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BIOTECHNOLOGICAL PRODUCTION OF AROMAS BY DE NOVO SYNTHESIS AND USE OF AGROINDUSTRIAL WASTE

PRODUÇÃO BIOTECNOLÓGICA DE AROMAS POR SÍNTESE DE NOVO E USO DE RESÍDUOS AGROINDUSTRIAIS

PRODUCCIÓN BIOTECNOLÓGICA DE AROMAS POR SÍNTESIS DE NOVO Y APROVECHAMIENTO DE RESIDUOS AGROINDUSTRIALES

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ABSTRACT
The biotechnological production of aroma compounds is an emerging field because, unlike traditional chemical synthesis, compounds produced by microorganisms are classified as natural, meeting consumer trends for healthy foods. As an agricultural country, Brazil is a great source of various residues that can have added value if used as raw materials for other processes. The use of agro-industrial residues makes the fermentation process for obtaining bioaromas more attractive by utilizing renewable sources, combined with a reduced environmental impact and consumer demand. Obtaining aromas through de novo synthesis (fermentation) leads to the formation of aroma compounds classified as natural by Brazilian, European, and American legislation. This chapter reviews the main aroma groups obtained through de novo synthesis and the use of residues as an alternative to making the biotechnological process feasible.

Keywords: Bioaromas. Waste. De Novo.

RESUMO
A produção biotecnológica de compostos de aroma é um campo emergente, pois diferentemente da tradicional síntese química, os compostos produzidos por micro-organismos são classificados como naturais, vindo de encontro à tendência dos consumidores por alimentos saudáveis. O Brasil por ser um país agrícola é uma ótima fonte de diferentes resíduos que podem ter valor agregado se utilizados como matéria-prima para outros processos. O uso de resíduos agroindustriais torna o processo fermentativo para obtenção de bioaromas mais atrativo utilizando fontes renováveis aliado a diminuição do impacto ambiental e a demanda do consumidor. A obtenção de aromas por síntese de novo (processo fermentativo) leva a formação de compostos de aromas classificados como naturais pela legislação brasileira, européia e americana. Esse capítulo revisa os principais

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grupos de aroma obtidos por síntese De novo e o uso de resíduos como uma alternativa para tornar o processo biotecnológico factível.

Palavras-chave: Bioaromas. Resíduos. De Novo.

RESUMEN
La producción biotecnológica de compuestos aromáticos es un campo emergente porque, a diferencia de la síntesis química tradicional, los compuestos producidos por microorganismos se clasifican como naturales, satisfaciendo así las tendencias de consumo de alimentos saludables. Como país agrícola, Brasil es una importante fuente de diversos residuos que pueden tener valor añadido si se utilizan como materia prima para otros procesos. El uso de residuos agroindustriales hace que el proceso de fermentación para la obtención de bioaromas sea más atractivo al utilizar fuentes renovables, combinado con un menor impacto ambiental y la menor demanda del consumidor. La obtención de aromas mediante síntesis de novo (fermentación) conduce a la formación de compuestos aromáticos clasificados como naturales por la legislación brasileña, europea y estadounidense. Este capítulo revisa los principales grupos de aromas obtenidos mediante síntesis de novo y el uso de residuos como alternativa para viabilizar el proceso biotecnológico.

Palabras clave: Bioaromas. Residuos. De Novo.

1 INTRODUCTION

The production of flavors has grown a lot in recent years due to scientific advances in several sectors related to the area, such as new extraction techniques and different (bio)production processes. One of the advances and focus of research is the use of microorganisms as an alternative to chemical synthesis and the extraction of plant materials, which are today the two most used classical methods to obtain these compounds (Berger, 2015; Bicas *et al.*, 2010; Ravi et al., 2025). Biotechnologically generated flavors are called natural, and are therefore more valued in the market than artificial chemical additives (Maróstica, 2007; Bicas *et al.*, 2010; Carvalho, 2011). In addition to the "natural" connotation, products obtained by microbial fermentation can be produced in the short term, at any time of the year, using low-cost substrates, with the advantage that Brazil is rich in by-products of this nature (Valduga, 2005). In this context, the article explores the use of agro-industrial waste as a necessary tool to ensure greater prominence in the production of natural flavors by biotechnological means, pointing out the main products obtained by *de novo synthesis*.

1.1 FLAVORS AND AGRO-INDUSTRIAL RESIDUES

Taste is a key aspect of perception, directly influencing consumer acceptance and preference. It arises from the combination of olfactory and gustatory sensations, with taste being determined by non-volatile molecules that interact with the receptors of the tongue, while aroma is perceived through volatile organic compounds detected in the nasal cavity. Among these, terpenes, esters, and aldehydes play a key role in defining the aroma of food, which significantly impacts the sensory appeal of food products and drives innovation in the food industry (Ravi, et al; 2025).

Flavors and aromas are widely applied in food, cosmetics, feed, chemicals, pharmaceutical, cosmetics, and personal care industries in order to complement, improve, or modify the original flavor/aroma of the product and, thus, play a major role in consumer acceptance of products (Akacha & Gargouri, 2015; Bicas et al., 2010; Burdock & Fenaroli, 2010; Palmerín-Carreño et al., 2015, Roy et al, 2020). The purpose of including flavors in the formulations of various products is to improve the sensory properties, ensuring acceptance and positive consumer evaluations regarding the performance of these products (Gupta et al., 2015; Medeiros et al, 2022).

It is estimated that flavors represent more than a quarter of the world market for food additives, with a large part coming from extraction from natural sources such as vegetables or through traditional methods such as chemical synthesis (Akacha & Gargouri, 2015). However, chemical synthesis can cause high environmental impact due to the emission of non-biodegradable residues in its process, while aromas extracted from plant sources are subject to seasonal variation, pest attacks and geographic effects (Carvalho, 2011). Thus, one of the alternatives is to invest in the use of microorganisms for the production of flavors, using biotechnological processes, including the fermentation and enzymatic routes, which are environmentally friendly, economically viable, low polluting, and attractive to a range of consumers looking for natural products (Ganesh, et al, 2022; Bergamo, 2010; Xiao and Xu, 2007).

Fermentation is a promising biotechnological technique for the production of natural flavors, and bacteria, filamentous fungi and yeasts can be used to carry out the bioprocesses, considering that the production of flavors by these microorganisms is advantageous both from a quantitative and qualitative point of view, also promoting sustainability (Soccol et al., 2008). The addition of precursors to substrates can increase the productivity of the fermentation process, increasing the production of compounds of interest (Szabo, et.al., 2021a)

The term bioaroma is used to designate aromas of enzymatic origin or produced via fermentation through biotechnological processes (Correia, 2015). Among the main advantages of using these types of processes, the following stand out: (i) high enantioselectivity, which allows obtaining a high purity aroma, (ii) continuous production throughout the year without interference from seasonality, (iii) the adoption of less rigorous process parameters, thus reducing energy costs and the use of reagents harmful to the environment and (iv) controllable and optimizable process conditions (Berger, 2015).

Aroma compounds result mainly from the secondary metabolism of microorganisms. Secondary metabolites are substances excreted near the end of the exponential growth phase or during the stationary phase and are not essential for growth and reproduction, whose synthesis depends on the cultivation conditions, especially in relation to the composition of the medium and are often overproduced (Brigido, 2000, Madigan, et al., 2010, Damasceno et al., 2003). Secondary metabolites contribute to the survival of the microorganism, and can competitively inhibit species that could occupy the same niche (Maróstica, 2006). One of the classic examples is the biotechnological production of esters, which would be related to the mechanism of removing acids and alcohols from the cell and the environment, which are toxic to the cell (Scharpf *et al.*, 1986). Many volatile substances are in this category, such as esters, alcohols, aldehydes, ketones, terpenes, and lactones (Berger, 2015; Carvalho, 2011).

The food industry inevitably produces large volumes of agro-industrial waste such as pomace, peels, and skins that still contain flavor precursors. Thus, the use of these residues as a fermentation substrate for the production of high-value aroma becomes relevant for the economic market (Berger, 2015).

The use of agro-industrial waste is part of the ecological movement coming against the growing consumer demand for green and ecological processes instead of chemical and industrial processes. In addition, the use of agro-industrial waste can be a cheap and natural substitute for the production of various high-value products (Sharma et al., 2020) as the waste is composed of carbohydrates, proteins, lipids, and various minerals. Their valorization as substrates for the production of value-added compounds is an approach to cost-effective production in the fermentation industry (XU, et al. 2023).

Brazil is a country whose economy is strongly based on agriculture, consequently, there is generation of large amounts of waste. For this reason, the interest in a more efficient use of agro-industrial waste such as cassava bagasse, sugarcane bagasse, coffee peels, cassava, malt bagasse, whey, banana peels, molasses, among others, has been in growing demand year after year. The application of agro-industrial residues in bioprocesses has been considered an excellent alternative for new substrates, in addition to helping to reduce environmental impact by being an environmentally friendly tool (Carvalho, 2011; Crescitelli, et al., 2020; Hernadez-Cruz, et al, 2024; Mirabella, et al., 2014; Ravi, et al. 2025; Sharma et al., 2020; Soccol and Vandenberghe, 2003, Szabo, et.al 2021; Matos et al; 2017; Xu et al, 2023).

The production of bioflavors has aroused a great deal of research interest. To meet the need of consumer preferences for natural compounds, biotechnological production has become a very attractive alternative to chemical production. Biocatalysts of natural origin, mainly microbial, have great potential to produce a wide variety of flavors, due to their great metabolic diversity to modify and add value to a variety of organic molecules (Akacha & Gargouri, 2015; Bicas et al., 2009; Kruis et al. 2019).

These bioprocesses based on microorganisms (bacteria, fungi) and their enzymes are part of white biotechnology. The principle of this technology consists of the use of renewable resources, clean production, less pollution, and more energy-efficient processes in biological systems, such as whole cells or enzymes, used as reagents or catalysts (Akacha & Gargouri, 2015).

The use of biotechnology for the production of bioproducts is encouraged by consumer awareness of labels with "clean" ingredients, as well as sustainability-oriented interests (Copetti, 2019).

According to ANVISA's Resolution No. 2, of 01/15/2007, flavorings are considered substances or mixtures of substances with odorous and/or savory properties, capable of conferring or intensifying the aroma and/or flavor of food, and can be classified as natural or synthetic, the former being obtained exclusively through physical, microbiological or enzymatic methods.

Based on their origin, aromas are classified as natural and synthetic, but there are also other special types, such as reaction or transformation and smoke. BRASIL (2007) defines that **natural flavorings** are those obtained exclusively through physical, microbiological or enzymatic methods, from natural raw materials. In this way, they are products of animal or vegetable origin, normally used in human food, that contain odorous and/or savory substances, either in their natural state or after appropriate treatment, such as roasting, cooking and fermentation, among others.

Natural flavors are subdivided into: essential oils; extracts; balsams; oleoresins; oleogomarresins and isolates. In turn, **synthetic flavorings** are chemically defined compounds obtained by chemical processes, which correspond to identical flavors to natural ones and artificial flavors. Reaction **or** transformation **flavorings** are products obtained according to good manufacturing practices, by heating at a temperature not exceeding 180ºC, for a period of less than fifteen minutes, where the pH may not exceed 8. They can be classified as natural or synthetic, depending on the type of raw material used and its production process. Generally, they are obtained from carbohydrate sources, protein nitrogen sources, lipid or fatty acid sources, among others. Finally, **smoke flavorings**, which are concentrated preparations used to give smoked aroma/flavor to food. They are obtained from the treatment of wood with one of the following procedures: controlled combustion, dry distillation and steam dragging. After that, their fractions are separated and aromatic components isolated (ANVISA, 2007).

The definition of natural flavoring is in line with the market trend in which consumers prefer products that contain natural raw materials in their formulation, to the detriment of chemical additives, differentiating these in the market (Berger, 2015; Carocho et al., 2015;

Carvalho, 2011).

Aroma compounds can be produced using three methods: (i) chemical synthesis, (ii) extraction from nature, and (iii) biotechnology. In the case of chemical synthesis, this method is marked by high yield and low cost. However, it generates low-quality products. This is because, considering its low region and enantioselectivity, a mixture of products is obtained at the end of the process. In addition, the scents obtained using this method cannot be labeled as natural, which represents a powerful marketing disadvantage. Another questionable point about this method refers to the process parameters, which generally require a high energy cost (high pressures and temperatures), in addition to generating environmental liabilities due to the use of large volumes of organic solvents (Akacha & Gargouri, 2014; Bicas *et al.*, 2010).

On the other hand, aroma compounds obtained by the method of direct extraction from nature or by biotechnology can be labeled as "natural". Thus, products obtained using such processes have an indisputable marketing appeal. However, the direct method of extracting from nature is fraught with challenges, among which we can highlight: (i) seasonality, (ii) ecological, social and political issues, and (iii) low yield, which results in a high price for products. Thus, the biotechnological production of compounds stands out as a very promising option to overcome the problems associated with these other production methods (Pinheiro & Pastore, 2004, Carvalho, 2011).

The biotechnological production of aroma compounds stands out as a promising option to overcome traditional methods of obtaining aroma. Among the main advantages, the following stand out: (i) high enantioselectivity, which allows obtaining a high purity aroma, (ii) continuous production throughout the year without interference from seasonality, (iii) the adoption of less rigorous process parameters, thus reducing energy costs and the use of reagents harmful to the environment and (iv) controllable and optimizable process conditions (Berger, 2015).

In this context, the production of flavors based on microbial biosynthesis or bioconversion has been studied as a differentiated way to obtain these compounds with high added value (Longo & Sanromán, 2006; Ravi, et al., 2025). Aroma compounds and fragrances obtained by biotechnological processes have great importance due to the increased consumer preference for natural additives (Akacha & Gargouri, 2015), and due to this preference, natural flavors produced by fermentation or enzymatic processes are growing worldwide.

1.2 BIOTECHNOLOGICAL PRODUCTION OF FLAVOURS: BIOFLAVOURS

The use of microorganisms to produce flavors in food has been around for hundreds of years. Initially, the fermentation process had as its main purpose the increase of shelf life, by releasing acids and other compounds, which ensured a greater durability of the product. However, in fermented foods and beverages, the action of microorganisms not only increased shelf life, but also played a fundamental role in the production of characteristic aromas of the product (Gatfield, 1995).

Based on the history and advanced experience of bioengineering and genetic engineering methods of industrial-scale fermentations of non-volatile flavors, such as organic acids and amino acids, flavor generation has received increased attention due to several factors. Among them, the following stand out: (i) the growing industrial demand for natural flavor compounds, (ii) the decreasing availability of natural flavors obtained from plant sources, due to agricultural or ecological problems in the producing countries; (iii) lack of convincing chemosynthetic alternatives and (4) consumer rejection of products containing unnatural additives (Additives & Ingredients, 2009).

Several compounds belonging to the classes of aldehydes, alcohols, esters and lactones have been identified (Lanza *et al*, 1976; Soares *et al.*, 2000; Bluemke & Schraderb, 2001; Bramorski *et al*, 1998; Christen *et al*, 1997). In 1976, Lanza and collaborators verified the production of aroma compounds with fruity characteristics by *Ceratocystis fimbriata*, where to this day, several works can be found related to this microorganism for the production of natural flavors.

In principle, there are two methods of producing aroma compounds biotechnologically: *de novo* synthesis and biotransformation/bioconversion. The first mode involves a comprehensive metabolic process, where the catabolism of proteins, lipids, and carbohydrates contributes to the formation of primary metabolites. These metabolites are then transformed into a mixture of compounds, which are critical for the production of flavorings (Melini, et al, 2024). When agro-industrial residues are used as substrates, sugar supplementation is often necessary to stimulate microbial growth in the early stages. An alternative approach to overcome nutritional limitations is to combine diverse residue substrates, creating a self-sustaining fermentation medium that does not require additional nutrients (Szabo, et al 2021b). A crucial strategy in de novo synthesis is the addition of metabolic precursors, which can induce the synthesis of specific aromas. For example, the incorporation of sugarcane juice and soybean oil in the fermentation of agri-food waste improves the production of ethyl hexanoate, a compound with a characteristic fruity aroma (Szabo, et al., 2021a).

The second refers to the synthesis of one or several aroma compounds by the addition of their precursors to the culture medium. While de novo synthesis uses the entire metabolic arsenal of the microorganism and generally produces a mixture of several aroma compounds that are important for the formation of the product's aroma or bouquet, biotransformation/bioconversion aims to obtain a main product. While biotransformation is able to catalyze the transformation of the substrate in a single step, bioconversion develops in two or more biochemical reactions, that is, bioconversion is a biotransformation that occurs in several steps. Microbial enzymes, however, both constructive and inductive, may be responsible for the formation of aroma compounds in a single reactive step (Maróstica, 2006; Berger 2015). Another way of obtaining aroma compounds is the use of plant cells (Petersen, 2006), which is less explored than microorganisms, but whose main advantage is a more complex enzymatic system, which can lead to specific products.

Overall, *de novo* synthesis and biotransformation offer interesting tools for the sustainable production of aroma compounds, using agro-industrial residues to generate high value-added molecules. The integration of advanced biotechnological strategies and process optimization will be essential to improve the efficiency, yield, and commercial viability of these sustainable bioflavor production methods (Ravi, et.al 2025).

The chemical compounds responsible for characteristic aromas are composed primarily of alcohols, acids, esters, ketones, aldehydes (Gatfield, 1995), and other complex molecules that result from the secondary metabolism of plants (Hamilton-Kemp *et al.,* 1996) or can be obtained from animal sources. Some fungi, such as *Aspergillus* sp.; *Fusarium* sp. and *Penicillium* sp., yeasts and bacteria, such as *Rodococcus* sp. and *Pseudomonas* sp, can also produce aroma compounds and fragrances due to secondary metabolism (Armstrong & Brown, 1994).

2 COMPOUNDS OBTAINED BY BIOTECHNOLOGICAL MEANS

2.1 ESTERS

Esters comprise one of the most important aroma groups and stand out for having a fruity aroma such as pineapple, strawberry, pear, peach, banana among others, floral, wine and cognac. They are found naturally in pineapple, strawberry, apple, kiwi, orange, mango, papaya, figs, grapes, cocoa, cheeses, alcoholic beverages, butter, vinegar, milk, beans, soybeans, corn, wheat, mushroom, soy sauce, cured meat, olives (Burdock & Fenaroli 2010). These compounds can be used in various products such as sweets, jellies, jams, bakery products, sake, wine, jellies, candies, confectionery, chewing gum, puddings, non-alcoholic beverages, dairy products (butter, rennet, yogurt, cheese) (Burdock & Fenaroli 2010, Longo & Sanromán, 2006) In addition, they play an important role in the formation of the *bouquet* alcoholic beverages, such as vodka (Enomoto, 2009), beer and wine, and is also important for the aroma of fruits and chocolate (Berger, 1995).

Biotechnological production of esters can occur by alcoholization of acyl-coA compounds or by esterification of an acid with an alcohol (Armstrong & Brown, 1994, Berger, 1995)

Some microorganisms have already been reported for their ability to produce fruity aromas such as apple, probably due to the formation of 3-methylbutyl-3-methyl butyrate, an ester with a strong fruity aroma (Janssens, et al. 1992) The production of ethyl hexanoate by *Neurospora* has been explored by different research groups, highlighting the production through the use of agro-industrial residues where a strong fruity aroma was detected (Carvalho 2011, Crescitelli et.al, 2020, Szabo,et al.; 2021a,b).

Short-chain esters can be produced through the bioconversion of appropriate precursors. The conversion of 3-methylbutanol and 2-methylbutanol, by *Hansenula miakii*, into their corresponding acetates with high yield, the final product being used as a natural banana flavor (Gatfield, 1988; Janssens, et.al. 1992; Welsh et.al; 1989).

The yeast *Williopsis saturnus* was shown to be able to synthesize some esters (such as branched-chain volatile esters), using affordable precursors, such as amyl alcohol, to improve the fermentation process (Vandamme, 2003) In addition to this biocatalyst, several other yeast strains are reported as ester-producing (Janssens, et.al. 1992).

Rossi *et al.* (2017) found that, through the fermentation submerged in sugarcane molasses by *Pichia fermentans*, the resulting volatiles revealed an intense banana aroma, evidenced mainly by the presence of esters such as isoamyl acetate.

The microbial production of several other esters was described in a review as the production of ethyl acetate, propyl acetate, isobutyl acetate by *Ceratocysis moniliformis* (Park, et.al, 2003) and esters such as ethyl butyrate, ethyl isovalerate and ethyl hexanoate, responsible for fruit aromas, can be produced by microorganisms of the genus *Pseudomonas* (Janssens, et.al. 1992).

A variety of flavors (peach, pineapple, banana, citrus, and rose) can be produced depending on the strain, medium, and growing conditions. *Ceratocystis fimbriata* synthesizes the esters ethyl acetate and isoamyl in citrus pulp. Orange pulp has also been found as a suitable substrate for ester production by Saccharomyces cerevisiae (Rossi, et al, 2017).

2.2 LACTONES

Lactones, cyclic esters, are compounds with pleasant aromas and of great industrial interest, generally associated with odors described as fruity, coconut, buttery, sweet or nutty.

These compounds can be produced by chemical synthesis, but the various advantages associated with biotechnological production have encouraged their production by fermentative methods, using fungi and yeasts (Janssens, et.al. 1992).

Several microorganisms are capable of producing lactones when they have a culture medium with the necessary nutrients. The yeast *Sporobolomyces odorus* (recognized as *Sporidiobolus salmonicolor*) has been described as producing lactones with aromatic notes similar to the aroma of peach. The major compound responsible for the aroma was identified as γ-decalactone, and its production was 1.6 mg. L^{-1} (cis-6-dodecen-4-olide) (Tahara & Mizutani, 1975) This lactone is widely used in flavors and fragrances due to its fruity aroma, with a threshold ranging from 1 to 11 ppb (Burdock & Fenaroli 2010).

The fungal strain Trichoderma *viridae*, for example, was the catalyst for the production of the compound 6-pentyl-2-pyrone, which reached about 170 mg. L-1. This lactone is characterized by an intense aroma similar to that of coconut (Welsh, et al. 1989) At high concentrations, it was observed that this compound was able to inhibit the microorganism employed in the process and, therefore, the authors proposed a pervaporation system, with a selective membrane, to continuously extract the lactone from the culture medium, avoiding this phenomenon of inhibition (Häusler & Münch, 1997).

Sarris & Latrasse (1985) reported that the microorganism *Fusarium poae* was able to produce a lactone with an aroma similar to that of a peach, characterized predominantly by the compound cis-6-dodecen-4-olide.

In addition, microorganisms of the genus *Ceratocystis* and yeast *Kluyveromyces lactis* They are also capable of producing a great diversity of terpenes and lactones, with a fruity and floral aroma of great impact (Pandey, et al, 2000) Meanwhile *Candida* sp. *Saccharomyces* sp. *Penicillium notatum, Cladosporium butyri, Cl. suaveolens* and *Sarcina lutea* produced lactones when grown in medium containing keto acids (Kempler, 1983).

It is known that the composition of the culture medium and age of the inoculum generally influence the formation of aroma compounds in *de novo synthesis*. Lee *et al.* (1999) reported the formation of γ-decalactone, when the *Sporidiobolus salmonicolor* crop grew in a medium supplemented with several nutrients.

The production of volatile lactones has proven to be successful industrially (Cardillo, et.al 1990) and some examples include γ-nonalactone, γ-decalactone and δ-decalactone (which have an annual consumption of approximately 16.5 tons), γ-undecalactone and β-methyl-γ-octalactone (whiskey lactone), and others.

2.3 ALCOHOLS

Although alcohols typically contribute less to the aroma, in appropriate concentrations they can contribute significantly to the aromatic profile of the product (Marques & Pastore, 1999) They can give the aroma of mushroom, lavender, earth, herbaceous and rose (Burdock & Fenaroli 2010).

They are found naturally in mushrooms, lavender, wines, apples, apricots, orange juice, berries, tomatoes, leaves, potatoes, ginger, honey, coffee, cocoa, butter, cheeses, breads, whiskey, rum, beer, cider, brandy and sake, olives, truffles, vinegar, etc... (Burdock & Fenaroli 2010).

They can be used in perfumes, beverages in general, gelatins, candies, oils, bakery products, chewing gums and cosmetics (Burdock & Fenaroli 2010).

Alcohols can be formed either from the primary metabolic pathway of a microorganism or by the reduction of a carbonyl to the corresponding alcohol. Alcohol production through amino acid metabolism can occur by transamination, decarboxylation and reduction or by deamination followed by decarboxylation and reduction (Heath & Reineccius, 1986).

Berger (2009) reported that these compounds can be produced biotechnologically to generate natural flavors of a commercial nature. In this way, the production of various alcohols such as 1-octen-3-ol, a mushroom impact compound, (Brigido, 2000; Uenojo, 2003) has been reported in several studies (Pastore, et.al, 1994; Yamauch, et al. 1991; Yoshizawa, et al, 1988).

One of the most important alcohols is 2-phenylethanol, which has a rose aroma. Martínez-Avila *et al*., (2021) studied 9 agro-industrial residues in the production of phenylethanol by Pichia kudriavzevii. In this work it was observed that the maximum production occurred in the fermentation of red apple waste, which is very useful in terms of process economy. Strains of *Kluyveromyces* are also known for the production of this compound, being widely used as a flavoring in the food industry and as a fragrance in the cosmetics and perfume industry. It is a compound that has considerable industrial interest (Fabre *et al*, 1998) and can be used to modify the composition of the aroma of ice cream, candies, jellies, puddings, chewing gum, among others (Mitri, et al.2022).

In addition to their importance as an aroma compound, alcohols are also important as precursors of esters and aldehydes which, in turn, collaborate with the aromatic profile of various compounds.

2.4 ACIDS AND ALDEHYDES

Acids are found naturally in fruits such as apples, grapes, pineapples, avocados, papayas, and more, in mint and mint oils (Burdock & Fenaroli 2010).

The production of acids by synthesis *De Novo* is highly commercially targeted, considering that the originated products can collaborate both with the aroma of the product and act as an intermediary for the production of new aroma compounds. As an example, some strains of *Acetobacter* sp. are capable of synthesizing methylbutyric acid, an important precursor for obtaining esters (Vandamme & Soetaert, 2002).

Armstrong *et al.* (1989) reported high yields of citric acid, considered one of the most industrially used products as an acidulant, imparting an acidic and refreshing flavor to foods and beverages, produced by strains of *Aspergillus niger*. The yeast *Geotrichum klebahnii* was able to produce a mixture of carboxylic acids and esters, which contributed to a pleasant fruity aroma (Janssens, et.al. 1992).

Methylbutyric acid can be found in the composition of various oils, such as mint and mint, and fruits (such as apples, grapes, papayas, and pineapples). This acid has a threshold between 10 and 60 ppb, with notes of fermented pineapple (Burdock & Fenaroli 2010). It has been reported that Acetobacter strains are capable of synthesizing methylbutyric acid. Despite its industrial interest, it can still be used as a starting material for obtaining other aromatic esters (Vandamme & Soetaert, 2002).

Aldehydes are found naturally in products such as cheese, peaches, black tea, almonds, apricots, among others. They are widely used in the food and perfumery industry (Burdock & Fenaroli 2010).

Aldehydes are usually formed *via* Strecker's degradation, in the involvement of amino acids in the Maillard reaction, containing one less carbon atom than the original amino acid.

However, some fungal strains have the potential to produce significant amounts of these compounds, where benzaldehyde (almond flavor) and vanillin (vanilla flavor) stand out as the most interesting and used industrially.

Vanillin (3-methoxy-4-hydroxybenzaldehyde) is a compound widely used in the food industry and in the preparation of some fragrances. Although the extraction of vanillin is possible from the vanilla beans (*Vanilla planifolia*), synthetic vanillin supplies the vast majority of the market. A promising alternative for obtaining it is through *way* Biotechnology through enzymatic extracts or purified enzymes, microorganisms and plant cell culture. Although the highest yields of natural vanillin are related to patented biotransformation/bioconversion processes of precursors such as ferulic acid and eugenol (Daugsch & Pastore, 2005), Some processes describe its obtaining from synthesis *Again*.

The use of agricultural waste as a substrate for fermentation has been widely explored for the synthesis of ferulic acid-based bio-vanillin, with substrates such as beet meal, rice bran oil, and fruit and vegetable by-products showing strong potential. The optimization of key fermentation parameters, achieved higher bio-vanillin yields of 0.476 g/100 g, compared to 0.029 g/100 g under non-optimized conditions (Mehmood, et al, 2022).

Benzaldehyde (almond odor) can be considered as a very important commercial aromatic compound for the food, beverage, flavor, and cosmetics industry. Due to its aromatic potential, with floral and sweet characteristics, this compound is widely used as a starting material for the production of a large number of fragrances (Burdock & Fenaroli 2010).

Benzaldehyde can be obtained from natural sources, such as extraction and distillation from botanical sources, or synthesized from benzyl chloride. In addition to these alternatives, several strains of basidiomycetes have been reported as producing this compound by de *novo synthesis,* such as *Pleurotus sapidus*, *Polyporus* sp., and others (Lomascolo, et al, 1999).

2.5 TERPENES

Terpenes and their derivatives represent one of the most diverse classes of substances in nature, extensively applied in industries such as aroma and fragrance compounds (Schrader & Berger 2001). They are often the most important components responsible for the characteristic aroma of essential oils. These compounds have interesting aromatic descriptors, namely: floral, fresh and fruity.

The vast majority of terpenes are produced by Ascomycetes and Basidiomycetes, which are suitable for biotechnological processes due to the fact that they grow in both solid and liquid media, both of simple composition (Janssens, et.al. 1992).

Ceratocystis represents the genus of microorganisms of great prominence for the production of terpenes by de *novo synthesis*, as for example, in the production of citronellol, geraniol, linalool, nerol and α-terpineol (Bluemke & Schraderb, 2001).

2.6 KETONES

Several butanone-type compounds are used in the flavor industry, such as phenyl 2-butanone (flower odor), 1-4-methoxyphenyl-2-propanone (ginger flavor, commonly recognized as gingerone) and 4-hydroxyphenyl-2-butanone (raspberry flavor, or recognized as raspberry ketone). Although the latter ketone can be chemically synthesized, its biotechnological production can be significant when mediated by species of *Nidularia* sp, grown in a suitable medium and preferably rich in peptone (Guentert, 2007).

Other compounds produced by de *novo synthesis* are diacetyl and pentan-2,3-dione (diketones vicinal), which have descriptors of characteristic aromas of buttery, honey or coffee, and can be perceived between the concentration of 0.1-0.14 mg. $^{L-1}$ (Marques & Pastore, 2000).

2.7 MARKET AND PATENTS

The global natural food and beverage market was valued at USD 79,137 million in 2016 and is estimated to reach USD 191,973 million in 2023. Brazil is currently the fourth largest market for healthy products. The natural flavors industry occupies a significant share of the natural ingredients market (Additives & Ingredients, 2019).

The global flavors and fragrances market was estimated to reach USD 32,265.6 million in 2024 and is projected to reach USD 52,388.2 million by 2033. The market is primarily driven by the increasing demand for processed and convenience foods, coupled with the increasing consumption of personal care products and cosmetics in emerging and developed economies. By product, the natural segment held the largest revenue share of 75.0% in 2024 in terms of value (Flavors and fragrances market, 2025)

The bioflavors market has been gaining space, due to the main fact that the products are considered natural. In addition to the search for natural products, consumers also crave products that are environmentally friendly. In this sense, one of the major problems is the generation of waste from agribusiness, which represents an important environmental problem (Rossi, et al, 2009; Soccol, & Vandenberghe, 2003, Szabo, et al 2021b). According to Timofiecsyk and Pawlowsky (2000), the term waste is used in a broad sense, encompassing not only solids but also liquid effluents and materials present in atmospheric emissions.

Industrial waste, after being generated, needs an appropriate destination, as it cannot be accumulated indefinitely in the place where it was produced. Their disposal in the environment should occur after the waste is treated and meets the standards established in environmental legislation (Pelizer, et al, 2007).

Concern for the environment leads to the feasibility of projects that lead to the sustainability of the industrial production system (Pelizer, et al, 2007). The extraction of high value-added products from agro-industrial waste is an important component for the development of a sustainable bioeconomy. Agro-industrial waste can be valued as raw materials through innovative and environmentally friendly bioprocesses for value-added products (Τέρπου, et al; 2021).

The use of waste in processes has become the target of study in various lines of research, such as use in animal feed, (Bourscheidt, , et al, 2010; Vieira, 2009) re-use for the elaboration of new products, as ingredients for the production of foods such as cookies (Wust, 2018) juice (Borges, et al.; 2004), or as a culture medium or substrate for biotechnological processes, aiming to obtain products with higher added value such as enzymes (Tacin et al, 2018) acids (Panesar & Kaur, 2015) flavors, protein (Gmoser et al., 2019), biofertilizer (Cajamarca,019), biogas (Sandrini and Souza, 2023), biopesticides (Ballardo et al., 2017), biodiesel (de Barros, 2020), beer (Τέρπου, et al, 2021), biopolymers (Fang et al., 2020), in addition to helping to reduce environmental impact. Despite the numerous uses of agro-industrial waste, we will focus in this article on the production of bioaromas.

The residues can be solid or liquid, and fermentation can be carried out in both, but they have different names. The term solid state fermentation (FES) can be defined as fermentation in which the growth of the microorganism on solid substrates occurs in the absence of liquid in free form. Free water, which is essential for the growth of microorganisms, is adsorbed on a solid support or complexed inside a solid matrix. The FES technique is known for its production of metabolites, in most cases at much higher levels than submerged fermentation. element. This process has been successfully applied to transform agro-industrial waste, such as sugarcane bagasse, coconut shells and other plant by-products into aroma and flavor compounds with high added value. With continued advancements, solid fermentation has the potential to reshape the future of flavor production by linking efficiency, sustainability, and sensory excellence (Ravi et al, 2025).

In submerged fermentation, the producing microorganism develops inside the fermentation medium, usually under agitation (Pereira, 2007). FES is generally characterized by cheaper processes and with a wide possibility of using agro-industrial, cellulosic or starchy residues, such as coffee husks, cassava bagasse, citrus pulp, among others (Pandey, 1992; Pandey, et al. 2001, Rossi; et al.; 2009). Several microbial strains are capable of producing key aroma compounds in liquid conditions, such as esters (fruity), aldehydes (nutty and floral), ketones (creamy), and alcohols (such as rose flavoring) (Ravi, et al, 2025).

Soccol et al. (1997) filed an Industrial Patent, demonstrating that the mixture of coffee husk and cassava bagasse is a viable alternative for the production of gibberellic acid by solid-state fermentation.

Pastore et al. (2011) filed a patent with the National Institute of Intellectual Property (INPI) where they used manipueira and malt bagasse as substrate for the production of ethyl hexanoate, demonstrating that the use of these residues for the production of fruit aroma is feasible.

Medeiros *et al.* 2000 used cassava bagasse and palm meal as substrate for the cultivation of *Klyveromyces marxianus* in order to produce aroma compounds. Both substrates were viable, and in the first, supplemented with glucose, the production of ethyl acetate, responsible for the fruit aroma, was higher, reaching a production of 1395 µmol/Lg, after 72 hours of fermentation.

Ceratocystis fimbriata has the potential to synthesize esters, is fast-growing, and produces a variety of aromas (peach, pineapple, banana, citrus, and rose), depending on the strain and growing conditions. Soares *et al*., 2000 used coffee peel supplemented with glucose and obtained the production of a strong pineapple aroma using *Ceratocystis fimbriata* and when supplemented with leucine they obtained the production of a strong banana aroma. Citrus pulp, a residue from the citrus juice industry, was suitable as a substrate for the production of aroma by *Ceratocystis fimbriata* , with isoamyl acetate, a high value-added banana flavor for the food industry, being the major compound, reaching a concentration of 1.66 µmol/L g (Rossi, et al; 2009).

Bramorski *et al.* (1998), using the line *C. fimbriata*, compared the production of fruit flavors in various agro-industrial residues as a culture medium (cassava bagasse, apple, amaranth and soybean). When the authors used a mixture between the residues, they found an intense fruity aroma and, although the compounds identified were mostly esters and alcohols, lower amounts of aldehydes, acids and ketones were still identified. The same microorganism using wheat bran, cassava bagasse and sugarcane as substrate with the use of precursors (glucose, leucine and valine) produced fruity aromas of banana, apple, pear (Christen, et al, 1997). Similarly, the microorganism *Rhizopus oryzae* produced several volatile compounds in a mixture of alternative media, about 80% of which was ethanol and small concentrations of aldehydes, esters and other alcohols (Christen, et al, 1997).

Five agro-industrial residues (apple, cassava, sunflower, sugarcane and large palm) were tested by solid-state fermentation, using statistical tools to evaluate the production of volatile compounds. The lineage *Kluyveromyces marxianus* produced a diversity of important compounds, mainly acetaldehyde, ethanol and ethyl acetate (Medeiros, et al, 2000).

As previously mentioned and discussed throughout this review, the application of agro-industrial waste in bioprocesses is a way to use alternative substrates and minimize pollution problems that they may cause. In addition to processes using agro-industrial waste, the interest in the production of natural flavors via synthesis *has* been demonstrated by the patents filed in recent years. Thus, a search was carried out in the patent databases Derwent, Espacenet and USPTO from the year 2000 aiming at an overview of the interest in aroma production via de *novo synthesis.*

Dias & Loh, (2005) deposited the invention where from the fermentation of whey it is obtained heat-stable compounds that can be used as a cheese flavoring in the preparation of food.

3 FINAL CONSIDERATIONS

The biotechnological production of flavors by de *novo* synthesis can directly contribute to the expansion of the natural flavors market, considering that the study of new flavor compounds is of great importance to allow innovations in the market and provide greater added value to the most diverse products.

To this end, production must be optimized, in such a way that the costs related to its production and purification can allow its commercialization. Several tools can collaborate with more competitive processes, such as the optimization of the fermentation process using statistical tools, the use of agro-industrial residues to minimize costs in contrast to synthetic culture media, the use of precursors, as well as the selection of new production lines and their genetic improvement study, based on genetic engineering. This last example may be responsible for the considerable improvement in the efficiency of the synthesis process *de novo* and the metabolic pathways achieved can lead to the obtaining of new aroma compounds.

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