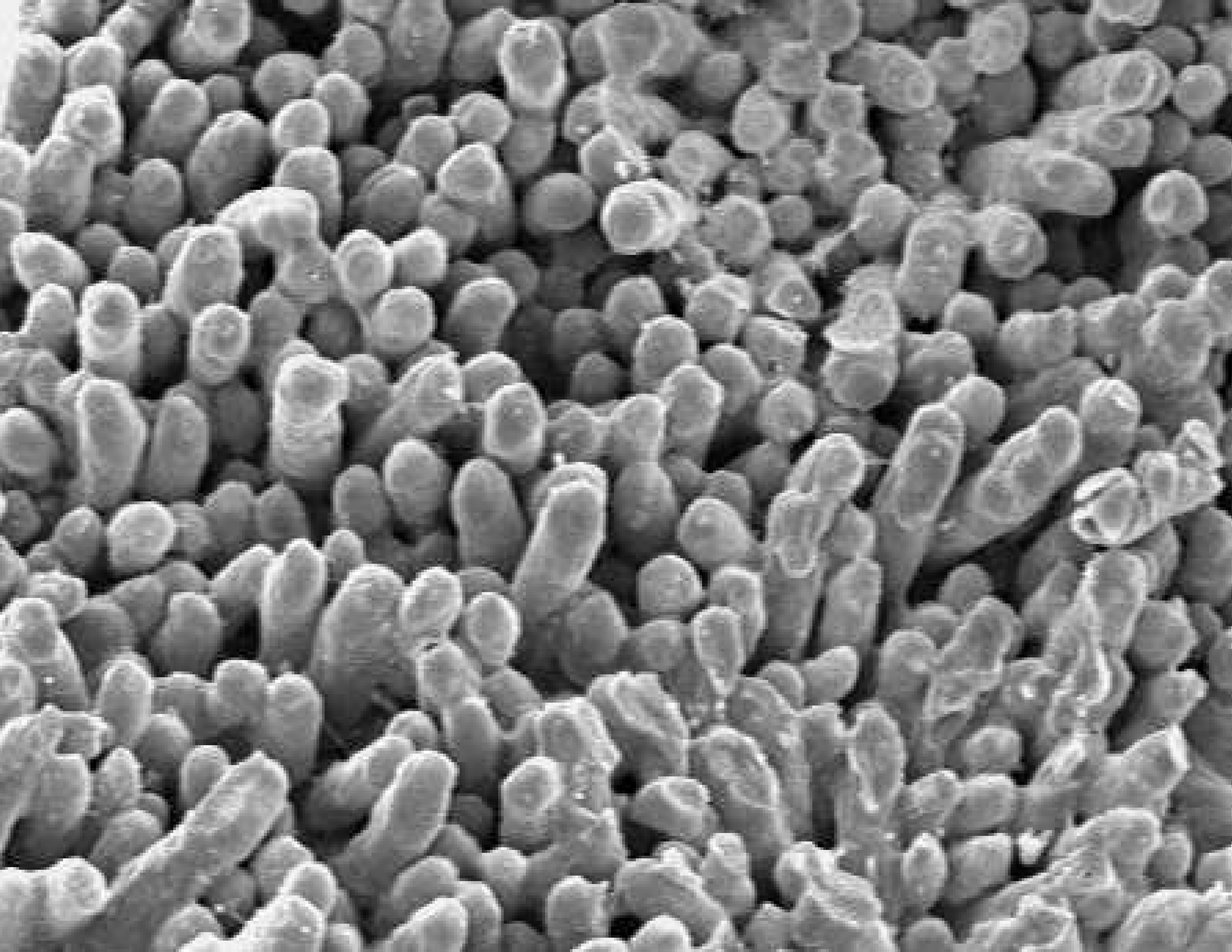

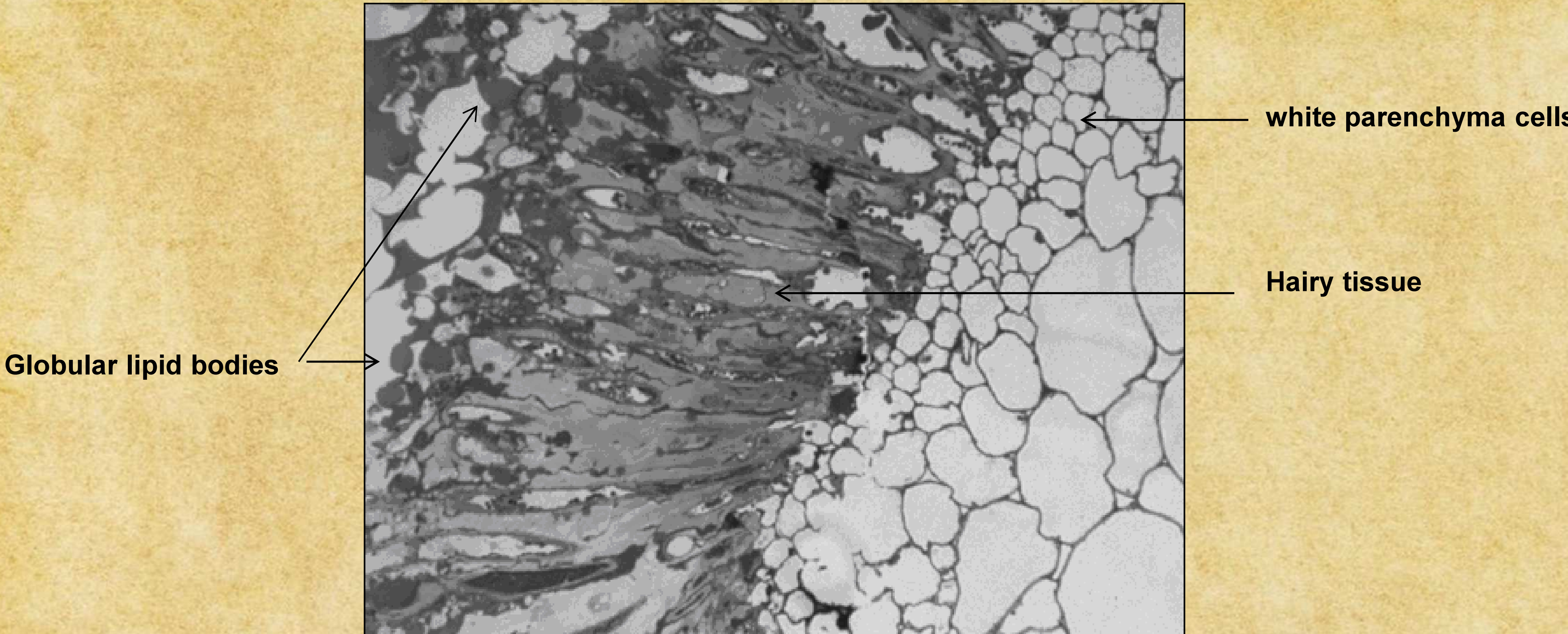

A magnified view (x 400) of cross section of green vanilla bean

The figure shows the inter-placental hairs (left) and white parenchyma cells in the fruit wall (right), which comprise the white portion of the fruit wall.

The hair-like cells contain enzymes in the vanillin biosynthetic pathway. These cells also release abundant globular lipid bodies seen as globular bodies (top left).

The outer fruit tissue contains hydrolytic and other degradative enzymes, essential for generating the prized vanilla flavor.

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Natural Sources of Vanillin

Vanillin is found in trace amounts in many plant tissues, in percentage fractions in potato skins, Siam benzoin resin, unicorn plant (*Proboscidea louisianica*) and *Narcissus* root.

However, the major natural source of vanillin is the cured bean of commercial *Vanilla* species (*V. planifolia* or *V. tahitensis*) where it is found in percentage levels (on dry weight basis).

Natural Occurrence of Vanillin in Plants

SPECIES	TISSUE	PERCENT DRY WEIGHT
Proboscidae cuisianica (unicorn plant)	Roots, pod	0.01
Solanum tuberosum (potato)	Tuber skin	0.01
Siam benzoin	Vascular tissue exudate	Traces
Narcissus (*Triandrus narcissi*, *Tazetta narcissi*)	Roots, basal plate	0.01 - 0.6
Hyacinth (*Hyacinthus orientalis*)	Roots, basal plate	0.2 - 0.5
Vanilla planifolia	Pod	1.0 - 3.0
Vanilla tahitiensis	Pod	0.5 - 1.0
Vanilla pompona	Pod	1.0 or less

Use of Plant Tissue Culture

- The metabolic potential of plants might be used in plant tissue or cell culture to synthesize useful compounds. An inherent problem with plant tissue culture is slow growth rate and particularly low level of formed products, which is reflected also in low production levels of vanillin. These phenomena put in doubt the commercial future of this approach.

- When plant tissue culture is used as a biotransformation system, feeding of vanillin precursors such as 3,4-dihydroxybenzaldehyde resulted in a complete uptake of applied compounds from the media and close to complete conversion to vanillyl alcohol (Havkin-Frenkel and Pederson, 2000; Herz, 2000).

- Vanillin is converted to vanillyl alcohol or vanillic acid. Plant cell cultures do not appear to have the ability to store vanillin.

- The precursors for vanillin are not easy to find from natural sources.

Degradation of Lignin to Vanillin

- Enzymatic degradation of lignin to useful compounds, including vanillin, has been the topic of many studies for the last 100 years.
- Lignin, a cell wall constituent in plants, harbor vanillin-like subunits in its polymeric structure and is an abundant by product of the paper industry (Janshekar and Fiechter 1983)
- Chemical breakage of lignin may yield around 4% vanillin.
- Companies utilizes enzyme-catalyzed oxidative degradation of lignin to obtain vanillin. The processes is carried out by extracellular enzymes from *Phanerochaete crysosporium,* a white rot fungus (Tien, M., & Kirt, T. K. 1983, Tien 1987).
- The lignin-degrading system does not readily attack native lignin but can degrade lignin fragmentation products obtained, for instance, by harsh chemical processing with sulfuric acid.
- The activity of lignin degrading enzymes depends also on supplementation with cofactors.
- The process yields around 1% vanillin as well as a vast array of other by-products.
- Degradable lignin fragments, obtained by harsh chemical treatments, may not be considered as a natural material, however.

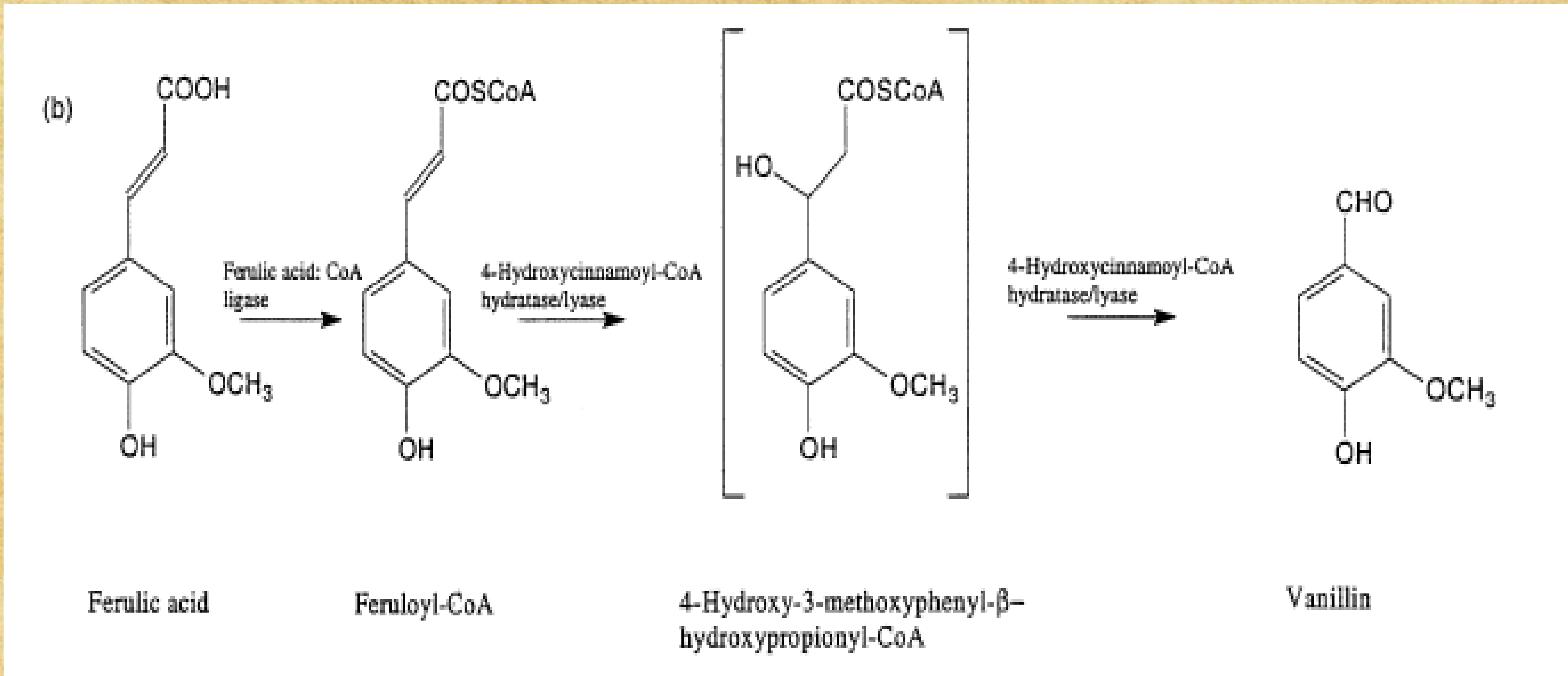
Hydrolytic Enzymes

- Beta glucosidase break glucovanillin to vanillin
- Cellulases and other hydrolytic enzyme lead to cell wall degradation

Bio-conversion of Ferulic Acid to Vanillin

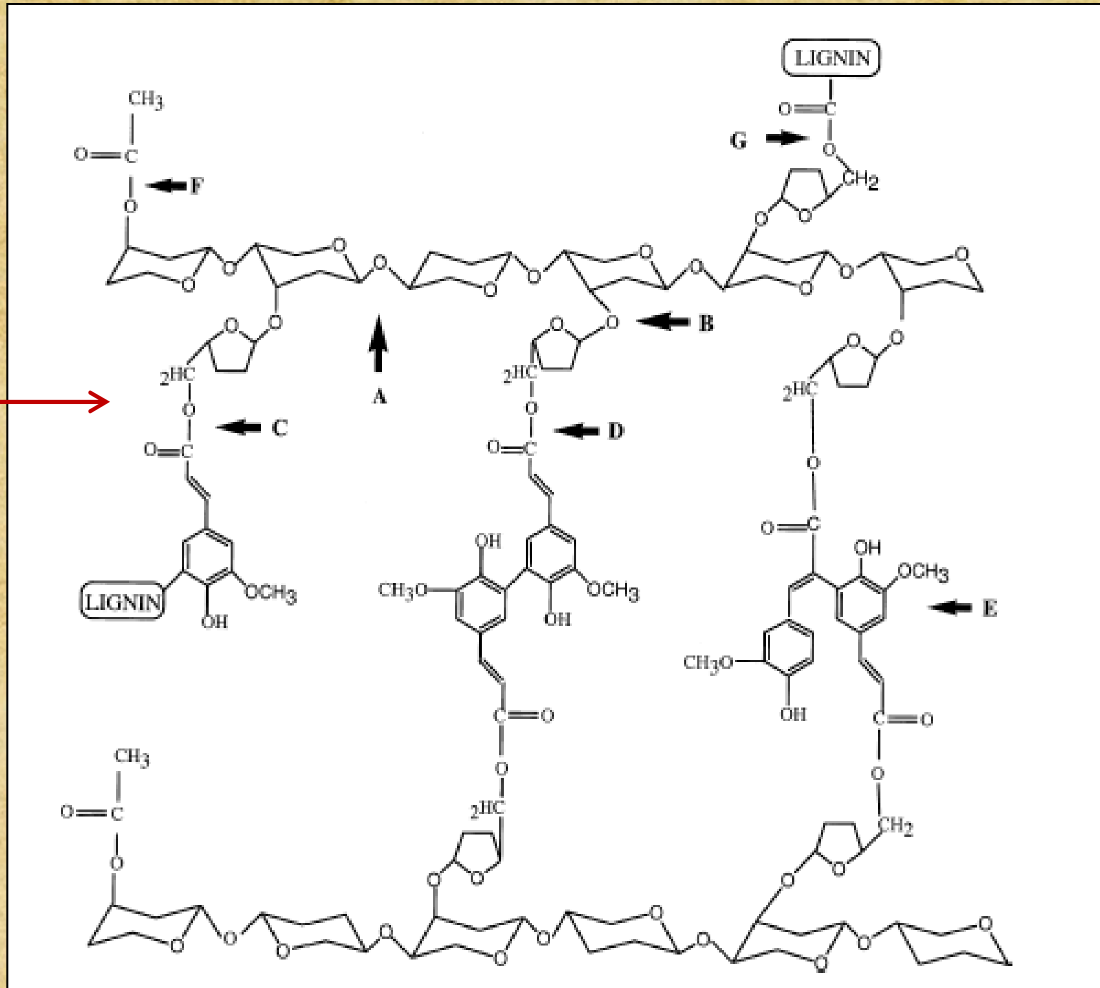
- Biosynthesis of vanillin by microbial and fungal fermentation is based on bioconversion or degradation but not on *de novo* synthesis in the manner found in plants.
- There is plentiful literature on the subject. Although there are many reports on successful laboratory-scale production of vanillin there are only a few companies producing vanillin on a commercial scale ($500-1000/kg vanillin), while other companies have abandoned this route for vanillin production because of the high production cost.

The route from ferulic acid to vanillin in *Pseudomonas* strains. 4-Hydroxy-3-methoxyphenyl-β-hydroxypropionyl-CoA is presumed to be an enzyme- bound intermediate. (Modified from Walton et al 2003).



Ester bond

Ferulate or diferulates can be released by enzymatic digestion (using esterase) or by alkaline hydrolysis (1 M NaOH)

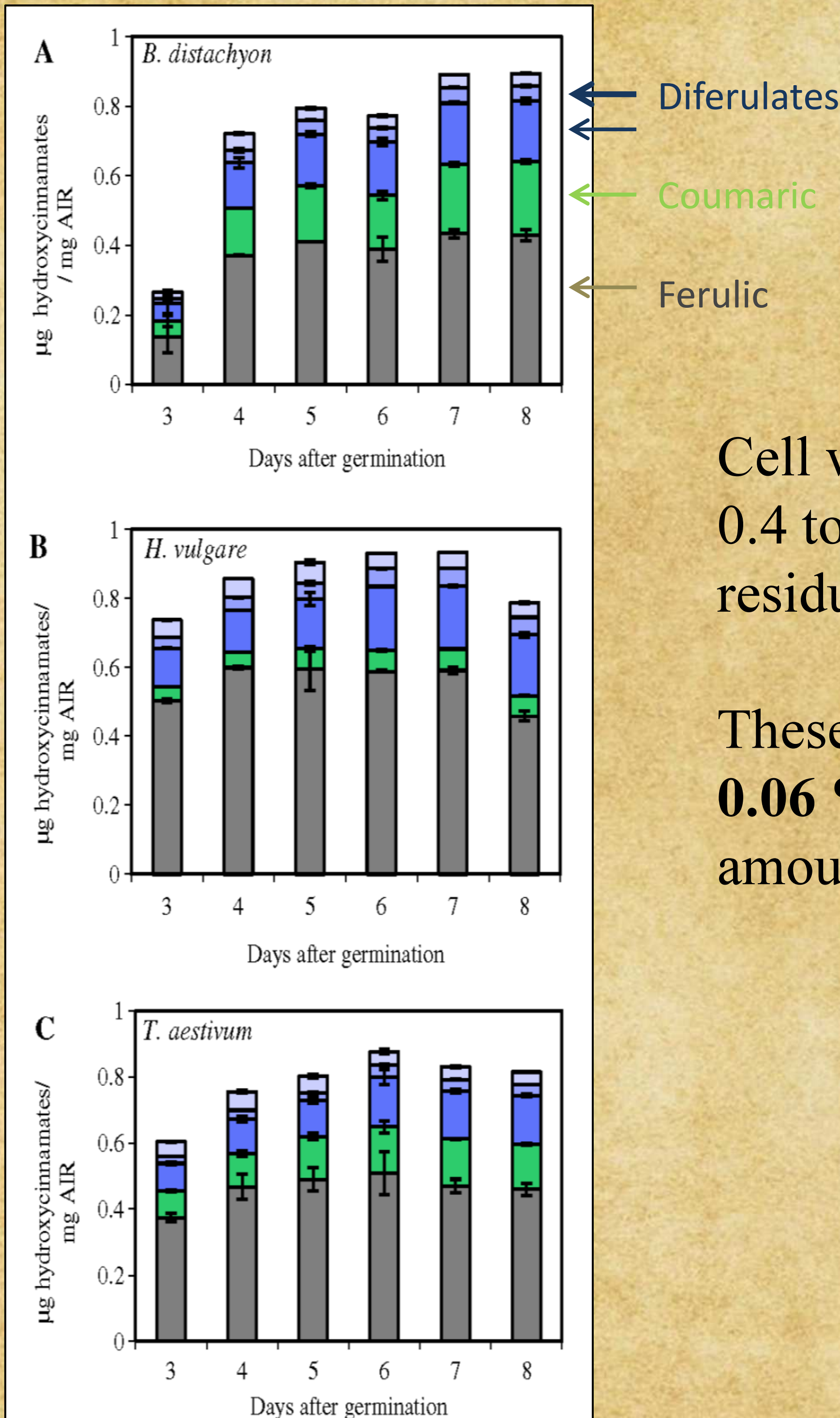


Note that most of the wall-bound feruloyl moieties are in the form of di-ferulate.

Generalized structure showing how a phenylpropanoid (ferulic acid) binds arabinoxylan (the main component of hemicellulose in monocots plants) to lignin.

A: β-(1-4) linked xylan backbone **B**: Xylose-arabinose linkage **C**: 5-O-feruloyl lignin
D: 5-O-diferuloyl group (5-5linked dimer) **E**: 5-O-diferuloyl group(8-5dimer)
F: 3-O-acetyl group, **G**: arabinose-lignin

***S Mathew (2004) Critical Reviews in Biotechnology*, 24:59–83**



Hydroxycinnamates of the cell wall of seedlings of (A) *Brachypodium distachyon*, (B) *Hordeum vulgare* (barley) *and* (C) *Triticum aestivum* (wheat)

Cell wall-bound ferulic acid comprises between 0.4 to 0.6 μg per gram AIR (alcohol insoluble residue), representing the total cell wall solids.

These amounts represent then between **0.04 to 0.06 %** of the cell wall solids (and 1/5 of this amount on a fresh weight basis).

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Christensen U et al (2009) Phytochemistry 71:62-69

De Novo Biosynthesis of Vanillin in Yeast

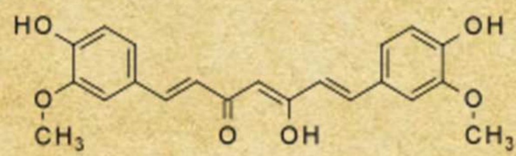
- A true de novo biosynthetic pathway for vanillin production from glucose has been established in *Schizosaccharomyces pombe*,
- Productivities were 65 and 45 mg/liter, after introduction of three and four heterologous genes, respectively, involved in lignin biosynthesis.
- The engineered pathways involve incorporation of 3-dehydroshikimate dehydratase from the dung mold *Podosporapauciseta*, an aromatic carboxylic acid reductase (ACAR) from a bacterium of the *Nocardia* genus, and an *O*-methyltransferase from *Homo sapiens*.
- Prevention of reduction of vanillin to vanillyl alcohol was achieved by knockout of the host alcohol dehydrogenase *ADH6*.

Hansen, E. H. *et al. De novo* biosynthesis of vanillin in fission yeast (*Schizosaccharomyces pombe*) and baker's yeast (*Saccharomyces cerevisiae*). Appl. Environ. Microb. 75, 2765–2774 (2009)

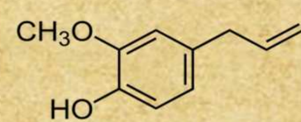
Commercial Sources of Vanillin

Commercial sources are synthetic vanillin, produced from lignin by degradation or by high-heat high-pressure cracking of curcumin or eugenol.

Between 12,000 to 14,000 tons of synthetic vanillin are produced annually.



Curcumin



Eugenol

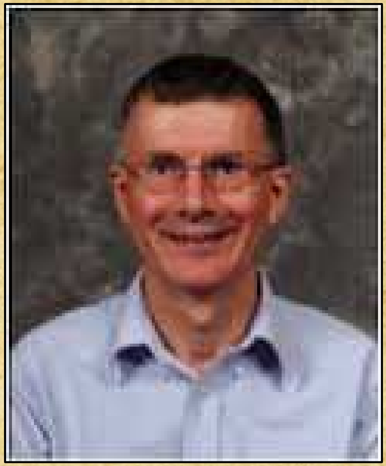
Recently, there is a thrust to produce natural vanillin by microbial biosynthesis or other biotechnology methods. It remains to be seen whether biotechnology would be price-competitive with current synthetic methods.

Conclusions and Future Outlook

- The sustainable supply of cured vanilla beans is often disrupted by economics, extreme weather, or disease. An economic incentive needs to be found to stimulate production. Also needed is a breeding program to increase the biodiversity of the vanilla, a clone, to create new, desirable traits and increase disease resistance.

- Biotechnology attempts to create vanillin, a major flavor component in vanilla. This effort is constrained by low yield, high cost, and often undesirable end products such as vanillyl alcohol or vanilic acid.

- The vanilla plant has a unique efficacy to synthetize and store vanillin. An understanding and manipulation of the vanillin biosynthetic pathways may resolve the chronic shortage in vanilla/vanillin.



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