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Microbial biotransformation for production of valuable aroma compounds: Current research and future challenges

Ajar Nath Yadav[1,2]*, Rajeshwari Negi[1], Tawseefa Jan[3], Babita Sharma[4], Tanvir Kaur[5], Naseer Ahmed[3], Sangram Singh[6], Sheikh Shreaz[7], Ashok Yadav[5], Paridhi Puri[8], Narinderpal Kaur[9], Neelam Yadav[10]

[1]Department of Genetics, Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India.
[2]Research and Innovation, Assam Down Town University, Sankar Madhab Path Gandhi Nagar, Panikhaiti, Guwahati, Assam, India.
[3]Department of Food Technology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India.
[4]Department of Microbiology, Akal College of Basic Sciences, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India.
[5]Department of Biotechnology, Graphic Era Deemed to Be University, Dehradun, Uttarakhand, India.
[6]Department of Biochemistry, Dr. Ram Manohar Lohia Avadh University Faizabad, Ayodhya, Uttar Pradesh, India.
[7]Desert Agriculture and Ecosystem Program, Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, Safat, Kuwait.
[8]University Centre for Research and Development, Chandigarh University, Mohali, Punjab, India.
[9]Chitkara Centre for Research and Development, Chitkara University, Baddi, Himachal Pradesh, India.
[10]Centre of Research Impact and Outcome, Chitkara University Institute of Engineering and Technology, Chitkara University, Rajpura, Punjab, India.

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ABSTRACT

Aroma and flavor represent the key components of food as they improve the organoleptic characteristics and enhance the acceptability of the consumers. Since ancient times, the concept of commercial production of aromatic and flavoring chemicals has lagged behind human habits, although coming from the industry's microbiological source. Microbial flavor compounds have garnered attention in recent decades due to their sustainable nature, especially because of their great biological activity and minimal toxicity. Fragrance chemicals are frequently found in medicinal products. The amount of scientific materials containing analytical and biological data on fragrance components is currently more than ever, despite debates regarding their use among academics in the fields of traditional and modern medicine. In addition, the food business, together with the highly significant perfume and cosmetic industries, supports the flavoring and preservation of food items through aromatic volatiles as well as the search for naturally occurring, pleasant-smelling raw components for these products. In addition, it serves as a de-foaming agent for ophthalmic solutions containing high surfactant concentrations. Fruits with mild processing are given a longer shelf life and increased safety by the use of natural fragrance components. The food industry's expanding demand for natural products has spurred incredible efforts to create biotechnological procedures for the synthesis of fragrance components. The present review assimilates the existing knowledge of microbial transformation to value-added products for their application in food, fragrances, agricultural, and pharmaceutical industries.

1. INTRODUCTION

Flavors and fragrances are extremely important for the food, feed, cosmetic, chemical, and pharmaceutical industries. Presently, flavors account for more than 25% of the global market for food additives, and the majority of them are produced using conventional techniques such as chemical synthesis or extraction from natural sources [1]. In the vicinity of now, they signify an industrial value of over $7 billion USD annually on a global scale, with an annual growth rate of 4.4%. This corresponds to 25% of the food additives market overall in terms of money. Natural products, as opposed to synthetic ones, are becoming more and more in demand. This rise is particularly noticeable for natural or bio-flavoring substances used as flavorings [2]. Flavors are produced in an undesired way by chemical synthesis. Their chemical compounds are elucidated by modern, sophisticated techniques such as mass spectroscopy and nuclear magnetic resonance (NMR) spectroscopy, followed by production on an industrial scale using chemical synthesis.

Nowadays, there is debate over the chemical synthesis of food fragrance because of problems with low yields, pollution, expensive production, poor selectivity that results in unwanted side effects, and

*Corresponding Author:
Ajar Nath Yadav,
Department of Genetics,
Plant Breeding and Biotechnology,
Dr. Khem Singh Gill Akal College of Agriculture,
Eternal University, Baru Sahib, Sirmour, India.
E-mail: ajarbiotech@gmail.com

low yields [3]. Chemical resolution of racemic mixtures is typically challenging. This results in decreased process efficiency and higher expenses down the line. Due to all of these reasons, flavor producers have been more inclined to concentrate on flavor compounds that have biological origins. Higher plants are typically responsible for providing the natural flavors [4]. However, these traditional agricultural sources have trace amounts of active ingredients, and how they are used depends on uncontrollably occurring natural variables such as weather and plant diseases [5]. In addition, they could be bonded, which makes it harder to isolate them and raises the price of flavor products. Natural resources can no longer meet the market's rising demand.

Plant essential oils contain natural fragrance components that are extracted using steam distillation of plant material and fractional distillation processes. Moreover, extraction has a number of issues. Because the necessary chemicals are frequently present in low concentrations in these raw materials, extraction is costly [6]. The aromatherapy and perfume sectors have been prompted by the growing demand for natural products to create novel biotechnology methods for obtaining fragrance molecules through natural means. Despite the fact that numerous biotechnological methods have been documented, the majority of them have not yet been used in the industrial synthesis of these chemicals because of their low yields and, consequently, high downstream processing costs [7]. The price of naturally occurring chemicals rises as a result, and it is now 10–100 times more expensive than that of synthesized ones. Microbial biosynthesis, also known as bioconversion, is a constituent of the alternate pathway employed for natural synthesis. Since the majority of natural flavors are made up of several hundred components, developing biotechnological processes for the synthesis of flavor and fragrance molecules could be a difficult undertaking.

The increased demand for petroleum-derived chemicals in the production of fuels, solvents, and materials, among other things, has made them indispensable in contemporary life. Many efforts have been made to create microbial strains that can produce a variety of chemicals and materials from renewable resources to address the major problems related to global climate change and the future scarcity of fossil resources [8]. The chemical, culinary, polymer, and pharmaceutical industries all depend on aromatic compounds for a variety of reasons. The bulk of aromatic compounds are chemically synthesized because biological manufacturing is inefficient or simply because there is no suitable bioproduction technology; however, bio-based processes for the production of a few aromatics have been commercialized. Yet, microbial synthesis of aromatic compounds has advanced significantly in recent years due to the quick development of systems metabolic engineering tools and techniques. This method can replace chemical methods that use petroleum-derived benzene, toluene, and xylene as starting materials for the synthesis of different aromatics with bio-based, sustainable, and eco-friendly techniques that use renewable resources [9].

Microbial biotransformation is a useful technique for producing high-value natural substances in controlled, eco-friendly environments. Natural aroma molecules made by microorganisms have benefits since they are recognized as natural flavors. To combat the exorbitant price of fragrance compounds and to ensure that consumer demands for natural components are fulfilled. Manufacturing with biotechnology is becoming an attractive possibility instead of manufacturing using chemicals. Natural biocatalysts, especially microbial cells, have a tremendous deal of promise for producing a diverse array of tastes [10]. They provide the food sector with a significant deal of economic possibilities in getting a variety of biomolecules that are of importance. Alternative approaches have emerged as a result of certain inherent challenges in the detection and synthesis of aroma molecules. The evaluation of aroma compounds has been transformed by biotechnological advancements and novel methods to enhance detection, separation, synthesis, and characterization. This has also created the possibility of obtaining natural extracts that may be useful [11] [Table 1].

Approximately 23,000 microbial fragrance compounds have been found, with the majority of these flavors and fragrance compounds originating from a very small number of different types of microbes. Microorganisms are essential for a variety of reasons, the main one being that biological systems are used to produce significant biomolecules. They have the ability to synthesize aromatic compounds and several natural aromas. Natural aromatic compounds produced by microbes can be used in foods as preservatives, flavor enhancers, nutritional supplements, emulsifiers, texturizers, or thickeners [12]. The microbial biotransformation approach aims to connect scientific innovation with market demands that leverage microorganisms' natural metabolic processes to convert simple substrates into complex, high-value products. Its application in producing nutraceuticals and aroma compounds aligns with several Sustainable Development Goals, particularly those addressing health, sustainable consumption, and industrial innovation.

2. BIOTRANSFORMATION SYSTEMS

The demand for natural flavor compounds developed through biotechnological means has pushed up the appeal of biotechnological generation, even though the traditional methods of chemical synthesis or separation of aroma compounds from plants are still viable [13]. A bioflavor's actual market price ranges from $100 to $500/kg, and then 100 flavor compounds, both complex flavor combinations and single constituents, are marketed. Microbial biosynthesis or bioconversion provides an alternate pathway for the creation of bioflavors. White biotechnology includes these bioprocesses that rely on microorganisms (fungi, bacteria, and yeasts) and their enzymes [14]. This technique worked on the principles of using clean, renewable resources, reducing pollution, and using biological systems such as complete cells or enzymes as reagents or catalysts with lower energy requirements.

In recent years, a number of microorganisms have been adopted for the biotechnological procedures used in flavor development [15]. Biotechnological strategies can be divided into two categories: microbiological and enzymatic methods. Biotransformation and *de novo* synthesis are two categories of microbiological techniques employed in the creation of natural compounds, particularly flavors [16]. In the first, aroma compounds were produced by metabolizing cells in basic culture conditions. In contrast, biotransformation is the process of employing microbial cells to specifically transform a precursor into the desired product [17]. Large-scale biotransformation of precursors into natural flavors can be accomplished with microbes. It is important to cultivate the chosen microbe in an environment that promotes the production of the required flavor components [18]. Cells typically have the redox system needed for flavor synthesis as well as the system needed for cofactor regeneration. Therefore, the challenges posed by the application of an enzyme-based redox system can be addressed by the utilization of entire cells in microbial transformation. In fact, the constant flow of metabolites is preserved by renewing the enzyme chains, necessary cofactors, and transporters. To minimize the inhibition of the producer cell by the substrate or product and

Table 1: Microbial production of aroma compounds.

Microorganism	Substrates	Aroma compound	Identification	References
Brevibacterium linens	Bacterial culture	Branched aldehydes, alcohols, and esters	Dynamic headspace technique coupled with gas chromatography-mass spectrometry	Deetae *et al*. [229]
Ceratocystis fimbriata	Coffee husk	Acetaldehyde, ethanol, isopropanol, ethyl acetate, ethyl isobutyrate, isobutyl acetate, isoamyl acetate, and ethyl-3-hexanoate	Gas chromatography	Soares *et al*. [230]
Ceratocystis fimbriata	Wheat bran, *Cassava bagasse*, and sugar cane bagasse	Aldehyde, alcohols, ketones, and esters	Gas chromatography headspace analysis	Christen *et al*. [231]
Chrysosporium pannorum	α-pinene	Verbenol and verbenone	Gas chromatography-mass spectrometric	Trytek *et al*. [232]
Chrysosporium pannorum	α-pinene	Verbenol and verbenone	Gas chromatography	Trytek *et al*. [233]
Fusarium oxysporum	Orange essential oil	α-terpineol	Gas chromatography-mass spectrometric	Maróstica and Pastore [234]
Kazachstania humilis	Pepper paste	Phenethyl alcohol, linalool, methyl salicylate, ethyl myristate, acetic acid, and ethyl hexanoate	Gas chromatography combined with olfactometry	Li *et al*. [235]
Kluyveromyces marxianus	*Cassava bagasse*	Isoamyl alcohol, isoamyl acetate, ethyl propionate, propyl acetate, ethyl isobutyrate and butyl acetate	Gas chromatography	Medeiros *et al*. [236]
Kluyveromyces marxianus	*Cassava bagasse* and palm bran	Alcohols, esters, and aldehyde	Gas chromatography headspace analysis	Medeiros *et al*. [237]
Lactiplantibacillus plantarum	Pepper paste	Phenethyl alcohol, linalool, methyl salicylate, ethyl myristate, acetic acid, and ethyl hexanoate	Gas chromatography combined with olfactometry	Li *et al*. [235]
Lindnera saturnus	Castor oil and crude glycerol	γ-decalactone	Gas chromatography	Soares *et al*. [238]
Microbacterium foliorum	Bacterial culture	Branched aldehydes, alcohols and esters	Dynamic headspace technique coupled with gas chromatography-mass spectrometry	Deetae *et al*. [229]
Penicillium digitatum	Orange peel oil	α-terpineol	Gas chromatography	Badee *et al*. [239]
Penicillium digitatum	Limonene	α-terpineol	Gas chromatography	Tai *et al*. [240]
Phanerochaete chrysosporium	Lignocellulosic substrates	Vanillin	Thin-layer chromatography and spectrophotometer	Karode *et al*. [241]
Phanerochaete chrysosporium	Green coconut agroindustrial husk	Vanillin	High-performance liquid chromatography	Dos Santos Barbosa *et al*. [242]
Picrorhiza kurroa	Bacterial culture	Menthol, phenylethyl alcohol, (+)-isomenthol, β-phellandrene, β-bisabolene, limonene, 3-pentanone, and 1-pentanol	Gas chromatography-mass spectrometric	Qadri *et al*. [243]
Polyporus brumalis	α-pinene and geraniol	p-menthane-3, 8-diol	Gas chromatography-mass spectrometric	Lee *et al*. [244]
Proteus vulgaris	Bacterial culture	Branched aldehydes, alcohols and esters	Dynamic headspace technique coupled with gas chromatography-mass spectrometry	Deetae *et al*. [229]
Psychrobacter spp.	Bacterial culture	Branched aldehydes, ketones, alcohols and esters	Dynamic headspace technique coupled with gas chromatography-mass spectrometry	Deetae *et al*. [229]
Saccharomyces cerevisiae	Ricinoleic acid	γ-decalactone	Gas chromatography-mass spectrometric	Rong *et al*. [245]

(*Contd*...)

Table 1: (*Continued*).

Microorganism	Substrates	Aroma compound	Identification	References
Sporidiobolus ruinenii	Ricinoleic acid methyl ester	γ-decalactone	Gas chromatography	Dufossé *et al*. [246]
Sporidiobolus salmonicolor	Ricinoleic acid methyl ester	γ-decalactone	Gas chromatography	Dufossé *et al*. [246]
Streptomyces sannanensis	Wheat bran	Vanillin	Thin-layer chromatography and high-pressure liquid chromatography	Chattopadhyay *et al*. [247]
Trichoderma viride	Sugarcane bagasse	Lactones, δ-octalactone, γ-nonalactone, γ-undecalactone, γ-dodecalactone and δ-dodecalactone	Gas chromatography-mass spectrometric	Fadel *et al*. [248]
Yarrowia lipolytica	Ricinoleic acid	γ-decalactone	Gas chromatography	Gomes *et al*. [249]
Yarrowia lipolytica	Methyl ricinoleate	γ-decalactone	Gas chromatography	Gomes *et al*. [250]
Yarrowia lipolytica	Castor oil and crude glycerol	γ-decalactone	Gas chromatography	Soares *et al*. [238]
Zygosaccharomyces bisporus	Pepper paste	Phenethyl alcohol, linalool, methyl salicylate, ethyl myristate, acetic acid, and ethyl hexanoate	Gas chromatography combined with olfactometry	Li *et al*. [235]

prevent the buildup of undesired metabolites, all phases in the microbial transformation process must be carried out under sterile conditions [19]. Thus, for an efficient manufacturing process, every stage, from fertilizer preparation to product downstream must be under control.

Approximately 200 of the approximately 4,000 known enzymes have been made available for commercial use, mostly in stereo-selective organic synthesis and the biotechnological development of flavor compounds [20]. A number of advantages, including high selectivity, high efficiency, fast reactions, and catalytic activity in both directions of reactions, occur from employing enzymes. When substrates of natural sources are utilized in flavor synthesis, enzymes can produce chemicals that may be marketed as "natural." These chemicals have been found to exhibit improved color and odor. When considering enzymatic conversion over direct plant extraction, large productivities of fragrance chemicals can also be obtained [21]. Enzymes have a global market of over $1 billion USD annually. In Europe, over 60% of all enzymes are generated. The primary source of flavor compound manufacturing is microbial enzymes [22]. The enzyme classes that are most frequently utilized in biotechnology operations are lyases, hydrolytic enzymes, transferases, and oxidoreductases [23]. The oxidoreductases that are employed in the production of flavor include vanillyl alcohol oxidase, lipoxygenase, alcohol dehydrogenases, and peroxidases such as chloroperoxidase. These enzymes are involved in improving the organoleptic properties and have been highlighted for their possible use in the synthesis of flavor molecules. These enzymes are involved in improving the organoleptic properties and have been highlighted for their possible use in the synthesis of flavor molecules. A few essential pathways are used to synthesize a wide variety of plant volatiles. Their variety is then attained by further modification events, including acylation, methylation, and oxidation/reduction once they emerge through these pathways.

3. METABOLIC ENGINEERING FOR BIOTRANSFORMATION

A biological process known as "biotransformation" or "biocatalysis" employs biological systems, such as whole cells, cellular extracts, and enzyme(s), to catalyze the transformation of one chemical component into its structurally related derivatives [24]. The production of these compounds, such as novel terpenes and terpenoids, which could eventually be used to produce scents in industry, is increasingly dependent on genetic and metabolic engineering because many of the compounds found in natural organisms are rather challenging to chemically synthesize and to extract in significant quantities [25]. Plant secondary metabolites are widely utilized in various industries, such as texturizing, flavoring, and coloring agents. Extraction of these compounds from plant material is typically challenging because plants frequently contain complex combinations of chemically closely related secondary metabolites. However, microorganisms engineered represent a possible substitute for plant metabolite production as they can be readily cultured in inexpensive culture conditions and reach rapid growth rates, producing high quantities of biomass [26]. Solid-state fermentation (SSF) and submerged fermentation (SmF) are two distinct fermentation processes that have been used to produce these bioactive substances on an industrial scale.

3.1. Solid-state Fermentation (SSF)

SSF is a process carried out with microbes that can grow on moist and solid substrates that serve as a nutrient source and promote microbial growth and development. It is employed in the manufacturing of products for the biochemistry, textile, food, pharmaceutical, and bioenergy sectors [27]. The microbes employed for fermentation are mostly fungi, and substrates are residues from the food and agriculture industry. This illustrates the benefits of SSF from an economic and environmental perspective [28]. SSF is thought to be an effective technique for converting agro-industrial wastes and by-products into value-added products, such as bioactive substances, biofuels, and bioplastics [29]. Mold and fungi are mostly used for SSF because they are less prone to bacterial contamination and require less energy to sterilize substrates. The SSF technique is especially suitable for filamentous fungi since it mimics their natural environment. The filamentous fungi are capable of synthesizing huge amounts of enzymes and other metabolic compounds under SSF conditions. Yeast and certain bacterial species, including *Bacillus subtilis*, *Bacillus thuringiensis*, and *Lactobacillus* spp., are thought to be the second-

best option due to their capacity to thrive in low water conditions. *Actinomycetes* spp. and *Streptomyces* spp. can also be used in SSF due to their resistance to harsh environments and ability to colonize solid residues in large quantities [30].

Lignocellulolytic enzymes such as cellulases, laccases, lignin, peroxidases, and xylanases produced in SSF by bacteria and fungi are utilized in various industries due to their ability to hydrolyze fibers and release polyphenolic compounds. In SSF bioreactors or other bioreactors, the enzymes are removed from the fermented solid by *in situ* recovery of aqueous solutions [31]. SSF has been used recently to produce a variety of metabolites. This process can produce a higher amount of aroma compounds as compared to SmF. In SSF processes, aroma substances can be generated in the headspace or solid matrix, but when aeration is necessary, they may be lost or stripped [32]. The most significant factors that impact the performance of the SSF process are substrate (particle size, chemical composition, and humidity), inoculum, the addition of external carbon and nitrogen, specific enzyme inducers for the growth of microorganisms, and the production of desired metabolite, temperature, pH, mixing, and O_2 concentration [33]. In an investigation, Premalatha *et al.* [34] studied the production of alpha-amylase enzymes from *Aspergillus tamarii* using the SSF process. In another investigation, Araujo *et al.* [35] reported the optimization of lipase production by *Penicillium roqueforti* through SSF using agricultural industrial residue. In a report, Paluzar *et al.* [36] studied the production and characterization of lipase from *Penicillium aurantiogriseum* under SSF.

3.2. Submerged Fermentation (SmF)

SmF is the process by which microbes grow and decompose substrates such as carbohydrates in the presence of an abundance of free water (liquid medium) [37]. Molasses and broth are examples of free-flowing liquid substrates that are employed in SmF. The byproducts of the fermentation process are released into the fermentation broth. The fermentation process in SmF requires a constant supply of substrate due to its high rate of substrate utilization. This method is well suited for the production of secondary metabolites from bacteria because the growth of bacteria demands high moisture content [38]. The three main modes used in SmF processes are batch, continuous, and fed-batch fermentation. In batch mode, the desired microbial culture ferments a sterilized nutrient solution in a closed vessel. This process has the benefits of being inexpensive and having an easy-to-use infrastructure for process control. However, the main drawbacks of this process are limited productivity and feedback inhibition [39]. In fed-batch fermentation, nutrients are added to the vessel after a specific period of time. In contrast, fresh medium is introduced to the culture with the corresponding withdrawal of grown microbes after a specific stage of growth [40]. In an investigation, Samanta and Jana [41] reported the optimization of cold-active amylase production by mesophilic *Bacillus cereus* under SmF. In another investigation, Premalatha *et al.* [34] studied the optimization of culture conditions for increased production of alpha-amylase using solid-state and SmF from *A. tamarii*. In a report, Ahmed *et al.* [42] demonstrated the production of α-amylase by using *Penicillium notatum* using the shaken flask technique of SmF.

4. TECHNIQUES USED FOR PRODUCTION OF AROMA COMPOUNDS

Due to their low volatility and restricted water solubility, aroma compounds are extracted from the matrix. The extraction technique primarily involves separating and extracting volatile aroma compounds from the non-volatile matrix, and water is a common practice, as the aroma generally makes up < 1% of the food matrix, with a few exceptions [43]. The significant increase in the ability to identify aroma components is achieved by separating volatile material from water and non-volatile substances. Various methods exist for analyzing aroma compounds in food. Every technique comes with its own set of applications, along with both advantages and disadvantages. It is important to acknowledge that no individual method can yield a flavor isolate that accurately reflects the entire flavor profile present in the food [44]. The selection of the method hinges primarily on the thermal stability, reactivity of the flavor compounds, volatility, and their interaction with the food system [Figure 1].

The taste constituents found in food are typically highly intricate, comprising anywhere in thermally processed items. The number of chemicals can range from 50 to 250 or even more [45]. Certain flavor compounds may be present at exceptionally low levels, falling below the detection threshold of currently available instruments. Flavor compounds exhibit significant variations in their physical and chemical properties. While some flavor compounds exhibit high volatility, others have elevated boiling points, such as vanillin. Some compounds exhibit notable instability or reactivity when extracted from food and concentrated for analysis. To release flavor compounds from food waste, it is common to apply techniques such as blending, crushing, homogenization, or grinding to the sample. In certain instances, enzymes can be employed to aid in the liberation of odorants, such as the utilization of amylase for the release of entrapped volatiles in starch-based food matrices [46]. Conversely, there are situations where enzymes present in foods must be deactivated to avoid changes in the flavor profile during prolonged isolation processes. Enzyme deactivation in aqueous samples is typically accomplished by applying heat.

Gas chromatography-mass spectrometry (GC-MS) constitutes a highly responsive method for analyzing fragrance compound identification, but it comes with specific constraints, such as the necessity for an extract containing minimal non-volatile material and preferably no water [47]. Solid-phase microextraction (SPME) is notable as one of the most frequently utilized methods for aroma extraction due to its user-friendly nature, making it particularly suitable for individuals unfamiliar with aroma analysis [48]. In the meantime, solvent-assisted flavor extraction is widely recognized as the method that produces an aroma extract that is most representative of the actual aroma in the food. In the end, solid-phase extraction, a technique extensively utilized in all aspects of domains of analytical science for analyte purification, holds considerable promise for analyzing highly polar aroma compounds in low-fat foods.

4.1. Solvent Extraction Technique

Within this extraction process, optimally sized raw materials undergo treatment with various organic solvents, absorbing not just the sought-after soluble components but also additional flavoring and coloring agents such as anthocyanins, which are acknowledged for their ability to combat cancer and reduce inflammation properties [49]. Typically, samples undergo centrifugation and filtration to eliminate solid residues; the derived extract can serve as an addition, nutritional supplement, or in the creation of foods enriched with nutrients [3]. To achieve favorable outcomes, enhancements have been made to the solvent extraction approach through the incorporation of alternative methods, which include microwave extraction, supercritical fluid extraction (SFE), Soxhlet, and ultrasound [50]. Extracting β-carotene and lycopene from tomato pomace involves the use of ethanol

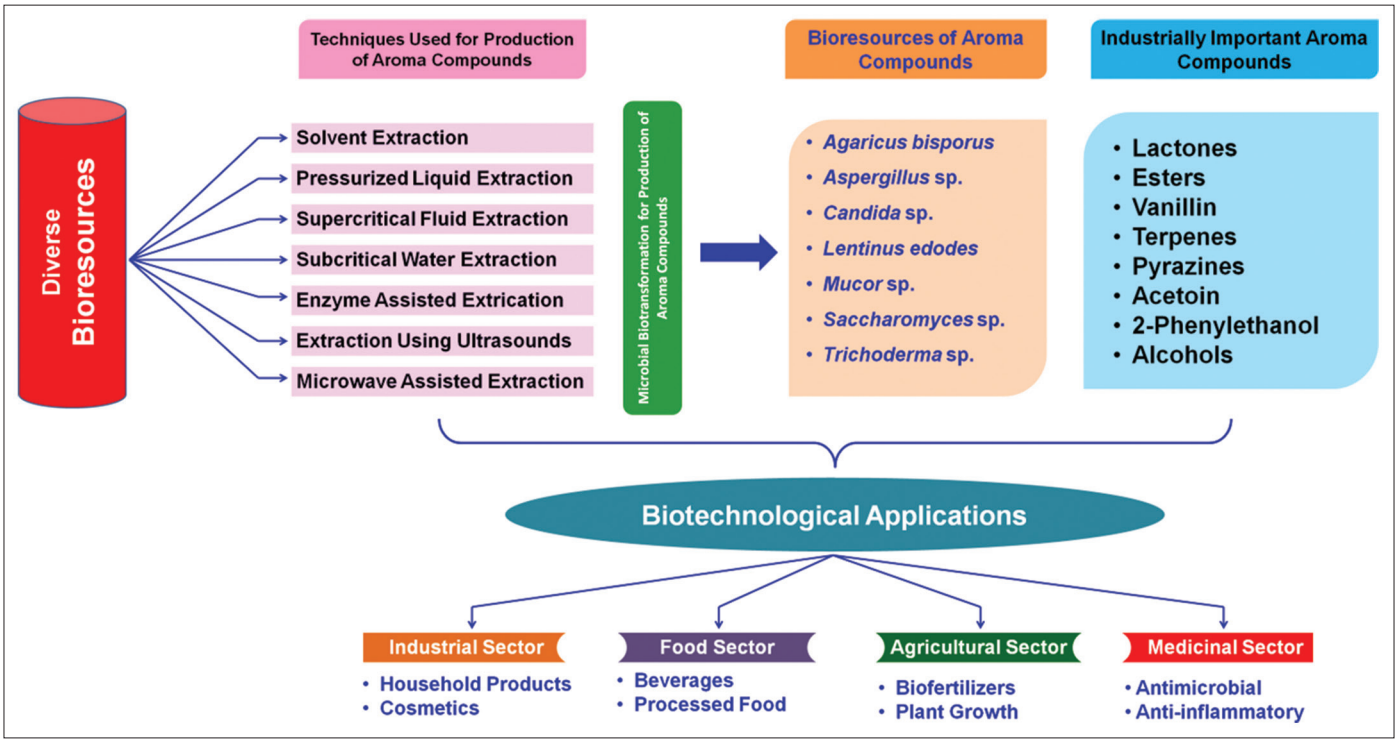

Figure 1: Microbial biotransformation for production of aroma compounds.

in combination with supercritical CO_2 [51,52]. The isolation of polyphenolic compounds from powders of Parkia speciosa pods involves the use of a 50% acetone solution. They observed that, in the retrieval of polyphenols from kinnow (*Citrus reticulata* L.) rind solvent extraction, the highest yield was obtained with a 50% acetone solution polyphenol content in comparison to ethanol, methanol, hexane, and ethyl acetate. For the highest amount of polyphenols, the extraction process, utilizing ultrasound assistance with 80% methanol, resulted in an extract with a content of 32.48 mg gallic acid equivalent (GAE)/g. In contrast, the maceration technique with 80% ethyl acetate yielded the lowest phenolic content at 8.64 mg GAE/g extract [53]. In the investigation of Bandar *et al.* [54], it was noted that among the organic solvents tested, ethanol demonstrated the highest efficiency, resulting in the maximum yield of extraction. Conversely, hexane exhibited the minimum yield when employed for obtaining bioactive compounds using these techniques. In addition, a higher yield of the compound obtained from extraction was noted with an extended extraction time.

4.2. Pressurized Liquid Extraction (PLE)

A novel extraction method has been introduced as a substitute for conventional solvent extraction, and it has been found to be extensive use in isolating natural bioactive compounds (NBCs). In this approach, organic liquid solvents are applied under elevated attaining a rapid extraction rate of compounds by applying temperatures ranging from 50°C to 200°C and pressures between 1450 and 2175 psi [55]. With increasing temperature, the solvent's dielectric constant experiences a decline, leading to a reduction in the solvent's polarity [56]. Increased pressure facilitates faster filling of extraction cells and compels liquid into the solid matrix. Numerous researchers have showcased the extraction of NBCs through PLE [57].

4.3. Supercritical Fluid Extraction (SFE)

In this extraction method, the container for extraction, furnished with temperature and pressure controllers, is employed to uphold the necessary conditions for positioning the raw material. Following this, pressurization of the extraction container occurs through the use of a fluid and pump. After the compounds dissolve, they are conveyed to separators, and the collection of products is carried out through a valve located at the bottom section of the separators. Subsequently, the liquid is either rejuvenated and circulated or discharged into the surroundings. The meticulous choice of supercritical fluids is pivotal for the efficient operation of this procedure, which involves a diverse array of compounds that can serve as solvents [58]. Significant extraction yield of naringin, a flavonoid, has been noted [59] when supercritical carbon dioxide (SC-CO_2) is modified with ethanol, the conditions being 9.5 MPa and 58.6°C. It has been employed in SFE for extracting procyanidins and polyphenols from grape seeds. In this process, methanol served as a modifier, and the use of the extraction of over 79% yield of epicatechin and catechin from grape seeds was obtained and achieved by utilizing CO_2 modified with 40% methanol [60].

The SFE method has also been utilized in the extraction of lipophilic compounds, including phytosterols, tocopherols, free fatty acids, and policosanols, from sorghum [61]. The investigation involved the study of extracting polyphenols (protocatechuic, ferulic, syringic, gallic, p-coumaric, and vanillic derivatives) and flavonoids (Incorporating quercetin and its derivatives) from sediment [62] by using SFE [63] the retrieval of phenolic compounds from the husks of black walnut (*Juglans nigra*) occurred during the extraction process performed at 68°C employing 20% ethanol in supercritical carbon dioxide (SC-CO_2) employing an ethanol modifier. Antioxidant compounds from *Crocus sativus* petals were also extracted using SC-CO_2 at a temperature of

62°C for a duration of 47 min under a pressure of 164 bar [64]. Under these optimized conditions, the extraction yielded a total phenolic measured 1423 mg/100 g, a total flavonoid content of 180 mg/100 g, and a total anthocyanins content of 103.4 mg/100 g.

4.4. Subcritical Water (SCW) Extraction

The extraction of phenolic compounds is effectively achieved through the use of SCW extraction from various food sources. It necessitates water within the temperature range from 100°C to 374°C and pressure maintained sufficiently elevated to maintain a liquid state, staying under the critical pressure threshold of 22 MPa. The study involves the utilization of SCW for extracting phenolic compounds from mango peels [65], which resulted in a more extensive extraction when compared to the Soxhlet extraction technique. SCW extraction emerges as a sustainable alternative to the technology designed for the extraction of phenolic compounds from agricultural residues. This method serves as a substitute for conventional approaches that rely on organic solvents. According to a study, quantities of extracted gallic acid and ellagic acid escalated as the SCW temperature increased, reaching a maximum of 180°C [66]. Simultaneously, the highest corilagin concentration was attained at 120°C while employing SCW for the extraction of polyphenolic compounds from *Terminalia chebula* Retz. Mangiferin, a bioactive component present in Mahkota Dewa, was extracted utilizing SCW extraction within a temperature range of 323–423 K and pressures ranging from 0.7 MPa to 4.0 MPa, and extraction durations spanning 1–7 h [67]. The production of specific phenolic compounds, including p-coumaric acid, caffeic acid, ferulic acid, and gentisic acid, can be heightened by adjusting temperatures within the range of 100°C to 220°C for 20 min. Likewise, altering the reaction duration within the range of 10–50 min at 160°C contributes to the enhanced production of these elements within the context of pumpkin leaves [68]. Gahruie *et al.* [69] explored SCW extraction as an environmentally friendly approach for obtaining phenolic compounds from *C. sativus* petals [70]. Utilizing two plentiful residues from coffee waste, we employed water at subcritical conditions extraction under conditions of semi-continuous flow to extract total phenolic compounds. The maximum recovery of total phenolic compounds (26.64 mg GAE/g coffee powder) was achieved at 200°C and 22.5 MPa.

4.5. Enzyme Assisted Extraction

Enzymes play a role in extracting bioactive components, and the existence of polysaccharides such as cellulose, hemicellulose, and pectins found in the cell walls of plants acts as a barrier, hindering the release of intracellular substances. Therefore, plant tissues are the primary reservoirs for extracting antioxidants. Numerous enzymes, including cellulase, pectinase, xylanase, β-glucosidase, and β-gluconase, assist in breaking down the structure of the unit of cell walls and depolymerizing polysaccharides within plant cell walls. This process facilitates the liberation of interconnected compounds [71]. Since water is employed instead of using the enzyme-assisted extraction technique, organic is a more environmentally friendly approach for extracting bioactive compounds and oil when employing a solvent [72]. The retrieval of bioactive compounds, including stevioside, from *Stevia rebaudiana* has been carried out using enzyme-assisted methods [71]. Enzyme-assisted extraction has been employed to create lycopene-rich extracts from utilizing pectinase (30 units/g) and cellulase (20 units/g) on the tomato peel at a temperature of 50°C for 60 min [71]. Existing research on enzyme-assisted extraction indicates that optimizing the liberation of compounds with bioactivity extracted from plant cells through cell disintegration and the process of extraction may be accomplished by employing preparations of enzymes, whether individually or in combinations. This optimization relies on the enzymatic ability to initiate reactions in aqueous solutions using mild processing conditions [73].

4.6. Extraction Using Ultrasounds

The method of ultrasound-assisted extraction is demonstrated to be a simpler and more effective approach in comparison to conventional extraction methods for isolating bioactive compounds from botanical products. Ultrasound enhances mass transfer by promoting increased solvent diffusion into cellular materials, thereby disrupting cell walls. This ultimately leads to the release of bioactive compounds [49]. The extraction yield is significantly affected by the ultrasound frequency, and this relies on the properties of the targeted plant material for extraction. The extraction of three dibenzylbutyrolactone lignans, specifically hemislienoside, arctiin, and tracheloside from *Hemistepta lyrata* was conducted using ultrasound-assisted extraction [74]. The study has investigated the ultrasound-assisted retrieval of isoflavone derivatives (glycitin, genistin, daidzin, and malonyl genistin) from soybeans [75]. The method of ultrasound-assisted extraction was utilized for the retrieval of anthocyanins and phenolic compounds from grape peel [76]. According to a study by Aliaño-González *et al.*, [77] extraction with the assistance of ultrasound was conducted and validated as a swift technique for extracting stilbenes from grape canes, applicable for use in the pharmaceutical and food sector and also examined the impacts of ultrasound technology on extracting polysaccharides soluble in water from by-products obtained from dried and milled *Agaricus bisporus* [78].

4.7. Microwave Assisted Extraction (MAE)

MAE integrates microwave technology with traditional solvent extraction methods, presenting an advanced approach to extraction. The extraction of mangiferin from *Curcuma amada* has been successfully conducted using MAE, with ethanol serving as the solvent [79]. They deduced that the mangiferin content rose up to 500 W but declined with further increases in microwave power. Studies have indicated that employing MAE for extracting saponins from chickpeas (*Cicer arietinum*) results in a greater yield of extracts compared to the extraction method using a Soxhlet apparatus [80]. Chickpea saponin, in its pure form demonstrated activity suppressing the growth of *Penicillium digitatum* and various filamentous fungi. Extracting mangiferin from the leaves of *Mangifera indica* has been accomplished through MAE, utilizing water serving as the solvent. The highest extraction yield, achieving 55 mg/g, was achieved with an extraction time of 5 min, a solid-to-solvent ratio of 1:20, and a microwave power of 272 W [81].

An advanced technique was utilized by Smiderle *et al.* [82]. The combined use of extraction methods involving PLE and MAE was employed to extract polysaccharides, specifically β-glucans with biological activity, extracted from the fruiting bodies of *Ganoderma lucidum* and *Pleurotus ostreatus*. A study reported that, employing response surface methodology, MAE was utilized to optimize the extraction of polyphenols from basil (*Ocimum basilicum* L.) [83]. They employed a mixture of using 50% ethanol, a microwave power set to 442 W, and an extraction period of 15 min. With these parameters, it was determined that the liquid extract of basil exhibited 4.299 g GAE/100 g of total polyphenols and 0.849 g catechin equivalents/100 g DW of total flavonoids. Hence, one can infer that the method assisted by microwaves presents numerous benefits in comparison to alternative methods, such as decreased extraction time,

increased extraction efficiency, enhanced extraction selectivity, and reduced labor demands. These attributes render it a favorable method for extracting bioactive compounds [50].

5. BIORESOURCES OF AROMA COMPOUNDS

A product's ability to be accepted or rejected is directly influenced by its flavoring ingredients. In light of their numerous uses in a variety of industries, including food, cosmetics, and medicines, they are regarded as crucial components in the industrial sector [84]. The increasing consumer concern and desire for flavors derived from natural materials necessitate the development of sustainable and affordable production solutions for these compounds. Although they often employ less damaging environmental circumstances, biotechnology procedures utilizing fungi are regarded as ecologically appropriate and sustainable among these alternatives [85]. Despite the strain and growth circumstances of filamentous fungi, such as basidiomycetes, which affect the ability to obtain bio aromas; their application in biotechnological processes has shown great promise.

5.1. A. bisporus

The fungus *A. bisporus* serves as a model for growth, adaptability, and persistence in humic-rich leaf litter [86]. In addition to its ecological importance *A. bisporus* has been a significant part of the human diet for more than 200 years. One of the most significant edible mushrooms in terms of commerce is *A. bisporus*, also known as the white button mushroom [87]. Mushrooms are rich in micronutrients that are often present in grains, vegetables, and meats, despite being biologically separate from both plants and animals [88]. These include the sulfur-containing amino acid ergothioneine, fiber-associated mono and polysaccharides, pantothenic acid, copper, phosphorus, selenium, and riboflavin. One of the few naturally occurring vegetarian sources of both Vitamin D, which is produced when ultraviolet (UV) radiation causes ergosterol to change into ergocalciferol, and vitamin B12, which is formed from bacteria, is found in mushrooms [89]. The beneficial associations have been shown between these bioactive chemicals and blood pressure, lipid profiles, immune system performance, gastrointestinal health, cancer, and cognitive function. They have also been positively connected to metabolism and weight management. The augmentation of cellular immunity to create immunomodulatory, anti-carcinogenic, anti-microbial, and hypocholesterolemic effects, as well as their impacts on the gastrointestinal microbiota, are thought to be primarily responsible for these positive health outcomes [90,91].

A. bisporus is a popular fungus in the global food industry that is 30% of all mushroom production worldwide. *A. bisporus* immature forms in two colors: brown and white. The brown one is referred to as a brown cap mushroom, whereas the white one is termed a button or common mushroom. Portobello mushrooms are mature *A. bisporus* mushrooms. Because *A. bisporus* lacks a cuticle layer to protect its skin, it is susceptible to both physical and microbiological harm [92]. According to reports, *A. bisporus* mushrooms have a shelf life of 1–3 days at room temperature (20–25°C), 5–7 days at 0–2°C, and around 8 days in a refrigerator [93]. Mushrooms have a short shelf life, which reduces their economic value. Mushrooms undergo a number of quality degradations throughout the postharvest phase, including moisture loss, discoloration, texture changes, off-flavor, and nutrition loss [94]. The moisture content of fresh mushrooms ranges from 85% to 95%. Because this much moisture promotes microbial growth, mushrooms should be maintained at low temperatures to reduce the amount of bacteria that may contaminate them [95]. It has frequently been stated in the literature that eight carbon aroma compounds, such as 1-octen-3-ol, 3-octanol, (E)-2-octenal, 2-octen-1-ol, and 1-octen-3-one, are the main characteristics of raw mushrooms. These compounds are typically formed through enzymatic or non-enzymatic lipid oxidations, with the breakdown of oxylipins by linoleic acid being the most common process. Among these, 1-octen-3-ol and 1-octen-3-one are thought to be marker fragrance compounds in a variety of mushroom species, giving off a distinct scent resembling that of mushrooms. Although more than 150 volatile chemicals have been found in various mushroom species, only a small percentage of these compounds actually give the mushrooms their distinctive perfume [96].

According to a study, the primary important odorants in both mushroom samples were 1-octen-3-one (mushroom-earthy), methional (boiled potato), and 1-octen-3-ol (mushroom). When the main odorants of the two varieties of mushrooms were compared, it was discovered that the oyster mushroom had higher terpene content than the champignon mushroom. Among these terpenes, the study identified (E)-caryophyllene, α-humulene, (E)-carveol, and (Z)-carveol as fragrance-active chemicals in mushrooms for the first time [97]. According to a study, *A. bisporus* extracts have good efficacy and may be a suitable natural raw material for cosmeceutical treatments that treat xerosis [98]. In a report, ABE has the potential to be a contender for creating a functional food component with anti-obesity qualities [99]. A study documented that omission experiments and odor activity value analysis allowed for the identification of seven essential scents. In addition, 1-octen-3-one was shown to be the primary odorant in cooked button mushrooms. The findings of the study may be useful for improving the flavor of cooked button mushrooms [100].

5.2. *Aspergillus* spp.

The asexual spore-forming structure known as the aspergillum is present in all *Aspergillus* species, with approximately one-third of them being known to possess a sexual stage [101]. Conidial fungi, or fungus in an asexual stage, are referred to as *Aspergillus*. A provisional classification of *Aspergillus* members into the Ascomycota can be made, nevertheless, since some of them are known to be in the Ascomycota and to have a teleomorph (sexual state) [102]. *Aspergillus* spp. was utilized for SSF after it was discovered to be a superior lipase producer in SmF. One of the main sources of bioactive compounds with fungal origins is the *Aspergillus* species. Aromatic butenolides are distinctive *Aspergillus* spp. metabolites that show a variety of biological properties, including antibacterial, cytotoxic, anti-inflammatory, and antioxidant properties [103]. The substrate with the highest lipase activity was wheat rava, followed by Bombay rava, oil cakes made of coconut, groundnut, sesame, and crushed soybeans. Ribeiro *et al.* [104] showed that, among the many filamentous fungi examined for the synthesis of xylanase, *Aspergillus clavatus* displayed the largest halos across all tested temperatures. After 48 h at 30°C, *Aspergillus terreus* produced large amounts of thermotolerant extracellular xylanase and exhibited little cellulase activity [105].

The quantity of CO_2 emitted during the biodegradation of petroleum hydrocarbons as a measure of *Aspergillus* spp. activity [106]. Zheng *et al.* [107] accomplished vanillin from the leftover rice bran oil waste by *Aspergillus niger* and *Pycnoporus cinnabarinus*. According to a study, *A. terreus* produces new natural aromatic buteolides that contain maleimide and asperimides A–D, and are anti-inflammatory. These discoveries broaden the chemical range and biological variety of aromatic buteolides [108]. A study concluded that the discovery of

phyA made it possible to establish a fungal cell factory that can produce protocatechuic acid from benzyl alcohol, benzaldehyde, benzoic acid, caffeic acid, cinnamic acid, cinnamyl alcohol, m-hydroxybenzoic acid, p-hydroxy-benzyl alcohol, p-hydroxybenzaldehyde, p-hydroxybenzoic acid, p-anisyl alcohol, p-anisaldehyde, p-anisic acid, p-coumaric acid, and protocatechuic aldehyde [109]. The primary products of *A. niger* and *A. baumanii* are coumarin and sinapic acid, which are aromatic compounds that can be processed into high-value goods [110].

5.3. *Candida* spp.

The genus *Candida* is classified as incerta sedis, or of unknown placement, and it is a member of the order *Saccharomycetales* in the phylum Ascomycota [111]. There are 314 species in the phylogenetically diverse genus *Candida* which occur in both natural and artificial environments. *Candida* are widely dispersed because of their damp and moist characteristics, high concentration of organic material (including ethanol and organic acids), wide temperature range, and high osmolarity of salt and sugar. There have been thousands of years of evidence linking some species to the transformation of foods and feeds [111]. *Candida* are beneficial for commercial and biotechnological activities because of their high biochemical potency. Many biotechnologically interesting chemicals, including higher alcohols, organic acids, esters, diacetyl, aldehydes, ketones, acids, long-chain dicarboxylic acids, xylitol, and glycerol, are produced using *Candida* yeasts. Additional products include D-β-hydroxyisobutyric acid, biotin, and nicotinic acid. The capacity to synthesize sophorosides when growing on substrates such as n-alkanes, alkenes, fatty acids, esters, or triglycerides is another characteristic displayed by certain strains of *Candida*. Moreover, the genus *Candida* has the ability to release highly valuable extracellular enzymes, including pectinases, β-glucosidases, proteases, invertases, amylases, and lipases [112].

Nonetheless, the fungus *Candida* is capable of producing a large range of secondary metabolites and adapting to a wide range of substances in their surroundings [113]. *Candida* spp. produces the metabolite 4-hydroxy-l-tetralone, also known as 4-hydroxy3,4-dihydro(2H) naphthalenone. The use of coconut oil cake as a solid substrate results in a potent substrate for the synthesis of lipase by *Candida mgosa* in solid-state fermentation (SSF). According to a study, the ortho-cleavage route is the mechanism by which *Candida oregonensis* yeast strains metabolize phenol. This is demonstrated by the identification of catechol 1,2-dioxygenase activity and the presence of cis and cis-muconic acid in the examined samples [114]. A study found that the use of *Candida parapsilosis* cells may be beneficial for the biodegradation of crude oil and maybe other aromatic chemicals that are related [115]. A study documented that 1H and 13C NMR analyses demonstrated the presence of significantly more aliphatic and significantly less aromatic compounds. Furthermore, the existence of amide groups, phenols, esters, and alkanes in *C. serrulata* ethyl acetate extract fraction 7a was verified by FTIR analysis. This study implies that fraction 7a exhibits strong anti-*Candida* activity against fungi that cause candidiasis. In addition, dose-dependent are fraction 7a's anti-diabetic and antioxidant properties [116].

5.4. *Lentinus edodes*

The second most common edible medicinal mushroom in the world, *Lentinula* edodes, is a shiitake fungus that is rich in flavor and nutrients. It is extensively grown throughout many Asian nations. Approximately 25% of all edible fungi produced worldwide is produced by *L. edodes*, whose production has expanded more quickly than that of any other mushroom [117]. Rich in vital amino acids, dietary fiber, vitamins, and minerals, yet low in calories and fat, *L. edodes* are important both as food and medicine and bioactive substances found in it, such as glycoprotein derivatives, polysaccharides, terpenoids, steroids, phenols, and nucleotides. Traditional Chinese medicine believed that *L. edodes* mushroom was useful for intestinal worms, lung disorders, and heart health [118]. It was also used as a tonic to combat the discomfort and exhaustion that come with aging. *L. edodes* has become more well-known recently because of its beneficial nutritional profile and bioactive ingredients [119]. In addition to being high in dietary fiber, shiitake mushrooms are an excellent source of provitamin D2, Vitamin B1, B2, B12, and niacin. Furthermore, the extract or ingredients have been used medicinally to treat conditions such as cancer, heart disease, hyperlipidemia (including high blood cholesterol), hypertension, infectious diseases, diabetes, hepatitis, bronchial inflammation, recurrent flu and cold episodes, fungal infections, cancer, and environmental allergies [120].

Several scientific studies have been conducted to determine which *L. edodes* active compounds provide the health advantages mentioned in conventional medicine. High-molecular-weight substances, including peptides and polysaccharides, have been identified as the majority of bioactive agents. On the other hand, pharmacologically active small compounds have also been detected. According to a study, *L. edodes* has significant levels of primary metabolites and bioactive substances with potent antioxidant activity [121]. Another study found that using GC-MS, 50 volatiles from various variable frequency drive stages were found in *L. edodes.* For fresh *L. edodes*, frozen *L. edodes*, and secondary dried *L. edodes,* the primary flavoring ingredients were alcohols, aldehydes, and volatile sulfur-containing compounds. After first drying, the predominant scent groups in *L. edodes* were aldehydes, ketones, and volatile sulfur compounds [122]. A study concluded that applying lignin at the ideal concentration of 0.10% could improve *L. edodes* nutritional and medicinal qualities in addition to promoting mycelial development and phenolic acid accumulation [123].

5.5. *Mucor* spp.

The filamentous fungus *Mucor* spp. is a member of the order Mucorales and the subphylum Mucoromycotina and can cause mucormycosis, also called zygomycosis, a serious infection that manifests in individuals with compromised immune systems [124]. *Mucor* spp. exhibited a high degree of industrial potential when it began to produce bioactive substances of interest, including sterols, carotenoids, and microbial lipids [125]. This makes carotenoids tetraterpenes that are generated from the 40-carbon isoprenoid phytoene. They are essential for photosystem health because they can absorb solar radiation and store energy. Strong scavengers of free radicals and antioxidants, carotenoids have the ability to influence the etiology of some malignancies and cardiac conditions. A study documented that *M. circinelloides* cultures can be used to create high-level, regulated production systems for sterols and carotenoids, two significant substances with a variety of biological functions [126]. The study also suggests that it is technically possible to produce biodiesel in treated *M. circinelloides* cultures without the need for an acid catalysis step. This is a step toward developing this process on an industrial scale [127]. A study found that MSEPS induced SGC-7901 cell death through apoptosis, as demonstrated by morphological examination and flow cytometry tests. As a result, MSEPS from *Mucor* spp. may be created as a possible anticancer medication [128].

5.6. *Saccharomyces* spp.

Due to its extensive use in food and beverage fermentation, where it has substantial economic value, *Saccharomyces* spp. has been an

essential component of human civilization. Around 30% of the one million tonnes of yeast produced in the European business each year is exported to other countries. From 2013 to 2018, the yearly growth rate of the global market was 8.8%. Regarding the beverage industry, *Saccharomyces cerevisiae* is used in the fermentation of numerous fermented drinks, such as wine, beer, and cider; distilled drinks, such as rum, vodka, whisky, brandy, and sake [129]. In addition, *S. cerevisiae* is used in the production of other alcoholic beverages from fruits, honey, and tea around the world [130]. Either the raw material microflora developing naturally or the addition of a pure yeast culture might cause fermentation. The role of *S. cerevisiae* in the fermentation processes of wine, bread, and cocoa is then discussed, emphasizing various points including the biochemical reactions that occur within the cell and whose byproducts define the end products, the characteristics that strains need to possess to be effective starters, and the possibility of using native strains in the industry [131].

Having a scent reminiscent of roses, 2-phenylethanol is one of the most important alcohols linked to aromas. It is still mostly made from toluene, benzene, styrene, or methylphenylacetate using petrochemical processes Nomura *et al.* [132], Yet, a costly procedure is primarily used to extract the natural 2-phenylethanol from rose petals. *Saccharomyces* spp. yeast strains have demonstrated a significant potential for the industrial manufacture of fragrance compounds, such as 2-phenylethanol, which is produced through bioconversion from 2-phenylalanine [133,134]. Numerous *Saccharomyces* have been found to contain b-glucosidases, which can be employed to liberate volatile terpenes and flavor precursors that are glycosidically bound and improve the scent of particular wines. For the fermentation of hexoses into ethanol, *S. cerevisiae* is the most prevalent microbe [135,136]. According to a study, *S. cerevisiae* has been shown to have more volatile fragrance molecules. Consequently, *S. cerevisiae* may significantly increase the amounts of polyphenols and volatile fragrance components in Nanfeng tangerine wines [137]. Another study found that the incorporation of *Pichia kudriavzevii* and *S. cerevisiae* into a mixed fermentation technique increased the amounts of phenylethanol, isoamyl alcohol, and ethyl esters, which improved the wine's fruity and rose-like flavor. This work offers a different strategy for enhancing wine aroma and phenolic profile [138].

5.7. *Trichoderma* spp.

The Ascomycota division comprises the genus *Trichoderma*, which is made up of around 100 species of filamentous fungi. They often appear on plant root surfaces, decomposing bark, and other organic components in soils throughout the world. *Trichoderma* members are known to be effective colonizers of their environments and possess powerful enzymatic machinery, including cellulases, chitinases, glucanases, and proteases, among others, for the breakdown and utilization of substrates found in soils, but particularly for the degradation of lignocellulosic material [139]. Members of this fungus have been studied in relation to a variety of physiological characteristics and biotechnological applications. Due to their ability to produce secondary metabolites known as novel antibiotics (polyketides, pyrones, terpenes, amino acid derivatives, and metabolite polypeptides) [140]. *Trichoderma* members are employed extensively in the food business, as a biological control agent, and for the commercial manufacture of lytic enzymes; nevertheless, their application in xenobiotics biodegradation is restricted. *Trichoderma* is a unique genus that exhibits a wide variety of substrate utilization, a high level of antibacterial chemical synthesis, and environmental opportunism [141]. Numerous high- and low-molecular-weight polycyclic aromatic hydrocarbons (PAHs), including naphthalene, phenanthrene, chrysene, pyrene, and benzo[a]pyrene, are known to be metabolized by a number of *Trichoderma* species. They are abundant in bioactive polyketides, non-ribosomal peptides, and terpenes. Peptaibols, gliotoxin 2, and 6-pentyl-2H-pyran-2-one 1 are the most researched and well-known secondary metabolites of *Trichoderma*. A study concluded that three metabolites were found during the pyrene degradation process: acetic acid, 3-hydroxybenzoic acid, and benzoic acid [142].

6. OPTIMIZATION OF THE PRODUCTION OF AROMA COMPOUNDS

Aroma compounds are intricate substances composed of various components, both volatile and non-volatile, with distinct physicochemical characteristics. These compounds play a role in generating odor perceptions in our brains. Both volatile and non-volatile components exhibit distinct properties; for instance, non-volatile components are accountable for taste, whereas volatile ones contribute to both taste and aroma [143]. Natural aromas are present in spices, essential oils, food items, as well as in flowers or plants. However, the current desire for natural products and the necessity for processes that are environmentally friendly have spurred the advancement of innovative processes for flavor synthesis through biotechnology. Flavors find extensive uses in food, cosmetics, fragrance, and in the pharmaceutical sector [7]. The majority of flavors are derived from either plant sources or synthesized chemically. Although naturally occurring aromas can be extracted, their high cost is attributed to the low concentration of the aroma compounds [144]. As a result, the exploration of alternative methods to acquire these compounds has gained significant interest in recent years [145]. Researchers have developed various biotechnological methods for generating aromatic compounds. The application of biotechnological approaches in the production of aromas is environmentally friendly and holds great promise as substitutes, owing to the capability of certain microorganisms to convert specific components of raw materials into aroma compounds. Aroma compounds can be generated by microorganisms through the fermentation of amino acids and sugars [146]. Bacteria, fungi, and yeasts are recognized for generating aroma compounds, yet the industrial utilization of specific microorganisms is restricted due to their widely considered generally regarded as safe (GRAS) status.

A study documented that two Kluyveromyces strains, specifically *K. marxianus* and *K. lactis*, were identified as contributors to the production of compounds such as isoamyl acetate and monoterpene alcohols (imparting a fruity aroma) produced in liquid fermentation [147]. Notably, *K. marxianus* falls within the GRAS category of microorganisms. SSF is deemed a suitable technique for the generation of aroma compounds because it employs agro-industrial residues for microorganism cultivation. It is considered advantageous in various aspects compared to liquid fermentation, particularly in bioprocesses where yeasts or filamentous fungi are engaged [148]. Yeasts and fungi have been cultured using SSF to generate aromas, such as in the case of *Neurosporas* [149], *Zygosaccharomyces rouxii* [150], *Aspergillus* spp. [151], *Trichoderma viride* [152], is grown utilizing pre-gelatinized rice, miso, cellulose fibres, and agar, respectively. Sulieman *et al.* [153] investigated generating a fruity aroma by *Ceratocystis fimbriata* through SSF with *Cassava bagasse* wheat bran and using sugar cane bagasse as the substrate. The cultivation of *Rhizopus oryzae* cultivated on residues from tropical agro-industry resulted in the production of acetaldehyde and 3-methyl butanol [154]. Kelly *et al.* [155] noted that the generation of aroma includes elements such as short to medium-chain free fatty acids, alcohols, esters,

dicarbonyls, aldehydes, lactones, methyl ketones, sulfur compounds, and phenolic compounds.

Lindsay *et al.* [156] conducted on investigation into the utilization of citrics pulp in solid-state cultures for producing volatile aromas by *C. fimbriata*. Their findings revealed that citric pulp, when enhanced utilizing sugarcane molasses, a mineral saline solution, and soya bran resulted in a distinctive fruity fragrance. Mantzouridou, and Paraskevopoulou [157] documented the generation of volatile bio-esters through the utilization of a commercial wine yeast strain (Vitilevure MT) in a SmF. This process involved orange peel (OP) supplemented with a nutrient-rich medium comprising yeast extract, salts, glucose, or hydrolysate from OP.

7. INDUSTRIALLY IMPORTANT AROMA COMPOUNDS

Aroma compounds are extremely important in many sectors since they are necessary ingredients in consumer goods such as cosmetics, meals, medications, and perfumes. Unfortunately, this substance is labile and volatile and is readily lost during the production process, storage, and use [158]. Aromatic compounds, including terpenes, esters, aldehydes, and alcohols, can be produced by bacteria, filamentous fungi, and yeasts by biotransformation or *de novo* synthesis. These substances have broad applications in food industries as natural or organic additives, known as bioaromas. A number of techniques, including improved recovery and purification procedures, product formulation, and the use of alternative substrates as fermentation media, have been developed to increase the production of natural additives from biotechnological processes [159]. At present, about 300 aroma compounds are available for purchase, with market prices ranging from US$ 100 to US$ 500/kg [10]. The aroma sector has been urged to use biotechnological procedures to create fragrance compounds due to the expanding market demand and consumer preference for natural products [160]. The food industry, which is of significant commercial importance, supports the flavoring and preservation of food items through aromatic volatiles, as well as the search for aromatic raw materials in nature for perfume and cosmetic products. In addition, it serves as a de-foaming agent for ophthalmic solutions containing high surfactant concentrations. They are also used to improve the self-life and safety of minimally processed fruits [161,162].

7.1. Lactones

Lactones are interamolecular or cyclic esters (or "R−C(=O)−O−R") created when the appropriate hydroxy acid is esterified between molecules. Most lactones are thought to be powerful scent molecules because they have low odor thresholds [163]. Lactones are aroma substances with a typical fruity smell and help in creating creamy sensations. They may be made by biotransformation of fatty acids or by chemical synthesis [22]. They can be categorized chemically as intramolecular esters of hydrocarboxylic acids with various ring diameters. Due to the durability of the ring structure, the most prevalent lactones are γ- and δ-lactones with five- and six-membered rings [164]. γ-decalactone contributes a strong fruity, particularly peach-like, smell that has a market value of several hundred tons per year. Natural 4-decanolide was an exceedingly rare and expensive natural flavor in the early 1980s. Similar to δ-decalactone, the closely related 5-decanolide can be found in numerous fruits and dairy products. It smells such as peaches and creamy coconut [2]. *Tyromyces sambuceus* and *Cladosporium suaveolens* efficiently produce the coconut flavored lactones (γ-decalactone and δ-dodecalactone) from ricinoleic acid and linoleic acid, respectively [165]. *Candida tropicalis* and *Yarrowia lipolytica* are examples of yeasts that break down ricinoleic acid into C-16, C-14, and C-12 acids. Interestingly, they additionally produce δ-decalactone, which has fruity and oily overtones that are crucial for producing fragrances of peaches, apricots, and strawberries. Because of its anti-inflammatory properties, dehydrocostus lactone, a naturally occurring sesquiterpene lactone, has been utilized to treat a number of illnesses. Recently, it has gained complete interest in investigators due to its anti-cancer properties in some types of carcinomas [166].

7.2. Esters

Esters are a diverse group of compounds with a wide range of uses, including flavors, scents, medications, cosmetics, green solvents, and cutting-edge biofuels. Although there has been a limited supply of natural esters, there is a growing global demand for them in the food, household cleaning, personal care, and perfume industries [167]. At low concentrations, ester has a nice, fruity scent but when present in large quantities, it is also thought to taste bad. The most volatile ester in food is ethylacetate [168]. The fatty acid acyl- and acetyl-Coenzyme A (CoA) pathways in yeast metabolism are primarily responsible for their production. CoA is used to activate intermediates during the production of medium-chain fatty acids, and it is an essential cofactor for several metabolic processes. The production of ethanol and acyl-CoA intermediates is achieved through estrification, which is facilitated by the activity of the enzymes esterase and transferase [169]. Utilizing a variety of yeasts, including *S. cerevisiae* and non-saccharomyces species such as *Candida*, *Hansenula*, and *Pichia*, whose unique enzymatic mechanisms enable the introduction of novel scents in wines, different yeasts have been employed to add complexity to wines through ester synthesis [170].

7.3. Vanillin

Vanillin, or 4-hydroxy 3-methoxy benzaldehyde, is a phenolic aldehyde that is aromatic and has distinct chemical characteristics as well as pharmacological effects. Owing to its strength, it serves as a model for drug discovery and development methods [171]. Vanillin can be synthesized through direct fermentation with glucose, chemical synthesis, extraction from vanilla plants, and microbial bioconversion of natural precursors. Nowadays, the majority of vanillin that is sold commercially is produced by extracting it from cured vanilla pods and then chemically synthesizing it with glyoxylic acid and guaiacol as the initial raw ingredients [172]. It is a white substance that dissolves in water and is the primary fragrance component that gives real vanilla its creamy, sweet smell. Due to high demand across several industries, the vast majority of vanillin used today is manufactured synthetically, and only less than 1% of the global vanilla market is derived directly from traditional natural sources [173].

Vanillin has three chemically modifiable functional groups (methoxy, aldehyde, and hydroxyl) and a distinct aromatic structure [174]. Recently, the bioactive properties of vanillin including its neuroprotective, antioxidant, anti-inflammatory, and anti-carcinogenic effects have drawn more attention and enhanced its possible applications [175]. This flavoring ingredient is valuable and widely utilized in the food and pharmaceutical industries. There are three different types of vanillin flavoring compounds: synthetic, natural, and biotechnologically produced. Food safety authorities consider vanillin developed from biotechnology to be identical to vanillin found in nature [176]. A possible anticancer chemical called ferrocenyl 4-trifluoromethylbenzoate 31 has been prepared using vanillin as a precursor [177].

7.4. Terpenes

Terpenes are derived from the 5-carbon precursor isopentenyl diphosphate (IPP), which is produced by plastids using the methylerythritol phosphate pathway from either pyruvate or glyceraldehydes [178]. These substances are not only found in plants but also in most other living things on Earth, such as bacteria, fungi, insects, marine organisms, mammals, and protozoa. They play countless structural and functional roles in these species [179]. Many biological characteristics of terpenoids are documented, such as their antifungal, antiviral, antibacterial, antihyperglycemic, anti-inflammatory, and antiparasitic qualities and their ability to prevent cancer by chemoprevention [180]. To recover the essential oils of particular aromatic plants, terpenes or terpenoids are extracted or steam distilled. These steam distillates are used to make high-quality perfumes, enhance the flavor and scent of food and beverages, and make phytopharmaceuticals or plant-based medications. Since the majority of these terpenoids have been shown to be high-value molecules in food, cosmetics, pharmaceuticals, biotechnology, and industrial crops, consumers' interest in natural products has grown in recent years [181]. Terpenes are mostly employed in the pharmaceutical sector as novel anti-cancer medications. For instance, element suppresses brain tumors, liver cancer, nasopharyngeal carcinoma, lung cancer, and other types of cancer naturally. It also has somewhat harmful side effects [182]. Furthermore, many terpenes are utilized in clinical medicine, including pinene and paclitaxel. In addition, terpenes can be synthesized into different products, including curing agents, adhesives, and pesticides, which are typically used in chemistry, agriculture, and other disciplines [183].

7.5. Pyrazines

Pyrazines are a class of nitrogen-containing volatile heterocyclic chemicals that give food items their nutty, baked, and roasted flavors. One crucial method of producing pyrazines is the Maillard reaction (MR). The production of pyrazines from the MR is anticipated to be encouraged during food processing, as they are desirable volatile chemicals [184]. Compounds based on pyrazine have a significant role in medical chemistry. Due to their heteroaromatic property, they combine the polar interactions of heteroatoms with the nonpolar interactions of aromatic moieties in a unique way [185]. Natural pyrazines are frequently used as flavorings in roasted and raw foods. These include mostly methyl- or ethyl-substituted forms. Alkylpyrazines, on the other hand, are employed as food preservatives because of their potent antibacterial properties. These naturally occurring pyrazines are extensively found in a variety of biological systems, including insects, plants, and mammals, each of which has a specific physiological function. Furthermore, fermentation and heat treatment are the processes by which pyrazines are produced in food [186]. Due to its ubiquitous use as a framework in the synthesis of bioactive components, catalysts, and reactions in various media, it has recently been the subject of a variety of approaches. Pyrazine compounds have a range of pharmacological actions, such as antipyretic, analgesic, antibacterial, anticancer, and antioxidant properties. It can be applied to several kinds of coupling reactions, such as oxidative, Suzuki, and Buchwald-Hartwig coupling reactions [187].

7.6. Acetoin

Acetoin is a high-value-added bio-based platform chemical that finds extensive applications in the chemical industry, food industry, cosmetics, and agriculture. It is a crucial precursor for the production of heterocyclic chemicals, liquid hydrocarbon fuels, and 2,3-butanediol. The biological synthesis of acetoin has gained more interest as a substitute for chemical synthesis due to the depletion of fossil resources [188]. Under specific conditions, various microbes, higher plants, insects, and higher mammals can synthesize acetoin through distinct enzymes and routes. Acetoin is an extremely active molecule that functions as a precursor to numerous other chemicals. Consequently, acetoin and its derivatives are commonly found when utilizing GC-MS for component analysis of a range of foods [189]. The first step in the biosynthesis of acetoin is the condensation of two pyruvate molecules into acetolactate, which is catalyzed by acetolactate synthase. Acetoin is produced when acetolactate is converted to acetoin by acetolactate decarboxylase. These two enzymes from *Lactococcus lactis* and *Escherichia coli*, respectively, have been installed to demonstrate acetoin synthesis from glucose in *E. coli* [190].

7.7. 2-Phenylethanol

2-Phenylethanol (2-PE) is a fragrant alcohol that has a rose-like fragrance. It is primarily produced through chemical synthesis and is extensively used in the food, cosmetic, and fragrance sectors [191]. Numerous international organizations, such as the Flavor and Extract Manufacturers' Association, the Joint Expert Committee on Food Additives, the Food and Drug Administration, and the Council of Europe, have approved this flavor. They consider 2-PE as GRAS (2858), which gives it an added value [192]. Conventionally, two main methods of producing 2-PE were chemical synthesis or extraction from plant sources. However, these approaches cannot satisfy the growing consumer desire for natural flavors. Because the biological synthesis of 2-PE is an environmentally beneficial technique and meets the definition of a "natural" product, it has become an attractive solution [193].

The production of natural 2-PE can be achieved using biotechnological methods or by extracting essential oils from different flowers, such as jasmine, hyacinths, and roses. As a matter of fact, the scarcity of naturally occurring 2-PE in flowers has resulted in an inability to meet the substantial market demand and a high selling price [194]. Improved 2-PE bioproduction has been greatly aided by recent metabolic engineering techniques in yeasts and *E. coli*. These techniques have reduced by-products, improved precursor transport, lessened feedback inhibition, and enhanced the activity of key enzymes [195]. Through the use of the Ehrlich and Shikmate pathways, yeasts that have undergone metabolic engineering can produce 2-PE from glucose or l-phenylalanine (l-Phe) as substrates [196]. 2-Phenylethanol (2-PE) and 2-Phenethyl acetate are frequently used in industry and are universally recognized as safe flavoring agents. These compounds have a rose-like odor, which makes them useful ingredients in polishes, personal care items, perfumes, and medications. Furthermore, because 2-PE has the potential to biocide, it is utilized in cleaning products, disinfectants, and pest control products [197].

7.8. Alcohols

One of the main ingredients in alcoholic beverages is alcohol. It has an impact on the sensory profile and is frequently applied to classify alcoholic products [198]. Some food items with a particular smell have been made with unsaturated alcohol. Various yeast species possess complex alcohols that possess an extensive range of organoleptic characteristics [199]. The main alcohol produced by lactic acid fermentation is thought to be ethanol. It is created by the breakdown of glucose and amino acid catabolism processes. In the ethanol synthesis pathway, glucose is broken down by ATP to produce CO_2, ethanol, and

lactic acid. Acetaldehyde is degraded during the synthesis of alcohol, which lowers the amount of acetaldehyde in the medium [200].

8. BIOTECHNOLOGICAL APPLICATION OF AROMA COMPOUNDS

Fragrances and flavors are pivotal elements within the food, cosmetic, feed, and pharmaceutical sectors. The process of obtaining flavors and fragrances from natural sources has roots in ancient times [201]. Historically, aroma compounds and flavor compounds were derived from both animal and plant sources. To address the substantial demand and cost considerations, many products are witnessing a growing introduction of synthetic chemicals. However, due to chemophobia and health concerns, consumers often find artificial flavors and fragrances unacceptable [13]. Biotechnological approaches offer more environmentally friendly alternatives to artificial flavors and fragrances. While most aroma products were traditionally synthesized chemically or extracted from plants, the utilization of innovative biotechnological processes has significantly risen in recent decades [202]. Food processing activities, spanning from premature harvesting to prolonged storage and physical treatments, can result in a loss of aroma. Although conventional methods such as synthetic production or extraction from plants remain Feasible, the biotechnological production of aroma compounds is gaining increasing appeal [203]. The biotechnological method provides supplementary benefits. Bioactive compounds are represented by flavors, and given the recognized influence of chirality on odor perception, employing biocatalysts is recommended [20]. Regulations regarding the assessment and adjustment of regulations governing flavoring and fragrance in Europe and the USA now include advancements in scientific and technological approaches for biotechnological aroma compound production. According to the guidelines stipulated in the European Code and the US Code of Federal Regulations, compounds obtained through enzymatic, microbiological, or physical processes using precursors isolated from animal, microbiological, or vegetable sources may fall under the classification of "natural" [204].

8.1. Industrial

The incorporation of flavor and fragrance compounds is deeply ingrained in our contemporary society. These compounds find widespread use in sectors such as the food industry (including processed food, beverages, and ready-to-eat meals), agrochemicals, household products (including soaps and detergents), cosmetics (toiletries, encompassing high-quality perfumes and body care products), and medications or drugs (including nutraceuticals and dietary supplements) [205]. The global market value for flavor and fragrance compounds was projected to reach USD 28.2 billion in 2017 and is anticipated to exhibit a compound annual growth rate of 5.3% until 2023. This expansion is motivated by the increasing demand for aroma compounds [206]. The majority of existing flavor compounds are currently manufactured through chemical synthesis or extraction. Despite numerous microbial processes being identified for generating intriguing flavors, their industrial applications are relatively constrained. In many instances, the primary factor behind this limitation is the low yield. Microbial flavors are frequently found in fermentation broths in relatively low concentrations, leading to elevated costs for downstream processing. Nevertheless, The market price of natural aromas, which is 100 times higher than that of synthetic aromas, offsets this [200]. As an illustration, natural source-derived γ-decalactone (flavor compound present in peaches) is valued at around US$6000/kg, whereas the synthetic version is priced at US$150/kg [13]. In this context, an increasing demand exists for biotechnology to offer alternatives in the production of natural flavorings and fragrances [207]. Even though aroma compounds generated through chemical synthesis are extensively used in industry which still holds a significant market share because of satisfactory yields, this approach is also associated with several environmental challenges. Moreover, it often lacks sufficient region- and enantio-selectivity toward the substrate, leading to the formation of a mixture of molecules [208]. Furthermore, the rising demand for products labeled as "natural" has prompted extensive research into the microbial production of what is known as 'bioflavors' [209].

8.2. Food

The aroma is a critical attribute of food directly linked to consumer product acceptance. Among various methods, the interest in aroma compounds produced through biotechnology has surged in recent decades to obtain these compounds. Considered a sustainable method of supplying natural additives for the food industry, this approach [22]. Hence, aroma has been recognized as a pivotal factor linked to the acceptance of food. It constitutes a significant portion of the market for food additives. In the food processing sector, biotechnology employs microorganisms for both food preservation and the creation of various value-added products, including flavor compounds, enzymes, vitamins, food ingredients, and microbial cultures [210]. The environmentally friendly nature of biotechnological aroma compound production is recognized, given that these bioprocesses occur under gentle conditions, eliminate the need for potentially harmful catalysts, and pose fewer challenges in waste management [211]. Aroma production through biotechnology is a meticulously managed process, providing a virtually limitless source of compounds that can be produced continuously. Consequently, The production of flavors through biotechnology is seen as a strategy that aligns with the three pillars of environmental, sustainability, social aspects, and encompassing economics [212]. Metabolic engineering focused on the production of aromatic compounds has been primarily centered on a limited number of microorganisms, notably *S. cerevisiae*, *E. coli*, *C. glutamicum*, and *P. putida* [213]. In the initial stages commercialization of bioprocesses utilizing *E. coli* paved the way for subsequent applications, thanks to regulatory considerations [214]. Baker's yeast, scientifically known as *S. cerevisiae*, boasts the lengthiest conventional practices in biotechnology, spanning for millennia in baking and brewing. It is also the most extensively examined eukaryotic microorganism [215]. *P. putida* exhibits notable tolerance to organic solvents, emerging as a favorable candidate for the bioremediation of aromatic compounds. The KT2440 strain, possessing GRAS status, has been extensively researched for potential uses in industrial biotechnology. This soil bacterium of the Gram-negative type adeptly handles xenobiotics and various commonly toxic substances [216]. A bacterium namely, *C. glutamicum,* classified as Gram-positive, is utilized as a GRAS organism widely used in the food and feed industries. It has a six-decade track record of safely producing amino acids [217].

8.3. Agricultural

As the population continues to grow, the environment undergoes significant changes, with agriculture being particularly susceptible to these shifts and encountering various challenges such as pollution, pathogenic attacks, salinity, drought, high and low temperatures, and more. These challenges inevitably impact productivity. To address such issues, eco-friendly approaches become crucial. Inevitable transformations in agriculture and food production are on the

horizon. Meeting the food demands of a growing population poses a considerable challenge, compounded by the necessity to alleviate the adverse environmental effects associated with conventional agriculture [218]. The key to delivering nutritious, safe, and healthy food, as well as producing chemicals and innovative materials with minimized resource inputs such as energy, land, and water, lies in industrial biotechnology. In addition, this approach offers the potential for achieving seasonal and geographical independence and reducing waste [219]. Agricultural sources, despite containing trace concentrations of active components, rely on natural factors that are challenging to manage factors such as weather conditions and plant diseases. Employing agricultural raw materials as a sustainable resource for both the chemical industry and fuel aligns with various expectations. As a result, these advancements are enthusiastically embraced, especially acknowledged by the agricultural community is the importance of industrial biotechnology [220]. In this context, biotechnological applications often involve the use of genetic engineering, selective breeding, or microbial consortia to regulate the synthesis generation, and emission of aroma compounds [221]. Considering the potential environmental and regulatory implications of this biotechnological intervention is crucial. In addition, it is frequently recommended to embrace a comprehensive and sustainable approach that combines biotechnological advancements with other agricultural practices for enduring benefits [222].

8.4. Medicinal

As of now, industrial biotechnology has reached its highest level of adoption in the pharmaceutical sector and fine chemicals, accounting for 15%, and is experiencing continued robust growth [223]. The pharmaceutical industry consistently requires new formulating aids to improve the deficient physical properties of new active ingredients and to reformulate existing drugs [224]. Antibiotics and their intermediates are regarded as highly significant fine chemicals, constituting a global market worth approximately 20 billion euros [225]. At present, biotechnological methods are increasingly substituting chemical modifications, offering notable economic and ecological advantages [226]. In the fine chemical industry, Lonza has led the way in a biotechnological process that initiates with 3-cyanopyridine, leading to the production of nicotinamide (niacin or Vitamin B_3), nicotinic acid and 6-hydroxynicotinic acid. At present, Industrial biotechnology is employed to produce these essential intermediate products crucial for various chemical syntheses [20]. The application of biotechnology in medicine seeks to harness the therapeutic properties of natural fragrances for various health-related purposes using aroma compounds [227]. Within the medical field, biotechnological applications of aroma compounds include enhancing their production, unraveling their mechanisms of action, and incorporating them into various therapeutic approaches to improve human health [228].

9. CONCLUSIONS

In the last several years, there has been a significant advancement in the development of techniques for producing natural flavor and aroma chemicals employing biotechnology techniques. Employing enzyme preparations or microbial cultures has a number of advantageous characteristics over conventional techniques. Through the implementation of biotechnological methods, natural flavors are produced, improving their suitability for ingestion. Exploiting the enantioselectivity of the enzymatic reactions involved in the biosynthetic pathways is one of the very novel procedures required in the field of bio-flavors to minimize production costs and improve the diversity of generated chemicals. It is also very important to make wise decisions about the type of reactor and its operating parameters. To achieve both economic and environmental performance, a number of criteria must be optimized. The most crucial ones are mass transfer, which is required for the progression of the reaction, the reactor design, the operating mode, the stabilization of the biocatalyst, and the recovery mode. For future scale-up, it is also critical to reduce capital and operating costs in many chemical bio-production processes. The synthesis and extraction of natural aroma compounds using a highly efficient continuous bioprocess is a viable and cost-effective method for producing bio-flavors on a big scale in the future.

10. AUTHOR CONTRIBUTION STATEMENT

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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14. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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16. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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