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Soy and Algae Combination Using Tempe Fermentation Method: A Proposed Opinion for the Development of Functional Food

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Abstract:

Backgrounds and Aims: Marine algae and plant-based protein have gained popularity among the most sought-after functional food ingredients and appeared as emerging trends for functional food. Combining ingredients that are well known to exert beneficial properties towards health can be considered an innovative strategy for developing novel functional foods. Each functional ingredient may contribute differently to health promotion and complement the beneficial properties of other components, thus increasing the overall health values of novel functional foods. In addition, these ingredients may exhibit synergistic activities that would improve the functionality of novel functional foods. Therefore, we propose that combining marine algae in the fermentation of tempe would be an innovative strategy to create a novel soybean-based functional food. This opinion-review article would provide a thorough insight into the conception, feasibility, and further research regarding the algae-tempe combination as a future functional food.

Results and Conclusions: The supplementation of marine algae in the fermentation of tempe would open a new horizon about novel soybean-based functional food. Introducing marine algae in tempe production would bring additional compounds that might not be naturally present in soybeans. These compounds are subject to mold fermentation. We suggest that marine algae would improve the nutritional value of tempe by providing additional carbohydrates and protein. We suggest algal supplementation in tempe fermentation could be done by incorporating freeze-dried algal powder into the pre-boiled soybeans and starters before fermentation. We also suspect that algal polysaccharides might affect the texture of the tempe and bind water required for mold growth during fermentation. Therefore, the fermentation parameters for this product would need optimizing. Furthermore, the organoleptic analysis should also be the primary consideration and be conducted to measure consumer acceptance regarding the product since marine algae might bring specific flavors that might not be acceptable to some consumers.

Keywords: Soy, Algae, Tempe, Fermentation Food, Functional Food, Soybean, Future Food, high value-added processing

1 Introduction

Functional food refers to food that provides specific beneficial functions beyond its nutritional values [1]. Today, consuming functional food is adopted by many people worldwide, as it is considered a trend and a complementary effort to a healthy lifestyle [2]. Concerning the continuously increasing global demand for functional food, there is a constant emerging need for developing novel functional food in the future. Marine algae and plant-based protein have gained popularity among the most sought-after functional food ingredients and appeared as emerging trends for functional food [3].

Marine algae have developed a good image globally as a functional food due to their richness in nutrients and bioactive compounds [4]. They are a rich source of iodine, glutathione, phlorotannins, fucoxanthin, and carbohydrates such as carrageenan, fucoidan, alginate, and agar [5]. Regular consumption of marine algae biomass has ameliorated the blood lipid profile *in vivo* [6]. Marine algae contain different phytochemicals or bioactive compounds, particularly as pigments, including chlorophyll, carotenoids, and phenolic compounds [5]. Phlorotannins, a type of polyphenol unique to marine sources and abundant in marine brown algae, exhibited potent antioxidant, anti-inflammatory, anti-diabetic, anti-tumor, anti-atherogenic, anti-allergic, and anti-bacterial activities [7]. Several studies have suggested that algal polysaccharides (chitin and fucoidan) could be important in preventing cardiovascular diseases, osteoarthritis, kidney, and liver diseases, and neglected infectious diseases [8].

Tempe, a traditional fermented soyfood from Indonesia, has been widely appreciated for its nutrients and qualities of health [9]. Due to its high protein content (20.8 g of protein per 100 g tempe) and the presence of vitamin B12, tempe is often regarded as a “vegan meat” [10]. Traditionally, tempe is made by fermenting pre-boiled soybeans with starters containing *Rhizopus* spp. (*R. oryzae*, *R. oligosporus*, *R. chinensis* and *R. arrhizus*) for 2-3 days at room temperature [11]. Different processes taking place during mold fermentation result in the increased bioavailability of nutrients (protein and lipid) and isoflavones, as well as the decrease of anti-nutrient compounds (such as phytic acid and trypsin inhibitor) [9]. In several studies, tempe has been reported to exert beneficial properties to human health, including antioxidant, immune boosting, gut-health promoting, anti-hypertensive, liver protecting, anti-diabetic, anti-microbial, and anti-aging activities [12]. Most of these properties are strongly linked to bioactive peptides and isoflavones in tempe [13]. Recently, tempe was suggested to be a future functional food due to its potential anti-cancer properties [14].

Combining ingredients that are well known to exert beneficial properties towards health can be considered an innovative strategy for developing novel functional foods [15]. Each functional ingredient may contribute differently to health promotion and complement the beneficial properties of other components, thus increasing the overall health values of novel functional foods. In addition, these ingredients may exhibit synergistic activities that would improve the functionality of novel functional foods [16]. Therefore, we propose that combining marine algae in the fermentation of tempe would be an innovative strategy to create a novel soybean-based functional food. This opinion-review article would provide a thorough insight into the conception, feasibility, and further research regarding the algae-tempe combination as a future functional food.

2 Tempe Fermentation Method

Tempe is usually made of soybeans fermented with *Rhizopus* spp. (*R. stolonifer*, *R. oryzae*, *R. oligosporus*, or *R. arrhizus*). Tempe production varies across different locations in Indonesia. The procedure includes soaking, dehulling, washing, boiling, draining, cooling, inoculating, packaging, and incubating [10,17]. The soaking step, usually the first step, lasts 6 to 24 hours and hydrates the soybeans, making the hulls easier to peel. Natural acidification can happen during this step (reaching pH 4.85), which can help inhibit the growth of pathogens and/or spoilage-causing microorganisms. Even though the dehulling method was traditionally done by hands or feet, for hygienic reasons, the dehulling process has been replaced with mechanical dehulling [10,17]. Dehulling is important because soybean hulls in finished tempe are considered contaminants, according to CODEX [18]. The washing step may be omitted because the boiling process is sufficient for successful fermentation. The boiling step, lasting for 20 to 30 min, is essential because it removes the raw flavor and eliminates pathogens and spoilage microorganisms. The draining step, which might include the drying process, reduces the water content in tempe as tempe fermentation requires an optimum level of approximately 62% humidity and 0.99 to 1.00 water activity with the desired temperature ranging from 25 to 38°C [10,17]. The inoculation step involves the dispersion of *Rhizopus* spp. sporangiospores (usually 10^4 CFU/g substrate) by packing the soybeans into containers with limited airflow. The sporangiospores grow into dense mycelium biomass without sporulation. The incubation step (done at 25 to 38°C for 18 until 72 hours) facilitates the growth of *Rhizopus* spp. before tempe can be harvested [10,17].

As a functional food (and nutraceutical) with well-respected health benefits and accessibility, the tempe product itself keeps being innovated. Some studies highlighted using soybean alternatives, such as jack bean, mung bean, red kidney bean, cowpea bean, and koro bean [19,20]. This shows that the tempe fermentation method is versatile and can be utilized with various ingredients. Regarding this point, we propose the potential synergistic activities of tempe with algae to improve their functionality. The following section will discuss the properties of marine algae.

3 Marine algae and their functional properties

Marine algae, as one of the ingredients for a superfood, are rich in bioactive components, such as sulfated polysaccharides, proteins, bioactive peptides, amino acids, polyunsaturated fatty acids, antioxidants, vitamins, and alkaloids, which can be used for enriching the nutrient properties in supplements or food to enhance their health benefits.

3.1 Sulfated polysaccharides

Polysaccharides are one of the leading marine algae's primary metabolites. Sulfated polysaccharides (SP), a major constituent of cell walls and the highest proportion of marine algae polysaccharides, possess multiple health benefits, functioning as antioxidant, antibacterial, antiviral, anti-cancer, immunomodulator, and prebiotic [21–23]. Ulvans are the major constituent for green algae (*Chlorophyceae*) cell walls (8 – 29 % of total dry weight), while galactans are the most prevalent in red algae (*Rhodophyceae*) (30 – 75 % of total dry weight) and alginates, fucoidans, and laminarans for brown algae (*Phaeophyceae*) (17 – 45 %, 5 – 20 %, and < 35 % of the total dry weight, respectively) [24]. As a prebiotic, when marine algae polysaccharides (MAP) are digested, beneficial metabolites are produced, such as the short-chain fatty acid (SCFA), which, when metabolized further, functions as an energy source, and also increases satiety, reduces gluconeogenesis, and lipid storage, improving insulin sensitivity and increasing adenosine 5′-monophosphate (AMP)-activated protein kinase activity [23].

3.2 Proteins, amino acids, and bioactive peptides

Proteins comprise around 5-47% of marine algae dry weight [25]. The highest protein concentration is in red algae, around 31-55% of its dry weight, and in microalgae, it can reach up to 77% of its dry weight [24]. Spirulina, for example, has high protein content, making it an ideal protein supplement choice [25]. Phycobiliproteins, a major protein in marine algae, are often used as a natural food coloring and gelling properties in food. In contrast, lectins, another major protein, have antimicrobial, antiviral, antitumor, and drug-targeting agents and are often incorporated into food products [24].

Around 42-48% of marine algae's amino acids are essential [25]. Marine algae also contain microspore-like amino acids (MAAs), which function as an antioxidant and anti-inflammatory and also protect cells from damage against UV rays [24].

Bioactive peptides, such as VECYGPNRPQF, polypeptide CPAP, Y2, VEGY, GMNNLTP, LEQ, and protein hydrolysates, are produced by algae as a result of contact with stress conditions. It consists of 2-20 amino acids and exhibits multiple properties, such as anticancer, antihypertension, immunomodulatory, and antiatherosclerotic effects [26].

3.3 Polyunsaturated fatty acids (PUFAs)

Marine algae are rich in omega-3 fatty acids, mainly DHA and EPA. *Schizochytrium* sp., for instance, is often used in the making of DHA-rich supplements. Another species, *Crypthecodinium cohnii*, produces purely DHA and no other PUFAs, making the purification process in making DHA-rich supplements easier [26].

3.4 Antioxidants Properties

Marine algae contain natural antioxidants, which reduce the reactive oxygen species (ROS), reducing oxidative damage to the cells. Some antioxidant compounds found in marine algae are carotenoids. It also has anti-aging, dietary, anti-inflammatory, antibacterial, antifungal, cytotoxic, anti-malarial, anti-proliferative, and anticancer properties [25].

3.5 Minerals and vitamins

Marine algae are rich in vitamins: A, C, B1, B2, B3, and B6 [25]. Spirulina, Chlorella, and D.Tertiolecta are rich in vitamin B12, while Dunaliella is rich in soluble vitamins [26]. Algae are a source of minerals, such as potassium, sodium, magnesium, and calcium, representing 97 % of total seaweed minerals [24].

With its numerous benefits, it is no wonder that algae are one of the most popular choices for a superfood [27]. However, it can be processed and innovated further to create an even more effective source of nutrients or combined with other products to create a newer, more advanced superfood.

4 Future directions and discussion

The supplementation of marine algae in the fermentation of tempe would open a new horizon about novel soybean-based functional food. Introducing marine algae in tempe production would bring additional compounds that might not be naturally present in soybeans. These compounds are subject to mold fermentation. We suggest that marine algae would improve the nutritional value of tempe by providing additional carbohydrates and protein. Furthermore, the fermentation process would help increase the bioavailability of algal carbohydrates and protein. *Rhizopus* spp. produce cellulase [28] that would digest the cell wall of marine algae, thus liberating their nutrients. Afterward, these molds also secrete different types of carbohydrates and proteases [28] that hydrolyze algal carbohydrates and protein, thus increasing the bioavailability of algal nutrients. In addition, the hydrolysis of algal protein also could lead to the formation of novel bioactive peptides with beneficial effects on human health. Interestingly, the presence of marine algae could also improve the amino acid profile of tempe. Lysine is the limiting amino acid in soybeans and tempe [29]. In contrast, marine algae contain a relatively high level of lysine [30] that can compensate for the lack of lysine in traditional tempe. Vitamin B12 is also present in a relatively low amount in tempe, and its formation is mainly due to the bacterial activity of *Klebsiella pneumoniae* appearing originally as contaminating bacteria in the fermentation of tempe [31]. Algal supplementation in tempe could provide vitamin B12 since many marine algae are rich in vitamin B12, mainly due to their symbiotic relationship with marine bacteria [32]. Tempe is also generally low in iron; supplementing some marine algae rich in iron would improve its nutritional interest [33]. Vegetable-based protein food, including tempe, often lacks bioavailable iron and vitamin B12, essential for preventing anemia [34,35]. Therefore, adding marine algae rich in iron and vitamin B12 would be a suitable strategy to alleviate the nutritional quality of tempe. Furthermore, a complex mixture of bioactive compounds in tempe and marine algae could exhibit desirable synergistic effects on antioxidant activity or other parameters. The proposed ideas are visualized in Figure 1.

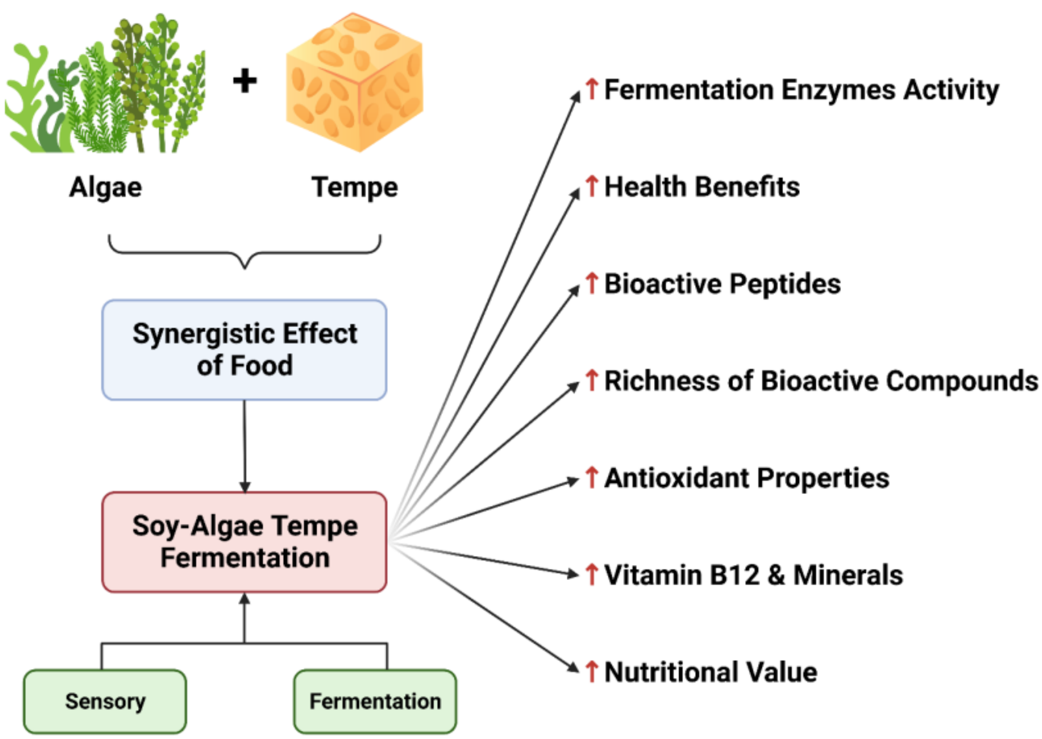

Figure 1. Added values from the incorporation of soy and algae through the tempe fermentation method

We suggest algal supplementation in tempe fermentation could be done by incorporating freeze-dried algal powder into the pre-boiled soybeans and starters before fermentation (Figure 1). We also suspect that algal polysaccharides might affect the texture of the tempe and bind water required for mold growth during fermentation. Therefore, the fermentation parameters for this product would need optimizing. Furthermore, the organoleptic analysis should also be the primary consideration and be conducted to measure consumer acceptance regarding the product since marine algae might bring specific flavors that might not be acceptable to some consumers.

5 Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest. Author FK was employed by the company PT Nutrifood Indonesia.

6 Author contributions

YAA, NM, RS, FN: Contributed to the conceptualization with the design of the critical opinion study. RS, NAT, WBG, YAA, MS, AAS, AW, FK, and FN: drafted the manuscript, edited-revised it, and approved the final version of the submitted manuscript. All authors and contributors contributed to the opinion article and approved the submitted version.

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9 Data availability statement

There is no data related to this opinion article. The data is only sourced from the literature listed in this article.

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Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.

Missing "," Review the rules for using punctuation marks.

Article Error You may need to remove this article.

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Frag. This sentence may be a fragment or may have incorrect punctuation. Proofread the sentence to be sure that it has correct punctuation and that it has an independent clause with a complete subject and predicate.

Sentence Cap. Review the rules for capitalization.

Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.

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Article Error You may need to remove this article.

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Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.

P/V You have used the passive voice in this sentence. You may want to revise it using the active voice.

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Proper Nouns You may need to use a capital letter for this proper noun.

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Dup. Did you mean to repeat this word?

Proofread This part of the sentence contains an error or misspelling that makes your meaning unclear.

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Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.

Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.

Prep. You may be using the wrong preposition.

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Article Error You may need to remove this article.

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S/V This subject and verb may not agree. Proofread the sentence to make sure the subject agrees with the verb.

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P/V You have used the passive voice in this sentence. You may want to revise it using the active voice.

Prep. You may be using the wrong preposition.

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