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Development of functional bioflavor based on Indonesian indigenous microbial fermentation products

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Abstract

Bioflavor and fermented foods in Indonesian cuisine were interesting for studying the relationship between fermentation products, microbial aspects, functional implications and biotechnological applications. The methodology employed in the literature review, including the sources used and inclusion criteria, demonstrates a meticulous approach to gathering and synthesizing information. Additionally, the factors influencing the perception of flavors on the tongue provide valuable insights into the complexities of taste perception, encompassing the role of specific amino acids and alkaloid compounds. The discussions on flavor production through microbial fermentation and the application of recombinant DNA technology in microbial flavor production showcase the strides made in biotechnology and their profound impact on flavor development. The escalating significance of natural ingredients and biocatalyst processes in producing flavor compounds aligns with consumer preferences for natural and sustainable options. Moreover, safety considerations for bioflavor products derived from biotechnology underscore the critical importance of ensuring consumer-friendly and safe products in this field. Functional bioflavor constraints provide practical considerations for developing and applying functional flavors, emphasizing the necessity for natural, safe and stable alternatives to conventional food additives. Overall, it offers a comprehensive and in-depth exploration of the multifaceted realm of flavor, integrating scientific, cultural and technological perspectives. It is an invaluable resource for researchers, industry professionals and enthusiasts engaged in flavor science and technology.

Keywords Bioflavor, Fermentation, Microbiology, Functional, Indonesia

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Introduction

The flavor is a complex sensory experience that engages multiple senses (smell, taste, sight and mouthfeel) when consuming food [1]. It comprises three fundamental components: smell, taste and mouth sensation [2]. To comprehend flavor, it is imperative to delve into the composition and compounds responsible for taste and smell and how they interact with receptors in our taste buds and olfactory organs, ultimately transmitting signals to the central nervous system [1–3]. Taste perception center around four primary tastes: sweet, bitter, sour and salty [4–6]. Additional nuances, such as sourness, spiciness, heat and coldness, can further influence this perception [5–7]. Taste cells undergo regeneration approximately every seven days. The human palate houses around 9–10 thousand taste buds, but their numbers decrease with age [7–10]. Variations in taste perception can be attributed to factors like age, gender and smoking habits, with heavy smokers exhibiting diminished responses [8, 9] Texture, encompassing smoothness, roughness, graininess and viscosity, significantly shapes the overall sensory experience [9–11]. Changes in viscosity can alter taste and smell by affecting the speed at which olfactory receptor cells and salivary glands are stimulated [11–13].

Functional flavors enhance the taste of food products and offer physiological benefits for overall well-being. These compounds, found in ingredients like ginger, herbs, green tea, ginseng and spices, impart desired tastes and confer health advantages, including antimicrobial and anti-inflammatory properties [12–15]. Flavor compounds are pivotal for the food and beverage industry, as they dictate product organoleptic properties and market appeal. They are categorized into two groups: indigenous compounds that arise from raw materials or during processing and intentionally added compounds, which can be natural or synthetic. These compounds are pivotal in defining product flavors and catering to consumer preferences [14–16].

While synthetic flavor compounds are often favored for their cost-effectiveness, increasing consumer apprehensions about safety and health have driven a surge in demand for natural flavor compounds [17, 18]. These not only intensify product flavor but also provide supplementary health benefits. Research endeavors in this domain aim to produce natural flavors more economically [19–21]. Adopting biocatalyst processes in flavor compound production is gaining traction as a sustainable alternative to chemical synthesis.

Indonesian cuisine relies largely on microbial fermentation, and it employs the extensive microbial biodiversity of the country to generate an extensive number of traditional fermented foods. A critical phase in the production of bioflavors, which are organic flavoring compounds derived from microbial metabolites, is microbial fermentation. During fermentation, microorganisms like yeast and bacteria can create bioflavor chemicals via biosynthetic processes. In order to generate an assortment of taste chemicals, they decompose lipids, proteins and carbs. The kind of taste chemicals generated can differ considerably depending on the microbe and substrate that are employed. Typical bioflavor substances include acids, alcohols, ketones and esters; each among these chemicals adds a unique flavor note, such as sour, buttery or fruity [1]. Microbial fermentation is in line with sustainable principles when it pertains to flavor production. It assists in creating an environmentally friendly food system through utilizing renewable resources and minimizing dependability on chemical processes [22]. Additionally, microbial fermentation is essential to the production of bioflavors since it allows a sustainable and natural approach to generate a variety of flavor components. In furtherance of encouraging the employment of renewable resources, this strategy meets customer demand for natural and eco-friendly products.

This review explores the microbial criteria in functional flavor, particularly fermented foods and their bioactive components within indigenous Indonesian cuisine. This exploration aims to shed light on the intricate interplay between microorganisms, flavors and the health-enhancing properties of these traditional foods.

Methods of scientific review

This literature review examined, synthesized and analyzed crucial information from various sources, encompassing books, journal articles and various published materials. These resources were distilled into a comprehensive overview of the current body of knowledge concerning bioflavor in fermented foods and their bioactive constituents within the unique context of indigenous Indonesian cuisine. The review also underscored areas where research gaps exist and proposed potential avenues for future inquiry. Specifically, this review delved into existing research on the proliferation of microorganisms in traditional Indonesian cuisine, their involvement in flavor generation and the associated technologies, including the application of recombinant DNA technology in microbial bioflavor production. It also explored the role of biotechnology in developing bioflavors, the safety considerations surrounding bioflavor products derived from biotechnology and the constraints related to functional bioflavors.

The information sources were collated from reputable academic research databases and search engines: Google Scholar, ScienceDirect, Scopus and JSTOR. Inclusion criteria encompassed studies published in peer-reviewed journals, proceedings and books, specifically focusing on microbial aspects, flavor, recombinant DNA technology, safety and functional constraints of flavors. Studies not available in either English or Indonesian were excluded from consideration. The keywords employed in the database searches included microbial flavor production, indigenous Indonesian cuisine, bioactive compounds, nutrition, food and culture, flavor production biotechnology and biotechnologically derived products' safety. The scope of the review was restricted to publications from 2000 to 2023. All relevant academic papers in the searches incorporated qualitative and quantitative data analysis methods.

Factors affecting the perception of bioflavor on the tongue

The high glutamic acid produces a strong taste when added to a food ingredient and can stimulate the nerves found on the human tongue. The properties of glutamic acid are utilized in the flavoring industry [20, 21]. The high content of glutamic acid produces a savory aroma and umami taste. In peptides, amino acids glycine, alanine, valine, leucine, tyrosine and phenylalanine will taste bitter [22]. According to Zhao et al. (2016) [23], arginine at concentrations below the threshold will increase the salty taste and give an umami taste. In large quantities, crab scallops can give a sweet taste and a distinctive seafood flavor [24]. Glycine and alanine are active flavor components that can give a sweet taste to food [24]. The sweet taste is caused by aliphatic organic compounds containing hydroxy groups (OH), several amino acids, aldehydes and glycerol.

According to Wongso & Yamanaka (2007) [24], the amino acid components that can give a bitter taste are valine, leucine and histidine, but they are not as bitter as phenylalanine. According to Stoeger et al. (2020) [25], the amino acid components that can give a bitter taste are glycine, alanine, serine and threonine have a sweet taste, whereas arginine, leucine, valine and methionine exhibit different flavor profiles. The content of several alkaloid compounds also causes a bitter taste. A proton donor causes a sour taste. The intensity of the sour taste depends on the $H+$ ions produced from the hydrolysis of the acid.

Temperature affects the ability of the buds to taste. Sensitivity will decrease if the temperature is greater than 20 °C and less than 30 °C, which will cause a slight difference in taste. For example, the taste of hot coffee will be less bitter when compared to cold coffee, and ice cream that has melted will taste sweeter when compared to ice cream that is still frozen. Too hot food will burn the tongue, damaging the taste buds' sensitivity, but damaged cells will be replaced within a few days. Cold food can anesthetize your taste buds so they are no longer sensitive.

The threshold is the lowest concentration limit for a taste, so it can still be felt. This threshold is not the same for everyone and is different for different tastes, for example, 0.087% NaCl and 0.4% sucrose. A person can experience taste blindness. To test whether a person tastes blind, testing can be done using the phenyl thiocarbamide (PTC) compound. If the person is blind to taste, this compound will taste bitter. Other taste components interact with primary taste components, which can increase or decrease taste intensity. The effect of this interaction is different at the level of concentration and threshold. Adding acid to the threshold concentration will add a salty taste to NaCl, while sugar will reduce the salty taste to NaCl and caffeine. Small changes in the chemical structure can change the taste of these compounds; for example, a sweet taste becomes bitter or bland. Adding a nitro group to the meta-position makes the compound very bitter. Substitution of methyl groups on iminos results in bland compounds.

Bioflavor functional concept in traditional fermented foods

Flavor compounds develop when microorganisms grow, and their enzymes break down basic ingredient components such as carbohydrates, proteins and lipids. The end products of metabolism found in traditional fermented food products can be elements such as amino acids, fatty acids and nucleotides, which provide certain taste characteristics [26]. Hydrophobic amino acids (for example, phenylalanine, leucine, isoleucine and methionine) produced by the action of proteolytic enzymes in the milk protein casein produce a bitter taste in Dadih and Dangke products. However, further metabolism of these compounds can produce a diversity of taste/aroma compounds: These compounds can be sulfur/cabbage resulting from the conversion of phenylalanine to methanethiol; sweet like honey, which is produced when phenylacetic acid is produced from phenylalanine; and fruit/banana/malt characteristics produced by the conversion of leucine to isovaleric acid, 3-methyl-1-butanol or 3-methyl butanal [27]. The breakdown of sugar (lactose) in dairy products usually results in the product being organic acids that produce a sour taste, but also alcohol and diacetyl, which gives a buttery aroma, or acetoin, which gives a fruity taste [28]. Methyl ketones and related secondary alcohols are produced from fatty acids and give the cheese its 'blue tone' [29]. All these characteristics have been described in blue cheese, and although individually, they may not always sound appealing; when combined, they provide desirable characteristics to the product. For example, the production of three volatile sulfur compounds, methanethiol, dimethyl disulfide and dimethyl trisulfide, is related to the desired flavor of cheddar cheese [30].

The types of bioflavor compounds produced through traditional fermentation processes and their concentrations depend not only on the composition of the food but also on the composition of the microbial population [31]. Each microorganism produces unique primary and secondary metabolite final products, but these can then be used by other microorganisms, which produce further final products. Production conditions determine the extent to which a particular group will continue to metabolize and produce the associated final product. Traditional spontaneous fermentation relies on native microorganisms introduced by the components [32]. However, this can result in poor product quality or even production failure if the right species are not present to provide certain desired characteristics. 'Back slopping' (using fermentation products as inoculum) can overcome this but can also perpetuate undesirable batches. The use of commercially produced starter cultures with known metabolic characteristics to initiate fermentation is widespread, and bacteria, yeasts and fungi are widely used in the food and beverage fermentation industry [29]. This produces a more uniform product but may only sometimes be the primary species influencing flavor formation.

Lactic acid bacteria such as *Lactococcus lactis* and *Lactobacillus* sp. are an important group of bacteria used in the dairy, fermented meat and fermented vegetable industries. These bacteria produce lactic acid as a final product from glucose but depending on the subspecies *Lactococcus lactis* or species *Lactobacillus* sp. Other end products that contribute to flavor may include ethanol, diacetyl and acetoin. In some products, certain species are used together to produce desired product characteristics. In yogurt fermentation, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are inoculated together. Both produce lactic acid, but together, this is better than each lactic acid because *Lactobacillus* sp. liberates valine through proteolysis, which stimulates the growth of *Streptococcus* sp. [33]. Streptococcus sp. produces the format required by *Lactobacillus* sp. Acetaldehyde and diacetyl are important flavoring volatiles produced to give yogurt its characteristic taste, with Lactobacillus being the main producers of these substances. The absence of the enzyme (alcohol dehydrogenase) in both species, which would convert acetaldehyde to ethanol, means the final product is yogurt-flavored and not an alcoholic drink [33].

In fermented meats such as salami, *Staphylococcus carnosus* and *Staphylococcus xylosus* are often added with a starter culture that produces lactic acid. Unusually, these organisms are not very tolerant of acids, so they do not grow when the pH begins to drop. However, the enzymes they produce are more tolerant, and essentially, the bacteria act as producers of enzymes that contribute to the breakdown of fats and proteins and, therefore, produce bioflavor compounds. Another important group in bioflavor production is yeast. Yeast is known for its alcohol production, but the proteolytic and lipolytic activities of certain species produce a variety of flavor compounds. *Yarrowia lipolytica* can break down tributyrin, producing butanoic acid, which has a cheese-like odor, and this is believed to be an important part of the development of bioflavor in several cheese varieties. Fungi also have proteolytic and lipolytic activities, which give them certain characteristics. *Penicillium roqueforti* imparts a characteristic 'blue' taste to cheeses such as Stilton and Roquefort [30].

Cheese products are a good example of products where the development of sensory characteristics is highly dependent on the balance of microorganisms present [29]. After initial fermentation with a starter culture, the cheese undergoes a ripening period, the length of which varies depending on the type of cheese. It is during this period that cheese becomes a complex dynamic ecosystem with the growth of many different microorganisms that contribute to the development of the product's flavor. In Stilton cheese, *Lactococcus lactis* and *Penicillium roqueforti* are two starters added by manufacturers. However, the final microbiota of mature cheese after 12 weeks contained many other bacteria and yeasts. Some of them have been proved to influence the characteristics of the flavors formed. Penicillium is added to allow the development of the characteristic flavor of blue cheese, primarily through the production of a methyl ketone. In model cheese studies using controlled flora composition, the presence of *Yarrowia lipolytica* with *Penicillium roqueforti* has been shown to enhance blue cheese flavor development through increased ketone production, compared to using *Penicillium roqueforti* alone, and produced sensory effects. It has characteristics equivalent to those of mature cheese that are not shared by the mold alone. This may be due to the highly lipolytic activity of yeast, which releases free fatty acids, which the fungus can then convert into ketones. Thus, the complete product characteristics desired by consumers may depend on the presence of these yeasts. However, these species are only present through chance introduction during processing, and therefore, their presence may vary from one batch to another, causing variability in the product [32].

Bioflavors resulting from microbial fermentation, such as monoterpenes, have been reported to show biological activity in vitro and in vivo against certain types of tumors and also have antimicrobial activity [34]. Terpene alcohol bioflavor compounds such as α-terpineol show antitumor and anticancer activity by reducing the expression of the nuclear transcription factor NF-B3 without undergoing lethal synthesis in the body's metabolism, making it safe for human consumption. Basidiomycete fungi such as *Ischnoderma benzoinum* have the potential to be a drug against influenza viruses and produce a spicy taste in submerged fermentation. This fermentation process follows two metabolic pathways in which L-phenylalanine is converted into two flavor compounds: one benzaldehyde (spicy taste) and 3-phenyl propanol (floral roselike aroma) [26].

Flavor production by microbial fermented food

Several microbes are used to ferment food and beverages to improve and even create new flavors different from the raw materials. Microbial fermentation produces flavor compounds through the metabolic activities of microorganisms such as bacteria, yeasts and fungi. During fermentation, these microorganism's catabolite the raw materials and transform them into a variety of chemical substances, including flavor compounds. The types of flavor compounds produced can vary widely depending on the strain of microorganism, the substrate they are fermenting and the conditions under which fermentation takes place.

Some of the common metabolic pathways that lead to flavor compound production are carbohydrate, lipid and protein metabolism. Glycolysis pathway will lead carbohydrate into glucose production, which is the source to produce alcohols, organic acids and esters (Fig. 1). For instance, yeast fermentation of glucose can produce ethanol and carbon dioxide, as well as other compounds that add flavor to bread, beer and wine. In addition, the hydrolysis of fatty acids can result in the formation of short-chain fatty acids and other metabolites that contribute to flavor such as methyl ketone and lactone. Moreover, catabolism products of protein, amino acids, present in the substrate form a wide range of flavor compounds, such as esters, alcohols and organic acids, which contribute to the taste and aroma of fermented foods (Fig. 2).

The production of flavor compounds is influenced by the microbial species involved, fermentation conditions like temperature, pH, oxygen availability and the substrate used. Fermentation can thus be tailored by selecting specific microbial strains and optimizing fermentation conditions to enhance the production of desired flavor compounds [35, 36].

The characteristic of fermented food or beverage serves the authentic flavor of each region. By the local community, some fermented products that are specific to an area, such as oncom, peuyeum (tapai), sticky tape, tauco, brem, shrimp paste, dadih, soy sauce, tempoyak, pickles and salted eggs, are used as business profit to be lead as souvenirs. Figure 1 shows local food and beverage fermentation in Indonesia. The flavor of fermented products gives a sense of a yearning desire for hometown food; therefore, many people buy these products. This shows that bioflavor from fermented food can have a socioeconomic advantage. In addition, the fermentation of food by bioflavor microbes has benefits such as increasing the nutritional value of food products and maintaining the food supply [21, 37–39].

Before 2000, studies of bioflavors explored the utilization of microbes to improve the taste of nourishment. The research motive in flavor branched out in the following years, even though there are still reports of findings of novel indigenous isolates that are characterized as flavored bacteria, such as *Lactobacillus* spp. from traditional beverages, Dadih, in Indonesia [11], *Kazachstania*

Fig. 1 Distribution of biodiversity fermented foods and beverages in Indonesia

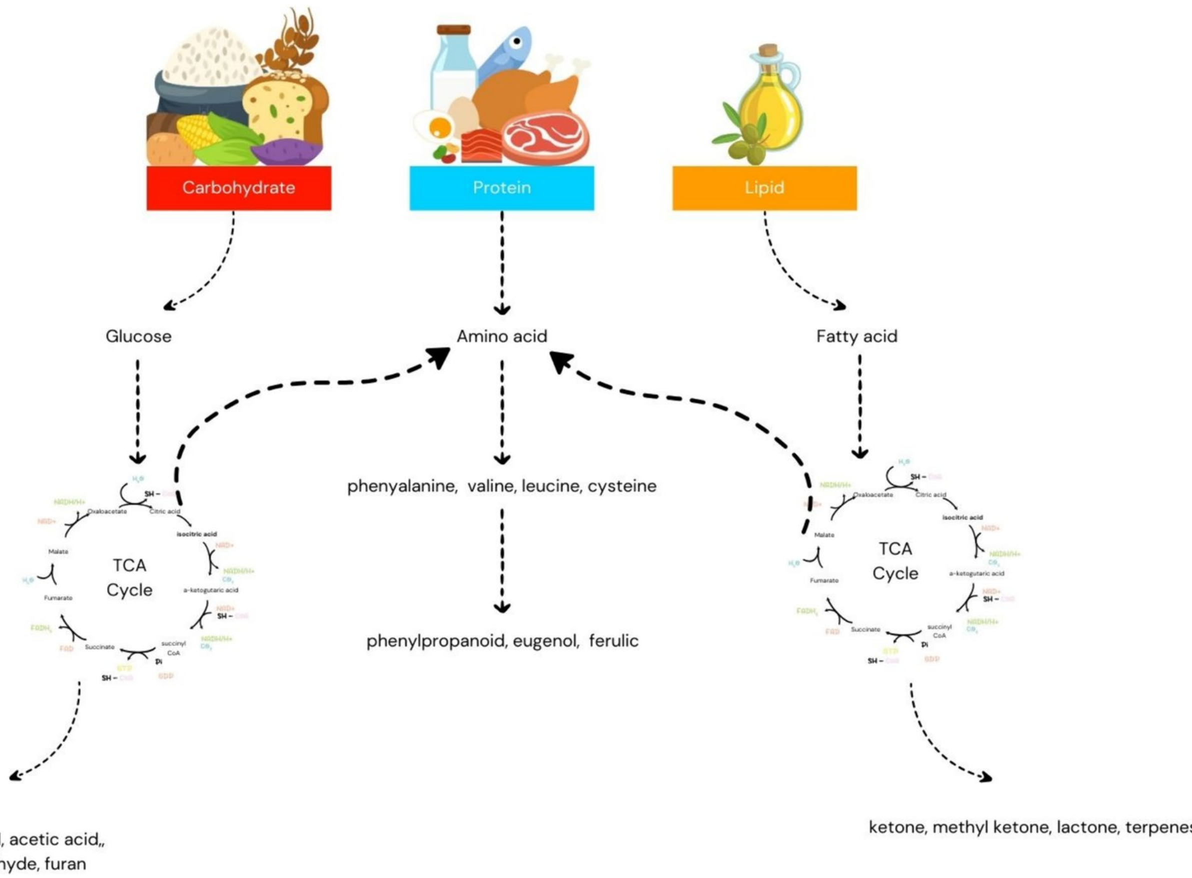

Fig. 2 Macromolecules catabolism in bioflavor transformation

Sinensis f.a., sp. nov from Thailand fermented fish [40]. Commencement in 2000, the accomplishment of technology to control the production of bioflavors began to be investigated. One of the research projects on the preservation technology of fermented food products to control microbial growth is the canning of the Mandai; the results of the research showed the flavor microbes can survive so that the Mandai commercialization process can be expanded [2]. Since microbial metabolism affects the flavor of the fermented product, it is a challenge to maintain the bioflavor content of fermented products.

The challenge for bioflavor research has developed regarding taste, added nutritional value, food preservation and the effects of bioactive compounds on health. Several research reports regarding the functional properties of Indonesian traditional foods and beverages fermented by bioflavor microbes (Tabel 1). Fermented products that have been explored regarding the benefits and active ingredients are Tempe. On the other hand, many traditional foods have not been disclosed regarding the health benefits of active ingredients. Moreover, the microbes responsible for pet shrimp fermentation have not been reported [39]. In addition, many active ingredients and bioflavor compounds from fermented food products have not been reported.

Therefore, the current research challenge is to study the identification of the active compounds and their benefits produced during fermentation by bioflavor microbes. A comprehensive study of a topic is essential to finding the breakthrough of a problem. It is hoped that bioflavor research will be reported in the scope of techno-economic socioeconomic research apart from scientific research. Nowadays, research innovations to meet bioflavors' needs, namely the production of roselike essential oil by mushrooms, have been reported [41]. Hereafter, the production and purification of bioflavor active compounds by fermentation on an industrial scale can be carried out to create unique bioflavors that benefit health.

The recombinant DNA technology applied on microbial bioflavor

Since 1970, recombinant DNA technology has played an important role in the biotransformation of flavor by microorganisms. For instance, this technology inserts a foreign gene into the vector and makes cloning to produce a specific target flavor. Many research studies were well documented [42–44]. The recombinant *Brettanomyces anomalus β-glucosidase* increased benzyl alcohol, eugenol, linalool and salicylate compared to wild types of other microorganisms [45]. Further, the potential of an engineered strain of *Ashbya gossypii* can produce limonene from xylose after the limonene synthase is overexpressed together with the native HMG1 gene [46]. Remarkably, vanillin, the common natural flavoring, is widely used in bioengineering. The study of overexpressing the pchF gene encoding vanillyl alcohol oxidase effectively induced 5.94-fold at 0.5 g/L vanillin [47].

A critical factor affecting the success of the recombinant DNA was well described by [48]. They highlighted that the expression of enzymes in *E. coli* was influenced by the sequences of genes involved in different stages of expression, the transcriptional promoter, the stability of the vector in host cells and the characteristics of the environment, such as the manipulation of culture media. At the same time, bioengineering of yeast such as *S. cerevisiae*, both the host strain's manipulation and precursors' manipulation, was needed to produce flavors [49]. Further, the recombinant enzymes needed for aroma (terpenoid) production in the mevalonate biosynthesis (MVA) pathway of *S. cerevisiae* [49, 50].

Lactic acid bacteria, which have a long history of fermented food, have the potential to be developed with recombinant DNA technology [51]. Most of them were used for biotherapeutic treatment. In comparison, the aroma from fermented products such as Indonesian local indigenous fermented food is diverse and can be used as a functional bioflavor. The recombinant DNA technology approach will assist in the transformation process [43]. It highlighted that the opportunity to use recombinant DNA technology in flavors would lead to a newly discovered pathway, improved quality and quantity of flavor, and new enzyme formation. Thus, it is both a challenge and an opportunity to capitalize on it.

The role of biotechnology in bioflavor development

Today, 'natural' ingredients related to food are used to meet consumer needs. The label 'natural' ('natural') is a powerful label used to market products that consumers need. 'Natural' products are believed to be safe for consumption. These products are included in GRAS (generally recognized as safe), so they are safe for consumption. Production of flavor compounds from plant extracts, biocatalyst processes and the application of genetic engineering to plants, as well as gene expression into bacterial or yeast cells, have started to be carried out commercially. Some commercially produced flavor compound products are given in Table 1.

The flavor industry mostly carries out the extraction and isolation of flavor compounds from plants to obtain' natural' flavor compounds. These flavor compounds have a higher economic value compared to synthetic flavor compounds. Difficulties in extraction or distillation occur when the content of flavor compounds is low, so the production of flavor compounds cannot be carried out by simple extraction and distillation methods. Thus, a higher-cost technology is needed to extract and isolate these flavor compounds. The development of science and technology leads to genetic engineering techniques for plants to produce higher-flavor components or express the genes responsible for producing these flavors so that they can be produced microbiologically with high productivity. Modern molecular biology and process engineering techniques, such as gene expression, mutagenesis, biocatalysts using microbial cells (whole-cell biocatalysis) and other engineering processes, can produce more biocatalytic processes for producing flavor compounds [52]. Industrial biocatalysis applications to produce 'natural' flavor compounds can be carried out to produce vanillin, ϒ-decalactone, carboxylic acids, C6 aldehyde compounds and alcohol compounds, ester compounds and 2-phenylethanol. In their review, Convetti et al. [53] discuss the link between biotechnology and the industrial application of these flavor compounds.

Flavor compound products, through processes using microbial cells or genetic engineering, compete with production processes by direct extraction from plants. Some of the considerations required for the application of biotechnology in the production process of flavor compounds are a combination of scientific and commercial considerations, such as (a) high-value flavor compounds contained in plants that cannot be carried out by classical extraction or distillation methods, (b) hazards of chemosynthesis products, consumers feel safer consuming 'natural' products. For example, in Europe, 90% of flavor compounds used in beverage products are 'natural' compounds (80% in the USA), (c) highly selective biocatalysts (chemo-, region-, stereo-) and (d) biocatalysts are accepted as 'natural' processes (white biotechnology) [54].

Glutamate in the form of monosodium glutamate (MSG) is one of the most widely produced flavor enhancers and is commonly produced in various countries. Most of these products are produced microbiologically by fermenting sugar-containing ingredients into glutamate using bacteria (*Corynebacterium glutamicum*). In Indonesia, several MSG companies use molasses as a substrate in the glutamate production process. With genetic

Table 1 Bioflavor as an active compound in fermented food

Raw material	Microbes	Fermented products	Bioflavor Volatile Compound	Future Challenges In Functional Properties	Problem	References
Soybean[a,b,c]	Rhizopus sp.[a,b,c]	Tempeh[a,b,c]	Ester, terpenoid, alcohol, aldehid, keton, furan[b]	Antioxidant[a] Antihypertensive[a] Antihypercholesterolemia[a] Antidiabetic[a] Anticancer[a,b]	Attractive packaging to increase consumer interest[c]	[a]Romulo& Surya, [3]; [b]Harahap et al. [5]; [c]Fibri and Frost [7]
Durian[a,b]	Lactobacillus sp. [a,b]	Tempoyak [a,b]	Thiol, ester, alcohols, aldehyde, alkane, ketone[b]	Probiotic[a]	Preservation product and large-scale production[a]	[a]Rajagukguk & Arnold, [4]; [b]Yuliana [6]
Buffalo milk[a,b,c]	Lactobacillus plantarum[a,b,c,d]	Dadih[a,b,c]	Fatty acids, ethanol, acetoin, acetic acid, butanone, diacetyl, acetaldehyde[c]	Probiotic[a] Antimicrobial[b], Hypocholesterolemic[b], Antimutagenic[b], Antioxidant[b], Immunomodulatory[b]	Availability of raw materials[b]; standardization of product quality and manufacturing processes[b]	[a]Collado et al. [8]; [b]Arnold et al. [9]; [c]Surono [1]; [d]Harnentis et al. [10]
Peanutted cake/ solid waste of soybean[a,b,c]	*Rhizopus oligosporus/ Neurospora sitophila*[a,b,c]	Oncom[a,b,c]	Amino acid[a]	Antioxidant, antimutagenesis[b]	Aflatoxin contamination[c]	[a]Andayani et al. [13]; [b]Matsuo [14] [c]Rohimah et al., [15]
Cassava tuber[a,b,c]	*Saccharomyces cerevisiae*[ab,c]	Peuyeum/Tapai/Tape[ab,c]	Alcohol[a] sugar[a,c]	Mycoprotein[d]	Standardization of product quality and manufacturing processes[b,c]	[a]Hidayat et al. [16] [b]Djunaidi et al. [17] [c]Asnawi et al. [18] [d]Sukara et al. [19]
Shrimp/Fish[a,b]	*Lactobacillus plantarum and* *Bacillus amyloliquefaciens*[b]	Terasi/Shrimp paste[a,b]	Amino acid, aldehydes, alcohols[b]	Antibacterial[a]	Preservation product[a]	[a] Prihanto & Muyasyaroh, [20]; [a]Prihanto et al. [21]
Shrimp[a,b,] Meat[c]	LAB[c]	Petis[a,b,c,]	Amino acid	Antiallergies[a,] antibacterial[c,]	Aflatoxin contamination[d]	[a]Tansy et al. [37] [b]Huda, [38] [c]Pramono et al. [39] [d]Handajani & Setiyaningsih, [90]
Black and white glutinous rice[a,b,c]	Saccharomyces cerevisiae, LAB [a,b]	Brem[a,b,c]	Acetic acid, ethanol, lactic acid[b]	Probiotic[b]	Distribution and license Regulation[c]	[a]Lenka et al. [41] [b]Mishra et al. [91] [c]Wisnumurti et al. [40]
Sweet Palm Sap[a,b,c]	C. tropicalis[b,c]	Tuak[a,b,c]	Alcohol[c]		Standardization of product quality and manufacturing processes[c]	[a]Lestari and Yusra, [92] [b]Hermansyah et al. [93] [c]Waisnawa & Sudana, [94]
Cow milk[a,b,c]	LAB [c]	Dangke[a,b,c]		Prevention of micropores in teeth[b], Probiotic [c]	Pathogenic bacteria contamination[a]	[a]Hatta et al. [95] [b]Samad et al. [96] [c]Zakariah et al. [97]
Fish[a]	LAB[a]	Pakasam[a]		Antibacteria[a]		[a]Rosanto et al., [98]

Table 1 (continued)

Raw material	Microbes	Fermented products	Bioflavor Volatile Compound	Future Challenges In Functional Properties	Problem	References
Tofu residue[a,b]	Rhizopus oligosporus[a,b]	Gembus[a,b]	Amino acid, fatty acid[a]	Antimicrobe[b] antiatherosclerosis[c] anticholesterol[d]		[a]Damanik et al. [99] [b]Noviana et al. [100] [c] Kurniasari et al. [101] [d]Sulchan & Rukmi [102]

[a] refers to the citations and references mentioned first in Table 1

[b] refers to the citations and references mentioned second in Table 1

[c] refers to the citations and references mentioned third in Table 1

[d] refers to the citations and references mentioned fourth in Table 1

engineering and engineered growth medium, these bacteria can produce glutamate in large quantities.

Many flavor compounds are being studied to be developed and applied to industrial processes. Vanillin is one of the flavor compounds that can be developed for microbiological production. Vanillin can be produced by extracting vanilla pods. Extraction of vanillin from vanilla pods requires a high cost, so synthetic vanillin production is still high. Annually, more than 10,000 tonnes of vanillin are chemically produced. This is triggered by the demand for vanillin, which continues to increase yearly. According to the 2012 Convetti et al. [53], vanillin flavor was in the top 10, and there was an increase in use (9%) from 2010 for beverage products This 9% increase is the same as the increase in apple flavor, which is the highest compared to other flavors used for drinks.

As a result of the high and increasing demand for vanillin flavor, the microbiological vanillin production business continues to be studied and developed so that vanillin production costs can be cheaper. At least the production costs are the same as the chemical synthesis process. The biotechnological production process is based on the bioconversion of ferulic acid, isoeugenol or eugenol by applying genetically engineered bacteria, fungi or other microorganisms. Microorganisms that are being studied intensively are *Amycolatopsis* sp. and *Pseudomonas* sp. Bacteria *Pseudomonas* sp. can convert eugenol to vanillin via ferulic acid by interfering with the vdh (vanillin dehydrogenase) gene, and these bacteria can convert eugenol to vanillin. Eugenol is a cheap and easy-to-obtain substrate in Indonesia. Because this process involves genetically modified organisms (GMOs), intensive research is needed so that the resulting vanillin product is safe for consumption.

Safety of bioflavor products from biotechnology

Health is still a priority for consumers when choosing food. The use of natural ingredients in food production has a market share that continues to increase yearly. Likewise, the use of 'natural' flavor compounds is still the people's choice before choosing synthetic flavor compounds. Consumer demand for natural flavor compounds is one of the factors triggering the increasing desire of the beverage industry to use natural flavors in their products [53] Biotechnology processes that can be applied in the industrial process of flavor production include direct extraction and isolation from plants, isolation of flavor compounds from the fermentation process and the use of genetically modified microorganisms to produce flavor compounds. Extraction and isolation of flavor compounds directly from plants or through a fermentation process is a process that is commonly carried out and has even been carried out traditionally for generations. Thus, this process can produce flavor compounds that are safe for the food industry and included in GRAS. Combining the fermentation process and extracting flavor compounds can increase the production of flavor compounds. In this case, the fermentation process can maximize the recovery of flavor compounds during the extraction process.

Microbiologically produced flavor compounds using GMOs are still being debated between the pros and cons. However, the development of biotechnology that leads to molecular biology is so rapid nowadays. This development led to transgenic foods or foods containing transgenic ingredients, including flavors. Flavor product development with processes that use GMOs needs to consider the health aspects of the product. The wise use of technology will result in a cheaper production process and a safer product.

Functional bioflavor constraints

The paradigm shift and acceptance of functional flavors by a broad spectrum of consumers provide the opportunity to create unique products. The biggest obstacle to using functional flavors is combining the concentration of components to provide the desired properties with the appropriate taste attributes. The sensitivity of human sensory organs to flavor attributes is not always consistent with active physiological abilities obtained at the same level. Often, our sensory threshold tendencies are much lower than the concentrations required for the active components to provide their benefits. Selecting certain flavor components and understanding with certainty the characterization of the activity of flavor components is a key factor in obtaining the physiological properties of functional flavors. Things that will be suggested when having to replace food additives that are commonly used with alternative food additives are:

1. Alternative food additives should come from natural sources (extracted from nature).
2. Alternative food additives are safe for the health of the human body. They are not harmful if consumed in the long term. (They are easily digested by the digestive system and do not leave harmful residues that can accumulate in the body.)
3. The alternative food additives is stable in food processing, packaging and storage.
4. Food additives should have functional added value in that besides improving sensory quality, it can also increase nutritional value and maintain health and fitness when consumed.

Bioflavor compounds (aroma and taste) are very important and determine the development of the food and beverage industry. Bioflavor compounds are included in food additives that improve the sensory quality of food. Bioflavor compounds are divided into two, namely natural bioflavor compounds and synthetic bioflavor compounds. In recent decades, natural bioflavors have been preferred due to consumer concerns about the dangers of synthetic bioflavors on health [52]. Natural bioflavor compounds can be obtained by extracting and isolating bioflavor compounds from plants, but this process often experiences several obstacles, namely high costs and low extraction yields [55]. The development of science and technology has led to molecular biology techniques and process engineering using microbial cells (whole-cell biocatalysis), which can produce more effective and efficient bioflavor compounds. The natural flavor products produced from this process are usually called flavors. One of the important flavor compounds is 2-phenylethanol [52].

The microbes that are often used to produce bioflavors are yeast. This is because yeast is a microbe that can convert simple carbohydrates into various complex molecules, including bioflavor compounds, through enzymatic catalytic reactions [42]. Various types of yeast are known to produce bioflavor compounds, one of which is *Kluyveromyces marxianus*, capable of producing 2-phenylethanol [56]. 2-Phenylethanol is an aromatic alcohol. This compound is naturally present in the essential oils of various flowers, for example, roses, daffodils, jasmine and lilies [52]. According to Fabre et al. [56], 2-phenylethanol tastes sweet and smells like roses. As a natural flavor, 2-phenylethanol can be applied to food products, such as soft drinks, candy, ice cream, gelatin, pudding, chewing gum and cookies [57]. 2-Phenylethanol can be produced from fermentation by various yeasts, including *Saccharomyces cerevisiae* [58], *Kluyveromyces marxianus* [59], *Pichia fermentans* [60], *Zygosaccharomyces rouxii* [61], *Yarrowia lipolytica* [62]. The advantages of production carried out by yeast are: (1) The product is a natural product whose safe use is permitted for food, (2) the raw material is more cost-efficient when compared to extracts from plants, (3) the production process is short, and (4) it is easy to control in the production process [63].

One of the yeasts chosen to produce 2-phenylethanol is *Kluyveromyces marxianus*. This is nonpathogenic yeast with a high potential to produce biotechnology products. It has a high specific growth rate and can use a broad spectrum of substrates [64]. According to Fonseca et al., K. marxianus is also a microbe with a safe status (Generally Regarded as Safe/GRAS). Production of 2-phenylethanol by yeast, including *K. marxianus*, is usually carried out by the biosynthesis pathway from L-phenylalanine catabolism via the Ehrlich pathway [65]. The resulting product, namely 2-phenylethanol, can poison the *K.marxianus* cells themselves. Fabre et al. (1998) [56] stated that the growth of *K.marxianus* cells was inhibited at a concentration of 2 g/liter. However, the sensitivity to 2-phenylethanol for each *K.marxianus* strain was different. Several strategies have been carried out to increase the production of 2-phenylethanol by *K. marxianus* to make it more effective and efficient, including screening superior strains [66], optimizing medium conditions [67], using in situ techniques: product removal (ISPR) to overcome cytotoxicity [59] or by carrying out genetic engineering to increase 2-phenylethanol production [68].

Isolation of bioflavor compounds

One of the stages that need to be considered in flavor production is the flavor isolation technique from other fermented products. The basic isolation methods that are often used are extraction, distillation and absorption. Currently, several methods have been developed to reduce flavor damage resulting from the interaction of flavor compounds with solvents. In general, flavors are composed of volatile compounds, so the extraction of flavor compounds can be done using the headspace method [69]. The principle of the headspace method is humidity of the compound, which can be conduct to replace the fermentation product in a closed bottle and then heat it to a certain temperature so that the volatile compounds can be separated and isolated [70].

Another method for isolating valuable organic compounds is to use the pervaporation method [71]. The advantage of pervaporation is that it can separate small amounts of volatile compounds in a mixture. Pervaporation is an acronym for permeation and evaporation, so this method requires a membrane to separate flavors from other compounds. Some examples of membranes used to separate aromatic compounds are polymeric membranes (polyvinyl alcohol, polyimide, polydimethylsiloxane), inorganic membranes (zeolite, silica and metal–organic framework), 2D membranes (graphene oxide, metal–organic framework and mixed-matrix membrane) [72]. The polymeric membrane is the cheapest pervaporation technology among others, so it is widely used in the scale-up industry but has weaknesses in terms of stability. The umami flavor which is dominated by peptide compounds can be purified using the nanofiltration membrane method [71].

In food industry, supercritical fluid extraction (SFE) is general method to purified natural compound. The basic principle of this method is the extraction of dissolved substances using high pressure. By passing high-pressure CO_2 through fermented sticky rice, the aroma of vinegar was isolated [73]. CO_2 gas under high pressure will become liquid so that it can extract volatile compounds in a fermented product. The choice as a solvent in the SFE method for food products is because this compound is inert, GRASS and easy to obtain [74].

Techniques for analysis and quantification of bioflavor compounds resulting from microbial fermentation

A number of instruments and methods are frequently employed in the identification and evaluation of bioflavors. The practice of identifying and analyzing chemicals produced by microorganisms during fermentation or other metabolic processes is known as bioflavor detection. It is crucial to remember that the particular flavor compounds of interest, the characteristics of the sample and the degree of detail needed for analysis all influence the choice of detection instrument.

Different analytical approaches are used to identify and quantify volatile chemicals that contribute to the overall flavor profile to detect bioflavors created by microbes. The analysis aims to analyze flavor compounds that play a role in food senses, both aroma and taste. The science related to this was previously known as sensomics. Sensomics analyzes the composition of aroma compounds that play a sensory role for analyzed compounds using gas chromatography–mass spectrometry (GC–MS) [75]. Samples are taken at critical points in the development or fermentation processes of microbial cultures, which are cultivated under well-regulated conditions [76]. Compounds that play a role in the sensory input of a fermentation product are called character impact compounds (CIP). CIP levels in the fermentation product in question are then accurately quantified using the stable isotope dilution analysis (SIDA) method. After knowing the CIP and its levels in the fermentation product being analyzed, then the levels that have been measured are mixed (recombination).

CIP analysis can be done using a gas chromatography–olfactory (GC–O) or a gas chromatography–mass spectrophotometer–olfactory (GC–MS–O). GC–MS–O is better used in bioflavor analysis because apart from determining CIP, it can also identify the type of bioflavor compound using MS connected to GC. At the same time, the compounds identified by MS were also analyzed for their odor descriptions by olfactometer. This is done by dividing the 2 end branches of the capillary column in the GC oven: one toward the MS and one toward the olfactometer. An olfactometer is used to smell the compounds coming out of the GC column, equipped with a wet airflow so that the assessor's nose, which describes the smell.

Using GC–MS–O, we can find the type of odor and target bioflavor compounds. To find out what types of compounds play a role in determining the aroma of a fermentation product, the aroma extract dilution analysis (AEDA) technique is carried out. This technique is carried out by analyzing the initial aroma extract with GC–MS–O or GC–O. After that, the extract was diluted twice and then analyzed by GC–MS–O or GC–O, and so on until there is no more odor coming from the olfactometer.

Untargeted bioflavor compounds also can be identified with the initial stage of extracting volatile compounds based on their type. In general, 2 extraction principles can be used based on the solubility and volatility of flavors in fermentation products. Typically, methods like dynamic headspace extraction or solid-phase microextraction (SPME) are used to remove volatile chemicals from the samples [77]. After being extracted, the volatile substances are introduced into a gas chromatography (GC) apparatus, where their volatility and other chemical characteristics are used to separate them. This method considers that it can extract pure flavors and does not involve nonvolatile compounds or matrices in the extract obtained. The extract must be concentrated or reduced using nitrogen gas if it contains solvent. The concentrated bioflavor extract can then be analyzed by GC–MS. The separated chemicals are then introduced into a mass spectrometer, where their mass-to-charge ratios are determined by ionizing and measuring them. MS gives details regarding each compound's identity and abundance. To process the GC–MS data, identify substances using mass spectra and calculate their concentrations, sophisticated software tools are used [75]. By comparing a compound's mass spectra with databases, it can be recognized, and using reliable standards can help with confirmation. On the other hand, high-performance liquid chromatography HPLC can be used to identify, quantify and segregate nonvolatile compounds in a sample. The examination of bioflavors that are challenging to evaporate often makes use of it.

Another approach of bioflavor compounds analysis resulting from microbial fermentation is by using an LC–MS–MS (liquid chromatography–mass spectrophotometer–mass spectrophotometer) instrument. Nonvolatile compounds contained in the extract are separated using an LC instrument. The respective compounds that have been separated are analyzed by MS first. In MS, the compound is first ionized, and then, the mass ion produced is selected; only the mass of the target compound, called the parent ion, is passed to the next stage. The parent ion is then passed to the collision cell, where the ionization process occurs. The formed ions are called daughter ions and then passed to the second MS. In the second

MS, the daughter ion, the parent ion of the target bioactive compound, is selected. If the target compound in question is present in the extract, the target compound's parent ion and daughter ion will be detected and can be quantified. LC-HRMS (liquid chromatography–high-resolution mass spectrophotometer) is used to determine the molecular mass with a more accurate detection limit, making it easier to identify bioflavor compounds. Suppose a new compound has not been identified in the LC–MS database. In that case, the compound is isolated until a pure compound is obtained and then identified using HRMS or NMR spectroscopy.

Utilizing the mass-to-charge ratio of a substance to identify and measure, it is possible with mass spectrometry [78, 79]. It frequently works in tandem with chromatography to provide thorough analysis. In sensory evaluation, real panelists taste and assess a product's flavor characteristics [80]. This subjective method is crucial to comprehending how flavors are perceived generally. Electronic gadgets with sensors designed to simulate human smell are called e-nose devices [81, 82]. In order to offer a flavor profile, they are able to identify and examine volatile chemicals in a sample. Compounds can be identified and measured using NMR spectroscopy according to their nuclear magnetic characteristics. It offers comprehensive structural details about the molecules present in a sample [83].

Direct thermal desorption is another approach for bioflavor analysis which is simple and rapid sample preparation. It does not require any solvent use. It is based on sparging volatiles from sample matrix and transferring them onto the chromatographic column. Heat treatment is usually applied to a matrix to extract volatile compound from sample. A cryofocussing unit or cold trap can be used to focus the volatiles at the head of the column [84].

Instrumentally analyzed volatile profiles may also be combined with sensory profiles. Usually, a trained sensory panel evaluates the most important sensory properties of a product in sensory laboratory (ISO 8589) conditions [85, 86], e.g., following a general sensory profiling protocol. When the sensory profile is connected to instrumental analyses, it is important to keep the sample preparation method as similar as possible in both methods. Different data matrices are relatively easy to combine, using multiregression statistical methods to identify the key volatile compounds contributing to smell or flavor [85]. However, it is necessary to determine the target, such as orthonasal odorants, retronasal odorants or flavor compounds, when selecting the correct method of analysis for instrumental measurement and human sensory evaluation.

Certain genes linked to the synthesis of bioflavors can be found using PCR methods. This molecular biology method is particularly helpful for researching the genetics of bacteria that produce flavor [87]. ELISA is an immunological technique that uses antibodies to identify substances, often known as antigens. It is adaptable enough to be used for taste component detection. The thorough examination of tiny molecules, or metabolites, in a biological sample is known as metabolomics [88]. It can be used for flavor analysis and offers information into the metabolic profile of bacteria. The microbial population in a sample can be examined using next-generation sequencing methods [89]. This facilitates comprehension of the variety of microbes involved in flavor creation.

Conclusion

The study of flavor has revealed the intricate interplay of sensory experiences with the key elements of flavor (smell, taste and mouth sensation), which are influenced by complex chemical compositions and interactions with receptors in sensory organs, ultimately transmitting signals to the central nervous system. Flavor compounds, crucial to the food and beverage industry, are categorized into indigenous and intentionally added compounds. As consumer concerns about safety and health rise, bioflavor compounds are gaining popularity, driving research into more economical production methods, such as biocatalyst processes. Microorganisms are crucial in shaping these traditional foods' flavors and health-enhancing properties. The production of flavors through microbial fermentation showcased economic and cultural significance, especially in the cesia. It highlighted flavor's potential to enhance socioeconomic development by commercializing unique, locally specific fermented products. Biotechnology's role in flavor development was discussed, emphasizing the growing demand for 'natural' flavor compounds. It highlighted various approaches, including genetic engineering, to produce high-value flavor compounds from natural sources, further blurring the lines between synthetic and natural flavors. Safety considerations for bioflavor products derived from biotechnology were addressed, emphasizing the importance of ensuring that GMO-based processes yield safe and consumer-friendly products. Finally, it touched upon functional flavors, showcasing their potential to enhance sensory experiences and confer health benefits. The challenge lies in balancing taste attributes with the desired physiological properties, ensuring consumers reap the full benefits of these functional flavors. Finally, this review provides a comprehensive overview of the multifaceted world of flavor, from its sensory components to its production through microbial fermentation and biotechnology. It emphasizes the potential for flavors to enhance culinary experiences and contribute to health and well-being. Future research in this field promises further innovations in flavor science and technology.

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