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Review Article

Valorization of Jack Bean as Raw Material for Indonesian Traditional Food Tempeh and Its Functional Properties

Andriati Ningrum, Sri Anggrahini and Widiastuti Setyaningsih

Department of Food Science and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Flora, No.1 Bulaksumur, 55281 Yogyakarta, Indonesia

Abstract

Jack bean is one of underutilized legume. In Indonesia jack bean tempeh is one traditional product from Indonesia, using jack bean tempeh as food ingredient. So far, scientific information on jack bean tempeh is scarce and thus this article aims to document the updated knowledge of jack bean tempeh. Tempeh is a sliceable, cake-like product made of dehulled cooked jack bean, penetrated and fermented by a mixed microbiota dominated by filamentous fungi. Jack bean as one type of pulses are a rich source of protein in the human diet and their consumption has been associated with the prevention of chronic diseases. The beneficial effect in human health has been related to their micronutrients, phytochemical bioactive compounds and recently BP (bioactive peptides).

Key words: Jack bean, tempeh, fermentation, bioactive peptides, Indonesia

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Corresponding Author: Andriati Ningrum, Department of Food Science and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Flora, No.1 Bulaksumur, 55281 Yogyakarta, Indonesia Tel: +6289680125630

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jack bean tempeh is one of the fermented legumes commonly it is jack bean product originally made by Central Javanese people through fermentation with mold with *Rhizopus* species. Although there is evidence of earlier fermentation of soy, historically, tempeh had appeared in the Central Javanese food pattern where mainly the raw material is made by soy bean[1]. Jack bean tempeh extensive use in main meals and also snacks.

Jack bean tempeh is commonly produced by small household industries in central Java (Fig. 1). The raw material jack bean or called with latin name *Canavalia ensiformis* (L.) DC is one of underutilized legume[2]. Originally jack bean is originated from south America and grown in the tropics and subtropics produces high yields especially in regions of low altitude, high temperature and also relative humidity[3]. Some countries, e.g., the westerns consume immature pods of jack bean as vegetables and the seeds are roasted and grounded to make coffee-like drink. The proximate analysis of jack bean seeds are 24-32% crude protein, 1.8-9.6% crude lipid, 4.7-10% crude fiber, 2-4.6% ash and 43-60% carbohydrates[3]. The powdered seeds of jack bean are used as an antibiotic and also antiseptic. Where the proteins of jack bean with complete sequence homology to bovine insulin especially in jack bean seed coat are used as antihuman insulin antibodies. This can be used as a treatment for diabetes, as it has been shown to significantly lower the blood glucose level *in vivo*. Several processing techniques can increase many bioactive compounds in jack bean that can enhance the potency as anti diabetic agent[3]. On the other hand, this seed also has a potency to decrease cholesterol level in hypercholesterolemia rats[3].

As a source of protein, jack bean tempeh is consumed as a good source for protein sources. Jack bean tempeh is not commonly consumed as a raw food but in the form of cooked tempeh and served as a delicacy or a side dish, often fried, boiled, steamed or roasted and also there are several culinary approach for developing several dishes made from jack bean tempeh[4].

During jack bean tempeh processing, there are valuable changes not only in the increase of nutritional values of some nutrients in jack bean, but also in the development of several phytochemical compounds. Jack bean as the raw material has been proven to have several bioactive compounds that can be beneficial for human health. On the other hand, there are several research in the tempeh field has shown its potential health benefit, possibly due to physicochemical and also biochemical changes during jack bean fermentation[5].

JACK BEAN TEMPEH PREPARATION AND FERMENTATION

There are four steps in the tempeh manufacturing process, soaking, boiling, inoculating with microbial and incubating at room temperature. Jack bean tempeh is fermented with *Rhizopus* sp. mold, especially *Rhizopus oligosporus*, *R. oryzae*, *R. arhizus*, *R. stolonifer* and *R. microsporus*. Traditional inoculum is prepared in Hibiscus or teak leaf and inoculum powder is prepared from cooked rice. Tempeh producers in Indonesia do not use the pure culture of *R. oligosporus* but they use a mixed culture of *Rhizopus* sp.

Figure 2 shows the principles of the tempeh-making process. Whole jack bean are soaked in water overnight at an ambient temperature of 28°C, after which they are dehulled. The dehulled split beans (cotyledons) are cooked in water for approximately 30-40 min, after which the cooked water is drained and the cotyledons are spread out to cool. During the cooling, the adhering water evaporates, enhancing the cooling and drying the cotyledons surface. At about 30°C the cotyledons are inoculated with inoculum. Then, tempeh is packed in plastic or banana leaves or other teak leaves and then incubated for several days. The incubation takes place in packages that offer limited access to atmospheric oxygen at 30-40°C during 1-2 days (the higher the temperature, the shorter the incubation) until the fungal mycelium is dense and binds the cotyledons firmly together into a solid mass. The jack bean fresh tempeh is then cut and sold fresh in Indonesia.

PHYSICOCHEMICAL AND BIOCHEMISTRY AND DURING JACK BEAN TEMPEH FERMENTATION

During the fermentation process, fungal metabolism causes changes in the composition of tempeh. Several research using several legumes for making tempeh e.g., soybean as main raw material for tempeh showed improvement of nutritional value during fermentation[6]. Due to protein degradation, water-soluble nitrogenous substances (peptides, amino acids) are increased[7].

Likewise, the lipid degradation results in decreased crude lipid and a concomitant increase of free fatty acids[8]. Such enzymic degradations generally result in increased amounts of low-molecular-mass water-soluble solids. The softening of the beans in tempeh is in accordance with decreased hemicellulose levels. On the other hand, dietary fiber levels are increased due to the formation of fungal mycelial polysaccharides including cellulose and chitin[9].

The protein content of jack bean from 24-32%, within these percentages and as the legume, the most abundant are

Fig. 1: Jack bean tempeh, traditional food from Indonesia

After 24 h miselia of *Rhizopus* hasn't been formed but it is fully formed after 36 h fermentation for all formulated tempeh with two different packaging. The miselia started to form brown miselia after 36 h fermentation

Fig. 2: Several steps in tempeh making

seed storage proteins (SSPs)[3]. Based on their solubility properties, SSPs are classified as globulins, albumins, glutelins and prolamins. Globulins are soluble in salt-water solutions, albumins in water, glutelins in diluted acids or bases and prolamins in ethanol-water solutions. This several protein can be a precursor of BPs[10].

BPs are defined as small amino acid sequences derived from food proteins that possess potential physiological properties beyond normal and adequate nutrition. Within the precursor proteins, the amino acid sequence conforming the BP is inactive forms, where once the peptide gets released it can display diverse biological activities e.g., anti-hypertensive effect, anti-diabetic, anti-diabetes, weight management, anti-oxidant, etc[10].

Proteolysis is necessary to generate BPs, which may occur through enzymatic digestion of the precursor protein, either *in vitro* or in the digestive tract (*in vivo*). Also, food processing and enzymes from micro-organism during fermentation like in tempeh making can cause proteolysis and release of potential BPs[5].

Table 1: Nutritional value of jack bean tempeh compared to soybean tempeh

Component	Jack bean tempeh	Soybean tempeh
Moisture	67.02	63.69
Ash	0.18	0.75
Fat	0.68	1.9
Protein	28.29	19.38
Carbohydrate by difference	22.83	12.59

From the textural characteristic as one of physicochemical characterization showed that jack bean tempe has a compact texture after fully fermented. After 24 h fermentation, jack bean tempeh has the hard texture and the hardness will increase along with increasing of fermentation time[11].

NUTRITIONAL VALUE OF JACK BEAN TEMPEH

There are several investigations to identify the nutritional value of jack bean tempeh[2,11]. Table 1 shows the nutritional value of jack bean tempeh compared to soybean tempeh.

Compared to soybean tempeh, the jack bean tempeh has more protein content. So that, jack bean tempeh is a good promising source of protein[11]. Jack bean also have several bioactive compounds that such give a promising effect on human health. Jak bean seeds contained total phenolic content approximately 12.98±1.63 g catechin equivalent/100 g extract. Phenolic components have a broad range of functions for human health e.g., anti-oxidants, anti-cancer, anti-microbes and other functions[3]. Several peptides from jack bean also have anti-pathogenic fungal and anti-insect activities[12].

FUTURE PROSPECT OF THE NUTRITIONAL VALUE OF JACK BEAN TEMPEH

Angiotensin-converting enzyme inhibitory activity: Inhibition of angiotensin-converting enzyme (ACE) results in an overall anti-hypertensive effect that has been exploited by several commercial drugs[13]. This fact has triggered the research for natural sources of ACE inhibitory agents, which in turn has led to the description of a large amount of BPs with presumably anti-hypertensive activity[14].

In general, the majority of ACE inhibitory peptides described from all kinds of food sources are relatively short sequences containing from 2-12 amino acids[15]. Other research has identified structural features from the C terminal tripeptide residue that play a predominant role in competitive binding to the active site of ACE. Such features are the presence of bulky hydrophobic residues, aromatic or branched side chains, proline at 1 or more positions, positively charged Arg and Lys residues in position 2, Tyr (Tyrosine), Phe (Phenylalanine) and Trp (Triptofan) residues and L-configured residues in position[16] 3.

The fermentation time is one of the important parameter to produce BPs. During the increase of fermentation time, it will increase the hydrolysis process. Commonly, the hydrolysates ACE-inhibitory potency increases with the hydrolysis time, implying thus that the smaller peptides are more active[17]. Fermentation has been investigated also to increase bioactive compounds and nutritional value in several traditional foods[18-21].

Now a days, the investigation of several BPs activity have been developed using holistic approach *in silico*, *in vitro* and *in vivo*. One of the approaches is *in silico* approach or called as computer-assisted approach. Computer-assisted databases are available for predicting BPs located within a parent protein[22]. Other databases predict the precursor protein of a BP from a known amino acid sequence. BIOPEP is a peptide sequences database integrated with a program that allows classifying food proteins as potential sources of BPs[23]. So even though *in silico* approaches enable the identification of BPs from the protein sequences, comprehensive assessment with *in vitro* and *in vivo* are the best method to search for the optimal hydrolysates activity and its use is obviously restricted to the search of BPs previously described. Our pre-preliminary result showed that canavalin as a good precursor of BPs from the jack bean as a raw material for jack bean tempeh as one of underutilized legume in Indonesia[24].

Quantitative structure-activity relationship (QSAR) modeling and substrate docking can be used to asses *in silico* numerous peptide structures for their bioactivity potential[25]. By using *in silico* approach using BIOPEP, ToxinPred and PeptideRank, it investigated that protein precursor in jack bean jack bean can be a precursor for BPs with high activity for ACE Inhibitor.

Several research has investigated ACE inhibitor activity using *in vitro* approach. The potency of ACE inhibitory activity is normally measured as an IC_{50} value, which indicates the concentration of inhibitory peptide or hydrolysates needed for 50% inhibition of ACE activity[26]. The next phase to establish if a BP is, in fact, hypotensive is through trials with small animals such as spontaneously hypertensive rats (SHRs). Finally, to reach the market bearing a health claim, BPs must be subjected to human clinical trials.

Anti-oxidant: Anti-oxidants (AOXs) can avoid or inhibit oxidation by preventing generation of reactive oxygen species (ROS) in the metabolism or by inactivating them[27-29]. Jack bean as raw material of jack bean tempe has several phenolic compounds that can act as anti-oxidant. Therefore, AOXs are of great importance in the human diet and health, as they can help the body to diminish oxidative damages.

Several processing technique e.g., roasting, frying, sprouting have been investigated to obtain comprehensive results of those process to the anti-oxidant activity of jack bean[3]. There is abundant literature information on AOX hydrolysates or peptides, derived from several plant proteins[30]. The production of anti-oxidant peptides through direct microbial fermentation rather than using purified enzymes is an integral part of healthy food production in many countries[31]. Natto and tempeh are fermented soybean products that contain antioxidant peptides by the action of fungal proteases[5]. The type, amount and activity of the peptides produced depend on the specific cultures used and *Rhizopus* spp. are some of the commonly used cultures[32].

Regarding the amino acid composition, it is known that basic amino acids possess the chelating capacity for metallic ions, while aromatic and sulfur counterparts have the capacity to donate protons to free radicals so that they can act for AOXs activity. About the position of the amino acids in the peptide chain, branched amino acids valine and leucine have more AOXs activity when they are found in the N-terminal position, as well as tryptophan and tyrosine in the C-terminal position[33]. However, the specific contribution of individual amino acid residues to the AOX activity of a peptide depends largely on the nature of free radical and the reaction medium[34].

Cancer preventing: Most of the research related to BPs and cancer has been investigated recently. Fraction <3 kDa from chickpea which is commonly used as raw material of tempeh inhibited human epithelial colorectal adenocarcinoma cells (Caco-2) and monocytic leukemia cells (THP-1). Interestingly, the authors determined the *in vitro* bioavailability and found a dramatic change in the inhibitory activity of the hydrolysates. Tempeh with soybean as a raw material has been shown to be capable of inhibiting proliferation and angiogenesis as well as triggering apoptosis in cancer cells in a dose-dependent manner[32].

Hypocholesterolemic: Jack bean seeds showed the positive result to reduce cholesterol[3]. On the other hand, fermentation of tempeh using soy bean as raw material showed a positive effect also to have hypocholesterol effect[35]. So its so promising in the future that the jack bean tempeh also has a positive effect for cholesterol-lowering effects have been explored for their potential in the prevention and treatment of hypercholesterolemia, one of the important risk factors for heart disease.

CONCLUSION

Jack bean tempeh is traditional fermented food from Indonesia. Jack bean as the raw material for making jack bean tempeh has been proved to have further bioactive compounds such as anti-oxidant activities, anti-oxidant activities, cancer-preventing effect and hypocholesterol activities. So that, it is very promising that jack bean tempeh also has those functions. This study discover the valorization of jack bean as a raw material for making jack bean tempeh that can be a source of valuable bioactive peptide that can be beneficial for human health. This study will help the researcher uncover the critical area of jack bean tempeh as a source of several bioactive compounds such as bioactive peptide that many researchers were not able to explore. Thus new theory on several precursors of protein in jack bean tempeh for a source of bioactive peptides may be arrived at. In the future, it is quite promising to investigate and diversify several processing parameters to improve the economic value of jack bean tempeh.

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