HAYATI Journal of Biosciences
ISSN: 1978-3019
EISSN: 2086-4094

Comparison Between the Potential of Tempe Flour Made from Germinated and Nongerminated Soybeans in Preventing Diabetes Mellitus

Made Astawan[1*], Inas Suci Rahmawati[1], Ananda Putri Cahyani[1], Tutik Wresdiyati[2], Sastia Prama Putri[3], Eiichiro Fukusaki[3]

[1]*Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Bogor, Indonesia*
[2]*Department of Anatomy, Physiology, and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, Indonesia*
[3]*Laboratory of Bioresource Engineering, Department of Advanced Science and Biotechnology, Graduate School of Engineering, Osaka University, Japan*

ARTICLE INFO

Article history:
Received April 1, 2019
Received in revised form August 2, 2019
Accepted August 10, 2019

KEYWORDS:
diabetes,
tempe,
antioxidant,
isoflavones,
α-amylase,
α-glucosidase

ABSTRACT

This study was aimed to compare the chemical characteristics of tempe flour made from nongerminated soybean (NST) and germinated soybean (GST), especially on their capacity in preventing diabetes mellitus (DM). Soybeans were germinated for 20 hours in the dark until 2.5-5.0 mm of the radicle emerged. The ungerminated soybeans and the germinated soybeans were then processed into tempe and tempe flour. The two types of tempe flour were subjected to proximate analysis, amino acid profiling, antioxidant capacity, total phenol content, isoflavone content, and α-amylase and α-glucosidase enzyme inhibition analyses. GST was superior in preventing DM in the protein content and antioxidant parameters, as these were significantly higher ($p<0.05$) than in NST. On the other hand, NST was superior in preventing diabetes in the isoflavon (daidzein, genistein, and total isoflavone) and α-amylase inhibition IC_{50} parameters which were significantly better ($p<0.05$) than in GST. On the contrary, the diabetes-preventing parameters total phenols, α-glucosidase inhibition IC_{50}, and insulinotropic amino acids (arginine, alanine, phenilalanine, isoleucine, leucine, and lysine) were not different ($p>0.05$). Therefore, GST and NST both have potential in preventing diabetes through different mechanisms.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia due to a defect in insulin production, insulin action, or both (American Diabetes Association 2010). Oxidative stress plays an important role in diabetes mellitus etiology and is responsible for damaging pancreatic β-cells. Therefore, controlling the blood glucose level, inhibiting oxidative stress (El-Kordy and Alshahrani 2015), and increasing body antioxidant defensive system (Shahidi *et al.* 2012) have been suggested to manage diabetes mellitus.

Plants high in antioxidants such as vitamin E, vitamin C, β-carotene, and polyphenols have been used as traditional medicine to treat diabetes in several regions. Antioxidants are compounds which actively bind reactive oxygen species (ROS) and protect pancreatic β-cells from oxidative stress (Firdaus *et al.* 2010). In addition to the ROS-binding mechanism, antioxidants can act as antidiabetic agents due to their ability to inhibit carbohydrate absorption. Inhibition of carbohydrate absorption occurs as antioxidants are able to inhibit α-glucosidase and α-amylase activities, inhibit gluconeogenesis, stimulate pancreas activity, and increase insulin sensitivity (Shahidi *et al.* 2012).

Acarbose is commonly used as an α–amylase and α-glucosidase inhibitor in diabetes treatment. Acarbose reduces the complex carbohydrate digestion rate which later decreases glucose absorption in the small intestines. However, acarbose has several gastrointestinal side effects, including flatulence, abdominal discomfort, and diarrhea (Setter *et al.* 2006). Phenolic extracts generally have slightly weak α-amylase inhibition but have a stronger ability in inhibiting α-glucosidase that does not give rise to significant side effects (Adefegha and Oboh 2016).

* Corresponding Author
E-mail Address: astawan@apps.ipb.ac.id

Bioactive compounds that show antidiabetic activities in legumes are isoflavones, an α-amylase inhibitor, and an α-glucosidase inhibitor (Gętek *et al.* 2014). A study regarding germination of soy demonstrated that it was able to increase the bioactive compounds (Puteri *et al.* 2018). During germination, the seeds undergo various biochemical and enzymatic reactions; therefore, increasing their nutritional value and digestibility compared to the seeds without germination process (Márton *et al.* 2010). Germination increases the amount of vitamin E, total isoflavones, antioxidant capacity, calcium, phosphorus, iron, and zinc content in germinated soy tempe flour compared to nongerminated soy tempe flour (Astawan *et al.* 2016a). In addition to the potential of germination soybean as antidiabetic has been evidenced by the presence of insulinotropic amino acids that play role in increasing insulin secretion such as alanine, arginine, phenylalanine, isoleucine, leucine, and lysine (Kanetro and Astuti 2013).

Tempe, a fermented soybean by *Rhizopus* spp mold originally from Indonesia, is an excellent protein source with high nutritional quality (Astawan *et al.* 2016b). Differences in the production process and unique fermentation condition in different regions result in varieties of tempe (Kadar *et al.* 2018). Tempe has higher nutritional values compared to soybeans because the fermentation process degrades carbohydrate, fat, and protein into their respective building blocks (Astawan *et al.* 2015). Aside from its increased digestibility, tempe contains higher aglycone isoflavones compared to soybeans (Sumi and Yatagai 2006). Fresh tempe is highly perishable and has a short shelf life. Processing tempe into flour is an alternative method to increase its shelf life (Astawan *et al.* 2017). This study was aimed to compare the characteristics of germinated soybean tempe flour (GST) and nongerminated soybean tempe flour (NST), especially the chemical compounds which act as antidiabetics.

2. Materials and Methods

2.1. Sample Preparation

The main ingredient used in this research was the local Grobogan variety soybean (*Glycine max)* collected from farmers in Grobogan, Central Java, Indonesia. The germination process was done by soaking the soybeans in water for 3 hours, then draining them, and incubating them for 20 hours in a dark condition. During the incubation process, the soybeans were sprayed with water every 4 hours to maintain their humidity (Puteri *et al.* 2018).

Tempe production was done according to Rumah Tempe Indonesia (Indonesian House of Tempe). Soybeans were soaked for 2-4 hours, boiled for 30 minutes, and soaked for 18-30 hours in clean water added with some of the boiled water used before. Through soaking, the pH would decrease (the target pH being 4.0-5.0). After that, the soybeans were ground, peeled, cleaned, rinsed with hot water, and drained at room temperature. After draining, the soybeans were inoculated with the commercial starter culture "RAPRIMA" produced by LIPI Bandung (2 grams per kilogram of dry soybeans) and then fermented for 48 hours. The first 24 hours was done at 30-34°C, while the second 24 hours at room temperature (below 30°C) (Astawan *et al.* 2017).

Tempe was further processed into flour according to the method by Astawan *et al.* (2015). Fresh tempe was sliced, steam blanched for 2 minutes, and then dried using an oven (GmbH 6072 Dreieich, West Germany) at 60°C for 8 hours. Dried tempe was ground using a disc-mill and sifted using a 60 mesh sieve. The flour was stored in a tightly sealed container. Tempe flour was used for chemical analysis consisting of proximate analysis, crude fiber, amino acids profile, isoflavones, and vitamin E analyses.

Tempe flour was further extracted with ethanol according to the Xu and Chang (2007) method with modifications. Flour was extracted with ethanol/water (70:30 v/v) at a ratio of 1:5 (b/v), shaken with a stirrer at room temperature for 3 hours, extracted for additional 12 hours in a dark condition, filtered through Whatman Paper No. 1, and followed by centrifugation (Centrifuge 5810R Eppendorf, Hamburg, Germany) 300 x g for 10 minutes. Ethanol was removed by a rotary evaporator (Rotavapor R-210 Buchi Labortechnik AG, Flawil, Switzerland) at 45°C. The extract was stored at 4°C in the dark until further analysis. This extract was used for antioxidant, total phenolics, and inhibition of α-amylase and α-glucosidase analyses.

2.2. Chemical Component Analysis

Moisture, ash, protein, fat, carbohydrate by difference, crude fiber, vitamin E, total isoflavone, and amino acids profile were determined using methods described by the Association of Official

Analytical Chemist (AOAC 2012). The total phenolics content was determined using the method described by Koh *et al.* (2012). A total of 1 ml tempe flour extract was placed in a test tube and mixed with 5 ml Folin-Ciocalteu reagent (1:1 with water). The mixture was allowed to stand at room temperature for 5 minutes. Then 4 ml of sodium carbonate solution (7.5% w/v) was added and mixed thoroughly. The tubes were placed in a dark condition for 2 hours and the absorbance was measured at 765 nm. Gallic acid (Sigma-Aldrich) was used as the standard and the amount of total phenolics content was calculated as a Gallic Acid Equivalent (GAE).

2.3. Antioxidant Capacity Analysis

The antioxidant capacity analysis was determined using method described by Hung and Nhi (2012). A total of 0.1 ml tempe flour extract was mixed with 3.9 ml of 0.075 mM DPPH (2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich) solution. The mixture was allowed to stand at room temperature in the dark for 30 minutes. The absorbance was measured at 517 nm. Ascorbic acid (Sigma-Aldrich) was used as the standard and the antioxidant capacity was expressed as an AEAC (Ascorbic Acid Equivalent Antioxidant Capacity).

2.4. Alpha-amylase Inhibition Assay

The alpha-amylase inhibition assay was conducted using method described by Thalapaneni *et al.* (2008). A total of 125 µl tempe flour extract was added to the same amount of sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 1 U/ml α-amylase (from *Aspergillus oryzae*) solution (Sigma-Aldrich). The mixture was incubated at 37°C for 10 minutes. Then, 125 µl of 1% corn soluble starch (Sigma-Aldrich) solution was added and the mixture was incubated again at 37°C for 10 minutes. The reaction was terminated by adding 250 µl dinitrosalicylic acid (DNS) solution and incubated in a boiling water bath for 5 minutes. The reaction mixture was then diluted by adding 5 ml of distilled water. The absorbance was measured at 540 nm.

2.5. Alpha-glucosidase Inhibition Assay

The alpha-glucosidase inhibition assay was done using method described by Sancheti *et al.* (2009). A total of 10 µl tempe flour extract was mixed with 50 µl of 0.1 m potassium phosphate buffer (pH 7.0), 25 µl ρ-nitrophenyl-α-D-glucopyranoside (dissolved in 0.1 m potassium phosphate pH 7.0), and 25 µl of 0.04 U/ml α-glucosidase (from *Saccharomyces cerevisiae*) solution (Sigma-Aldrich). The mixture was incubated at 37°C for 30 minutes. The reaction was terminated by adding 100 µl of 0.2 m sodium carbonate. The mixture was then diluted by adding 1 ml of distilled water. The absorbance was measured at 405 nm.

3. Results

Soybean germination before processing into tempe flour changed the content of several components analyzed. The result of the proximate analysis and crude fiber analysis are presented in Table 1. The germination process increased moisture ($p<0.01$), protein and crude fiber contents ($p<0.05$) of the tempe flour yielded, while the carbohydrate content decreased ($p<0.01$). Fat, and ash contents demonstrated no change.

As shown in Table 2, most amino acids did not change significantly and only a few suffered a decrease. The germination process significantly decreased the aspartic acid level ($p<0.01$), followed by threonine, valine, and leucine ($p<0.05$). Among the essential amino acids, leucine was the highest in amount in both tempe flours, while the lowest in amount was methionine. In addition, the content of insulinotropic amino acids such as arginine, alanine, phenylalanine, isoleucine, and leucine tends to decline due to the germination process of soybeans.

Table 3 presents the result of the antioxidant analysis and the components which were expected to be involved in the antioxidant activity. The antioxidant capacity of GST was higher than NST ($p<0.05$). However, total isoflavones content in GST was decreased ($p<0.01$), while the total phenolics content did not show significant change.

Germination decreased the inhibition of α–amylase and α-glucosidase ($p<0.05$) that shown by the increased of their IC_{50} value. The IC_{50} value of α–amylase was significantly lower (74.8 mg/ml) in NST as compared to GST (92.0 mg/ml). The similar result was also observed in α-glucosidase, where NST had IC_{50} value significantly lower (85.3 mg/ml) as compared to GST (115.9 mg/ml), as shown in Figure 1.

4. Discussion

Various biochemical and enzymatic reactions occur during the germination process that breaks down macromolecules into their respective building blocks. Macromolecule breakdown increases the

Table 1. Proximate analysis and crude fiber content of tempe flours

Parameter	Tempe flour from	
	Nongerminated soybean	Germinated soybean
Moisture (% wb)	3.92±0.82[a]	5.98±1.03[b]
Ash (% db)	2.08±0.05[a]	1.89±0.11[a]
Fat (% db)	25.94±1.35[a]	25.65±0.99[a]
Protein (% db)	50.69±0.53[a]	52.98±0.55[b]
Cabohydrate (% db)	17.36±1.70[b]	13.50±2.46[a]
Crude fiber (% db)	5.05±0.06[a]	6.59±0.10[b]

*Different superscripts in the same row means statistically different (p<0.05)

Table 2. Amino acid profile of tempe flours

Parameter (%, db)	Tempe flour from	
	Nongerminated soybeans	Germinated soybeans
Aspartic acid	6.14±0.06[b]	5.64±0.05[a]
Glutamic acid	10.17±0.23[a]	9.49±0.09[a]
Serine	2.76±0.03[a]	2.48±0.15[a]
Histidine	1.18±0.01[a]	1.18±0.08[a]
Glycine	1.93±0.03[a]	1.97±0.06[a]
Threonine	2.15±0.03[b]	1.97±0.04[a]
Arginine	3.70±0.08[a]	3.42±0.04[a]
Alanine	2.34±0.04[a]	2.15±0.01[a]
Tyrosine	1.86±0.01[a]	1.70±0.03[a]
Methionine	0.58±0.05[a]	0.61±0.04[a]
Valine	2.66±0.01[b]	2.48±0.01[a]
Phenylalanine	2.89±0.06[a]	2.75±0.02[a]
Isoleucine	2.66±0.03[a]	2.50±0.00[a]
Leucine	4.21±0.07[b]	3.94±0.06[a]
Lysine	2.60±0.58[a]	3.39±0.08[a]

*Different superscripts in the same row means statistically different (p<0.05)

Table 3. Antioxidant analysis of tempe flours

Parameter (per 100 g db)	Tempe flour from	
	Nongerminated soybean	Germinated soybean
Antioxidant capacity (mg AEAC)	186.819±17.34[a]	249.79±5.06[b]
Daidzein (mg)	53.56±1.02[b]	35.96±3.59[a]
Genistein (mg)	62.46±0.56[b]	44.16±5.26[a]
Total isoflavones (mg)	116.02±1.58[b]	80.13±8.86[a]
Total phenols (mg)	426.74±58.88[a]	483.92±37.60[a]
Vitamin E (mg)**	4.75	4.87

*Different superscripts in the same row means statistically different (p<0.05)
**Data were obtained from one analysis only

digestibility of germinated soybeans (Márton *et al.* 2010). The moisture content tends to increased after germination. Soybean absorb water during the germination process for its metabolism. Increased water absorption occurs due to the increasing number of cells to becoming hydrated seed. So that there were increased in the moisture content in tempe flour (Warle *et al.* 2015).

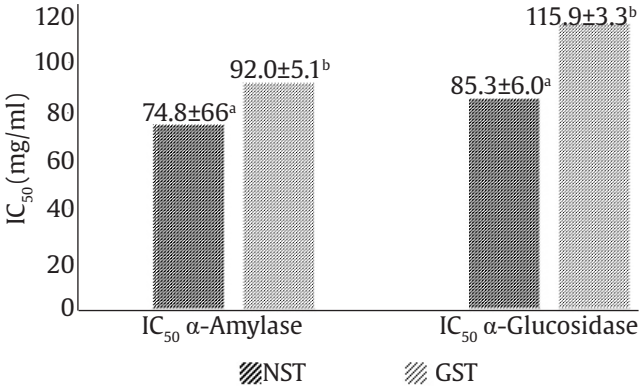

Figure 1. IC_{50} value of α-amylase and α-glucosidase in tempe flours. NST=nongerminated soybean tempe flour; GST=germinated soybean tempe flour. Different superscripts means statistically different in each enzyme (p<0.05)

Germination tends to decrease ash content since the soaking and cleaning process leaches the mineral content to the water. The soaking process decreases Mg and Zn content (Márton *et al.* 2010). Ash content tends to decrease represents mineral loss during germination and washing, but some essential mineral such as Ca and Cu could rise during germination (Jiang *et al.* 2013). However, a study conducted by Astawan and Hazmi (2016) showed that the germination process can increase the ash content of soybean flour. The difference in results may be due to the calcium oxide solution added during the germination process in that study.

Carbohydrate and fat are hydrolyzed to provide the energy needed to synthesize protein (Shi *et al.* 2010). Decrease fat level is due to the use of fat deposits as an energy during the germination process. Decrease fat levels are also followed by a decrease in carbohydrate. Carbohydrate molecules will be broken down during the germination process to provide nitrogen absorption for the formation of amino acids (Joshi and Kanika 2016).

The increase in protein content may be caused by protein synthesis and reduction of other components from the leaching during boiling or they were used by the tempe mold for its growth (Astawan *et al.* 1994). Protein synthesis occurs during imbibition to produce enzymes, hormones, and other components needed for radicle growth. Despite the fact that throughout the germination process protein is broken down into amino acids, it seems that the protein synthesis exceeded the protein breakdown.

The crude fiber content was increased in tempe flour from germinated soybean (GST). In germinated soybean, the amount of crude fiber was contributed by the presence of seed layer, like epidermis, hypodermis, and parenchyma that content fibers such as cellulose, pectin, galactomannan, and glycoprotein (Krisnawati and Adie 2008). Some studies have shown that intake of fibers especially water soluble fiber can also help to control glucose level. Fibers are able to absorb water and bind glucose, thereby reducing glucose availability. Insufficient dietary fiber also resulted in increased digestibility of carbohydrates (Santoso 2011).

Villegas *et al.* (2008) studied the relationship between soy consumption and the risk of type 2 diabetes mellitus in Chinese women aged 40-70 years. That study showed a higher consumption of soy decreased the occurrence of diabetes. The protective effect of soy was likely due to fiber, protein, polyphenols, and the low glycemic index of soy. It is suggested that the protective effect was not caused by a single component, but was due to the interaction of several components (Sugano 2006).

Soy protein inhibits body fat accumulation through the inhibition of insulin secretion, inhibition of lipogenesis, and enhancement of insulin sensitivity. These mechanisms are affected by the amino acid profile and isoflavones bound to the proteins. In experimental rats given soy protein, there was an increase in insulin sensitivity (Noriega-López *et al.* 2007). Reduction of body fat accumulation decreases the obesity risk which is one of the risk factors for diabetes mellitus.

Another study showed that soy protein and germinated soy protein were able to stimulate insulin secretion in diabetic rats. Germinated soy protein contains free amino acids which can stimulate insulin secretion better than nongerminated soy protein. Several amino acids show a stronger insulinotropic effect, such as arginine, leucine, and phenylalanine (van Loon *et al.* 2003). However, the germination process in this research did not increase the content of insulin-stimulating amino acids (Table 2). A decrease in leucine content was observed instead. The availability of amino acids is not necessarily a reference, but the bioavailability affects the effectiveness of the use of these insulinotropic amino acids.

The mechanism of several amino acids in stimulating insulin release are varied. The results of the study using alanine amino acid showed that the addition of 10 mmol/l alanine in basal glucose concentrations increased insulin secretion three times in cells β-BRIN-BD11 and 1.6 times on islet. Leucine amino acid stimulate the release of insulin in the cells of β pancreas through a process that includes increased mitochondrial metabolism through the activation of GDH, increased production of ATP through leucine transaminases into α-toxicity and subsequently enter the TCA cycle through acetyl CoA (Newsholmes *et al.* 2005).

Arginine affecting the wound healing, cause arginine as the one of the nitric oxide-forming materials (NO) that would help the collagen synthesis in the wound area. Another studies showed that NO will regulate the metabolism of glucose, fatty acids and amino acids, so that the consumption of arginine would decrease fat mass in the obese and diabetic rats. Nitric oxide also enhances glucose transport, lowers glucose, glycogen synthesis, stimulates release of insulin and inhibit the rate of pancreatic β-cell damage (Utari *et al.* 2011).

Studies about the amino acid profile in germinated and nongerminated soybeans demonstrated different results. Pomeranz *et al.* (1977) only found a little difference in amino acid composition between commercial soybean flour and germinated soybean flour. Different results were observed by Mostafa and Rahma (1987) where soybean germination relatively increased the essential and non-essential amino acid content. The highest increase was observed in leucine, tyrosine, phenylalanine, and glutamic acid, while methionine content decreased slightly. The different results obtained might be due to different germination conditions, different soybean varieties used, and different analysis methods.

The fermentation process also affected the amino acid profile of tempe flour. According to the study by Gibbs *et al.* (2004), fermentation of tempe for 24 hours decreased the content of aspartic acid, glutamic acid, lysine, tyrosine, and serine, while glycine, isoleucine, leucine, proline, and threonine increased. Some amino acids, for example, alanine, valine, arginine, histidine, phenylalanine, cysteine, methionine, and tryptophan tended not to change in content. This is affected by the peptidase activity of *R. oligosporus.*

Germination caused increase production of bioactive compounds with potent antioxidant properties. Studies demonstrated that germination can alter the level of bioactive compounds of soybean by

increasing lunasin, isoflavone and saponins. Analysed soybeans after 48 h of germination, concluding that germination reduced tannins while increased total phenolics (Vernaza *et al.* 2012). The phenolics content of legumes tends to have a positive correlation with its antioxidant capacity. This current study showed that the increase in antioxidant capacity in GST was likely not due to the increase in phenolics as the phenolics content remained constant and the isoflavones decreased. Nevertheless, non-phenolics compounds, including ascorbic acid, tocopherols, phytic acid, carotenoids, and saponins, could also contribute to the antioxidant activity (Lee *et al.* 2011). The increase in antioxidant capacity may be affected by the increase in protein content in GST. Soy protein has been shown to potentially act as an antioxidant. Antioxidant amino acids and vitamin E work in synergy as antioxidants in the body.

Park *et al.* (2008) stated that several peptides and amino acids act as antioxidants in soy hydrolysates. The peptide with the highest antioxidant properties was found to have a low molecular weight (<3 kDa) and was mostly comprised of hydrophobic amino acids such as phenylalanine, alanine, and proline. Phenylalanine was the main component found and was believed to be the main active component. When exposed to hydroxyl radicals, phenylalanine is converted into tyrosine which has radical scavenging activity (Park *et al.* 2008). In Table 2, both tempe flours were observed to have no significantly different phenylalanine and tyrosine content ($p>0.05$). Proline, one of the abundant amino acids found in antioxidant peptides, was not analyzed here; therefore, the proline content was not known.

Yuan *et al.* (2009) has been reported that germination changed the distribution profile of isoflavone. The results showed that after germination, the glycoside contents decreased and the total aglycone content increased. In line with Shi *et al.* (2010) that malonyl genistin and malonyl daidzin are decreased, but daidzin, genistin, daidzen, and genistein are increased during germination. Zhu *et al.* (2005) studied the effect of germination on the isoflavone profile of two different soybean varieties, *Hutcheson* and *Caviness*. After soaking, the total isoflavone content of both varieties increased to the maximum amount (*Hutcheson* 2,491 mg/g db and *Caviness* 2,780 mg/g db). After this stage, a decrease in the isoflavone content was observed. Another possible cause for the decrease in the isoflavone content may be contributed to the conversion of isoflavones to other flavonoids.

Isoflavone conversion may happen not only during germination but also during fermentation. Soybean flour fermented with *Aspergillus oryzae* underwent a decrease of total isoflavones after 48 hours of fermentation. Various reactions such as hydroxylation, methylation, glycosylation, and acetylation can occur and change the structure of isoflavones (da Silva *et al.* 2011). Isoflavone structure is important in determining its antioxidant activity. The antioxidant activity of flavonoids is determined by the arrangement of the functional group bound to the structure such as the hydroxyl group, methyl, double bound, and carbohydrate moieties, and also the degree of polymerization (Heim *et al.* 2002).

Genistein was the most abundant isoflavone found in both tempe flours. Products high in aglycone isoflavones have higher efficiency in preventing chronic diseases because of their higher absorbability. El-Kordy and Alshahrani (2015) studied the effect of genistein on streptozotocin-induced diabetic rats. Administration of 20 mg/kg/day of genistein showed a protective effect on pancreatic β-cells against ROS.

The α-amylase and α-glucosidase is an enzyme that plays an important role in converting carbohydrates into glucose. Soybean flour known can significantly inhibits the work of enzymes by reducing the increase of postprandial blood glucose (Subramanian *et al.* 2008). The anti-diabetic actions of the plant is suggested by inhibiting α-amylase and α-glucosidase which could decrease postpandrial blood glucose level, and also by repairing the damage of pancreatic beta cells, thus enhancing the insulin secretion directly (Febrinda *et al.* 2014). In this study, no improvement of inhibition activity on starch digestion enzymes was observed due to soy germination. In addition, a decrease in α-amylase inhibition (increase in IC_{50} value) was detected in GST. GST was expected to have lower glycosides due to its phenolics degradation during the germination process. Free phenolics were shown to have lower inhibition activity on α-amylase compared to their bound forms (Ademiluyi and Oboh 2013). Bound phenolics which exist mostly in glycosides are hydrophilic and since the enzymes work in an aqueous phase, the direct enzyme-inhibitor interaction is expected to be higher in NST. Compared to the study by Ademiluyi and Oboh (2013), the α-amylase and α-glucosidase inhibition of soybeans was higher than both tempe flours. This result may be likely because the tempe flours underwent various heating process.

Heating process decreases the enzyme inhibition property of soy (Gętek *et al.* 2014).

GST and NST were found to have several different compositions; therefore, they were expected to have different antidiabetic properties. With higher a protein content and antioxidant capacity, GST was expected to have a higher potential in increasing insulin sensitivity in a high-fat diet and in preventing pancreatic β-cell destruction. NST had a higher leucine content, hence it was expected to increase insulin secretion in a high blood glucose condition. In addition, NST might be more promising in inhibiting starch digestion since it had a higher α-amylase and α-glucosidase inhibition values (lower IC_{50} values than GST). Further study to obtain the optimum germination conditions is needed to increase the bioactive content in germinated soybean tempe.

Conflict of Interest

The authors have no conflict of interest regarding the results of this research.

Acknowledgements

The authors would like to thank the Directorate of Research and Community Service, the Directorate General for Research and Development, Ministry of Research, Technology and Higher Education, Republic of Indonesia, through "Penelitian Berbasis Kompetensi 2019" scheme under the name of Made Astawan.

References

[ADA] American Diabetes Association. 2010. Diagnostic and classification of diabetes mellitus. *Diabetes Care* 33: 562-569. DOI:10.2337/dc10-S062

Adefegha SA, Oboh G. 2016. Antioxidant and inhibitory of *Clerodendrum volubile* leaf extracts on key enzymes relevant to non-insulin dependent diabetes mellitus and hypertension. *J TU Sci* 10:521-533. DOI:10.1016/j.jtusci.2015.10.008

Ademiluyi AO, Oboh G. 2013. Soybean phenolic-rich extract inhibits key-enzymes linked to type 2 diabetes (α–amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) *in vitro*. *J Exp Toxicol Pathol* 65:305-309. DOI:10.1016/j.etp.2011.09.005

[AOAC] Association of Official Analytical Chemist. 2012. *Official Method of Analysis of the Association of Official Analysis Chemist*. Gaithersburg: AOAC.

Astawan M *et al.* 1994. Changes in protein-nutritional quality of Indonesian dried-salted fish after storage. *Journal of the Science of Food and Agriculture* 66:155-161.

Astawan M *et al.* 2015. Pengaruh konsumsi tempe kedelai Grobogan terhadap profil serum, hematologi, dan antioksidan tikus. *J Teknol dan Industri Pangan* 26:155-162. DOI:10.6066/jtip.2015.26.2.155

Astawan M, Hazmi K. 2016. Karakteristik fisikokimia tepung kecambah kedelai. *J Pangan* 25:105-112.

Astawan M *et al.* 2016a. Karakteristik fisikokimia tepung tempe kecambah kedelai. *J Gizi Pangan* 11:35-42.

Astawan M *et al.* 2016b. Application of vacuum packaging to extend the shelf life of fresh-seasoned tempe. *Int Food Res J* 23:2571-2580.

Astawan M *et al.* 2017. *Tempe: Sumber Zat Gizi dan Komponn Bioaktif untuk Kesehatan*. Bogor: IPB Press.

da Silva LH *et al.* 2011. Effect of the fermentation of whole soybean flour on the conversion of isoflavones from glycosides to aglycones. *Food Chem* 128:640-644. DOI:10.1016/j.foodchem.2011.03.079

El-Kordy EA, Alshahrani AM. 2015. Effect of genistein, a natural soy isoflavone, on pancreatic β-cells of streptozotocin-induced diabetic rats: Histological and immunohistochemical study. *JMAU* 3:108-119. DOI:10.1016/j.jmau.2015.03.005

Febrinda AE *et al.* 2014. Hyperglycemic control and diabetes complication preventive activities of Bawang Dayak (*Eleutherine palmifolia* L. Merr.) bulbs extracts in alloxan-diabetic rats. *International Food Research Journal* 21:1405-1411.

Firdaus M *et al.* 2010. Prevention of endothelial dysfunction in streptozotocin-induced diabetic rats by *Sargassum echinocarpum* extract. *Medical Journal of Indonesia* 19:33-35.

Gętek M *et al.* 2014. The active role of leguminous plant components in type 2 diabetes. *J Evid Based Complementary Altern Med* 1-12. DOI:10.1155/2014/293961

Gibbs BF *et al.* 2004. Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food. *Int Food Res J* 37:123-131. DOI:10.1016/j.foodres.2003.09.010

Heim KE *et al.* 2002. Flavonoid antioxidants: chemistry, metabolism, and structure-activity relationships. *J Nutr Biochem* 13:572-584. DOI:10.1016/S0955-2863(02)00208-5

Hung PV, Nhi NNY. 2012. Nutritional composition and antioxidant of several edible mushrooms grown in Southern Vietnam. *Int Food Res J* 19:611-615.

Jiang S *et al.* 2013. Food quality improvement of soy milk made from short-time germinated soybeans. *Foods* 2:198-212. DOI:10.3390/foods2020198

Joshi P, Kanika V. 2016. Effect of germination and dehulling on the nutritive value of soybean. *Nutrition and Food Science* 46:595-603. DOI:10.1108/NFS-10-2015-0123

Kadar AD *et al.* 2018. Gas chromatography coupled with mass spectrometry-based metabolomics for the classification of tempe from different regions and production processes in Indonesia. *J Biosci Bioeng* 126:411-416. DOI:10.1016/j.jbiosc.2018.03.020

Kanetro B, Astuti S. 2013. Profile of amino acid for stimulation of insulin secretion in the extract after protein removal of local legumes sprout. *AGRITECH* 33:258-264.

Koh SP *et al.* 2012. Nutritive value between fermented and germinated soybean: γ-aminobutiric acid, amino acids content, and antioxidant properties. *Borneo Science* 31:143-151.

Krisnawati A, Adie MM. 2008. Varieties morphology of seeds from some soybean germplasm genotypes. *Germplasm Bulletin* 14:14-18.

Lee JH *et al.* 2011. Comparative analyses of total phenols, flavonoids, saponins, and antioxidant activity in yellow soybeans and mung beans. *Int J Food Sci Technol*

46:2513-2519. DOI:10.1111/j.1365-2621.2011.02775.x
Márton M *et al.* 2010. The role of sprouts in human nutrition: A review. *Acta Univ Sapientiae Almentaria* 3:81-117.
Mostafa MM, Rahma EH. 1987. Chemical and nutritional changes in soybean during germination. *J Food Chem* 23:257-272.
Newsholmes P *et al.* 2005. New insights into amino acid metabolism, β-cell function and diabetes. *Clinical Science* 108:185-194.
Noriega-López L *et al.* (2007). Pancreatic insulin secretion in rats fed a soy protein high fat diet depends on the interaction between the amino acid pattern and isoflavones. *J Biol Chem* 282:20657-20666. DOI:10.1074/jbc.M701045200
Park SY *et al.* 2008. Purification and characterization of antioxidant peptides from soy protein hydrolysate. *J Food Biochem* 34:120-132. DOI:10.1111/j.1745-4514.2009.00313.x
Pomeranz Y *et al.* 1977. Flour from germinated soybean in high protein bread. *J Food Sci* 42:824-827. DOI:10.1111/j.1365-2621.1977.tb12613.x
Puteri NE *et al.* 2018. Characterization of biochemical and functional properties of water-soluble tempe flour. *Journal of Food Science and Technology* 38:147-153. DOI:10.1590/fst.13017
Sancheti S *et al.* 2009. Chaenomeles sinensis: a potent α- and β- glucosidase inhibitor. *Am J Pharm and Toxicol* 4:8-11. DOI:10.3844/ajptsp.2009.8.11
Santoso A. 2011. Food fibre and health benefits. *Magistra* 75:35-40.
Setter SM *et al.* 2006. Diabetes. In: Helms RA, Quan DJ, Herfindal ET, Gourley DR (Eds.). *Textbook of Therapeutics: Drug and Disease Management*. Philadelphia: Lippincott Wiliams and Wilkins. pp. 1042-1075.
Shahidi F *et al.* 2012. Antioxidants and human health. In: Yu L, Tsao R, Shahidi F (Eds.). *Cereals and Pulses: Nutraceutical Properties and Health Benefits*. Oxford: J Wiley. pp. 273-308.
Shi H *et al.* 2010. Comprehensive profiling of isoflavones, phytosterols, tocopherols, minerals, crude protein, lipid, and sugar during soybean (*Glycine max*) germination. *J Agric Food Chem* 58:4970-4976. DOI:10.1021/jf100335j
Subramanian R *et al.* 2008. *In vitro* α-glucosidase and α-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide. *Acta Biochimia Polonica* 55:391-398.
Sugano M. 2006. Nutritional implications of soy. In: Sugano M (Eds.). *Soy in Health and Disease Preventing*. Boca Raton: CRC Pr. pp. 1-16.
Sumi H, Yatagai C 2006. Fermented soybean components and disease prevention. In: Sugano M (Eds.). *Soy in Health and Disease Preventing*. Boca Raton: CRC Pr. pp. 251-278.
Thalapaneni NR *et al.* 2008. Inhibition of carbohydrate digestive enzymes by *Talinum portulacifolium* (Forssk) leaf extract. *J Complement Integr Med* 5:1-10. DOI:10.2202/1553-3840.1120
Utari D *et al.* 2011. Potency of amino acid in tempeh for improving lipid profile and diabetes mellitus. *Journal of National Public Health* 5:166-170.
Van Loon LJC *et al.* 2003. Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. *Diabetes Care* 26:625-630. DOI:10.2337/diacare.26.3.625
Vernaza MG *et al.* 2012. Antioxidant and anti-inflammatory properties of germinated and hydrolysed Brazilian soybean flours. *Food Chemistry* 134:2217-2225. DOI:10.1016/j.foodchem.2012.04.037
Villegas R *et al.* 2008. Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai Women's Health Study. *Am J Clin Nutr* 87:162-167.
Warle BM *et al.* 2015. Effect of germination on nutritional quality of soybean (*Glycine max*). *OSR-JESTFT* 9:13-16. DOI:10.9790/2402-09421316
Xu BJ, Chang SKC. 2007. A comparative study on phenolic profiles and antioxidant activities of legume as affected by extraction solvents. *J Food Sci* 72:159-166. DOI:10.1111/j.1750-3841.2006.00260.x
Yuan JP *et al.* 2009. Changes of isoflavone profile in the hypocotyls and cotyledons of soybeans during dry heating and germination. *J Agric Food Chem* 57: 9002-9010. DOI:10.1021/jf902248b
Zhu D *et al.* 2005. Isoflavone contents in germinated soybean seeds. *Plant Food Human Nutr J* 60:147-151. DOI:10.1007/s11130-005-6931-0