

## Review

# Research Progress on Bioaugmentation Technology for Improving Traditional Chinese Fermented Seasonings

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**Abstract:** Chinese traditional fermented seasonings, essential to the culinary heritage of China, are produced through fermentation, resulting in a diverse range of unique flavors and aromas. The microorganisms involved in fermentation play significant roles in shaping the quality of these traditional fermented seasonings. The production of traditional fermented seasonings is affected by various biological and abiotic factors, presenting challenges concerning product quality and safety. This review investigates the impact of bioaugmentation technology on key Chinese traditional fermented seasonings, such as vinegar, soy sauce, *sufu*, *doubanjiang*, *dajiang*, and *douchi*. Additionally, the challenges and constraints linked to the implementation of bioaugmentation technology are discussed. The potential of bioaugmentation is highlighted by its ability to shorten the fermentation time, optimize raw material utilization, improve nutritional value, and enhance the quality parameters of these seasonings. This paper demonstrates an interesting convergence of traditional culinary heritage and contemporary technological advancements.

**Keywords:** bioaugmentation; fermentation; fermented seasonings; soy sauce; vinegar



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## 1. Introduction

Fermentation, an ancient technique for food processing and preservation, entails the conversion and decomposition of complex food components, such as carbohydrates, proteins, and fats, by various microorganisms and enzymes [1–3]. As a result, fermented foods are defined as “foods prepared through the growth and enzymatic transformation of food components by specific microorganisms” [4].

A seasoning is a prepared food compound that contains one or more spices that enhance the flavor of food [5,6]. Fermented seasonings are part of the various traditional fermented foods produced by diverse microorganisms [7]. Traditional Chinese fermented seasonings typically undergo technological processes, such as koji preparation and the fermentation of raw materials, both of which are susceptible to biotic and abiotic factors, including the environment and microorganisms, due to the semi-open fermentation method. Consequently, ensuring the quality and safety of products has become a challenging task. Therefore, the industry has prioritized improving the quality of fermented seasonings and reducing potential safety hazards.

Bioaugmentation is a method of enhancing efficiency through the introduction of specific strains [8]. Many studies have investigated microorganisms with specialized functions to enhance the koji-making and fermentation processes, aiming to improve the flavor compounds, optimize raw material utilization, reduce the contents of harmful substances, and ensure overall product quality [9–12]. A study by Zhang [13] showed that fermenting soy sauce with *Tetragenococcus halophilus* and *Candida versatilis* significantly increased the presence of aroma and taste-active substances, particularly the amino acids that contributed to the umami taste. In addition, the inoculation of marine yeast and flour yeast, known for their robust ability to degrade biogenic amines, into soy sauce resulted in a significant reduction in the biogenic amine content and an overall safety improvement [14].

Microbial bioaugmentation positively influences the production of fermented seasonings, offering a potential solution to deficiencies in traditional fermented food processing. This review specifically investigates the application of bioaugmentation technology in the production of key Chinese fermented seasonings, such as vinegar, soy sauce, *sufu*, *doubanjiang*, *dajiang*, and *douchi* (Figure 1). By examining the role of microbial bioaugmentation in traditional fermented seasonings, this review aims to establish a theoretical framework for advancing the fermented food industry.



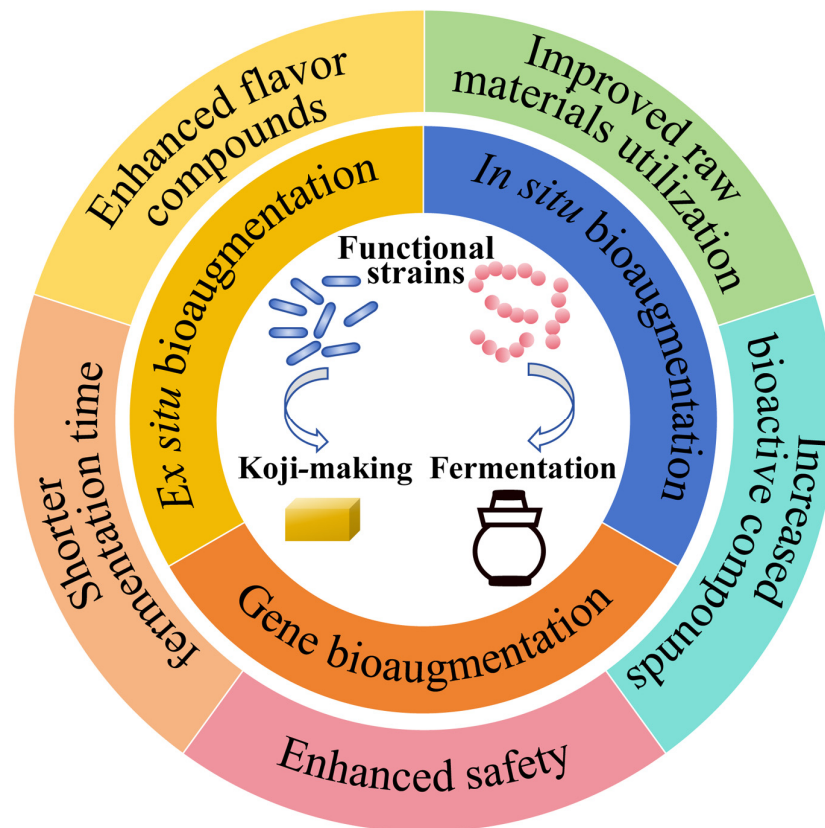
**Figure 1.** Representative traditional Chinese fermented seasonings.

## 2. Bioaugmentation Technology

Bioaugmentation, rooted in ancient food preparation [15], is generally categorized into three types: *in situ* bioaugmentation, *ex situ* bioaugmentation, and bioaugmentation with genetically engineered microorganisms. *In situ* bioaugmentation involves the application of microorganisms screened from specific fermented foods to the same food; *ex situ* bioaugmentation entails the use of microorganisms from different sources in the fermentation of foods; and bioaugmentation with genetically engineered microorganisms involves the use of genetically modified microorganisms carrying specific enzyme-encoding genes to facilitate food fermentation (Figure 2) [16,17]. Notably, it is essential to verify the safety of genetically engineered functional strains, and their direct introduction into food is prohibited in certain countries.

In traditional fermented foods, *in situ* bioaugmentation technology is often employed, which entails incorporating a small portion of materials from the previous batch of fermentation during the initial stage. This process leads to an improved product during the subsequent fermentation cycle. However, modern bioaugmentation, achieved by introducing specific microorganisms to the native microbial community, has demonstrated a greater efficacy [18]. Successful bioaugmentation relies mainly on the precise selection of bacterial strains, prioritizing safety by opting for nonpathogenic strains that do not pose a risk to human health. Additionally, the careful consideration of the activity and viability of the strains in the environment as well as their interactions with the native

microbiota is essential [15]. In recent years, there has been a gradual increase in research on the application of bioaugmentation in the field of fermented foods.



**Figure 2.** Efficacy of bioaugmentation technology on traditional Chinese fermented seasonings.

### 3. Traditional Fermented Seasonings and Their Associated Microorganisms

#### 3.1. Vinegar

Vinegar, a traditional seasoning of global consumption, is particularly esteemed in China, with notable traditional varieties being Shanxi aged vinegar, Zhenjiang aromatic vinegar, Sichuan Baoning vinegar, and Fujian Monascus vinegar [19,20]. The production of traditional Chinese vinegar involves solid-state fermentation, employing grain as the main raw material and Daqu as the starter. The fermentation encompasses three stages: starch saccharification, alcoholic fermentation, and acetic acid fermentation, typically lasting 20–30 d. Following leaching, vinegar requires a period of aging to enhance its distinctive flavor profile [21,22]. Various molds, including *Aspergillus* and *Monascus*, play key roles in vinegar fermentation by converting starchy materials into fermentable sugars [23,24]. This starch saccharification process creates optimal growth conditions for the microorganisms involved in alcohol and acetic acid fermentations. During alcohol fermentation, yeast are crucial for converting fermentable sugars into ethanol and generating aroma compounds, like esters [25]. In acetic acid fermentation, acetic acid bacteria and lactic acid bacteria are the predominant bacteria. Acetic acid bacteria are capable of converting ethanol into acetic acid [26]. Various microorganisms exhibit distinct physiological and biochemical functions, yielding diverse metabolites during fermentation, thereby significantly influencing the vinegar quality. Table 1 presents the microorganisms found in typical traditional fermented seasonings.

**Table 1.** Representative microorganisms in traditional Chinese fermented seasonings.

| Seasoning   | Fungi  | Bacterium  | Reference  |
|-------------|--|--|------------|
| Vinegar     | <i>Aspergillus</i> ,<br><i>Saccharomycopsis</i> , <i>Pichia</i> ,<br><i>Alternaria</i> , <i>Candida</i> ,<br><i>Issatchenkia</i> , <i>Monascus</i>   | <i>Lactobacilli</i> , <i>Acetobacter</i> ,<br><i>Gluconacetobacter</i> ,<br><i>Komagataeibacter</i> , <i>Weissella</i> ,<br><i>Bacillus</i> , <i>Staphylococcus</i> ,<br><i>Enterobacter</i> , <i>Pseudomonas</i> ,<br><i>Clostridium</i>  | [21,27–33] |
| Soy sauce   | <i>Candida</i> , <i>Pichia</i> ,<br><i>Zygosaccharomyces</i>   | <i>Weissella</i> , <i>Tetragenococcus</i> ,<br><i>Staphylococcus</i> , <i>Bacillus</i> ,<br><i>Lactobacilli</i>  | [34,35]    |
| Sufu        | <i>Simplicillium</i> , <i>Verticillium</i> ,<br><i>Actinomucor</i> , <i>Candida</i> ,<br><i>Debaryomyces</i> ,<br><i>Trichosporon</i> , <i>Rhizopus</i> ,<br><i>Monascus</i> , <i>Debaryomyces</i> ,<br><i>Rhodotorula</i> | <i>Weissella</i> , <i>Lactococcus</i> ,<br><i>Enterococcus</i> , <i>Kurthia</i> ,<br><i>Tetragenococcus</i> , <i>Lactobacillales</i> ,<br><i>Enterococcus</i> , <i>Enterobacter</i> ,<br><i>Leuconostoc</i> , <i>Pseudomonas</i>   | [36–39]    |
| Doubanjiang | <i>Aspergillus</i> , <i>Trichosporon</i> ,<br><i>Zygosaccharomyces</i> ,<br><i>Fusicolla</i> , <i>Candida</i> , <i>Pichia</i> ,<br><i>Milleriozyma</i>   | <i>Staphylococcus</i> , <i>Weissella</i> , <i>Bacillus</i> ,<br><i>Lactobacilli</i> , <i>Lysinibacillus</i> ,<br><i>Enterococcus</i> , <i>Escherichia-Shigella</i> ,<br><i>Sphingomonas</i> , <i>Leuconostoc</i><br><i>Tetragenococcus</i> , <i>Weissella</i> ,<br><i>Lactobacilli</i> , <i>Leuconostoc</i> ,<br><i>Tetragenococcus</i> , <i>Pediococcus</i> | [40–43]    |
| Dajiang     | <i>Penicillium</i> , <i>Aspergillus</i>  |  | [44,45]    |
| Douchi      | <i>Debaryomyces</i> , <i>Fusarium</i> ,<br><i>Pichia</i> , <i>Aspergillus</i> ,<br><i>Saccharomyces</i> , <i>Petromyces</i> ,<br><i>Rhizopus</i> , <i>Penicillium</i>  | <i>Staphylococcus</i> , <i>Pediococcus</i> ,<br><i>Bacillus</i> , <i>Weissella</i> , <i>Lactobacilli</i>   | [46–49]    |

### 3.2. Soy Sauce

Soy sauce, produced through the microbial fermentation of wheat and soybeans or defatted soybeans, is a liquid seasoning known for its distinct color, aroma, and taste. It has become a vital seasoning in various Asian countries [50]. The production processes for different soy sauce products are similar, but their technical requirements, such as the ratio of raw materials, fermentation time, and temperature, differ, leading to variations in flavor and composition [51]. Soy sauce can be categorized into Chinese and Japanese types based on the proportions of soybeans and wheat in the raw materials. Chinese-style soy sauce contains a greater proportion of soybeans and a lower amount of wheat, and it is consumed predominantly in China, Indonesia, Malaysia, the Philippines, Singapore, and Thailand. Japanese soy sauce is made up of equal parts soybeans and wheat and is primarily manufactured in Japan and Western countries [52]. Soy sauce fermentation involves two primary methods: low-salt solid-state fermentation and high-salt liquid-state fermentation [53]. Both methods involve a two-step fermentation process, including koji fermentation and mash (moromi) fermentation. Koji fermentation, triggered by the introduction of molds like *Aspergillus oryzae* to steamed soybeans, is a fundamental stage in the creation of top-quality soy sauce. The fermentation of moromi, which comprises koji, sea salt, and brine, typically takes several months to finish [54]. During the koji fermentation stage, molds generate proteolytic enzymes that hydrolyze proteins into peptides and amino acids, as well as amylase to convert starch into fermentable sugars. These nutrients support the growth of bacteria and yeast during the moromi fermentation stage, contributing to the distinctive flavor of soy sauce [51]. In the moromi stage, salt-tolerant lactic acid bacteria and yeast dominate due to the inhibitory effect of high-concentration brine on the growth of *Aspergillus* [55].

### 3.3. Sufu

*Sufu*, a traditional Chinese fermented soybean product, shares similarities in shape and fermentation process with cheese, yet distinguishes itself with a unique taste and flavor profile [56]. Widely utilized as a seasoning and appetizer in China and other Asian countries, its distinct taste, flavor, and nutritional benefits make it a popular choice [57]. *Sufu* is abundant in proteins, carbohydrates, vitamins, and bioactive compounds that mitigate antinutritional factors, thus promoting human health [58]. The production process of *sufu* includes bean curd production, preliminary fermentation, pickling, and post-fermentation [36]. The fermentation process occurs in a semi-open environment and is facilitated by intricate microbial communities comprising fungi and bacteria [37]. Initially, pehtze is obtained by introducing spores of *Mucor*, *Aspergillus*, or *Rhizopus* as the starter on the surface of the tofu cubes, or by using microorganisms naturally occurring in the environment for fermentation. The resulting pehtze is then salted for 24 h, transferred into wide-mouthed bottles, and aged in a dressing mixture for 60 d [38,59]. Throughout the fermentation of *sufu*, the predominant bacteria are mainly *Tetragonococcus*, *Bacillus*, *Acinetobacter*, *Lactococcus*, and *Enterobacter* [60,61]. The formation of flavor substances in *sufu* primarily involves lipolysis and proteolysis. Through the action of microbial lipases and proteolytic enzymes, macromolecules, such as proteins and lipids, are broken down into smaller peptides, amino acids, and fatty acids, providing *sufu* with its unique flavor profile [39].

### 3.4. Doubanjiang

*Doubanjiang* (broad bean paste), a prominent bean-based fermented food in China, is widely utilized as a seasoning in Chinese cuisine [62]. The type originating from Pixian county in Sichuan Province holds particular renown, being revered as the quintessence of Sichuan cuisine [40]. Its preparation involves the use of broad beans, wheat flour, red pepper, and a high-concentration brine [63]. The conventional manufacturing process involves three phases: first, the fermentation of broad beans with 12~14% (*w/w*) salt to produce *doubanjiang*-meju; second, the fermentation of red peppers with 14~16% (*w/w*) salt to obtain red pepper moromi; and third, the aging fermentation of the mixture of *doubanjiang*-meju and red pepper moromi for over six months in a semi-open environment to enhance flavor [64]. The microorganisms participating in the traditional fermentation process of *doubanjiang* comprise *Leuconostoc lactis*, *Staphylococcus xylosus*, *Staphylococcus succinus*, *Amylomyces rouxii*, *Mucor genevensis*, *Absidia corymbifera*, *Issatchenkia orientalis*, *Basidiomycete yeast* sp., and *Metschnikowia pulcherrima*, which are responsible for imparting unique flavors to *doubanjiang* [65].

### 3.5. Dajiang

*Dajiang*, also referred to as *doujiang* or soybean paste, is a traditional seasoning produced through the fermentation of soybeans and wheat flour. With its rich color, moderate viscosity, fresh, and mellow characteristics, and balanced salty-sweet taste, it serves as a valuable flavor enhancer for various dishes [44,66]. This staple seasoning holds significant importance in Asian culinary traditions and has gained global popularity [67]. The production of *dajiang* involves two primary stages: koji production and fermentation. As a starter of traditional fermented soybean products, koji is produced through soaking, steaming, crushing, and molding [68], serving as a source of nutrients and flavor. The natural fermentation process to acquire mature koji may take 4~5 months, involving the joint action of microorganisms, including fungi, yeast, and bacteria [67]. Subsequently, the mature koji is combined with brine and fermented for over two months to achieve the distinctive flavor of *dajiang* products.

### 3.6. Douchi

*Douchi*, a traditional fermented black soybean product originating in China, has a long history [69]. It is produced through two stages: the initial koji-making stage and the subsequent fermentation stage. To make *douchi*, black soybeans are treated with the 'house



flora’ that initiates the koji-making process. At this stage, a koji inoculum from a later stage is added to the black soybeans, which are then subjected to 7 days of koji fermentation until a white mold covers them. Following this, the black soybeans are mixed with salt to inhibit microbial growth and are transferred to sealed fermentation tanks to exclude oxygen. The fermentation process continues for 15 d at approximately 55 °C, after which the resulting product is dried in an oven [70]. *Douchi* can be categorized into four types based on variations in the microorganisms involved in the fermentation process: bacteria, *Aspergillus*, *Mucor*, and *Rhizopus*, with *Aspergillus*-type *douchi* being the most prevalent in China [71]. Microbial enzymes, including proteases and amylases, play pivotal roles in decomposing soybean proteins and starches during fermentation, leading to the production of essential nutrients and flavor compounds. The volatile components produced by different types of *douchi* vary significantly in type and content, owing to differences in the production process, microorganisms, and environment [72].

4. Effect of Bioaugmentation on Traditional Fermented Seasonings

4.1. Enhancement in the Key Flavor Substances

The fermentation process of traditional fermented seasonings is intricate, involving protein hydrolysis, starch saccharification, fat hydrolysis, acid production, alcohol fermentation, enzymatic browning, and the Maillard reaction. The progression of these reactions depends on the participating microorganisms and fermentation conditions, which are also pivotal for the generation of complex flavor compounds [73]. However, the traditional fermentation process is typically conducted in a semi-open environment, where fluctuating microbiota and environmental conditions can significantly impact product quality, particularly flavor composition [74].

Flavor serves as a crucial parameter for assessing traditional fermented seasonings [75,76]. Bioaugmentation involves the use of functional strains capable of generating particular flavor compounds or the requisite enzymes to enhance the synthesis of flavor compounds. During the production of Sichuan bran vinegar, the introduction of Hongqu, obtained from fermenting steamed glutinous rice with *Monascus purpureus*, along with traditional Daqu (koji) as a starter, lead to an increase in the relative abundance of acetic acid bacteria. This, in turn, causes a 1.95-fold increase in the concentrations of organic acids, a 2.30-fold increase in aromatic esters, and a 3.55-fold increase in alcohols within the vinegar [24]. Wang et al. [77] investigated the impact of three aroma-producing strains (*Wickerhamiella versatilis*, *Candida sorbosivorans*, and *Starmerella etchellsii*) on raw soy sauce during a 30-day fermentation period. The study found that *W. versatilis* increased the levels of esters, alcohols, and aromatic compounds, while *S. etchellsii* enhanced the contents of 2,6-dimethylpyrazine, methyl pyrazine, and benzeneacetaldehyde. Additionally, soy sauce bioaugmented with *C. sorbosivorans* exhibited elevated concentrations of furfuraldehyde, methane, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, and maltol, resulting in sweet and caramel aromas. The effects of bioaugmentation technology on enhancing flavor compounds in other traditional fermented seasonings are summarized in Table 2.

Table 2. Efficacy of bioaugmentation technology on the flavor of traditional Chinese fermented seasonings.

| Seasoning            | Microorganism                     | Bioaugmentation Strategy  | Efficacy on Flavor   | Reference |
|----------------------|-----------------------------------|---|--|-----------|
| Shanxi aged vinegar  | <i>Pichia manshurica</i> Y14      | <i>P. manshurica</i> Y14 was inoculated (7%, v/v) in the Daqu-based fermentation.<br>A new Daqu prepared by combining <i>B. amyloliquefaciens</i> -bioaugmented Daqu and traditional Daqu without Chinese herbs at a 1:1 (v/v) ratio was used as a starter. | The contents of ester compounds increased from 15.3 to 21.5 g/L.                                       | [25]      |
| Sichuan bran vinegar | <i>Bacillus amyloliquefaciens</i> |   | The contents of ethyl acetate and tetramethyl pyrazine increased by 191.84% and 123.17%, respectively. | [23]      |

Table 2. Cont.

| Seasoning                                     | Microorganism   | Bioaugmentation Strategy   | Efficacy on Flavor  | Reference |
|---|---|--|---|-----------|
| Zhenjiang aromatic vinegar                    | <i>Lactocaseibacillus casei</i> (formerly <i>Lactobacillus casei</i> ) M1-6, <i>Acetobacter pasteurianus</i> G3-2                                     | Same number of <i>Ls. casei</i> M1-6 and <i>Acetobacter pasteurianus</i> G3-2 were inoculated.   | The contents of acetoin, ethyl acetate, ethyl lactate, and Chuanqiongqin increased by 102.4%, 146.6%, 91.7%, and 52.1%, respectively.   | [78]      |
|   | <i>Lactiplantibacillus plantarum</i> (formerly <i>Lactobacillus plantarum</i> ) M10-1, <i>Ls. casei</i> (formerly <i>Lactobacillus casei</i> ) 21M3-1 | One liter of each strain supernatant ( $10^{12}$ CFU/mL) was inoculated into 164 kg vinegar <i>Pei</i> .   | The presence of <i>Ls. casei</i> 21M3-1 led to a four-fold increase in L-lactic acid production, whereas <i>Lp. plantarum</i> M10-1 enhanced the contents of both L-lactic acid and D-lactic acid by one-fold.  | [22]      |
| Soy sauce                                     | <i>T. halophilus</i> , <i>Zygosaccharomyces rouxii</i> , <i>Torulopsis versatilis</i>   | After 15 d of moromi fermentation, <i>T. halophilus</i> ( $2 \times 10^5$ CFU/mL) was inoculated. <i>Z. rouxii</i> ( $10^6$ CFU/mL) was then inoculated on day 30, followed by the inoculation of <i>T. versatilis</i> ( $10^6$ CFU/mL) on the 45th day. During moromi fermentation, co-inoculation with <i>T. halophilus</i> and <i>Z. rouxii</i> , or inoculation firstly with <i>T. halophilus</i> , followed by the sequential inoculation of <i>Z. rouxii</i> . | The fruity, saucy, alcoholic, and caramel-like flavors increased by 64.3%, 22.7%, 43.1%, and 36.2%, respectively, while the saline taste increased by 64.3%.  | [79]      |
|   | <i>T. halophilus</i> , <i>Z. rouxii</i>   |  | The promotion of alcohol formation obtained through bioaugmentation led to the development of more intricate aroma characteristics.   | [80]      |
| High-salt liquid-state fermentation soy sauce | <i>Millerozyma farinosa</i> CS2.23, <i>Z. rouxii</i> CS2.42, <i>Candida parapsilosis</i> CS2.53   | Each strain ( $10^7$ cell/mL) was inoculated in high-salt liquid-state moromi fermented for 45 d.  | The volatile esters content inoculated with <i>M. farinosa</i> CS2.23, <i>Z. rouxii</i> CS2.42, and <i>C. parapsilosis</i> CS2.53 increased by 108.85%, 166.71%, and 113.61%, respectively.   | [81]      |
|   | <i>Wickerhamomyces anomalus</i> ZMS55, <i>W. anomalus</i> ZMS102  | Following the fermentation of high-salt liquid-state moromi to a pH of 5, it was inoculated with each strain ( $2 \times 10^6$ cells/g).   | The production of esters showed increased diversity, accompanied by significantly higher yields of ethanol, acids, and aldehydes.   | [82]      |
|   | <i>Z. rouxii</i> QH-25, <i>C. versatilis</i>  | On the first day of the high-salt liquid-state moromi fermentation, <i>Z. rouxii</i> QH-25 was inoculated, followed by the inoculation of <i>C. versatilis</i> on the fifth day.   | The concentrations of volatile substances, including ketones, esters, phenols, and alcohols, increased by 3.07-, 1.91-, 1.36-, and 1.22-fold, respectively. Characteristic components, such as ethyl octanoate, 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone, 4-ethyl-2-methoxy-phenol, and 3-methyl-1-butanol, exhibited increases by 3.99-, 3.29-, 1.63-, and 0.70-fold, respectively. | [83]      |
|   | <i>Leuconostoc mesenteroides</i> F24  | <i>L. mesenteroides</i> F24 was inoculated in the mixture of brine and yellow tofu serofluids at approximately $10^6$ CFU/mL.  | The contents of esters, alcohols, aldehydes, acids, and aromatic compounds increased.   | [84]      |
| Gray sufu                                     | <i>Weissella confusa</i> M1   | <i>W. confusa</i> M1 was added to the mixture of brine and yellow tofu serofluids at approximately $10^6$ CFU/mL.  | The contents of 13 free amino acids increased, particularly aspartic acid and glutamic acid.  | [85]      |

Table 2. Cont.

| Seasoning                              | Microorganism  | Bioaugmentation Strategy   | Efficacy on Flavor   | Reference |
|--|--|--|--|-----------|
| Doubanjiang                            | <i>T. halophilus</i> , <i>W. confuse</i> ,<br><i>Z. rouxii</i>   | <i>Lactobacillales</i> ( <i>T. halophilus</i> and <i>W. confuse</i> ) and <i>Z. rouxii</i> were inoculated into the mixed <i>Pei</i> at $10^6$ CFU/g and $10^5$ CFU/g, respectively. | The contents of amino acids, like glutamic acid and aspartic acid, along with volatile flavor compounds, such as esters, carbonyls, and phenols, increased.  | [41]      |
|  | <i>Z. rouxii</i> Y-8   | <i>Z. rouxii</i> Y-8 was inoculated at the beginning of <i>Pei</i> fermentation at $10^6$ CFU/g <i>Pei</i> .   | The total concentrations of volatile flavor compounds increased from 4767.22 to 72,813.09 $\mu\text{g}/100$ g dry <i>Pei</i> , with the presence of 33 new volatile flavor compounds, including alcohols, esters, acids, and carbonyl compounds. | [86]      |
| <i>Aspergillus</i> -type <i>douchi</i> | <i>Meyerozyma Caribbica</i> ,<br><i>Meyerozyma guilliermondii</i> , <i>Candida etchellsii</i> , <i>C. versatilis</i> | Following a 3-day culture period, the purified strains were co-inoculated with <i>A. oryzae</i> to produce <i>Aspergillus</i> -type <i>douchi</i> .                                  | The contents of amino acids, unsaturated fatty acids, and organic acids increased.   | [87]      |

#### 4.2. Improvement in Raw Material Utilization

Bioaugmentation offers a promising solution for low starch utilization in vinegar production. Zhang et al. [88] demonstrated this by introducing *Pediococcus lactis* AAF5-1, a strain known for its resilience to acidity and heat, into the initial acetic acid fermentation process of Tianjin Duliu aged vinegar. This intervention resulted in an elevated abundance of amylase-producing *Lactobacilli* strains, leading to an increase in starch utilization from 79% to 83%. In the production of Sichuan Baoning vinegar, Liu et al. [89] utilized *Aspergillus niger* AS 3.758 to prepare bioaugmented bran Qu and observed a lower starch content of 5.49% in vinegar *Pei*, compared to 7.88% starch content in the non-bioaugmented group. Moreover, Peng et al. [90] introduced *Komagataeibacter europaeus* JNP1 during the acetic acid fermentation stage and noted a significant increase in the expression of genes related to sugar metabolism. This led to a notable decrease in the reducing sugar content, indicating the enhanced utilization of the starchy raw material through bioaugmentation.

During *doubanjiang* fermentation, the enzymes produced by microorganisms degrade proteins, starches, and other raw materials into smaller molecules, like peptides, free amino acids, and fermentable sugars, thereby enhancing the raw material utilization [91]. Studies by Gupte and Verma demonstrated the positive impact of fungal co-cultures on hydrolase production [92,93]. *Aspergillus* was found to be particularly influential in enhancing enzyme activities during *doubanjiang* fermentation [94]. Therefore, Tang [12] selected *A. oryzae* QM-6, known for its high neutral protease production, and *A. niger* QH-3, known for its high acid protease production, and co-cultured these strains at a specific ratio. The resulting inoculation of the co-cultures into *doubanjiang* koji revealed a significant enhancement in proteolytic enzyme activity compared to the inoculation of *A. oryzae* QM-6 alone. This co-cultured approach resulted in an improved protein utilization and a higher amino acid content.

#### 4.3. Shorter Maturity Time

Shortening the fermentation time has a significant impact on the cost of producing fermented foods. Feng et al. [95] developed a starter by combining *Kocuria kristinae* F7, *Micrococcus luteus* KDF1, and *Staphylococcus carnosus* KDFR1676, which were isolated from Kedong *sufu*, and applied it at a 2:1:2 ratio to the surface of tofu. The use of this mixed microbial starter led to a 60-day reduction in the maturity period of *sufu*, while still meeting the national standards for physicochemical properties, compared to tradi-



tional back-slopping *sufu*. Similarly, Feng et al. [96] investigated the impact of introducing *Kocuria rosea* KDF3, isolated from traditional Kedong *sufu*, on the bioaugmentation of *sufu* fermentation. Following a 120-day fermentation period, the bioaugmented *sufu* exhibited significantly higher levels of peptides, total free amino acids, and 14 specific free amino acids in comparison to the non-bioaugmented group, which had undergone 150 d of fermentation. Additionally, compared to the non-bioaugmented sample, the bioaugmented sample showed significantly elevated levels of amino acid nitrogen and water-soluble proteins. The sensory evaluation revealed no significant difference between the bioaugmented group fermented for 120 d and the non-bioaugmented group fermented for 150 d. These findings indicated that bioaugmented Kedong *sufu* reached maturity 30 d earlier than their non-bioaugmented counterpart.

#### 4.4. Producing Bioactive Compounds

As the demand for healthy foods increases, consumers are seeking more functional seasonings. The selection of strains that yield high levels of bioactive compounds for fortifying seasonings can enhance the bioactive compound content in the resulting products. Tetramethyl pyrazine, the primary bioactive compound in vinegar and a main component of the Chinese herbal medicine Chuanxiong, demonstrates therapeutic efficacy against cardiovascular and cerebrovascular diseases, diabetes, liver injury, headache, and dizziness [97]. The concentrations of acetoin, a tetramethyl pyrazine precursor, can be significantly increased by introducing lactic acid bacteria and acetic acid bacteria [78]. During soy sauce fermentation, a combination of *A. oryzae* HG-26 and *A. niger* HG-35 was chosen for koji production. Higher levels of total phenols, total flavonoids, and three soybean isoflavone glycosides were observed during brine fermentation compared to koji produced with *A. oryzae* HG-26 alone, along with a notable enhancement in the antioxidant activity [98].

Studies have shown that *Limosilactobacillus reuteri* (former *Lactobacillus reuteri*) can increase the vitamin B12 content in soybean products [99]. In response to the variability in the vitamin B12 content in *sufu*, Bao et al. [100] investigated the effect of *Lm. reuteri* inoculation. The results revealed that the growth of microorganisms harboring complete genes for vitamin B12 synthesis, such as *Streptococcus*, *Enterococcus*, and *Lactobacilli*, was stimulated. The vitamin B12 content in *sufu* inoculated with *Lm. reuteri* (141.7 ng/g) significantly exceeded that in the control group (36.0 ng/g). *Levilactobacillus brevis* (former *Lactobacillus brevis*) demonstrates a notable capacity for  $\gamma$ -aminobutyric acid (GABA) production [101]. Consequently, Bao et al. [59] introduced *Lv. brevis* into the *sufu* fermentation. The findings revealed that the samples inoculated with *Lv. brevis* exhibited a substantially higher GABA concentration 10 d after ripening in comparison to the control group.

#### 4.5. Improving Safety

Traditional fermented seasonings are susceptible to safety issues due to their production in a semi-open environment and the prolonged production process. Mycotoxins, particularly aflatoxins, may be present during fermentation, and the concentrations of biogenic amines often exceed the standard limits. These challenges can be partially addressed through the application of bioaugmentation technology.

Aflatoxins, potent carcinogens, pose great threats to human health [102] as they easily contaminate grains, beans, and other raw materials and can therefore be found in fermented foods. Various physical, chemical, and biological methods can be employed to remove aflatoxins [103,104]. The selection of microorganisms capable of degrading aflatoxins to enhance the fermentation process undoubtedly offers a safe and economical solution. During the production of Sichuan *doubanjiang*, Feng et al. [105] inoculated a co-culture of *Lp. plantarum* DPUL-J5 and *Pichia kudriavzevii* DPUY-J5 in a brine fermentation system containing *Bacillus subtilis* DPUL-J2. Following fermentation, the *doubanjiang* product fermented by the three strains exhibited a 65% reduction in aflatoxin B1 levels compared to the product solely inoculated with *B. subtilis* DPUL-J2. Similarly, the co-inoculation of *Lp. plantarum* DPUL-J8 and *P. kudriavzevii* DPUY-J8 during the fermentation of northeast

doujiang resulted in an approximately 70% decrease in the AFB1 content compared to the uninoculated control group [106].

Biogenic amines are nonvolatile, low-molecular-weight nitrogen-containing organic compounds primarily formed by the decarboxylation of the corresponding amino acids. They are frequently found in fermented foods [107]. The raw materials used in fermented bean seasonings are rich in protein, which can be easily hydrolyzed into large amounts of free amino acids and peptides. Microorganisms, such as lactic acid bacteria, can produce amino acid decarboxylase, leading to higher levels of biogenic amines. The consumption of foods containing high concentrations of these substances may result in food poisoning, characterized by symptoms such as headache, nausea, and fluctuations in blood pressure [108]. Consequently, there is widespread concern regarding the management of biogenic amines. Several studies have improved the fermentation process by introducing strains capable of degrading biogenic amines, thereby reducing their production during fermentation (Table 3). For instance, Feng et al. [109] introduced two mixed-culture starters, named Starter I and Starter II, into the production of Kedong *sufu*. Starter I consisted of *K. kristinae* F7, *K. rosea* KDF3, *M. luteus* KDF2, and *M. luteus* KDF4, while Starter II comprised *K. kristinae* F8, *K. rosea* KDF1, *M. luteus* KDF1, and *M. luteus* KDF3. The utilization of Starter I and Starter II led to reductions of 27% and 35%, respectively, in the total biogenic amine content in *sufu*, as compared to the traditional back-slopping fermentation method.

**Table 3.** Efficacy of bioaugmentation technology on the safety of traditional Chinese fermented seasonings.

| Seasoning           | Microorganisms  | Bioaugmentation Strategy   | Efficacy on Safety  | Reference |
|---------------------|---|--|---|-----------|
| Soy sauce           | <i>Staphylococcus piscifermentans</i> QR19                              | <i>S. piscifermentans</i> QR19 was inoculated into the fermentation mash at the beginning of fermentation.   | The biogenic amine content decreased by 63.25% compared to soy sauce without <i>S. piscifermentans</i> . Additionally, they were 81.19% and 71.87% lower, respectively, than two commercial soy sauces.               | [110]     |
|                     | <i>Z. rouxii</i> , <i>T. halophilus</i>                                 | During brine fermentation, <i>T. halophilus</i> ( $2.5 \times 10^6$ CFU/g) and <i>Z. rouxii</i> ( $2 \times 10^6$ CFU/g) were inoculated into moromi.                    | The biogenic amine content was reduced by 52.36–55.05%.   | [111]     |
| Cantonese soy sauce | <i>T. halophilus</i> CGMCC3792, <i>Z. rouxii</i> CGMCC21865             | At the beginning of the brine fermentation, <i>T. halophilus</i> ( $2.1 \times 10^6$ CFU/g) and <i>Z. rouxii</i> ( $1.6 \times 10^6$ CFU/g) were inoculated into moromi. | The biogenic amine content was reduced by 67.68%.   | [112]     |
| <i>Sufu</i>         | <i>Lv. brevis</i> (formerly <i>Lactobacillus brevis</i> )               | <i>Lv. brevis</i> ( $3.8 \times 10^6$ CFU/mL) was added to the mixture of brine and yellow tofu serofluids.  | The biogenic amine content was reduced significantly.   | [59]      |
| Doubanjiang         | <i>Lp. plantarum</i> (formerly <i>Lactobacillus plantarum</i> ) DPUL-J5 | <i>Lp. plantarum</i> DPUL-J5 (2%) was inoculated into brine containing 2% <i>B. subtilis</i> DPUL-J2.  | The biogenic amine content was reduced significantly.   | [105]     |
|                     | <i>B. amyloliquefaciens</i> 1-G6, <i>Bacillus licheniformis</i> 2-B3    | Each strain ( $10^6$ CFU/g) was inoculated on the third day of fermentation.   | Inoculation with <i>B. amyloliquefaciens</i> 1-G6 led to a 29% reduction in the biogenic amine content, while inoculation with <i>B. licheniformis</i> 2-B3 resulted in a 16% decrease in the biogenic amine content. | [113]     |

Table 3. Cont.

| Seasoning | Microorganisms  | Bioaugmentation Strategy   | Efficacy on Safety   | Reference |
|-----------|---|--|--|-----------|
| Dajiang   | <i>Lp. plantarum</i> (formerly <i>Lactobacillus plantarum</i> ) HM24  | The <i>Lp. plantarum</i> HM24 supernatant (4%) was inoculated into a mixture of koji and brine.  | The degradation rates of tryptamine, phenethylamine, putrescine, cadaverine, histamine, and tyramine were 35.31%, 43.14%, 30.18%, 33.44%, 32.74%, and 39.91%, respectively.  | [114]     |
|           | <i>S. carnosus</i> M43, <i>Pediococcus acidilactici</i> M28   | A mixed bacteria solution of each strain at $10^7$ CFU/g was prepared at a ratio of 1:1 and inoculated for fermentation.   | The biogenic amine content decreased by 39.69%.  | [115]     |
|           | <i>Lp. plantarum</i> DPUL-J8, <i>P. kudriavzevii</i> DPUY-J8  | <i>Lp. plantarum</i> DPUL-J8 and <i>P. kudriavzevii</i> DPUY-J8 were co-inoculated.  | The biogenic amine content decreased by 67.15%.  | [106]     |
| Douchi    | <i>Bacillus tropicus</i> A11, <i>Bacillus siamensis</i> D11, <i>B. subtilis</i> T2, <i>B. subtilis</i> U2   | Each strain was inoculated into soybeans at 3% ( <i>v/m</i> ).   | Through the mono-fermentation of <i>B. tropicus</i> A11, <i>B. siamensis</i> D11, <i>B. subtilis</i> T2, and <i>B. subtilis</i> U2, the contents of biogenic amines decreased by 74.38%, 61.85%, 82.13%, and 65.43%, respectively. | [116]     |
|           | <i>Mucor racemosus</i> (M1), <i>Mucor wutunqiao</i> (M2), <i>Actinomucor elegans</i> (M3), <i>A. oryzae</i> 2339 (A1), <i>A. oryzae</i> 41380 (A2), <i>A. oryzae</i> 40188 (A3) | Each strain was cultivated in potato-dextro agar at 28 °C for 3 d. Subsequently, 1 mL of sterilized water was added to the agar to obtain the spore suspension, which was then incorporated into the bran medium and incubated at 28 °C for 3 d. Following this, a mixture of 0.3~0.5% ( <i>w/w</i> ) of the bran medium containing the strains was mixed with steamed soybeans. | The biogenic amine content decreased by 38.76%, 32.11%, 36.27%, 21.44%, 25.06%, and 21.27% for <i>douchi</i> inoculated with A1, A2, A3, M1, M2, and M3, respectively.   | [117]     |

## 5. Conclusions and Prospects

The increasing demand for high-quality fermented seasonings has drawn significant attention to their safety and nutritional attributes. Bioaugmentation technology shows great potential in addressing these concerns. This review examines the primary microorganisms in six types of traditional Chinese fermented seasonings and evaluates the impact of bioaugmentation on product quality. The findings demonstrate the effectiveness of bioaugmentation technology in enhancing the flavor, nutritional value, and safety of fermented seasonings. Furthermore, this approach contributes to a better utilization of raw materials and a reduced ripening time, thereby enhancing the economic sustainability of the products. These results offer a theoretical basis for the industrial advancement of bioaugmentation in the field of fermented foods.

The variations in raw materials, production processes, fermentation conditions, and geographical environments among different fermentation seasonings are noteworthy. As a result, the selection of bioaugmentation strains should be guided by the specific attributes and product requirements of the fermentation products. Additionally, the assessment of the strain performance, such as safety, enzyme and ester production, and salt resistance, is crucial for identifying the most suitable strain. The limitations of using a single strain for bioaugmentation are evident as it can only enhance one aspect. In contrast, a naturally occurring microbial consortium is more robust to environmental challenges, has a reduced metabolic burden, and exhibits more complex functions [118]. These advantages can be

harnessed in the development of fermented seasonings through the construction of tailored microbial consortia, thereby addressing the diverse requirements for product enhancement [119]. Limited research has been conducted on the bioaugmentation mechanism of particular functional strains, which is essential for the precise control of the fermentation process in the production of fermented foods. Moreover, the inoculation strategy, including method, order, and timing, yields diverse effects on bioaugmentation. Therefore, it is crucial to explore the optimal bioaugmentation parameters to ensure the quality and safety of fermented foods.

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