PREVENTIVE POTENTIAL OF EXTRACT FROM GERMINATED SOYBEAN AGAINST DIABETES

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ABSTRACT

Long-term type 2 diabetes can lead to numerous biological complications, such as hypertension and cardio-vascular disease. The key enzymes involved in the enzymatic breakdown of complex carbohydrates; pancreatic α-amylase and intestinal α-glucosidase, have been targeted as potential avenues for modulation of type 2 diabetes-associated post- prandial hyperglycemia through mild inhibition of their enzymatic activities which to decrease meal-derived glucose absorption. This study investigates to access the inhibitory activities of phenolic-rich extracts from germinated soybean fermented with *Bacillus subtilis*, *Lactobacillus lantarum* and *Lactococcus lactis*. Free phenolic extract of the bean was obtaining by extraction with 80% ethanol, while that of the bound phenolic extract was done by extracting the alkaline and acid hydrolysed residue with ethyl acetate. The inhibitory action of these extract on the enzyme activities as well as their antioxidant properties was assessed. Both phenolic- rich extract inhibited α- glucosidase in a dose independent pattern. However, the free phenolic extract exhibited significantly ($p < 0.05$), had high α- glucosidase inhibitory activity. Furthermore, the enzyme inhibitory activities of the phenolic- rich extracts were not associated with their phenolic content. Phenolic- rich extract of bean could inhibit key enzyme link to type 2 diabetes. Results indicated that *B. subtilis* could utilize fermented legume to generate certain α-glucosidase inhibitor more effectively than *L. plantarum* and *L. lactis*.

INTRODUCTION

Non- insulin- dependent diabetes mellitus or Type 2 diabetes affects approximately 150 million people worldwide (Lee & Lee, 2011). Postprandial hyperglycemia is a primary risk factor in the development of Type 2 diabetes. α-glucosidase inhibitors that reduce postprandial hyperglycaemia have a key role in the treatment of Type 2 pre-diabetic states and also have the potential to reduce the progression of diabetes (Toeller, 1994). A recent study showed that risk for Type 2 diabetes mellitus was decreased with consumption of whole grains of bean. soybeans are an important component of Asian diets and have been consumed for thousands of years. Various soy products are available, including soy flour, soy protein, tofu, soy milk, soy sauce, and soybean oil (Lee & Lee, 2001). Soybeans contain antioxidants and phytonutrients that have been linked with various health benefits, while could be reduced risk of cardiovascular disease, type II diabetes, and some cancers (Watanabe et al., 1997).

However, the original content of some functional compounds are very low (Tokunaga & Matsuoka, 1990). Various reports indicate that there are not enough for people to meet nutritional needs and for manufacturer to produce functional products and dietary supplements by directly using the raw (non- germinated) beans as food materials for consumption. Germination process can improve the chemical compositions of the whole grain (Kim et al., 2000), because the biochemical activity produces essential compounds and energy, for the formation of the seed growth. Fermentation is one of the processes to improve nutritional composition of the cereals. Fermented cereals are mainly carried out by lactic acid bacteria of lactic acid fermentation process (Noat et al., 1989). Many reported, *Bacillus subtilis* is an important starter culture for fermented soybean foods like Japanese nutto. In addition, the characteristics of the starter *B. subtilis* also play an important role in the properties and functionality of the fermented product (Katina et al., 2007). During fermentation, hydrated seeds contain living cells and different bio-catalytic activities, and micro-organisms start to modify the grain constituents. Many biochemical changes occur during fermented, which affect product properties such as structure, bioactivity, flavor, stability and digestibility.

In this work, thus aim to Improvement the percentage inhibition of α- glucosidase with increasing antioxidant activities of germinated soybeans by fermentation process.

EXPERIMENT

Samples:

Soybean, was purchased from Marketing Organization for Farmers, Bangkok, Thailand. Soybean seeds were cleaned by removing the dirt and then dried to a moisture level about 10%. The dried samples were kept in a refrigerator (11±1°C) prior used.

Microorganism:

Lactobacillus plantarum, *Lactococcus lactis*, and *Bacillus subtilis*, were supplied by the Thailand Institute of Scientific and Technology Research (TISTR).

Germination process:

Soybean seeds was soaked in distilled water in water bath at 40°C for 12 h. After that, distilled water was drained at the end of soaking. The soaked soybean was incubated on nylon net and place in plastic box under room temperature in the dark place. After germinated, soybean was dried, milled and passed through a sieve of 0.8 mm and stored at 4°C in desiccator's cabinet (Watcharaarparpaiboon et al., 2010).

Fermented preparation:

Control bean (non-germinated) and germinated bean were fermented with three lactic acid bacteria. Germinated bean (200 g) was added 10 mL distillated water and sterilized in autoclave for 15 min. The sterile bean samples were inoculated with three lactic acid bacteria (10 mL of inoculum). Samples were allowed to ferment at 37°C for 0 (control), 12, 24, 48 and 72 h. After fermentation process, samples were dried with hot air oven at 55°C, milled and passed through a sieve of 0.8 mm. All samples were stored in desiccators at 4°C prior analysis.

Measurement of Total Phenolic Content: Total phenolic content was determined according to the Folin-Ciocalteau method. A sample of 50 µL was added to 0.95 mL distillated water, mixed and added to 5 mL (10%) Folin-Ciocalteau reagent. After incubation for 5 min, 4 mL of (7.5%) sodium carbonate was added. Samples were incubated for 90 min in the dark at room temperature and measured absorbance at 750 nm. A standard curve was prepared with gallic acid (10-60 mg/mL) and results were expressed as gallic acid equivalents/ 100 g dried sample.

Determination of DPPH radical-scavenging activity (1,1-diphenyl- 2-picrylhydrazyl): One milliliter of each bean hydrolysate solution (100 µL of sample + 900 µL of distilled water) was added to 2 mL of 0.1 mM DPPH dissolved in 95% ethanol. The mixture was shaken and left for 30 min in the dark at room temperature, and measured the absorbance at 517 nm. The scavenging effect was expressed as percentage disappearance of DPPH.

α-Glucosidase inhibitory assay: The α-glucosidase inhibitory activity of the extracts was determined using a modified procedure reported by Apostolidis (2007). The α-glucosidase (EC 3.2.1.20) isolated from Baker's yeast and the substrate 4-nitrophenyl-a-D-glucopyranoside was purchased from Sigma–Aldrich Australia. The initial concentration of the enzyme solution was 0.444 U/mL in a sodium acetate buffer (pH 4.5) and the initial concentration of the substrate solution was 4.44 mM in the same sodium acetate buffer. The enzyme solution (45 µL/microtitre plate well) was mixed with the samples or controls (10 µL/well) in a clear 96-well microplate (flat bottom) and the reaction was started by addition of substrate solution (45 µL/well). The plates were incubated at 37°C for 30 min after shaking and the reaction was stopped by addition of 0.2 M Na_2CO_3 (100 µL/well). Enzyme inhibition was determined by the absorbance of 4-nitrophenol (product) at 405 nm, as measured with a microplate reader (Wallac Victor 2 1420 Multilabel Counter). Background absorbance was determined using a non-enzyme control microplate containing the sodium acetate buffer (45 µL/well) and was subtracted from the absorbance of samples and controls.

ANALYSIS

Total phenolic content

The antioxidant activities and total phenolics of three beans are shown in Table 1. Significant differences were observed for TPC from processes soybean (non-germination, germination and fermentation. Non-germinated soybean has lowest total TPC about 25.63 mg/100g when compared with germination and fermentation processes because phenolic content in non-germinated soybean was found in bound phenolic content (Moongngarm and Saeting 2010), that make difficult to analyst. After germinate for 36 h, the effect on the phenolic content, is shown in Table 1. The phenolic content, for germinated soybean was increased significantly by 29.04 mg/100g. Soybean was more effective in increasing TPC, compared with non-germinated soybean. The addition in TPC of cereals seed, by germination, has been frequently reported. The total phenolic was increased because seeds subjected to stress conditions initiating to produce accelerated substances to protect themselves (Katina et al., 2007). Germinated cereals fermented with *L. lactis*, *B. Subtilis and B. longum* at 12, 24 48 and 72 h, level of phenolic in germinated soybean fermented with *B. Subtilis* at 72 h (45.37 mg/100 g) are highest.

The levels of TPC can be modified during fermentation by the metabolic activity of microbes. Fermentation induced structural breakdown of cereals cell walls might also occur

leading to liberation or synthesis of various TPC. The type of microbes and type of raw materials were shown to be important in determining the extent of these changes. When germinated grains were introducing as a fermentation substrate, the microbial community and the type activity of enzyme in the grain were change. Germinated grain could be considered as packages of enzyme and nutrients, the enzymes originating from the grain itself or from indigenous microbes presented during germination and fermentation. This might be due to considerably higher pH of fermentations (pH 4.5–6.0), which might provide an optimum pH for cell wall degrading enzymes originating from the cereals kernel or for additional microbial enzymes synthesised during germination (Zielinski, 2000)

Table 1 Total phenolic content of germinated soybean fermented with: *Lactococcus lactis*, *Bacillus subtilis*, and *Bifidobacterium longum*.

soybeans	**Total phenolic (mg/100g)**
control	14.85±0.11^{e}
germinated	29.04±0.16^{d}
germinated fermented with *L. lactis* 12hr	38.37±6.42^{c}
germinated fermented with *L. lactis* 24hr	39.62±0.13^{c}
germinated fermented with *L. lactis* 48hr	41.67±0.11^{bc}
germinated fermented with *L. lactis* 72hr	42.24±0.02^{b}
germinated fermented with *B. subtilis* 12hr	42.30±0.03^{b}
germinated fermented with *B. subtilis* 24hr	42.75±0.06^{b}
germinated fermented with *B. subtilis* 48hr	44.80±0.04^{ab}
germinated fermented with *B. subtilis* 72hr	45.37±0.03^{a}
germinated fermented *with B. longum* 12hr	37.09±0.05^{c}
germinated fermented *with B. longum* 24hr	40.53±0.02^{bc}
germinated fermented *with B. longum* 48hr	42.58±0.04^{b}
germinated fermented *with B. longum* 72hr	43.15±0.03^{b}

a, b, c,.. Means in the separate processes (non-germinated, germinated and fermented soybean) with different letters are significantly different ($p \square 0.05$).

Antioxidant activities

The effected of germination and fermentation on the antioxidant by DPPH of soybean are summarized in Table 2. In the DPPH test, the color stable DPPH radical is reduced in the presence of an antioxidant or a hydrogen donor into non-radical DPPH, and the reduction in color is monitored over time. The color intensity of DPPH radicals with no antioxidants or grain extracts was stable over the test time with an average absorbance of 1.944. This average absorbance was used for the calculation of DPPH scavenging capacity. The antioxidant extracts from soybean, germinated soybean and fermented germinated soybean were different. The non-germinated soybean extracts showed percent inhibition at 25.63 mM Trolox. After soaking (germination process) in water 12h (6.5 pH), germinated soybeans showed the higher DPPH radical scavenging than non-germinated soybean approximately 1.02 fold or about DPPH radical scavenging about 29.04 mM Trolox. Normally, the germination combined with fermentation could increase chemical compound in plant, wherewith hydrolytic enzyme activates chemical compounds such as carbohydrate protein and fatty acid (Katina et al., 2007).

Table 2 Antioxidant activitycontent of germinated soybean fermented with *Lactococcus lactis*, *Bacillus subtilis*, and *Bifidobacterium longum*.

soybean	**Antioxidant activity by DPPH (mM trolox)**
control	25.63 ±2.58^{e}
germinated	29.04±0.14^{d}
germinated fermented with *L. lactis* 12hr	43.69 ±0.03^{c}
germinated fermented with *L. lactis* 24hr	45.38 ±0.29^{b}
germinated fermented with *L. lactis* 48hr	47.90 ±0.13^{b}
germinated fermented with *L. lactis* 72hr	51.71 ±0.11^{a}
germinated fermented with *B. subtilis* 12hr	45.41 ±0.02^{b}
germinated fermented with *B. subtilis* 24hr	44.41 ±0.38^{bc}
germinated fermented with *B. subtilis* 48hr	52.20 ±0.54^{a}
germinated fermented with *B. subtilis* 72hr	54.78 ±0.44^{a}
germinated fermented *with B. longum* 12hr	43.74±0.02^{c}
germinated fermented *with B. longum* 24hr	43.90 ±0.05^{c}
germinated fermented *with B. longum* 48hr	44.71 ±0.08^{bc}
germinated fermented *with B. longum* 72hr	46.63 ±0.04^{b}

a, b, c,.. Means in the separate processes (non-germinated, germinated and fermented soybean) with different letters are significantly different ($p \square 0.05$).

Anti-α-glucosidase activities

Table 3 shows the α-glucosidase inhibitors of non-germinated soybean, germinated soybean and fermented germinated soybean at 72 h. The samples of germinated soybean extracts showed significant higher anti α-glucosidase activity (58.82%) than non-germinated soybean (47.72%). During germination process, several enzymes have been activated and some of non-protein nitrogen substances could be produced the α-glucosidase inhibitor activity (Lee & Lee, 2011). The highest anti-α-glucosidase activity was observed in germinated soybean fermented with *B. subtilis* (92.12 %). This could be due to the different ability of the type of bacterial to generate active compounds from the soybean during fermentation to possess significant α-glucosidase inhibitory activity (watanabe et al., 1997).

Table 3 α-glucosidase inhibition of germinated soybean fermented with *Lactococcus lactis*, *Bacillus subtilis*, and *Bifidobacterium longum*

Soybeans	α-glucosidase inhibition (%)
control	47.72±1.30
germinated	58.82±2.70
fermented germinated with *L. lactis*	61.33±0.90
fermented germinated with *B. subtilis*	92.12±0.75
fermented germinated with *B. longum*	83.67±1.43

CONCLUSION

This study has demonstrated that bioprocesses such as germination and fermentation processes have significantly enhanced the antioxidant properties. Moreover, upon germination the concentrations of these antioxidant activities were increased and fermentation of germinated soybean had the highest antioxidant activities. In addition, cereals anti-α-glucosidase activities were the highest when beans were subjected to germination combination with fermentation process. This study showed that it might be possible to develop innovation to food supplement.

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