



THE USE OF ENZYMES IN VANILLA EXTRACTION

Vanilla is one of the most universally used flavours in the food, pharmaceutical and cosmetic industries. Traditionally vanilla flavourings are extracted from the matured pods or beans of luminous celadon coloured orchids, *Vanilla planifolia*. The distinctive flavour and aroma comes mainly from the phenolic compound vanillin (Figure 1.0) and other aromatic compounds, which make up less than 2% of the vanilla bean.

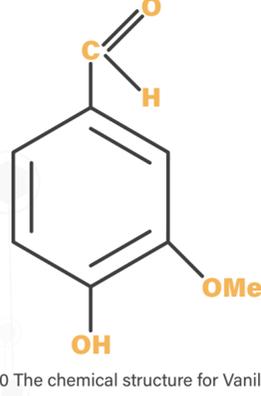


Figure 1.0 The chemical structure for Vanillin.

The supply of vanilla extract can be volatile due to its reliance on a good crop of vanilla beans. A poor season can see prices inflate and push food producers to consider using less of this popular flavour in their food products. This isn't ideal for the producer or the consumer.

To combat this problem, flavour houses and vanilla extract producers need to ensure the maximum amount of vanilla extract is produced from each production run. The more vanillin produced (with less waste) the better, making limited quantities of vanilla beans go as far as possible. This can be achieved by using enzymes in the vanilla extraction process.

Flavour houses and vanilla extract producers using enzymes to assist the extraction process can expect a significant increase in yield (normally around 2-4 fold) and quality when compared to the natural curing process. This technical bulletin will look at the process of using enzymes in vanilla extraction in detail, giving recommendations to achieve the best possible extraction and in turn dilute out potential supply issues.

BACKGROUND

Vanilla plants were cultivated by the Aztecs, who used it to flavour their cocoa based drink, xocolatl. Considered an aphrodisiac, it was so rare that it was reserved for royalty. Today, around 12,000 - 16,000 tonnes per year of vanillin is used in various flavourings around the world although less than 1% of this amount is natural vanilla from beans.



Natural vanilla is in relatively short supply because it is produced by a long and laborious process; therefore the price of natural vanilla tends to be very high. The orchid blossoms open only once a year and must be pollinated by hand. The vanilla pods then take 8 to 12 months to mature and must be hand-picked.

The mature green pods do not have the characteristic flavour or aroma that is produced by 'curing' the bean. The curing process can take between 5 weeks and 5 months. First the beans are 'killed' by heat (e.g. 20 seconds in boiling water or 48 hours in an oven) or freezing. The pods are then laid out and heated in the sun before being wrapped in blankets and allowed to sweat overnight, this is followed by drying and conditioning. This process is repeated for 1-2 weeks. During this process enzymes naturally present in the beans (glycosidases, proteases and oxidases) ferment the beans, which shrink by up to 400% and turn their characteristic brown colour.

The best grades of beans develop a visible white coating of vanillin.

The most common types of beans used are:



Tahitian vanilla beans - Intensely aromatic, though not as flavourful as the other varieties.

Bourbon Madagascar vanilla beans - Rich, sweet and the thinnest type of vanilla bean. About 75% of the world's vanilla beans come from Madagascar.

Mexican vanilla beans - Smooth rich flavour, in some cases they may contain coumarin.

Methods for the production of vanilla flavouring vary considerably around the world and are regulated differently in countries such as the USA, Great Britain and France. For example, in the USA definitions for vanilla flavourings are regulated under Code of Federal Regulations, Title 21 - Food and Drugs, Part 169 - Food Dressings and Flavourings.

VANILLA POWDER

Vanilla powder is made by grinding dried vanilla beans to a powder. The flavour from vanilla powder does not evaporate when heated as readily as vanilla extract making it useful for baked goods. Under FDA standards vanilla powders may contain blending agents such as sugar and anti-caking ingredients such as calcium silicate.

VANILLA EXTRACT

Vanilla extract is the most common form of vanilla flavouring used. It is made by macerating chopped beans in an alcohol-water solution. The mixture is usually aged for several months to produce a clear brown liquid with a strong vanilla flavour and fragrance. Heating the mixture may speed up the process but this may cause some of the more volatile flavour components to be lost altering the flavour. A variety of manufacturers recommend a slower 'cold' extraction process using recirculation of the menstruum over the beans solution to minimize loss of volatile compounds. To meet FDA regulations a 'vanilla extract' must contain at least the sapid and odorous principles extracted from one unit weight (13.5 ounces per gallon) of vanilla beans by an aqueous alcohol solution of not less than 35% (v/v) ethanol. Commercially available double and triple strength vanilla extracts are usually based on multiples of the legal minimum unit weights - e.g. a two-fold extract is extracted from 26.7 ounces of vanilla pods per gallon. Vanilla extract may contain additives such as glycerin, PEG, sugar, dextrose or corn syrup.

ARTIFICIAL VANILLA

Artificial (imitation) vanilla is produced by a chemical process that converts by-products (such as wood pulp from the paper industry) into vanillin. It lacks many of the flavour components extracted from vanilla pods and often has a harsh quality that may leave an aftertaste. Artificial vanilla is usually less than half the cost of natural vanilla. The production of artificial vanilla has nothing to do with the use of enzymes to produce natural vanilla.

ENZYME ASSISTED EXTRACTION PROCESS

The addition of enzymes to the extraction process can significantly improve the yield (2-4 fold) and quality of the vanilla extract, when compared to the natural curing process. The enzymes work primarily by macerating the tissues of the vanilla pods, breaking down the plant cell walls to release bound flavour compounds. Plant cells walls are highly complex structures containing interwoven strands of cellulose, xylan, pectin etc. Hence, successful degradation of the plant cell wall will require a cocktail of enzyme activities such as cellulase, xylanase, pectinase etc. rather than a single enzyme. Glycosidases in particular have been shown to be useful in releasing flavour compounds.

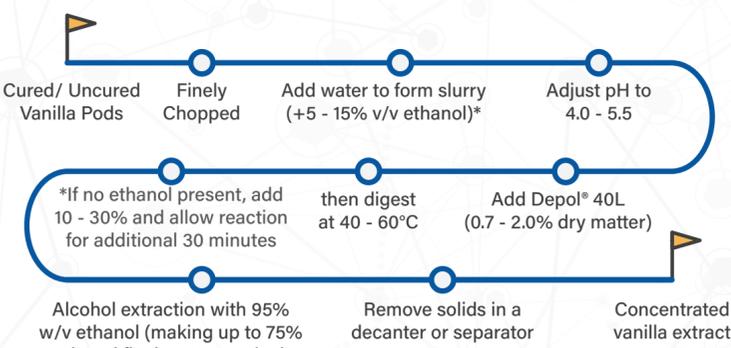
In addition to the macerating activities, Depol® 40L contains a wide range of exo-glycosidase activities which are particularly useful in releasing active flavours from their bound inactive forms. This preparation allows a more efficient extraction of valuable plant components, increasing the extracted yield of flavours. It is active over a broad range of pH and temperatures. In particular the enzymes are capable of hydrolysing the precursor, glucovanillin, to vanillin, the desired product.

Depol® 40L from Biocatalysts Ltd is a unique mixed carbohydrase enzyme which has been specially developed to aid the extraction of natural vanilla extracts from vanilla pods.

Specific conditions will depend on the type of vanilla pods available, the processing equipment available and a variety of other factors. We recommend that customers should always run trials to determine the enzymes activity under their specific processing conditions.

PROCESS FOR ENZYME ASSISTED VANILLA PRODUCTION

The cured or uncured vanilla pods are first finely chopped. The chopped material is then dispersed in a mixture of water and from 5-15% (v/v) aqueous alcohol. Water without alcohol can be used at this stage, but it is preferable to use a



mixture of alcohol and water because considerably less alcohol will then be required to bring the mixture up to 70% (v/v) alcohol at the later extraction stage.

The mixture should be acidified to a pH of 4.0 to 5.5 and the temperature controlled at 40 - 60°C. Depol® 40L should be added at 0.75 - 2 % based on dry matter content followed by incubation with agitation for between 3 -16 hours. The exact time will depend on the material being extracted and amount of enzyme dosed. Longer incubation times (up to 48 hours) may be required in some instances.

In trials, samples can be removed at periodic intervals to determine the effectiveness. Ethanol can be added to give a final concentration of 10-30% (v/v) (the exact amount depends on trials) & the reaction allowed to proceed for a further 30 minutes.

Once the digestion is complete, the pH is adjusted to 7.0 - 7.5 and alcohol is added to a final concentration of at least 70% (v/v). The alcohol solubilizes vanillin and other phenolic compounds and precipitates non-valuable components. Solid debris and insoluble residuals are then separated from the more valuable soluble components in a decanter or separator.

COMMON PROBLEMS ENCOUNTERED IN VANILLA EXTRACTION



Problem	Enzyme Solution
Digestion taking longer than predicted.	1. Ensure vanillin slurry is warm so that temperature of the bath does not drop thus reducing enzyme efficiency. 2. Try a higher dose of Depol® 40L.
Enzyme not working.	Ensure no direct heat is de-naturing enzyme solution and rendering it ineffective.
Too high a volume of alcohol needs to be added at the extraction stage.	Try increasing the percentage of alcohol used to make the initial slurry.
Low extraction of vanillin and other sapid components.	Ensure concentration of alcohol in the final extraction stage exceeds 70% (v/v).



Developing #BiobasedValue

Contact Biocatalysts' scientists to learn more about enzymes used in Vanilla production or to create something bespoke to your application.

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