

# Berkala Hayati

*by* Budhi Setiawan

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17  
1 **Potential improvement in the safety and quality of traditional fermented soybean**  
2 **products: A narrative review**

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3  
4 **ABSTRACT**

5 Soybean is one of the important crops in the world because of its nutritional value. It has been  
6 processed into various fermented soybean products. During conventional soybean fermentation,  
7 microorganisms are involved, biochemical reactions occur, and bioactive components are produced.  
8 With the improvement of people's living standard, consumers pay more attention not only to the flavor  
9 and nutrition but also the safety and quality of fermented soybean foods. Public health issues related  
10 to fermented soybean foods include quality inconsistency, mycotoxins, formation of biogenic amines,  
11 and high salt content. This paper reviews the common concerns associated with the safety and quality  
12 of fermented soybean foods and discusses the plausible efforts for the improvement. Attempts and  
13 methods have been proposed to ensure the safety of the fermentation process and food quality.  
14 Official regulations, the use of suitable microorganisms, high-quality cultivars utilization,  
15 administration of additives are plausible alternatives for safety and quality improvement. We  
16 conclude that implementing International food standard, guidelines and code of practice such as The  
17 Codex Alimentarius for fermented soybean products regulations and the use of scientific novel  
18 methods (eg. starter combination, high-pressure processing, or low-dose gamma irradiation, additives  
19 usage, low salt fermentation technique) are the potential solutions to mitigate the issues and improve  
20 the safety and quality of the products.

21 **Keywords:** Fermentation, soybean, safety, quality, food, nutrition,

29  
23 **INTRODUCTION**

24 Soybean (*Glycine max* [L.] Merrill) is a legume with high economic values and one of the affordable  
25 primary protein sources for the human diet in the world, which can be used as meat substitution  
26 (Xiang et al. 2019). Soy-based foods are known as non-fermented (soymilk, okara, tofu, yuba, soy

Commented [A1]: Is there Any direct correlation between health issue with quality inconsistency?

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Commented [A3]: Too wide perception, make it more specific, what are the standard regulations and scientific novel methods

Commented [A4R3]: Some examples have been added

Commented [A5]: Please make it be a better organized introduction

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27 nuts, etc.) and fermented (miso, tempe, sufu, natto, etc.) (Liu 2008). Several studies have reported  
28 that fermentation of soybean results in numerous beneficial properties. The fermentation process can  
29 reduce cooking time, improve the nutritional quality of soybean, increase the bioactive components,  
30 such as phytoestrogens, and make soybeans more digestible (Chen et al. 2016). Some fermented  
31 soybean products (FSPs) are commonly used as condiments to raise the appetite and enhance the  
32 flavor of foods (Xiang et al. 2019).

33 It is well known that antinutritional factors present in whole soybean exert negative impacts.  
34 However, Whole soybean also contains anti-nutritional factors such as trypsin inhibitor, lectins,  
35 phytic acid, oligosaccharides, which raise concern for its consumption (Thakur et al. 2019). Anti-  
36 nutritional factors in foods may cause negative effects associated with the absorption and utilization  
37 of nutrients and the destruction of vitamins (Thakur et al. 2019). The deleterious influence on the  
38 absorption of these anti-nutritional factors might can trigger deficiency nutrients, and result in various  
39 human health-related issues especially in animals. The trypsin inhibitor activity in unfermented  
40 soybean affects negatively on Broiler chicken's growth performance and pancreas weight (Hoffman  
41 et al. 2019; Hemetsberger et al. 2021).

42  
43 Studies have shown that microorganisms used in conventional fermentation could produce toxins that  
44 contaminate FSPs (Chen et al. 2016). As an example, *Rhizopus microspores*, a common species used  
45 as soybean fermentation starter, can produce two types of mycotoxins, namely rhizoxins and  
46 rhizonins. These mycotoxins can be found in tempe (Indonesian fermented soybean cake) and  
47 fermented bean curd (Rohm et al. 2010). *Aspergillus oryzae*, another strain frequently used in  
48 traditional soybean fermentation, produces also toxic secondary metabolites, such as cyclopiazonic  
49 acid, aspergillomarasmine, 3-nitropropionic acid, kojic acid, maltoryzine, and violacetin (Blumenthal  
50 2004).

51

**Commented [A9]:** If you mention non fermented and fermented one, (to connect the previous sentence to the next one) then add 1 more sentence to explain why you prefer to write more the fermentation one

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**Commented [A11]:** Is it a whole soybean? Or just the fermented soybean?  
Again, please write in a good organization (smooth shifting among sentences/paragraph)  
If you talk anti-nutrition, then add sentence(s) to talk the nutritional content in the previous

**Commented [A12R11]:** A sentence has been added and whole soybean is written also

**Commented [A13]:** This is a review, it means that every statement has to be strongly supported by references (not only one), then please dont use 'prediction' (use might, may be etc) statement

**Commented [A14R13]:** It has been adjusted with an example of negative effect of antinutrients

52 Biogenic amines (BA), harmful substances generated during the fermentation process are often found  
53 in fermented soybean products from Korea, China, Japan, and Indonesia with significant  
54 concentrations (Park et al. 2019). Some fermented soybean products, such as fermented bean curd,  
55 soy sauce, douchi and dajiang are usually preserved without sterilization, but through the use of high  
56 salt addition. This technique prevents spoilage and prolongs the product's shelf life (Chen et al. 2016;  
57 Liu et al. 2020). However, an excessive amount of salt intake may cause an expansion in circulating  
58 volumes and lead to an increase in blood pressure in humans (Komnenov et al. 2019). Therefore, this  
59 narrative review aims to examine the problems associated with the safety and quality of common  
60 fermented soybean products and discuss the potential solutions.

61

## 62 INCONSISTENCY OF QUALITY IMPROVEMENT

63 The making of traditional fermented soybean foods usually uses traditional fermentation methods.

64 There are common different techniques used to produce indigenous soy-based foods (figure 1). Asian  
65 countries have diverse preparations for fermented soybean products according to indigenous recipes

66 (table 1). ~~Standard Quality control is not might be difficult to be appropriately~~ applied on the  
67 conventional fermentation process so which results in inconsistent outcomes of the fermented

68 soybean products. Consistency of quality for particular fermented soybean food is determined by  
69 several factors. For instance, the quality of tempeh (Indonesia fermented soybean cake) might be

70 affected by soybeans, process water, yeast, fermentation time, and fermentation temperature (Novita  
71 & Abidin 2020). In daily practice, the process of fermentation is complicated and the product quality

72 mostly depends on several conditions, such as geographical location, environment, weather, season,  
73 etc (Liu et al. 2020). ~~As an example, there is a possibility in Indonesia that the small-scale food~~

74 ~~industry of fermented soybean could not vary in meeting the national quality and safety of food~~  
75 ~~standards provided by the government due to environmental factors and raw materials (Anggriawan~~

76 ~~2018; Kadar et al. 2021). In Indonesia, it has been found that protein content in commercial products~~  
77 ~~of soy sauce was 1% less than the national standard (2.5%). Furthermore, many institutions cannot~~

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Commented [A17]: It is not the case. You can control everything, if you want to.

Commented [A18R17]: The sentence has been adjusted

Commented [A19]: This can be a mislead statement. To make it real, you need to support it with data

Commented [A20R19]: It has been adjusted with references

Commented [A21]: Is that commercial product produced by traditional method? We cannot make it as general conclusion, if the case is the protein content less than the standard, then it just need to 'play' with formulation (reduce the water/non protein material used in the formulation)

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79 **Table 1.** Countries distribution and variety of fermented soybean products (Tamang et al. 2020).

Country	Local name	Organoleptic characters, culinary and additional substrate
India	Bekang	58 line; sticky, paste, curry
	Tungrymbai	Alkaline; sticky, curry, soup
	Hawajjar	Alkaline; sticky
Japan	Miso	Alkaline; paste
	Natto	Alkaline; sticky, breakfast
Indonesia	Kecap	Liquid, wheat
	Ketjap	Syrup; black
	Tauco	Alkaline; paste, use as flavoring agent
Korea	Chungkokjang (or jeonkukjang, cheonggukjang)	Alkaline; sticky, soup
	Doenjang	Alkaline; paste, soup
	Gochujang	Hot-flavored seasoning, red-pepper
	Meju	Alkaline; paste
	Kanjang	Soya sauce; meju; salt, water
China	Furu	Mild-acidic soybean-curd
	Yandou	Alkaline; sticky, salted, snack
Taiwan	Jiang-sun	side dish
China, Taiwan	Douchi	Alkaline; paste
	Meitauza	Liquid
India, Nepal, Bhutan	Kinema	Alkaline; sticky; curry
Japan, Korea, China	Shoyu	Alkaline; liquid, seasoning
Indonesia (Origin), Netherlands, Japan, USA	Tempe	Alkaline; solid, cake
Country	Local name	References
India	Bekang	Chettri and Tamang (2014); Singh et al. (2014a, 2014b)
	Tungrymbai	Chettri and Tamang (2015); Singh et al. (2014a, 2014b)
	Hawajjar	Singh et al. (2014a, 2014b)
Japan	Miso	Tamang et al. 2016
	Natto	Liu 2012
Indonesia	Kecap	Alexandraki et al., 2013
	Tauco	Kindosi FM et al., 2012
Korea	Chungkokjang (or jeonkukjang, cheonggukjang)	Hong et al. 2012
	Doenjang	24 g et al. (2013); Kim et al. (2016a)
	Gochujang	Nam et al. 2012
	Meju	Sanni et al. 1991
	Kanjang	Shin, D. H. 2012
China	Furu	Lin et al. 2016
Taiwan	Yandou	Chen, Y. S. 2010
China, Taiwan	Jiang-sun	Chen, Y. S. 2010
	Douchi	Yang et al. 2019a, 2019b
India, Nepal, Bhutan	Meitauza	24
	Kinema	Chettri et al. (2016); Chettri and Tamang (2015); Singh et al. (2014a, 2014b)
Japan, Korea, China	Shoyu	Sugawara, E. (2010),
Indonesia (Origin), Netherlands, Japan, USA	Tempe	Frias et al. (2017)

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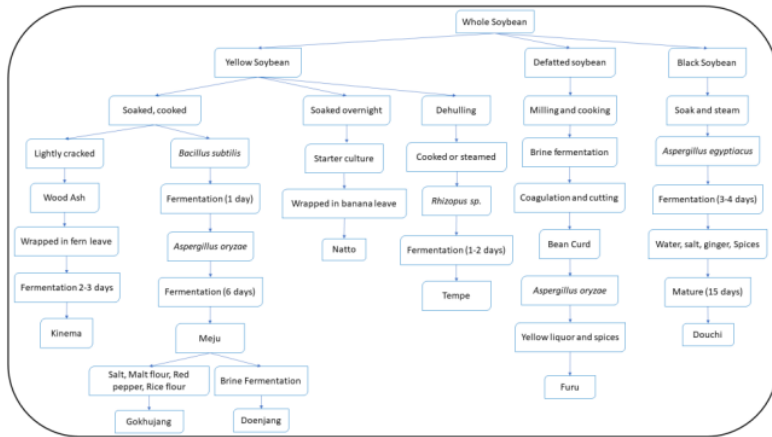
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Figure 1. Different methods of soybean fermentation process.

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## 86 REGULATORY IMPLICATION

87 Although fermented soybean products are <sup>36</sup>considered as safe, there are still health risks associated  
88 with inappropriate fermented soybean products, which are produced under inadequate hygienic  
89 standards and uncontrolled conditions, especially in household industry settings. The potential risks  
90 of adverse events are greater therefore higher hygienic standards need to be considered. To improve  
91 hygienic standards and preserve community health, various food safety assurance systems should be  
92 applied (Tamang et al. 2020). Because of the significant nutritional value of various fermented  
93 soybean foods, many of these products have been studied in order to improve their quality and safety.  
94 Urgent monitoring <sup>7</sup>is required to improve the quality of the components and integrate safety  
95 management systems for every industrial purpose. The manufacturing process needs to be <sup>26</sup>  
96 standardized to produce consistent quality, especially in small-scale industry settings (Anal 2019).

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Commented [A26]: This sentence does not make a sense; safe but still health risks

Commented [A27]: You should support this statement with data (previous reports of the cases; what the inadequate hygienic practice problems and what the health risks?

Commented [A28]: Another statement that needed data to support

Commented [A29]: For example?

Commented [A30]: ???

Commented [A31]: ?? please write in detail what is the urgent monitoring?

97

98 There are several certification schemes, such as Good Manufacturing Practices (GMP), Good  
99 Agricultural Practices (GAP), Good Hygienic Practices (GHP), Hazard Analysis Critical Control  
100 Points (HACCP), and International Organization for Standardization (ISO) standards. These  
101 certifications should be implemented and supported by government policies in order to reduce public  
102 health risks caused by fermented soy products. Having Codex Alimentarius registration for fermented  
103 soybean products such as Kimchi, Gochujang and Doenjang will improve the quality standard  
104 especially in international trade and commerce (Lee et al. 2012). Food safety and quality audits are  
105 also used widely to ensure food safety systems and programs are compliant and effective. Evaluation  
106 of management system, assess the condition of premises and products, and certification to certain  
107 food safety and quality standards are the common application of the audits (Kotsanopoulos &  
108 Arvanitoyannis 2017). However, the objective of food safety and quality audits should be considered  
109 as one of constant improvement rather than onetime assessment results or policing exercise  
110 (Bradford-Knox 2017).

#### 112 ANTI-NUTRITIONAL FACTOR

113 Several processing methods may diminish the anti-nutrient effects, such as soaking, pressure cooking,  
114 blanching, germination, fermentation, autoclaving, genetic manipulation, and other methods (Thakur  
115 et al. 2019; ten Brink et al. 1990). By using these diverse methods alone or in combinations, it is  
116 possible to lessen the content of anti-nutrients in foods (ten Brink et al. 1990). It has been suggested  
117 that autoclaving could be a better method to reduce levels of several anti-nutritional factors than other  
118 processing methods (Samtiya et al. 2020). However, comprehensive research is still needed to invent  
119 removal methods for heat-stable anti-nutritional factors existing in various foods without changing  
120 the quality of nutritional value. Heat-stable anti-nutritional factors include phytic acid, tannins,  
121 alkaloids, saponins, non-protein amino acids, and heat-labile group are lectins, cynogenic glycosides,  
122 protease inhibitors, and toxic amino acids (Thakur et al. 2019). When these substances present at low

Commented [A32]: All of them or either one?

Commented [A33]: ? in what?

Commented [A34]: Those statements are general things, you need to be more specific application in soy product fermentation. What is the standard and how to comply with that standard

Commented [A35]: You suppose to write, what are the anti-nutritional factors problems found in the traditional fermented soy products? How many (the content) of each, then write why the antinutritional factors in that amount is not good for human health, and how the recent ways to solve /reduce the problem

Commented [A36]: ? again, this is a review. You should not just make 'guessing'. Explain one by one how soaking reduce anti nutrient, how pressure cooking etc. and how many % the anti-nutrient is successfully decreased by each method

Commented [A37]: Again, support with data (of previous reported in journals)

Commented [A38]: From what point of view?

Commented [A39]: are all of these compounds found in soy?

123 levels, some anti-nutritional factors and their metabolites may have positive health effects but limited  
124 knowledge and information on their properties can result in health risks (Gemedo & Ratta 2014).

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125  
126 Innovative fermentation methods have been conducted in order to reduce the level of anti-nutritional  
127 factors in fermented soybean products such as soybean meal. Bacillus fermentation on soybean meal  
128 protein using *Bacillus siamensis* isolate JL8 has shown a significant reduction in major anti-  
129 nutritional factors (ANFs) in soybean meal (SBM) namely glycinin,  $\beta$ -conglycinin, and trypsin  
130 inhibitor by 86.0%, 70.3%, and 95.01% respectively (Zheng et al. 2017). A similar study using solid-  
131 state *Bacillus subtilis* fermentation method was able to reduce levels of beta-conglycinin subunits,  
132 glycinin subunits, and trypsin inhibitors by 70%, 50%, 58% respectively in soybean meal after 24  
133 hours (Seo and Cho 2016). Additionally, soybean meal fermentation using the suitable proportion of  
134 *Bacillus subtilis*, *Bacillus lactis*, and *Saccharomyces cerevisiae* at 35°C may improve the levels of  
135 free amino acids, small peptides and reduced the activity of protease inhibitor (Zhang et al. 2017).

Commented [A42]: What is the meaning of innovative fermentation?

### 137 MYCOTOXIN CONTAMINATION

138 The microorganisms involved in FSPs, especially in traditional spontaneously fermented products,  
139 are mostly unknown. However, predominant microorganisms used as in common fermentation have  
140 been described (Figure 2). Mycotoxins are secondary metabolites of molds that may harm the health  
141 of humans or animals after being consumed and it could contribute also to unwanted effects on crops  
142 (Komnenov et al. 2019). If ingested, mycotoxins can cause acute illness or chronic episodes of illness,  
143 called mycotoxicosis. The word Mycotoxin is derived from Greek word "mykes" means fungus,  
144 and Latin word "toxicum" means poison or toxin (Kirovska & Velickova 2021). Mycotoxin is  
145 produced during fungal growth and can be found in the hyphae and spores of organisms. Several  
146 mycotoxins such as aflatoxins, ochratoxins, deoxynivalenol (DON), trichothecenes, zearalenone,  
147 citrinin were reported to be present in fermented food products (Omotayo et al. 2019; Sivamaruthi et  
148 al. 2019). Some trichothecene mycotoxins, such as DON and T-2 toxins (a trichothecene produced

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Commented [A45]: How much the minimum (concentration) limit to cause the illness

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Commented [A46]: Remember your focus is fermented soybean products. Are all those mycotoxin presence in the fermented soy products?



149 by *Fusarium* spp) can directly impair mucosal tissues through disruption of the intestinal epithelial  
150 barrier and further enable translocation of intestinal commensal microbiota, and pathogens. The result  
151 could be the elevation of microorganism infection and inflammation (Nazhand et al. 2020). Aflatoxin  
152 (AF) is a common example of mycotoxins synthesized by *Aspergillus flavus*, *A. parasiticus* and *A.*  
153 *nomius*. Aflatoxins are chemically derived from difuranocoumarins with bifuran-based coumarin  
154 nuclei groups and lactone rings (Aflatoxin Gs) or pentanone rings (Aflatoxin Bs and Aflatoxin Ms).  
155 During the biosynthesis of AF in plants by *A. flavus* and *A. parasiticus*, the main substrate of hexanoyl  
156 is converted to polyketide using a polyketide synthase and two fatty acid synthases (Nazhand et al.  
157 2020).

158  
159 It has been reported that 97.5% of tested samples of 40 domestic and imported (from Japan) soy  
160 sauces sold in China were contaminated with DON. The mean incidence rate of DON contamination  
161 in domestic soy sauces was 97.1% with a concentration range from 4.5 to 1245.6 µg/l, whereas those  
162 imported from Japan were 100% in a range of 30.5 – 238.3 µg/l (Zhao et al. 2013). Meju prepared  
163 without additives may result in isolation of *Aspergillus ruber* which ability to release aflatoxin B<sub>1</sub> and  
164 the ochratoxin A (Shukla et al. 2017). *Aspergillus oryzae* and *Aspergillus sojae* are commonly used  
165 in the soybean fermentation process such as soy sauce, and miso. These strains are closely associated  
166 with the aflatoxigenic species *A. flavus* and *A. parasiticus*. Although these fermented soybean  
167 products have rarely been shown to produce aflatoxin, they may contain homologs of several aflatoxin  
168 biosynthesis pathway genes (Shukla et al. 2017). Doenjang prepared through inoculation with a  
169 natural starter is commonly contaminated with aflatoxin. The fungal community present during  
170 traditional production is found to be the aflatoxigenic *Aspergillus flavus* (Zain 2011).

## 172 MYCOTOXIN MITIGATION

173 Official regulation and policy play an essential role in the mitigation of mycotoxin levels in foods  
174 among many countries. According to FAO data, at least 99 countries regulate mycotoxins content

Commented [A47]: Why do you need to have this sub chapter specific just for mycotoxin? While there is no sub-chapter of mitigation of other items

175 for food and/or feed in 2003<sup>27</sup>. The number of countries shows an increase of about 30 percent  
176 compared to 1995. The total population in these countries represents about 87% of the total world  
177 population (“Worldwide Regulations for Mycotoxins in Food and Feed in 2003” n.d.). On top of that,  
178 perhaps special approach for the reduction of mycotoxin level in fermented soybean product need to  
179 be explored. As an example, fermentation of Meju using plant extracts<sup>22</sup> (*Nelumbo nucifera* leaves,<sup>40</sup>  
180 *Ginkgo biloba* leaves, and *Allium sativum* cloves) may significantly influence fungal microflora,<sup>22</sup>  
181 leading to its quality improvement. This outcome could be due to the diminishing effects of the<sup>22</sup>  
182 extracts against toxin-producing fungal pathogens (Shukla et al. 2017).

Commented [A48]: Is there any newest data?

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#### 183 184 <sup>42</sup> **BIOGENIC AMINES**

185 Biogenic amines (Bas) are low molecular weight, non-volatile nitrogenous<sup>25</sup> bases with an aliphatic,  
186 aromatic, or heterocyclic structure, which have been found in fermented foods, such as fish products,  
187 cheese, wine, beer, miso, chunjang, jajang, kimchi, sufu etc. (Komprda et al. 2007; Pachlová et al.<sup>33</sup>  
188 2012; Renes et al. 2014; Alvarez & Moreno-Arribas 2014; Bai et al. 2013; Lee et al. 2019; Shukla et  
189 al. 2010; Guan et al. 2013; Byun & Mah 2012). The name of a biogenic amine is ascribed to the name<sup>5</sup>  
190 of the amino acid from which it is derived. For instance, histamine is derived from the decarboxylation  
191 of histidine, and tyramine is formed from tyrosine through the same reaction (Santos 1996). The  
192 formation of biogenic amines in foods is mainly due to the presence of bacteria that are able to<sup>5</sup>  
193 perform decarboxylation to certain amino acids (Renes et al. 2014; Bover-Cid et al. 2003; Buňková<sup>44</sup>  
194 et al. 2013).

Commented [A50]: Again, remember that your focus is fermented soybean products – therefore, everything you write here should only relate to soyfoods

195  
196 The bacteria with amino acid-decarboxylase activity are *Pseudomonas*, *Clostridium*, *Bacillus*,  
197 *Photobacterium*, *Enterobacteriaceae* family (*Escherichia*, *Klebsiella*, *Citrobacter*, *Proteus*, *Shigella*,<sup>5</sup>  
198 and *Salmonella*), *Micrococcaceae* family (*Staphylococcus*, and *Micrococcus*), *Enterococcus*,<sup>5</sup>  
199 *Lactobacillus*, *Carnobacterium*, *Pediococcus*, *Lactococcus*, and *Leuconostoc* (Santos 1996; Stadnik  
200 and J. Dolatowski 2010).

Commented [A51]: One paragraph should contains at least two sentences

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201  
202 <sup>2</sup> In low amounts, the intake of BA in the human diet is usually not harmful to health because it can be  
203 detoxified by amine oxidase enzymes present in the intestine. These enzymes namely monoamine  
204 oxidase (MAO), diamine oxidase (DAO), and histamine N-methyltransferase (HNMT) can  
205 metabolize dietary BA in healthy people (Fogel et al. 2007). However, BA can turn into toxic  
206 <sup>2</sup> metabolites that can threaten human health when the amount in the diet is too high or when the  
207 detoxification ability is inhibited or impaired in humans (Anderson 2008; Ruiz-Capillas & Herrero  
208 2019). Since biogenic amines accumulation might occur if there are uncontrolled microbial enzymatic  
209 reactions (Halász et al. 1994), therefore the presence of BA in food <sup>5</sup> is always undesirable because if  
210 absorbed at high concentration, it can cause headache, respiratory distress, palpitation, hypo or  
211 hypertension, and allergic reactions (Restuccia et al. 2015).

Commented [A53]: How low is low? Add data

Commented [A54]: How high is too high? Add data

Commented [A55]: Again, scientific writing always supports every single qualitative word with data (quantitative)

## 212 213 **BIOGENIC AMINE MITIGATION**

214 The formation of BA in fermented soybean products is a complex phenomenon that depends on  
215 several factors, such as BA-producing strain or species, salt concentration, and high temperatures  
216 (Bai et al. 2013; Chun et al. 2020). Different concentrations of salt may affect the formation of BA.  
217 <sup>50</sup> This happens due to high salt levels can inhibit the growth of microorganisms that produce biogenic  
218 amino acids, so that the production of decarboxylase enzymes decreases (Chun et al. 2020; Chin &  
219 Koehler 1986). It has been shown that thermal breakdown of BA can occur during high-temperature  
220 treatment such as cooking. After 10 minutes of frying at 200°C, there is approximately a 40%  
221 reduction in the <sup>37</sup> levels of tryptamine and b-phenylethylamine. While other Bas namely cadaverine,  
222 histamine, tyramine, spermidine, and spermine show decreases ranging from about 10% to 20% (Bai  
223 et al. 2013).

Commented [A56]: Data?

Commented [A57]: In what product?

224  
225 Up to date, most efforts on regulatory standards for monitoring biogenic amines are more focused on  
226 fish and seafood products than fermented soybean foods (Park, Lee & Mah 2019). The suggested

Commented [A58]: In where? What level? Global? Regional? National?

227 toxic limit of BA content in fermented soybean products is 1000 mg/kg (Santos 1996) and 30 mg/kg  
228 for  $\beta$ -phenylethylamine, 100 mg/kg for histamine, and 100 mg/kg for tyramine (ten Brink et al. 1990).  
229 Attempts have been made to reduce the formation of BA in foods such as handling and processing  
230 under sanitary conditions, using some amine-negative starter cultures, adding some probiotic  
231 bacterial strains alone or in combination with the starter cultures, high-pressure processing, or low-  
232 dose gamma irradiation (Oh et al. 2014; Kim et al. 2005a; Mendes et al. 2005; Zhang et al. 2013).  
233  
234 In order to reduce BA concentration in fermented soybean foods, the use of a selected starter culture  
235 containing bacteria with less decarboxylase activity is one of the common ways to reduce the  
236 development of BA in soybean fermented foods (Alvarez & Moreno-Arribas 2014). The application  
237 of *Bacillus subtilis* and *Bacillus amyloliquefaciens* strains are able to reduce histamine levels in vitro  
238 by 27 – 46% and 70%, and tyramine contents by 42 – 59% and 71% respectively (Kim et al. 2012).  
239 Besides that, the use of *Lactobacillus plantarum* as a starter culture could decrease histamine and  
240 total BA content by 58 – 100% and 27%, respectively (Lee et al. 2016; Kung et al. 2017). Besides  
241 the application of BA low producing microorganisms for fermentation, the use of additives has been  
242 proposed as an alternative method for this purpose. The addition of ethanol to the dressing mixture  
243 during soybean fermentation has shown BA content reduction. It might be due to ethanol's ability  
244 to inhibit the degradation of water-soluble protein during the ripening stage (Qiu et al. 2018). Another  
245 additive such as nicotinic acid may reduce tyrosine decarboxylase activity of *Enterococcus faecium*  
246 isolated from cheonggukjang. It suggests that nicotinic acid could be used as tyrosine decarboxylase  
247 inhibitor to reduce tyramine content in vitro and in situ (Kang et al. 2018). Additionally, the  
248 application of gamma irradiation may not only reduce the usage of salt but also lessen biogenic amine  
249 levels during soybean fermentation (Kim et al. 2005). Though studies have described several methods  
250 to decrease BA content in vitro and in situ, practical application is still crucial to ensure the production  
251 of safe fermented food products (Park et al. 2019).

Commented [A59]: Give example

Commented [A60]: Range of pressure applied?

Commented [A61]: Range of dose?

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## 253 HIGH SALT CONTENT

254 Fermented soybean products might use different methods and some of them may require a certain  
255 amount of salt. In general, the salt content is above 18% in soy sauce, 10-15% in douchi, and over  
256 10% in dajiang (Liu et al. 2020). Sodium Chloride is able to control water activity, affect microbial  
257 growth and fermentation speed, make the fermented soybean products more savory and extend their  
258 shelf life (Liu et al. 2020). Salt is an essential seasoning used for making traditional fermented soy  
259 paste such as doenjang (Korea), miso (Japan), dajiang (China), and thua nao (Thailand) as well as  
260 ganjang, a soy sauce made with fermented soybean in Korea (Shim et al. 2016; Chun et al. 2020). As  
261 an example, a traditional Korean doenjang is made by fermentation of doenjang-meju bricks soaked  
262 in high solar salt concentrations (approximately 16 -18%) for one to two months (Jeon et al. 2016).

263 Different concentrations of salt (NaCl) and temperature may influence microbial development and  
264 organoleptic quality fermented soybean products (Shim et al. 2016). Salt can inhibit the enzyme  
265 maturation process in the hydrolysis of proteins and lipids. The lower salt content in sufu (fermented  
266 tofu) can result in a decrease in hardness, elasticity, and the degree of degradation of proteins and  
267 lipids. Reducing the salt content can speed up the cooking time of the sufu, but it will also cause the  
268 sufu to spoil during the ripening stage with less salt content equal to 50 g kg<sup>-1</sup> (Han et al. 2003).

269  
270 Salt addition may reduce essential bioactive components that contribute to antioxidant properties  
271 during soybean fermentation, such as isoflavones. The amount of total isoflavones during the process  
272 sufu making can lose 68.7% of total isoflavones from soybeans due to leaching from materials. The  
273 change in isoflavones content is mainly during the preparation of tofu and salting of pehtze.  
274 Furthermore, Nacl concentration can inhibit isoflavones hydrolysis by beta-glucosidase activity  
275 during the fermentation (Li-Jun et al. 2004). Similarly, higher concentrations of NaCl in douchi  
276 (Chinese fermented black soybeans) production process may decrease isoflavone level and inhibit  
277 enzyme activity. It has been shown that total isoflavones content in douchi fermentation process  
278 reduced by 61% from raw soybeans during pre-fermentation (43%) and post-fermentation (18%)

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279 when the sodium content was 10% (Wang et al. 2007). Salt also can inhibit <sup>29</sup>  $\beta$  glucosidase activity so  
280 that the conversion of isoflavone glucosides to isoflavone aglycones decreased. As a result, there is  
281 concern that high salt intake is associated with increased consumption of fermented products  
282 soybeans due to high salt is one of the major risk factors for hypertension (Komnenov et al. 2019).  
283 Therefore, it is necessary to make efforts to maintain the <sup>49</sup> physicochemical properties and sensory  
284 quality of fermented soybean products by reducing the addition of salt, which appears as a problem  
285 in the field.

286  
287 Despite this, the increase of government oversight and control in response to salt reduction, such as  
288 setting up the maximum amount of salt per-serving target, it is also necessary to engage with  
289 producers to reduce salt use in fermented soybean products. Salt reduction in fermentation has been  
290 the subject of studies. One common strategy is to identify the lowest salt concentration under which  
291 the growth of the fermenting microorganisms, as well as <sup>46</sup> the flavor and texture of the fermented  
292 soybean products, will not be adversely affected. Several novel methods have been developed to  
293 produce low salt, or no salt <sup>43</sup> fermented soybean products, such as natto, tempe, and kinema (Wang et  
294 al. 2007).

#### 296 Selection and use of high-quality soybean cultivars

297 <sup>4</sup> Food-grade soybeans are the raw materials for soy food products, with or without fermentation  
298 <sup>4</sup> including tofu, soymilk, miso, natto, soy sprouts, and tempeh. Most food-grade cultivars share several  
299 common traits. They are non-GMO, have a high seed quality lacking mottling, cracking, etc. and  
300 because most soy food products require a soaking step, thus rapid water uptake is essential (Anderson  
301 et al. 2019). Besides these traits, the advance of soybean breeding and cropping system provides not  
302 only better chemical composition but also yields preservation through resistance improvement toward  
303 weed, pathogen, insect pest, and abiotic stress (Anderson et al. 2019). Selection and use of cadmium  
304 (Cd) safe soybean cultivars for fermentation may avoid the undesirable metal accumulation in the

**Commented [A68]:** This subchapter is going everywhere, please make it more focus on the list of salt content of several fermented soy foods, and write of why do fermented soy foods contain high salt, what the possible effect of those high salt on the human health, then how to solve the problem?

**Commented [A69]:** What is this? Part of what?

**Commented [A70]:** Is there any non-food grade soybean??

**Commented [A71]:** For example (name of variety?)

**Commented [A72R71]:** )

**Commented [A73]:** ?????? so GMO soy is not food grade??? Reference?????

**Commented [A74]:** ?? is the cultivar available everywhere globally?

305 human body from Cd contaminated soil (Zhi et al. 2020; 2015). A particular cultivar of soybean might  
306 be more suitable for specific fermented soybean products, for example, Saedanbaek cultivar provides  
307 better quality standards than other cultivars (Jinpung, Daepung 2, Pyeongwon, Cheonga and  
308 Saeolkong) for the fermentation process of the soy paste (Shin et al. 2019). This cultivar has shown  
309 an ability to produce higher amylase and protease activities, amount of viscous substance, and amino-  
310 type nitrogen contents (Shin et al. 2019).

311

### 312 **Conclusions**

313 Fermented soybean products (FSPs) have been the attention of scientific studies and consumers due  
314 to their nutritional values, bioactive components, and flavor profiles and texture. Even though FSPs  
315 are considered as safe foods, along with the development of society, people have become more aware  
316 of food-borne illness possibilities. Urgent attention is required to improve the safety and quality of  
317 fermented soybean products to prevent unwanted public health problems in the future. Anti-  
318 nutritional factors, pathogenic microorganisms and mycotoxins, the production of biogenic amines,  
319 the use of high concentration of salt during the fermentation process, and other safety hazards are still  
320 significant issues in fermented soybean production. In order to promote nutritional quality and safe  
321 fermented soybean foods, the following aspects are essential and should be taken into consideration.  
322 First, food regulation policy and standards need to be implemented, especially in industrial  
323 fermentation. Second, the application of pure, or excellent suitable strains under a controlled  
324 environment will produce a better conventional soybean fermentation process.

325

### 326 **Acknowledgments**

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328 Indonesia has supported this paper through internal grant scheme (No. 22/LPPM/UWKS/2021).

329

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