Review Article

Production of Food Flavouring Agents by Enzymatic Reaction and Microbial Fermentation

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Abstract
Rising trends in the consumptions of food flavour compounds lead to motivation in the production of food flavours. The conventional techniques of flavour production are insufficient to produce flavour compounds according to the ascending demands of the market in terms of quantities and varieties. The current flavour production methods utilize chemical synthesis, which can produce a greater numbers of flavours with less time. However, the demand for natural products in consumables have created a necessity for new methodologies to produce flavour compounds with the label of "natural" origin. Emerging techniques in biotechnologies have enabled industries to produce compounds that can be considered natural. This review provides insights into the classification of flavour compounds and their production using microorganisms and enzymes in an eco-friendlier manner. The compounds produced by these techniques can be labelled as "natural" and can increase the market size of food flavours.

Keywords: Food flavour, Microbial fermentation, Enzymatic reaction, Platform chemicals

1 Introduction

One of the widely attractive research topics nowadays is regarding the perception of smell and taste in connection with food. Smell and taste are mainly dependent on the flavouring chemicals of food. These chemical flavours can impart aroma and taste for the food, which will attract more consumer's interests. The market size of food flavour was estimated to be $14.6 billion in 2019 and it is expected to attain $17.4 billion in 2027

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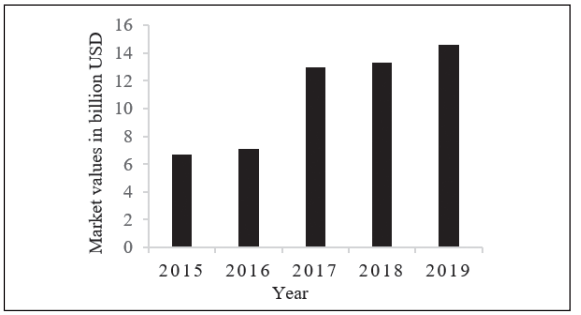


Figure 1: Global market size of food flavour compounds during 2015–2019.

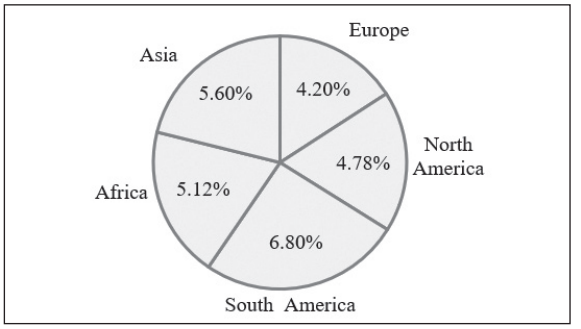


Figure 2: Market value's growth rate of food flavours estimated in different world regions during 2020–2025.

[1]. The graphs in Figures 1 and 2 depicts the increase in market size of food flavour compounds over the past few years and in different regions of the world, respectively. The market share of food flavours is distributed equally in different continents. These flavours play important roles in the food industry to develop new food product or to change the existing taste of the food. In addition to the continuous innovation in the food flavour industry, it can also flourish as there is an increased demand for new flavours from the fast-food sector.

In general, almost all food products available in the market have flavours additives either natural flavour or synthetic flavour. Practically, during cooking and food processing, the natural taste, and smell of raw materials in foods are partially lost and entirely modified. Therefore, food additives, especially food flavouring chemicals are added to maintain their original aroma, texture, or taste, and to imitate the senses of natural foods. These flavours can be broadly divided into artificial flavours and natural flavours. Natural flavours are extracted from natural sources, such as plants or animals. Whereas artificial flavours

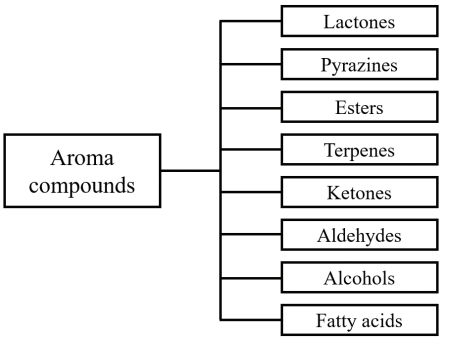


Figure 3: Classification of aroma compounds.

are produced by chemical synthesis.

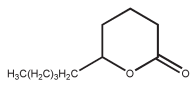
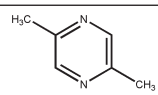
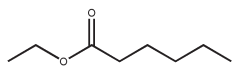
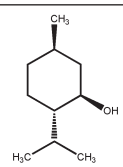
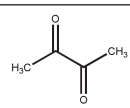
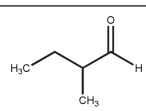
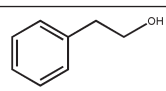
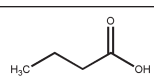
The first synthetic flavour and fragrance compounds made available for use in the food industry were coumarin and vanillin in the years 1868 and 1874, respectively [2]. Initially, the flavours were directly extracted from natural sources like plants. With the advancements in analytical procedures during the mid-twentieth century, the extraction of flavours was much easier with supports of new technologies. Eventually, the production of flavours shifted to chemical synthesis using techniques like ultrasound-assisted extraction, microwave-assisted extraction, distillation. Currently, most of the flavours are produced in the market using the most advanced biotechnological methods, utilizing bacteria and enzymes [3]. This review focuses on flavour and aroma compounds in terms of their classifications and different production methods. Also, this review can provide insight into how the technologies drive the development of these products in conventional markets.

2 Classification of Flavour Compounds

Odour or smell in the food mainly occurs due to the presence of aroma compounds in it and is sensed by human sensory receptors in the nose. These aroma compounds are also known as odorants.

Each odorant has different characteristic and properties. Aroma compounds are mostly present in low quantity in foods. They are mostly low molecular weight compounds, which are highly volatile [4]. These aroma compounds can be classified, based on the functional groups present in them, to be lactone, pyrazine, ester, terpene, ketone, aldehyde, alcohol, and fatty acid (Figure 3). Some examples of each class of aroma compound were listed in Table 1.

Table 1: Classification of flavour compound

Class	Example	Structure	Taste	Reference
Lactones	δ-decalactone		Fruity	[5]
Pyrazines	2,5-dimethyl pyrazine		Nutty	[13]
Esters	Ethyl hexanoate		Aniseed	[13]
Terpenes	Menthol		Minty flavour	[12]
Ketones	Diacetyl		Butter flavour	[5]
Aldehydes	2-methylbutanal		Chocolate like flavour	[14]
Alcohols	Phenyl ethyl alcohol		Rose	[15]
Fatty acids	Butyric acid		Butter flavour	[16]

2.1 *Lactones*

Lactones are cyclic carboxylic esters of γ- and δ-hydroxy acids [21]. These molecules were first described as lactic acid derivatives. Their aroma expresses as coconut, sweet, creamy, fruity, and nutty flavours [2], [21]. Lactones are naturally present in fresh vegetables, such as celery, apricot, plum, raspberry, tomato, roasted almonds etc. Biotechnological methods to synthesize lactone was discovered in the 1960s by using microbial fermentation processes with several species of microbes that can produce varieties of lactones. Few microbes among them are *Trichoderma viride*, *Tyromyces sambucus*, *Cladosporium suaveolens* and *Candida tropicalis* [5]

2.2 *Pyrazines*

Pyrazines are heterocyclic aromatic compounds, with nitrogen atoms in the aromatic ring. It provides flavours of nuts or roasted nuts [2]. Naturally, they are formed by Maillard reaction during the conventional cooking or roasting of foods [21]. One of the pyrazine derivatives, 2,5- dimethyl pyrazine was identified in grilled chicken. It was responsible for the nutty and roasted aroma in grilled chicken, especially where there is caramelization [6]. Some groups of bacteria, such as *Bacillus subtilis* [7] and *Corynebacterium glutamicum* [8] can also produce pyrazine compounds during fermentation.

2.3 *Esters*

In nature, many types of scents are normally identified as ester compounds which are generated from reactions between carboxylic acids and alcohols. Esters generally express fruity smells [24]. Ester compounds are added in many products, especially foods and beverages, including candies, jellies, jams, yoghurt, milk, soft

drink, cheese etc. [2]. Various strains of lactic acid bacteria were demonstrated to be able to synthesize ethyl esters and thioesters, such as *Lactococcus lactis*, *Saccharomyces*, *Pichia anomala*, *K. marxianus* [2], [9].

2.4 *Terpenes*

Terpenes are volatile compounds containing unsaturated hydrocarbons and is composed of isoprene units [21]. Terpenes are the building block of terpenoid compounds, which are major groups of bioactive compounds in herbs. They are found mainly in plants as important components in essential oils and provides a wide variety of scents [21]. *Ascomycetes* and *basidiomycetes* are the major fungus species that produce terpenes in nature [5]. Among aroma terpenes, limonene is one of the main terpenoid precursor, which is widely studied as food additives with flavour or aroma property [10]. Limonin was identified as the compound imparting bitterness in Thai lime cultivars like *Citrus aurantifolia* and *Citrus latifolia* [11]. Compounds like terpinolene and linalool have been detected in Thai lime juice samples as key odorants [11]. Carvone, linalool, nootkatone, eucalyptol etc. are few other terpenoid flavouring compounds [12].

2.5 *Methyl ketones*

Methyl ketones are used mainly to impart fruity and cheesy flavour [2]. It can also cause stale flavour in UHT (Ultra High Temperature processing) milk. Methyl ketones can be naturally produced by mammals and fungi by decarboxylation of beta-keto acids. The odour and taste in ripened cheese are due to the presence of methyl ketones produced by oxidation of aliphatic hydrocarbons by bacterial metabolism [2]. Diacetyl is an important ketone that provides buttery flavour in dairy products [5]. Along with lactic acid bacteria, some fungus like *Aspergillus niger*, *P. roqueforti* etc. can also produce ketones [5].

2.6 *Aldehydes*

Aldehydes are key flavour compounds in many foods. Vanillin, benzaldehyde, 3- methyl butanal, 2-methyl propanal are few examples of flavour compounds belonging to aldehydes [13], [14]. Aldehydes, which possess a -CHO group, can be easily reduced to alcohol or oxidized to carboxylic acids. Hence, their natural presences as aldehydes are relatively low [14]. Aldehydes are found to be major aroma compounds in Japanese grilled chicken samples [6]. Vanillin is one of the most studied flavours because its natural form obtained from vanilla pods are expensive. Many research works focus on converting its precursor molecules like ferulic acid, eugenol or isoeugenol to vanillin using microbial fermentation [5]. Similarly, studies are being carried out to elucidate new methods to synthesize natural flavour compounds.

2.7 *Alcohols*

Many microbes can produce alcohol during anaerobic fermentation as part of their normal metabolism. Alcohol can also be produced by bacterial fermentation, such as *Zymomonas mobilis* [2]. Many aroma alcohol compounds are of great interest in the flavour industry, such as 2- phenyl ethanol, isoamyl alcohol etc. These compounds and their ester derivatives have organoleptic properties, which can be used in the flavour industry [5].

2.8 *Fatty acids*

In plants, many volatile compounds are derived from saturated or unsaturated fatty acids. These fatty acids undergo α- or β- oxidation to form alcohols, aldehydes, esters methyl ketones and lactones [17]. Lactic acid-producing bacteria like *Lactococcus*, *Lactobacillus* can produce fatty acid compounds like lactic acid, butyric acid which imparts dairy flavours to food [18]. In addition to lactic bacteria, some fungal species belonging to *Rhizopus* sp. can also produce lactic acid [19]. Fatty acids imparting dairy flavours are important in the food and beverages industry [13].

3 Flavour Production

In the past decade, the flavours were produced from plants by various extraction methods [20]. However, these compounds are most volatile at room temperature, so it was difficult for efficient extraction and recovery. Moreover, the concentrations of these aroma compounds present in the plants may be exceptionally low, or they may be present in bound form with other compounds, which makes extraction even more difficult [20].

In addition to this, the plant metabolisms for formations of these aroma compounds can vary depending on several factors, such as season, humidity, growth phase, light intensity [20]. In due course, the knowledge about their chemical structures helped in producing these flavours by chemical synthesis [21]. This kind of synthesis was more effective for the production of flavour than extraction from natural sources. This also can be processed for commercialization. However, the chemical synthesis process sometimes produces undesired by-products making more complexity in separation, purification and waste treatment. Furthermore, the increased concern of people worldwide over their health created market demand for natural products. This scenario led to the requirement of more innovative techniques to obtain the flavour compounds naturally.

The potential method for synthesizing flavour products was then identified in microbes through microbial biosynthesis or bioconversion [21]. Microbes can synthesize these compounds as their secondary metabolites during fermentation in the nutrient medium. Flavours can also be produced with the catalytic activities of enzymes, such as lipases, proteases etc. These enzymes assist the conversion of the precursor molecule to the desired product. Based on these methods, flavour production can be categorized into three groups detailed below.

3.1 *De novo synthesis of flavour compounds*

Some microorganisms can consume varieties of substrates and convert them to amiable aroma compounds by their metabolic activities [22]. This was even used as a parameter for classifying microbial species in the 19th century [23]. Researchers were able to produce and isolate many volatile compounds from bacteria and fungi even before biotechnology advancements. Emerging new techniques have helped in identifying more odours and compounds to be isolated from microorganisms. De novo synthesis of aroma compounds utilizes the metabolic pathways of microorganisms like glycolysis, amino acid degradation pathway to produce flavour compounds from substrates.

De novo synthesis of flavours can be defined as the compounds being produced from substrates such as sugars, amino acids, or other simple biomolecules via metabolic pathways of microorganisms. For example, some esters with a fruity smell, such as ethyl acetate, can be produced by *Candida utilis* grown on nitrogen free medium with ethanol added in it [24].

Another example is 4-decalactone with a strong peach smell that is produced by *Sporobolomyces* odorus from modified Yeast Nitrogen Base with 0.6% ricinoleic acid and castor oil hydrolysate [25]. Few other examples of flavour compounds produced by microbes are given in Table 2. The concentrations of aroma compounds produced through de novo synthesis pathways are normally low, and mostly less than 100 mg/L. This is because metabolite is produced as one of the hundreds of metabolites within microbial cells [10]. The low quantity of metabolite production poses a major limitation for its industrial application.

Table 2: Flavour production by microbes via de novo synthesis

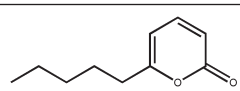
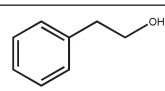
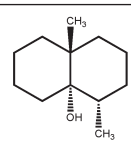
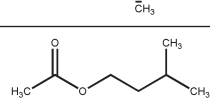
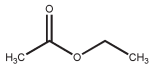
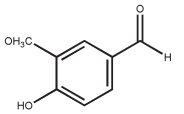
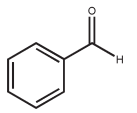
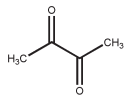
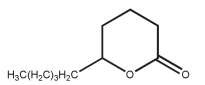
Microorganism	Chemical Name	Chemical Structure	Flavour	Reference
Trichoderma viridae	6-pentyl-2-pyrone		Coconut aroma	[2]
Yarrowia lipolytica	2-phenyl ethanol		Rose	[27]
Streptomyces griceus	Geosmin		Earthy smell	[2]
Kluyveromyces marxianus	Isoamyl acetate		Fruity	[28]

Table 2: *(Continued)* Flavour production by microbes via de novo synthesis

Microorganism	Chemical Name	Chemical Structure	Flavour	Reference
Kluyveromyces marxianus	Ethyl acetate		Fruity	[28]
Pycnoporous cinnabarinus	Vanillin		Vanilla	[29]
Ischnoderma benzoinum	Benzaldehyde		Cherry	[29]
Lactobacillus casei	Diacetyl		Buttery	[30]
Cladosporium suaveolens	δ-dodecalactone		Coconut	[31]

3.1.1 De novo synthesis of flavours by bacteria

Several research attempts have been carried out to produce flavour compounds by bacterial fermentation. Genetically engineered bacteria are also used in many studies for the productions of flavour compounds. A study by Escamilla *et al.*, [26] had found that strains of *Lactobacillus acidophilus* and *Pediococcus pentosaceus* can produce more diacetyl derivatives in starch-based media. The diacetyl is mainly responsible for butter flavour. Aromatic ethanol, 2-Phenylethanol, which imparts rose like odour was produced from recombinant *E.coli.* Since the compound was present in too low concentration in nature, also extraction from plants was ineffective. Hence researchers have genetically engineered *E.coli* by cloning it with kdc (encoding phenylpyruvate decarboxylase) and adh1 (encoding alcohol dehydrogenase) from *Pichia pastoris* and *Saccharomyces cerevisiae*, respectively to establish a synthetic pathway for 2-Phenylethanol production [32]. Similarly, limonene which expresses orange like the flavour was produced by *E.coli* after being genetically engineered with genes for precursor molecules of limonene and *E.coli* was constructed with monoterpene biosynthetic pathway [33]. A new strain of *E.coli* was also genetically engineered by deleting six genes that encoded aldo-keto reductases and alcohol dehydrogenases. This genetically engineered strain of *E.coli* was capable of producing vanillin from glucose [34].

3.1.2 De novo synthesis of flavours by fungi

In addition to bacteria, the fungus was also able to naturally produce many flavours. *Ceratocystis moniliformis* was identified as a potential candidate to produce fruit like aroma essence. By changing the carbon and nitrogen sources in the medium, *C. moniliformis* was able to produce different aroma compounds and the quantity of aroma varied depending on the compositions of the growth medium [35]. *C.tropicalis* and *Y. lipolytica* were demonstrated to degrade ricinoleic acid to smaller chain acid and stored δ-decalactone, which provide fruity and oily aroma notes for peach, strawberry and apricot smell [21]. A study was carried out by eleven yeast strains, which belonged to genus *Candida*, *Hanseniapora*, *Metschniowia*, *Pichia Schizosaccharomyces*, *Zygoaccharomyces* and *Saccharomyces*, to screen for acetate ester productions. The results revealed that Hanseniapora and Pichia showed different specificities for certain types of substrates but were able to produce different derivatives of ester including ethyl acetate, geranyl acetate, isoamyl acetate and 2-phenyl ethyl acetate. Other compounds, including 2-phenyl ethyl

acetate and isoamyl acetate, were produced strongly by *Hanseniapora guillierondii* and *Pichia anomala* [36]. Furthermore, vanillin, an important flavouring agent, can also be de novo synthesized by Baker's yeast from glucose after being genetically modified [37].

3.2 *Flavour production by biotransformation*

Flavour production level through de novo synthesis by microbes is still very less and hence it poses difficulty in its industrial application [10]. Another method that could be considered to produce flavour compounds on a commercial scale is biotransformation. This new method could produce more quantity of product. Unlike the de novo synthesis where flavour compounds are produced by using the complete metabolism of microorganisms, biotransformation uses only a single specific reaction to produce a flavour compound [38]. It can be defined as specific chemical reactions catalysed by functions of either enzymes or microorganisms [24]. Biotransformation can be used on a commercial scale as it has a high potential to produce food flavour compounds [21]. For example, benzaldehyde recognized as the almond flavouring compound can be produced by the cultivation of *Ischnoderma benzoinum* supplemented with L-phenylalanine. 158 mg/L of benzaldehyde and 25 mg/L of 4-methoxy-benzaldehyde were synthesized from *I. benzoinum* CBS 311.29 in 28 days. Moreover, 146 mg/L of benzaldehyde and 360 mg/L of phenyl ethanol were produced from *I. benzoinum* ATCC 26314 in 28 day- periods. However, *I. benzoinum* CBS 752.83 could not generate benzaldehyde more than 16 mg/L. *I. benzoinum* CBS 311.29 could therefore provide the highest amount of benzaldehyde among these three strains. It was found that glucose was preferred to be used as a carbon source and pH 6 was an optimum working condition for producing almond flavour. *I. benzoinum* ATCC 26314 also provided 450 mg/L of phenyl ethanol (an intermediate of conversion of L-phenylalanine to benzaldehyde) giving a rose-like aroma [39].

Vanillin, another important flavour compound could be produced by biotransformation of eugenol by *Bacillus safensis*. Vanillin yield could reach 0.055 g/L within 96 hours of biotransformation by growing 4% *B. safensis* in 10% culture media with 500 mg/L eugenol at 37°C and pH 7, shaking rate at 180 rpm [40]. Terpene is also another group of aroma compounds that have been widely studied to be produced by biotransformation of substrates by microbial activities. For instance, limonene is a monoterpene, extracted mainly from citrus fruit peel. In the year 2011, Preito *et al.*, studied biotransformation of limonene by *Penicillium digitatum* DSM 62840 to produce (R)-(+)-a-terpineol. (R)-(+)-a-terpineol is used in perfumes, cosmetics, and fragrance industry to produce lavender aroma. The team could produce 1864 mg/L of (R)-(+)-a-terpineol from 14.7 mM limonene in malt yeast broth with pH 3.5. The broth was inoculated with induced spores of *P.digitatum* DSM62840 which are in the early stage of exponential growth [41]. Apart from this, there are several other flavours produced by microbial biotransformation, which are listed in Table 3.

Table 3: Flavour production by microbial biotransformation

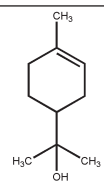
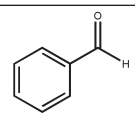
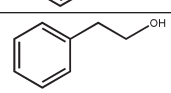
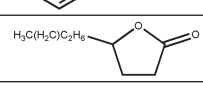
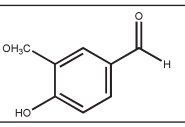
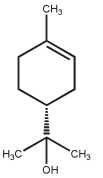
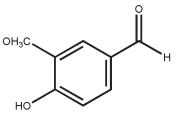
Substrate	Microbe	Product	Chemical Structure	Flavour	Reference
Limonene	*Sphingobium* sp.	α-terpineol		Lilac odour	[42]
L-phenylalanine	*Ischnoderma benzoinum* CBS 311.29	Benzaldehyde		Bitter almond aroma	[39]
Cinnamyl alcohol	*Colletotrichum acutatum*	2-phenyl ethanol		Roselike odour	[43]
Oleic acid	*Micrococcus luteus*	γ-dodecalactone		Peach and strawberry flavour	[44]

Table 3: *(Continued)* Flavour production by microbial biotransformation

Substrate	Microbe	Product	Chemical Structure	Flavour	Reference
Ferulic acid	*Pycnoporous cinnabarinus*	Vanillin		Vanilla flavour	[45]
R-(+)-limonene	*Fusarium oxysporum*	R-(+)-α-terpineol		Lilac odour	[46]
Isoeugenol	*Bacillus pumilus*	Vanillin		Vanilla flavour	[47]

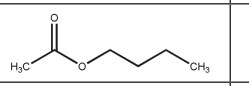
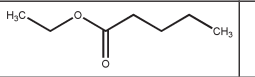
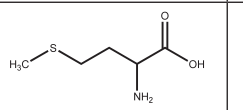
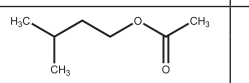
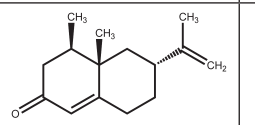
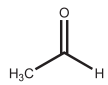
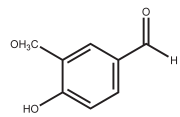
3.3 *Flavour production by enzymes*

In addition to the production of aroma compounds by using the de novo synthesis pathway and biotransformation pathway assisted by microorganisms, purified enzymes can also play important roles in flavour production. Enzymes accelerate the conversion reactions of substrates to targeted products, especially flavour compounds. Lipases, proteases, glucosidases, esterase are examples of the enzymes involved in flavour production [21].

Lipases are commonly used in the production of esters, and it is currently used in biodiesel productions in much research [48]. Lipase catalyses the esterification reaction between carboxylic acid and alcohol with the rate of reaction depending upon the alcohol or acid chain length [21]. pH, temperature, lipase concentration and emulsion content are important parameters that are needed to be controlled for increasing the production of flavour and fragrance [49]. Lipase produced from *Staphylococcus simulans* was used by Chaabouni *et al.*, [50] to produce green apple flavour and pear flavour. The flavour for green apple and pear was produced by ethyl valerate and hexyl acetate, respectively. To produce these flavours, crude *S.simulans* lipase was immobilized on a calcium carbonate medium. Using 200 IU of immobilized enzyme and 20%(w/w) water in the reaction mixture (containing valeric acid to ethanol molar ratio as 1) at 37°C, 51% conversion of ethyl valerate was obtained. On the other hand, 41% conversion of hexyl acetate was possibly obtained with 100 IU immobilized lipase, 10% (w/w) water and acetic acid: hexanol molar ratio of 1 at 37°C [33].

Another study focused on the synthesis of flavour in a solvent free condition [51]. Methyl butyrate and octyl acetate were generated under solvent-free conditions through immobilized *Rhizopus oryzae* NRRL 3562 lipase interposed transesterification. The effect of transesterification factors, such as alcohol molarity, reaction time, temperature, agitation, water addition, and the amount of enzyme on molar conversion (%), was analysed. An agitation speed of 200 rpm and water addition (0.2%) for the reaction time of 12 and 14 h was used in the study. The study also included transesterification factors as 2 M octanol in vinyl acetate and 0.6 M methanol in vinyl butyrate using 60 U and 80 U immobilized lipases at 36°C and 32°C for octyl acetate and methyl butyrate. In these conditions, the study could provide maximal molar conversion of 92.35 and 70.42%, respectively. Relative activity (more than 95%) using an immobilized enzyme greatly maintained up to five and six recycles for methyl butyrate and octyl acetate, respectively [51]. Proteases enzyme is a group of enzymes used in the hydrolysis of protein to shorter chains of amino acids, which can be used to enhance food flavour. These protein hydrolysates can cause bitterness in the food [52]. Protease has been used widely in cheese production [53]. Protease enzyme of bacterial origin has also been used in cheese ripening and flavour improvement [54]. Glucosidases are used in the wine industry for flavour enhancement of wine by releasing volatile terpenes or flavour precursors from their glycosidic bonds [21]. A study conducted on Muscat wine used different types

Table 4: Flavour production by enzymatic catalytic conversion

Enzyme	Source	Flavour Compound	Flavour Produced	Chemical Structure	Reference
Lipase	*Rhizopus oryzae*	Butyl acetate ester	Pineapple		[56]
Lipase	*Burkholderia cepacia*	Ethyl valerate	Green apple		[57]
Aminotransferase	*Lactobacillus casei* IFPL731	Methionine	Cheese		[58]
Lipase	*Rhizomucor meihei* / *Candida antartica*	Isoamyl acetate	Banana		[59]
Oxygenase	*Pleurotus sapidus*	Nootkatone	Grapefruit flavour		[60]
Threonine aldolase	*Lactobacillus bulgaricus* / *Lactobacillus acidophilus*	Acetaldehyde	Yoghurt flavour		[61]
Eugenol oxidase	*Rhodococcus jostii*	Vanillin	Vanilla flavour		[62]

of enzymes including β-glucosidase, α-arabinosidase and α-rhamnosidase immobilized on acrylic beads to release conjugated monoterpenes and nor isoprenoids, which are the aromatic precursors. These enzymes obtained from *Aspergillus niger* could increase the unbound monoterpenes from 1119 µg/L to 2132 µg/L [55]. Likewise, there are many enzymes obtained from microorganisms and being used in flavour production, some examples are given in Table 4.

3.4 *Solid state fermentation*

Many industries use solid state fermentation to produce microbial metabolites instead of using a liquid medium. Generally, oilseed cakes, brans of grains etc. are used as solid support. This method reduces the amount of waste and liquid effluent generated during the normal flavour production process [63]. Initially, the fungus was used for fermentation, later yeasts and bacteria have also been used in the fermentation process. It has been used in bread making, cheese ripening, pickling and in much other food processing, also it has been used for food flavour production [63].

The fruity flavour was generated by the solid-state fermentation of glucose supplemented coffee husk by using *Ceratocystis fimbriata*. An aroma chemical with pineapple flavour was generated at 6.58 and 5.24 mmol/L per gram total volatiles (TV) by using solid media supplemented with 20 and 35% of glucose, respectively. In the headspace of microbial culture, ethanol, isopropanol, acetaldehyde, ethyl isobutyrate, isobutyl acetate, isoamyl acetate, ethyl-3-hexanoate and ethyl acetate were detected. Furthermore, the production of TV, especially for isoamyl acetate and ethyl acetate, not only could be enhanced but also strong banana flavour was measured by addition of leucine. Adding soybean oil could not enhance the number of volatile compounds, instead, mineral salts could inhibit this biosynthesis [64].

Volatile compounds could be generated by the fermentation of manipueira (cassava wastewater) using *Geotrichum fragrans*. This aerobic fungus obtained from cassava wastewater was resistant to cyanide during respiration. The fermentation of manipueira by *G. fragrans* could generate fruity volatile compounds. The volatile compounds that were produced included

1-butanol, 3-methyl 1-butanol (isoamylic alcohol), 2-methyl 1-butanol, 1-3 butanediol and phenyl ethanol; ethyl acetate, ethyl propionate, 2-methyl ethyl propionate and 2-methyl propanoic and were detected after 72 h of cassava wastewater fermentation [65]. *Kluyveromyces marxianus* was solid state-fermented on cassava bagasse to produce aroma compounds. Packed bed reactors and two different aeration rates tests were also included. The culture growth was measured by respirometric analysis. Gas chromatography was applied in the analysis of cultured headspace and found 9 out of 11 produced compounds. The major compounds were produced as ethyl acetate, ethanol, and acetaldehyde. The production of total volatile (TV) was increased by a lower aeration rate (0.06 L/h/g of initial dry matter). At this aeration rate, the production rate was also enhanced, and the maximal concentrations of TV (0.12 L/ h/g) was achieved at 24 h and 40 h [66].

Soya bean meal and rice husks were fermented by *Rhizopus oligosporus* USM R1 to generate benzaldehyde known as a bitter cherry almond flavour. Light microscope and scanning electron microscope (SEM) was used to identify *R. oligosporus* USM R1. The optimal conditions for Solid State Fermentation (SSF) were 40% (v/w) (water content), 1×10^5 spores/g (substrate inoculum size), 0.7 mm (particle size of substrate), 30°C (incubation temperature), 50:50 (ratio of soybean meal) and 7 g (substrate weight). After 48 h of agitation and 96 h of fermentation, the maximum production of benzaldehyde was obtained. Moreover, after 96 h of fermentation, the highest amount of benzaldehyde was produced as 5.47 mg g^{-1} substrate. Carbon and nitrogen source in the medium could also enhance the growth and the production of benzaldehyde. The highest amount of benzaldehyde was received with L-phenylalanine supplement (precursor of benzaldehyde synthesis) providing 38.69 mg benzaldehyde/g substrate. This amount is 6-times higher than substrates without the addition of L-phenylalanine [67].

The butter-like flavour was generated by 120 h-mixed cultures cultivation of *Pediococcus pentosaceus* MITJ-10 and *Lactobacillus acidophilus* Hansen 1748 grown in semisolid maize-based media and applied with orthogonal factorial design. Hot lime-treatment with partial defatting and cooking was conducted to pretreat maize substrate. The culture media was supplemented with yeast extract and used for culturing the mixed cultures of *P. pentosaceus* and *L. acidophilus*. It was observed that when the culture grew with the specific growth rate at 0.47 h^{-1}, the native microorganisms were dominated by Lactic acid bacteria (LAB). Diacetyl compound was found to be the major aroma compounds produced from LAB at the maximum concentration of 4,800 mg/kg during the long stationary phase. This process could be beneficial to produce natural aromatic compounds for food applications [68].

Flavour compounds were produced by solid state fermentation of orange peel (OP) using a specific industrial yeast strain. Sterile OP was autoclaved to remove D-limonene and natural microflora then yeast viability, nutrient consumption and capability of flavour compound production were investigated. The advantages and disadvantages of the sterilized process were evaluated on non-sterile OP. The growth performance of yeast cells under sterilized process conditions was better than that one under non-sterilized process conditions. The results after 72 h of improved de novo synthesis for fruity esters in the first case were analysed as isoamyl acetate (48.7 mg/kg of fermented OP), ethyl dodecanoate (25.2 mg/kg of fermented OP), decanoate, (9.3 mg/kg of fermented OP), octanoate (6.3 mg/kg of fermented OP) and phenyl ethyl acetate (4.5 mg/kg of fermented OP), respectively. The synthesis of ethyl hexanoate at 48 h was accelerated as 154.2 mg/kg OP by yeast cells. In this experiment, natural aroma compounds bioconversions were also evaluated. These processes for high yields of industrial volatile aroma esters production (total of ~250 mg/kg OP) were applied in a sustainable biorefinery to valorise OP waste [69].

Another study has used, *Cerevisiae* (Pasteur Red-UCD#904) in winemaking for fermentation and to understand the role of a yeast strain in the extraction of phenolics and how they affect the sensory characteristics of wine [70]. Mangosteen pericarp was fermented by using two yeast strains namely, Montrachet UCD#522 and Pasteur Red-UCD#904. The study results showed that with higher pericarp content of mangosteen, yeast could influence the production of the aroma of the wine. Pasteur Red was responsible for fruitier and more floral aroma in the wine [70]. Coconut flavours could be originated from the solid-state fermentation of sugarcane bagasse using *Trichoderma viride* EMCC-107. The highest amount of total carbohydrates was found as 43.9%

(w/w) compared to other constituents in sugarcane bagasse. The highest intensity of odour and maximal amount of volatile compounds was achieved on the 5th day of fermentation. During 12 d of induction period, the unsaturated lactone; 6-pentyl-α-pyrone (6-PP) and saturated lactones; δ-octalactone, γ-nonalactone, γ-undecalactone, γ-dodecalactone and δ-dodecalactone were determined in coconut aroma compounds. Increasing the concentration of sugarcane bagasse could significantly enhance biomass ($p < 0.05$) and 4.5 g of sugarcane bagasse could provide high production of 6-PP. Under the same conditions of fermentation, the concentration of 6-PP (3.62 mg/g DM) was higher than in prior studies. An increment of biomass and concentration of sugarcane bagasse might also increase the concentration of sugar [71].

4 Production of Aroma Compound: A Case Study

Vanillin is a costly flavour compound widely used in the food and perfume industry. Natural vanillin market size was valued at 11.5 million USD in 2015 and this industry is expecting that the consumption of it would surpass 500 tons by 2023 [72]. Global synthetic vanillin market size is expected to grow more in the coming years. Consumers being more conscious about their health has increased the demand for natural vanillin. Nevertheless, extraction of vanillin from orchid pods is highly laborious and recovery yield is very less [72]. This scenario has generated an immense interest in many industries to research upon producing vanillin as a natural flavour using new technologies at low cost. Various studies are focused on producing vanillin flavour using biotechnological methods.

Vanillin can be synthesized by different routes, including de novo synthesis, biotransformation, and enzymatic catalysis. In 2005, a group of researchers, isolated a novel strain of *Bacillus fusiformis* from garden soil and studied its ability to convert isoeugenol to vanillin. The study showed that the isolated strain of *B.fusiformis* produced vanillin when 60% isoeugenol (v/v) was used as substrate. The biotransformation could produce 32.5 g/L of vanillin over 72 h [73]. A study by Yan *et al.*, 2016 [74] produced vanillin from ferulic acid by biotransformation using *Bacillus subtilis* in stirring packed bed reactors filled with carbon fibre textiles. The team could get a maximum vanillin yield of 0.047 g/L/h and a molar yield of 60. 43% at a ferulic acid concentration of 1.5 g/L in 35°C, for 20 h at a pH of 9.0 and 200 rpm stirring speed.

Vanillin, primarily acquired from *Vanilla planifolia*, could also be synthesized from the fermentation of ferulic acid esters in wheat bran or the other agricultural waste using *Streptomyces sannanensis* MTCC 6637. A study demonstrated a system to produce vanillin through biotransformation using *S.sannanensis* from agro residues. Thin layer chromatography and high pressure liquid chromatography was applied in the analysis of major enzymes related to vanillin production. Response Surface Methodology (RSM) was also used for the optimization of vanillin production conditions. 10% w/v of de-starched wheat bran, 0.2% w/v of sucrose, 1% w/v of peptone (pH 7.5), 220 rpm of agitation, 28°C for temperature and 5 days continuous fermentation were the optimal conditions for producing vanillin as 708 mg/L. Ferulic acid ester was converted into ferulic acid with the support of ferulic acid esterase using this *S. sannanensis* MTCC 6637. Coenzyme-A dependent non–β-oxidation pathway (retro-aldol reaction) of ferulic acid was applied in the catabolic process. Ferulic acid was converted into vanillin using Feruloyal Coenzyme-A synthetase and Enoyl-Coenzyme-A hydratase/aldolase. [75].

Furthermore, another biotransformation experiment also produced vanillin from ferulic acid. The study showed that ferulic acid was obtained from the enzymatic hydrolysis of wheat bran using a mixture of Fungamyl Super AX and Celluclast BG enzymes. Engineered *E. coli* strain JM109(pBB1) carrying catabolic cassette required for converting ferulic acid into vanillin was applied in the hydrolysed mixture. This enabled the conversion of ferulic acid into vanillin. This study could achieve a 50% conversion yield of vanillin but most of the vanillin was degraded to vanillyl alcohol. Hydrolysate purification with an ionic exchange resin to remove carbohydrates was shown to enhance bioconversion yields up to 70% and decreased vanillin reduction [76]. Vanillin was also generated by the coalition between *Pycnoporus cinnabarinus* CGMCC1115 and *Aspergillus niger* CGMCC0774 on the novel bioconversion of ferulic acid obtained from the rice bran oil waste. The comparison of different ferulic acid concentrations in cultures showed that *A. niger* CGMCC0774 in a 25 L-fermenter using 4 g/L of ferulic acid could provide the highest yield of vanillic acid as 2.2 g/L. The culture of *A. niger* CGMCC0774

was filtered and concentrated after and *P. cinnabarinus* CGMCC1115 turned vanillic acid into vanillin. [77]. Therefore, these works are a demonstration of the possibility to produce vanillin from agricultural by-products via biotransformation.

White-rot fungus *Pycnoporus cinnabarinus* I- 937 was also used for biotransformation of ferulic acid to produce vanillin. The maximal concentration of vanillin was provided as 64 mg/L (27.5% molar yield) during the production stage of secondary metabolism (after 6 days of growth). Furthermore, the propanoic acid (side chain of ferulic acid) was cleaved to vanillic acid and subsequently decarboxylated to 2-methoxyhydroquinone. Additionally, two reductive pathways were also deduced concerning the biotransformation of ferulic acid and vanillic acid into coniferyl and vanillyl alcohols, respectively. Unfortunately, *P. cinnabarinus* could release laccase, therefore it was difficult to control the biotransformation process. In the presence of this enzyme, the ferulic acid was polymerized to lignin-like polymers, instead of producing vanillin [78]. Similarly, several studies have been carried out to produce vanillin from ferulic acid, eugenol, isoeugenol, vanillic acid and lignin. Most of the studies could show that ferulic acid as a more potential candidate to produce vanillin [79]. These new processes are considered cost effective with reduced unwanted by products. Hence this could become a trend in research interest. In addition to this, these techniques also allow labelling the product as "natural" which can attract more consumers.

5 Conclusions

Most of the flavours used in the food and perfume industry were synthesized from a chemical process. Advancements in technology have led to a change in the production process of flavours due to the increased demand for natural products. Scientists are even able to alter the genes of microbes and manipulate different metabolic pathways to produce the compound of interest in large quantities. These new techniques can also provide industries with the benefit of labelling their product as natural. This can increase the market for flavour compounds. Moreover, these techniques which use microbes is more environmentally friendly and can reduce unwanted products also. More research is needed in the field to identify new precursors and pathways to produce different flavour compounds.

Acknowledgement

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