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Vanilla flavor production methods: A review

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ABSTRACT

The biosynthesis of vanillin and other vanilla flavor compounds is still not completely understood. A better understanding of the pathways and how they are regulated may lead to better control of the production of the flavor and thus increase the commercial value of the pods. Non-synthetic vanilla flavor consists of over 250 components, with vanillin being the major one in terms of amount in the pod. Synthetic pure vanillin lacks complex flavor notes, and thus cannot replace vanilla in high quality products. Although customers prefer non-synthetic flavors, non-synthetic vanilla extract is approximately 200 times more expensive than its synthetic counterpart. To produce non-synthetic vanillin, fungi are being used for the biotransformation of vanillin precursors into vanillin. All plants contain several fungal endophytes which do not cause any symptoms of diseases; some endophytes are plant-specific and are thought to play a role in the interaction of the plant with its environment. Certain fungal endophytes have been reported to produce secondary metabolites previously thought to be produced by the plant; such fungi, particularly those isolated from pods, may play a role in the biosynthesis of vanillin and vanilla flavor compounds in the plant. This could be in the form of complete pathways, or parts of the vanillin pathway, interacting with the plant's vanilla flavor compounds biosynthesis machinery. Furthermore, studies on a possible role for endophytes may help to elucidate aspects of the vanillin biosynthetic pathway that still are under debate. Terroir effects on flavor, as observed for vanilla pods, may be due to the presence of microorganisms in the rhizosphere or in the plant itself. Based on this analysis, it is proposed that vanilla plant endophytes are studied and investigated for the presence of possible vanillin and vanilla flavor biosynthetic reactions. As a first step, fungal endophytes have been isolated from vanilla pods from Reunion Island and found to be involved with the development of the vanilla flavor.

1. Introduction

The orchid genus *Vanilla* (Family: *Orchidaceae*) consists of 110 species, three of which are cultivated for their flavor-related commercial value (*Vanilla planifolia* Jacks. ex Andrews, *Vanilla tahitensis* J.W. Moore, and *Vanilla pompona* Schiede, Family: *Orchidaceae*) (Soto-Arenas and Cribb, 2010). Vanilla originated in Central America where it was also used as a flavoring agent; the Spanish brought it to Europe around 1520 and vanilla production on Reunion Island started around 1819 (Table 1).

Apart from environmental concerns, chemical synthesis of vanillin, the major vanilla flavor metabolite, is expensive given the cost of ferulic acid (the precursor for a one-step reaction) whereas agricultural waste is a cheap source of ferulic acid and can be used directly for microbial vanillin synthesis (Chakraborty et al., 2017; Kaur and Chakraborty, 2013). Additionally, there is a lack of substrate specificity in the reactions due to competing by-product formation in the chemical synthesis of vanillin, reducing the yield of the product (Chakraborty et al., 2017; Kaur and Chakraborty, 2013) and making the process less efficient. Producing vanillin through tissue culture of vanilla plants is also not cost effective given the slow growth rate of the plant and the absence of active vanillin biosynthesis. Using microorganisms to produce vanillin is thus a favored alternative.

Fungi reportedly have the highest vanillin yield compared to other microorganisms (Table 1) and vanillin yield may well be associated with the ability of microorganisms, in general, to tolerate vanillin toxicity; at toxic concentrations, vanillin would be converted into the less toxic products vanillyl alcohol and vanillic acid, which reduces vanillin yield (Kaur and Chakraborty, 2013). Glycosylation of vanillin is another method of reducing vanillin toxicity to microorganisms and increasing vanillin yield. This was engineered by incorporating genes involved in glycosylation in vanillin-producing microorganisms like

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Table 1
Important events in the vanilla flavor history.
Adapted from: Firmenich, 2017; Givaudan, 2016 Ni et al., 2015; Perfumer and Flavorist, 2014; Kaur and Chakraborty, 2013; Hansen et al., 2009; Zheng et al., 2007; Rabenhorst and Hopp, 2002; Lesage-Meessen et al., 2002; Thibault et al., 1998; Rasoanaivo, 1998; Toms and Wood, 1970.

Year	Events
Ca. 1520	- Vanilla plants in Europe from Mexico. - Absence of insect pollinator in Europe, no pods obtained.
1819	- Vanilla plants in Reunion Island. - Artificial pollination developed in Reunion Island, pod commercialization follows world-wide.
1858	- Nicholas-Theodore Gobley isolates the vanilla flavor metabolite (vanillin) from vanilla pods.
After 1920s	- Synthetic vanillin synthesized from lignin, followed by commercialization. - Environmental issues with synthetic vanillin synthesis process leading to non-synthetic vanillin synthesis.
1970	- Synthetic ferulic acid biotransformation by *Pseudomonas acidovorans* (den Dooren de Jong) Wen (bacteria) to non-synthetic vanillin.
1998, 2002	- Agricultural byproduct (19,900 mg/l ferulic acid) biotransformation by *Amycolatopsis* sp. Rabenhorst (actinomycete) to non-synthetic vanillin (11,500 mg/L).
2002, 2007	-Synthetic ferulic acid biotransformation by *Aspergillus niger* Tiegh. (fungus) and *Pycnoporus cinnabarinus* (Jacq.) P. Karst. (fungus) to non-synthetic vanillin (300–2800 mg/L, depends on fungal strains, addition of adsorbent resins[a]). - Isotope ratio $\delta^{13}C_{PDB}$ shows non-synthetic and synthetic vanillin differ.
2009	- Non-synthetic vanillin *de novo* synthesis (45-65 mg/L) from glucose by *Schizosaccharomyces pombe* Lindner (yeast) and *Saccharomyces cerevisiae* Meyen (yeast). - Vanillin synthesis only after a novel biosynthetic pathway was genetically engineered in both yeasts.
2011	- World vanilla flavor demand exceeds vanilla pod supply. - To meet demand, substitutes of vanilla extract developed by flavor companies.
2014	- Vanillin from green vanilla pod fermentation by microorganisms. - Complex sensory profile without off-notes. - US patent application for process submitted by Givaudan flavor company.
2015	- Non-synthetic *de novo* vanillin synthesis from glucose by *Escherichia coli* (bacteria). - Only possible after genetic engineering of *E. coli* towards a novel biosynthetic pathway. - Vanillin yield from experiments with other genetically modified organisms: 97.20 mg/l from l-tyrosine, 19.30 mg/l from glucose, 13.30 mg/l from xylose, 24.70 mg/l from glycerol.
2017	- Low cost vanilla produced by Firmenich.

[a] Adsorb produced vanillin away from microorganisms improving total vanillin production.

yeast (Kaur and Chakraborty, 2013). Moreover, vanillin exists in a glycosylated form (glucovanillin) in green vanilla pods, where fungal endophytes responsible for vanillin production may reside.

To date, there are no reports of *de novo* vanillin biosynthesis in microorganisms. However, this has been achieved by genetically modifying microorganisms to engineer the lacking biosynthetic steps for *de novo* vanillin biosynthesis (Hansen et al., 2009; Ni et al., 2015). Nevertheless, there are several issues associated with *de novo* vanillin synthesis in genetically engineered organisms. For instance, production of contaminating by-products like isovanillin was observed in the modified *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 8739™) strain (Kaur and Chakraborty, 2013). Additionally, for the latter, the production of vanillic acid as an intermediate reduced the vanillin yield. In the case of engineered yeast, vanillin reduction to vanillyl alcohol was inhibited through gene knockout of the host alcohol dehydrogenase ADH6 enzyme (Hansen et al., 2009). However, an increase in vanillin toxicity in the culture was noted.

1.1. Components of the vanilla flavor

The commercial value of vanilla is in the flavor of the cured pods. Vanillin is the major compound influencing the vanilla flavor, contributing 1–2% w/w of the cured pod (Sinha et al., 2008); however, a series of additional minor compounds from the pods also contribute and are responsible for the differences between the vanilla flavor and pure chemical vanillin. The aroma of cured vanilla beans includes descriptors such as sweet, vanillin, floral, prune/raisin, spicy, woody, and tobacco-like (Ranadive, 2011).

Vanillin has organoleptic properties that have been defined as one-dimensional, whereas the vanilla flavor is not one-dimensional; however, vanilla flavor has also been described as subtle (Dunphy and Bala, 2012a, b) and is highly complex (Gleason-Allured, 2011), as a consequence of the latter, vanilla pods, the source of vanilla flavor, are considered to be a spice (Cameron, 2011). The non-synthetic vanilla flavor is dependent on changes in the growth conditions of the plant (Dunphy and Bala, 2012a, b). According to Alwahti (2003), a key feature of the vanilla trade is quality, i.e., the correct mixing of flavor compounds to obtain the unique complex vanilla flavor from pods.

The organoleptic quality of the flavor is due to the qualitative and quantitative metabolite composition of the pods. More than 250 compounds are now known to influence vanilla flavor and the quality of the cured vanilla pods and the extract thereof is dependent on the relative abundance of these compounds, including vanillin. Consequently, it is important to understand how the concentrations of the vanilla flavor components influence sensory detection. For instance, vanillyl alcohol is detected in the cured pods by olfactory-GC analysis as intensely as vanillin, despite being present at a considerably lower concentration (1000 times less) (Hoffman and Zapf, 2011). Vanillin itself is present in all pods, signifying that it does not contribute significantly (less than 12%) to vanilla flavor variability across cured pods (Hoffman and Zapf, 2011).

Some putative precursors of vanillin also contribute to the aroma and flavor of vanilla, including *p*-hydroxybenzaldehyde - Organoleptic property: sweet (Sigma-Aldrich, 2014), vanillic acid - Organoleptic properties: chocolate, creamy, grape, nutty, wine-like (Sigma-Aldrich, 2014), *p*-hydroxybenzoic acid - Organoleptic property: phenolic (Sigma-Aldrich, 2014), and vanillyl alcohol - Organoleptic properties: mild, sweet, balsamic, and vanilla-like odor (Burdock, 2010). Other components influencing flavor include volatiles such as monoterpenes, sesquiterpenes, ethers, arenes, phenolics, and lactones (Zhang and Mueller, 2012; Toth et al., 2011). However, some of these are volatiles that disappear if exposed to high temperatures, causing vanilla flavor to change upon baking (Kennedy, 2015). Volatiles are more concerned with olfactory receptors, whereas non-volatile components are more concerned with taste receptors; consequently both have different effects on flavor and aroma.

1.2. The need for alternative sources of the vanilla flavor

Vanilla is one of the most labor-intensive crops worldwide (Dignum et al., 2001, 2002). As a result, vanilla pods are expensive and the price fluctuates according to political and climatic conditions. This price instability is one reason for the shift to artificial vanilla flavor or pure vanillin by the food and beverage industry. Additionally, non-synthetic