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A Review

Production of Bioflavour from Microbial Sources and its health benefits

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Aroma and flavour are the important part of food that increase the organoleptic properties of a food and makes the food more acceptable among consumers. Flavour and aromatic compounds are the most essential components in food, feed, cosmetics, pharmaceutics, and toiletries products. Commercial production of flavour and aroma compounds from the microbial source in the industry is a modern approach but the concept behind it is in human practices since time immemorial. However, the health-promoting benefits of microbial bioprocesses products are numerous ranging, from antibiotics to fermented functional foods are among the most appreciable one. This review includes the verity of flavour production from various types of microorganisms and its application in the food industry, and a brief discussion about its health benefits among the consumers.

Keywords: Bioflavour, *Bacillus lichiniformis*, Citronella, *Geotrichum fragans*, Geraniol, Methyl salicylate, Nootkatone

Intoduction

Flavour is an essential attribute of food that helps in enhancing the organoleptic properties of food and hence gives the consumer a pleasurable satisfaction. It clicks the olfactory receptors of the vital sense organs such as smell and taste, which give consumers a better knowledge and justification of food acceptability. Volatility is an elite characteristic of every flavour and fragrance compounds because of the low molecular weight, lower than 400 Da. Flavouring agents comes under the food additive category and added in very minute concentration during food processing. Most of them are the chemical formulations of hydrocarbons, aldehydes, ketones, esters or lactones. But a synthetic flavour compound may undergo lethal synthesis when incorporated in the body metabolic pathway to form a toxic compound thus leading to many complex chronic disorders. To solve this problem, researchers searched for bioflavour obtainable from natural sources like plants, animals and microorganisms. For food industries, extensive research has been done on bioflavours from plant origin. However, that have drawbacks such as they are usually expensive, delicate, and can't able to withstand the harsh conditions during food processing and storage. However, there are lots of advantages of bioflavours originated from microbial sources such as bacteria and fungi, as they are robust, thus can sustain fluctuating temperature, pH, gasses and osmotic pressure during industrial processing. Microbial aroma compounds fascinate the interest of pharmaceutical and food industries not only for their technological properties but also for other functional features such as health-promoting benefits. Likewise, research on biocolour production by the microorganism such *Spirulina platensis* shows health benifits[1]. Moreover, production of bioflavours from microorganisms by the use of food industrial waste is as a better way of waste management and very economical and feasible in many cases. A good example, is the production of ɤ-decalactone by microbial fermentation reduces the production cost from US$ 20000/kg to US$ 120/kg during the year from the 1980 to 1995 as reported by researchers[2]. This review discuses the current scenario, brief history, classification, and benefits of microbial originated flavours.

Current scenario of flavours used in processed food

Considering the current scenario, most of the industrially processed food such as ice-cream, pastry, custard or pudding and filling in cream sandwich biscuits, all used chemical flavour agents to give the product a unique blend of fruity, buttery and chocolaty flavours. It's true that chemical synthesis of flavour is economic, thus having a high yield and low-cost, but also having low quality and does not have good region-enantio selectivity[2]. Processed salty

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snacks food such as potato chips and variety of namkeens use various spices as flavouring agents. Thus microbial originated bioflavours required special research attention. However, dairy-based food such as buttermilk, cheese, yogurt, *etc*. having *in situ* bioflavour originated from the inoculated microbial strain. But in case of *ex situ* flavour production, such as fruity, flowery and nutty flavour production by specific microbial strain followed by flavour extraction and its application in processed food remains a big research lacuna. Moreover, bioflavour or bioaroma production by using the different agro-industrial waste is a better choice for waste management and it's having the enantio-selectivity[2]. The market study of the aroma industry in 2015, showed that it is worth of US$ 3.85 billion. While, globally prediction of aroma industry from 2016 to 2024 the compound annual growth rate (CAGR) is of 6.2%[3]. Figure 1, represents the current scenario of food flavour additive used in industrially processed food and its origin of production.

Background of microbial flavour production

Aroma and flavour producing microorganism are ubiquitous in nature, like an earthy aroma from soil comes after the first rain, the pungent smell from a damp room and a nasty smell from slaughterhouse all because of ubiquitous microorganisms. While considering commercial production of flavour and aroma compounds by the microorganisms in the industry is the latest approach, but the concept behind it is in human practices since time immemorial. The delicacy of wine and its production by fermentation is known to human around a hundred years ago. Over 180 years ago the first flavour compound benzaldehyde was identified that having characteristic almond-like flavour[4]. One of the pioneer concepts in microbial aroma production was given by Omelianski in the year 1923[4]. That pioneering work explained the formation of pleasant aromatic products by the action of microorganisms in a certain food. It also explains the changeable character of the flavour producing microorganisms that depends on a substrate, time of incubation and temperature. According to Omelianski, the microorganism produces specific aroma or flavour given an epithetic name such as *aromaticus*, *aromafaciens*, *odorus*, *odoratus*, *esterifaciens*, *fragi*, *fragariae*, *nobilis*, *etc*. The list of few microbial species that produces bioflavour compounds with structure and it resembles with natural flavour compounds is given in the review (Fig. 2).

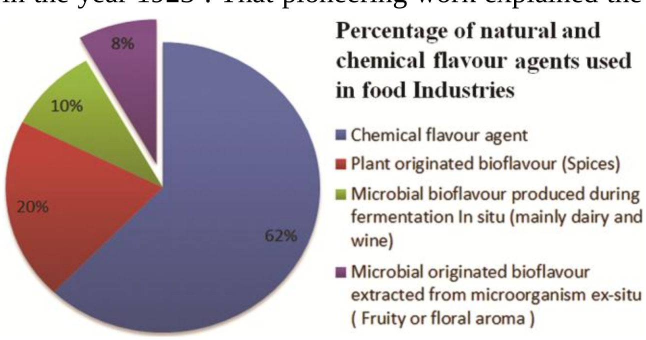

Fig. 1 — Current scenario of food flavour additive used in industrially processed food and its origin of production

Flavour in dairy products such as buttermilk, cheese, yogurt, dahi, *etc*., is because of *in situ* microbial culture such as bacteria of genus *Lactobacillus*. Other than buttery flavour, various organisms also reported to produce fruity and floral aroma in dairy products. One of the famous fungi from the genus *Ceratocystis*; name as *Ceratocystis fimbriata* was reported to produced intense fruity aroma in Solid State Fermentation (SSF) by using different agro-industrial waste material such as wheat bran, cassava bagasse, and sugar cane bagasse, *etc*. as

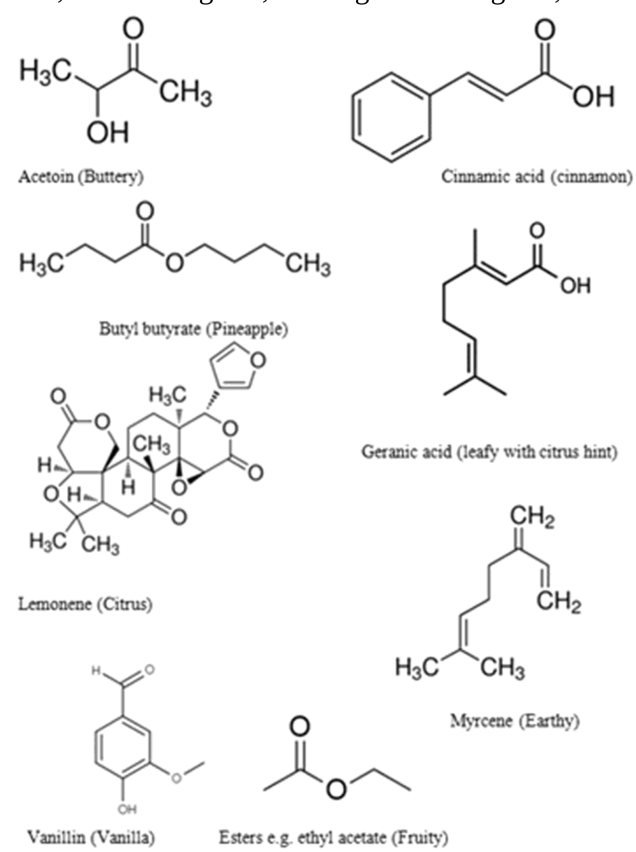

Fig. 2 — Bioflavour molecules resemble with natural flavour compounds

substrates[5]. Another edible fungi *Rhizopus oryzae* also produces fruity aroma during SSF by use of similar kind of agro-industrial and food-industrial waste[6]. It is predicted that the industrial production of bioflavours and bioaroma can be cost effective. As the raw material used for the production is a waste of other food industry. Thus the production of microbial bioflavours is a good alternative for chemical flavour agents. That can carry out consumers demands for natural flavour and minimizing the exorbitant cost of the production[7].

Classification of bioflavour production according to the source microorganism

The earlier review and research work done in microbial bioflavour, classification and categorization were done according to flavour compounds. But in this review classification has done according to the source of microorganisms regarding three major classes, bacteria, fungi, and algae. Most of the microorganisms produce aromatic volatile compounds during the stationary phase of fermentation, thus aroma compounds are categorized as secondary metabolite. Researchers studied that most of fungal species produce natural flavours through *de novo* pathway than bacterial species. However, genetic engineering of bacteria is easier than the fungus. Thus incorporating flavour producing gene in bacteria is much easier. Whereas, fungus being an eukaryotic species, thus genetic engineering in fungus remains a challenging task. However, the microorganisms can produce bioflavours both by either *de novo* pathway (by the producing specific enzymes), or by biotechnology methods of gene editing. Table 1, presents the classification of flavours according to the source of the microorganism.

Bacteria originated bioflavours

Vanilla is the choicest ice-cream flavour, traditionally being produced by Vanilla plant. The active compound responsible for this unique flavour is vanillin, that can be produced from bacteria *Nocardia iowensis* by *de novo* synthesis and also by microbial biotransformation[8,9]. Other than this, bacteria such as

Table 1 — Classification of flavour according to source of microorganism

Bacterial originated bioflavour			Fungal originated bioflavour		
Name of the organism	Molecule name and flavour/aroma	References	Name of the organism	Molecule name and flavour/aroma	References
Pseudomonas gladioli	α-terpineol	13	*Ceratocystis fimbriata*	Esters *e.g.* ethyl acetate (Fruity)	30
Escherichia coli & *Lactococcus lactis*	Acetoin & Diacetyl (Buttery)	24	*Geotrichum fragans*	Esters *e.g.* ethyl acetate (Fruity)	20
Bacillus subtilis	Acetoin (Buttery)	25	*Saccharomyces cerevisiae*	Butyl butyrate (Pineapple)	31
Nocardia iowensis	Vanillin (Vanilla)	26	*Pichia pastoris*	Benzaldehyde (Cherry & Almond)	32
Pseudomonas putida	Geranic acid (leafy with citrus hint)	27	*Rhizopus oryzae & Candiada tropicalis*	Lemonene (Citrus)	33
Streptomyces setonii	Vanillin (Vanilla)	28	*Trichoderma viride*	6-pentyl-α-pyrone (coconut)	34
Streptomyces sp. V-1	Vanillin (Vanilla)	11	*Kluveromyces marxianus CBS 600*	2- phenylethanol (Rose and honey like)	35
Corynebacterium glutamicum	Tertamethylpyrazine (Nutty and roasted)	18	*Ceratocystis moniliformis*	Isobutyl acetate (banana), Geraniol (rose)	7
Arthrobacter globiformis	Vanillin (Vanilla)	10	*Pycnoporous cinnabarinus*	Vanillin (Vanilla)	36
Streptomyces griseus	Geosmin (Earthy)	15	*Ischnoderma benzoinum*	Benzaldehyde (Nutty & Almond)	18
Pediococcus pentosaceus & *Lactobacillus acidophilus*	Acetoin & Diacetyl (Buttery)	29	*Nidula niveo-tomentosa*	4-(4-Hydroxyphenyl)-2-butanone (Raspberries)	4
Pseudomonas oleovorans	Methyl ketones (Butterscotch)	4	*Aspergillus niger*, *Penicillium* sp., *Aureobasidium pullulans*	Methyl ketones (Butterscotch)	4

Pseudomonas putida, *Corynebacterium glutamicum*, *Arthrobacter globiformis* and *Serratia marcescens* synthesize vanillin by bioconversion of euginol and isoeuginol[10]. Another bacteria *Streptomyces* sp. V-1, also produces 19.2 g/L vanillin after 55h of fermentation at 30°C and at pH 7.2 in broth medium when supplemented with 45 g/L of ferulic acid and 8% macroporous absorbent resin DM11[11]. Vanillin also can be produced by chemical synthesis from lignin, a residue of paper and pulp industry[2]. However, two main precursors from the petrochemical industry such as guaiacol and *p*-cresol are preferably used for synthetic vanillin production[12]. Bioflavour that present in essential oils such as α-terpineol was reported to produce by *Pseudomonas gladioli* in quantities of 1 g/L[13]. Another bacteria *Bacillus lichiniformis* also produces isoamyl acetate form isoamyl alcohol and *P*-nitrophenyl acetate[14]. Few soil bacteria of genera *Enterobacteriaceae* produces characteristic grapefruit aroma known as ‘nootkatone’[4,15], that have a high market demand and used in fruity flavored beverages and perfumes. Some unique aroma producing bacteria was reported by Janssens and co-researcher that produce camphor-like smell, chemically known as borneol and isoborneol produces from *Pseudomonas pseudomallei*[4]. The common earthy aroma known as ‘Geosmin’ evolve after first rain is being produced by *Streptomyces citreus*[16]. Some species of *Bacillus* and *Pseudomonas* produce a nutty and roasted peanut flavour, compounds known as pyrazines[17]. Tetramethylpyrazine derivate of pyrazine having musty and nutty flavour synthesized by *Corynebacterium glutamicum* from amino acids[18].

Fungal originated bioflavours

A wide range of the fungal species reported to produce bioflavours naturally and by biotransformation. It includes yeasts and molds. Brief discussion on two major categories of yeast and mold with respect to bioflavour production is given below:

Kluyveromyces marxianus a highly studied flavour producing yeast, studied for optimization of the fruity flavour production in solid-state fermentation by factorial design and response surface methodology (RSM)[19]. Fungi *Geotrichum fragans* produces various secondary metabolites such as alcohol, acids, and esters. Hence esters such ethyl acetate and ethyl butyrate give strong fruity aroma such as pineapple[20]. Fungal mold such as *Fusatium oxysporum*, *Penicillium digitatum*, and *Cladosporium* sp. extensively studied for optimized α-terpineol production by the different researcher in different years[21]. The highest α-terpineol produces by *Cladosporium* sp. around 1.0 g/L concentration is obtained[21], and lower production by *Fusatium oxysporum* in amount around 500 mg/L[22]. Other than this fungi, the phylum ascomycetes and basidiomycetes also synthesized terpenes, one of the good examples is *Ceratocystis variospora*[22]. Researchers studied that few fungi like *Ceratocyctis moniliformis* having potential to synthesize several aromatic compounds such as isobutyl acetate, isoamyl acetate, propyl acetate, geraniol, and citronello[15,18].

Algal originated bioflavours

Synechococcus elongates, unicellular cyanobacterium (blue-green algae) has been reported to produce buttery bioflavour through acetone synthesis metabolic pathway[23]. Other than this some algae also produce earthy aroma of Geosmin by *de novo* synthesis.

Consumer choice for microbial bioflavours and its health benefits

Most of the flavour agents used by food processing industries are of chemical originated mainly the chemical formulation of different chemical compounds such as amyl acetate, benzaldehyde, ethyl butyrate, methyl salicylate, fumaric acid, *etc*. that impart various fruity flavour in food (Fig. 2). But it is being reported that chemical food flavours additive incorporates with the human body metabolism and thus undergoes lethal synthesis that causes mutagenic effects. Some time it alters the human genome and causes mutation, thus acts as a culpable homicide for the consumer. The good effect of bioflavour and the bad effect of chemical flavour are given as a pictorial representation in (Fig. 3) as a resultant health effect on consumer.

It was reported that bioflavours such as monoterpenes show both *in vitro* and *in vivo* biological activity against certain types of tumor and also having antimicrobial activities. Terpenes alcohol such as α-Terpineol shows

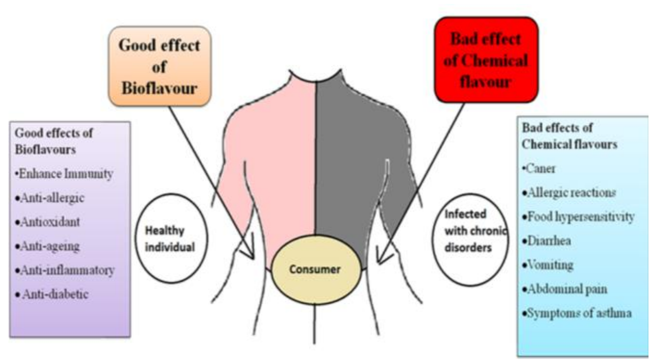

Fig. 3 — Bioflavour health effect on consumer

antitumor and anticancer activities by reducing the expression of the nuclear transcription factor NF-κB[3] without undergoing lethal synthesis in body metabolism. Thus graded as food safe for human consumption. Moreover, basidiomycete fungi such as *Ischnoderma benzoinum* have the medicinal potential against influenza virus and also produce nutty flavour in submerged fermentation. This fermentation process follows two metabolic pathways in which L-phenylalanine was converted in to two flavour compounds one benzaldehyde (nutty flavour) and 3-phenylpropanol (flowery, rose-like aroma)[4,18].

Conclusion

The emerging consumer demands for the natural flavours and aroma compounds gives biotechnologists and microbiologists a new challenge for bioflavour production and its optimization. As it is 'natural' thus will not have harmful health effect and will be beneficial to health. It will be a better option for food industrial waste management also. The most important criteria are to select the strain and the microorganism that can give desirerd flavour at a high level and can withstand the industrial processing. Using microbial bioprocess in flavour production can be economical. In future large scale production of bioflavours along with biocolors from microorganisms, will promote sustainability and good health to the consumers.

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