Original Article

Anti-diabetic and anti-hypertensive potential of sprouted and solid-state bioprocessed soybean

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Long-term type 2 diabetes can lead to numerous biological complications, such as hypertension and cardiovascular disease. 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 so as to decrease meal-derived glucose absorption. Further, inhibition of hypertension-linked angiotensin I-converting enzyme (ACE) was targeted as a potential approach for modulation of diabetes-linked hypertension. Water-soluble extracts of soybean optimized for phenolic content via sprouting or bioprocessing by dietary fungus (*Rhizopus oligosporus*, *Lentinus edodes*) were investigated for inhibitory activity against porcine pancreatic α-amylase (PPA), yeast α-glucosidase, and rabbit lung ACE *in vitro*. PPA was allowed to react with each phenolic-optimized extract and the derivatized enzyme-phytochemical mixtures obtained were characterized for residual amylase activity. α-Glucosidase and ACE activities were determined in the presence of each phenolic-optimized extract. All of the soybean extracts possessed marked anti-amylase activity, with extracts of *R. oligosporus*-bioprocessed soybean having the strongest inhibitory activity, but only slight anti-glucosidase activity. The anti-amylase activity of each extract seemed associated with extract antioxidant activity. Anti-enzyme activity was slightly associated with total soluble phenolic content per se, but seemed more associated to the length of sprouting or bioprocessing of the soybean substrate. Short-term sprouting or bioprocessing seemed to improve anti-amylase activity, while long-term sprouting or bioprocessing seemed to aid anti-glucosidase activity. While ACE activity was strongly inhibited by all of the soybean extracts (44-97%), only sprouting was found to increase this inhibition and bioprocessing of soybean with *L. edodes* decreased inhibitory activity of soybean extract. The results suggest that sprouting and dietary fungal bioprocessing of soybean improve the anti-diabetic potential of soybean extracts, potentially through modulation of the phenolic profile of the extract, and further suggest that enzyme inhibitory activity may be linked to phenolic antioxidant mobilization during spouting and/ or bioprocessing. The significance of food-grade, plant-based enzyme inhibitors for modulation of carbohydrate breakdown and control of glycemic index of foods in the context of preventing hyperglycemia and diabetes mellitus complications such as hypertension in the long-term is hypothesized and discussed.

Key Words: alpha amylase, alpha glucosidase, angiotensin I-converting enzyme, soybean, *Rhizopus*, *Lentinus*, hyperglycemia, hypertension, diabetes, obesity.

Introduction

Hyperglycemia, a condition characterized by an abnormal excess of sugar in the blood, has been linked to the onset of type 2 diabetes mellitus and associated cardiovascular complications including hypertension.[1-3] Although a few synthetic anti-diabetic drugs are available to combat the impaired insulin secretion, insulin resistance, and hyperglycemia that characterize type 2 diabetes mellitus, some of these drugs can have negative side-effects at high doses.[4-8] A major focus of current anti-diabetic research is the development of anti-hyperglycemic agents that are safe and free of negative side-effects.

As a number of anti-hyperglycemic agents have been found in plants, research into understanding the scientific basis for plant-based traditional medicines from various cultures has increased as scientists search for clues to discovering new therapeutic drugs for type 2 diabetes mellitus.[9-11] Traditional Indian medicines have long used plant and herbal extracts as anti-diabetic agents.[12] These plants are typically rich in phenolic compounds, which are known to interact with proteins and can inhibit enzymatic activity.[13,14] A number of medicinal plant and herbal extracts have been found to inhibit the enzymatic activity of α-glucosidase and α-amylase, and therein may have potential as dietary anti-diabetic agents to improve the control of post-prandial hyperglycemia.[15-18]

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One of the complications of long-term diabetes is hypertension, or high blood pressure. Hypertension is potentiated by angiotensin II, a potent vasoconstrictor agent.[19] Angiotensin II is formed from angiotensin I, a histidyl-leucine dipeptide, by the action of angiotensin I-converting enzyme (ACE).[20] Anti-hypertensive drugs have been isolated from bovine beta-lactoglobulin and a number of plant extracts.[21-24]

In this study, we investigated the anti-diabetic and anti-hypertensive potential of aqueous extracts of soybean enriched for phenolic content by sprouting or bioprocessing by dietary fungus (*Rhizopus oligosporus* or *Lentinus edodes*). Soybean is a rich source of isoflavonoid phenolics, especially genistein and daidzein which have been shown to have numerous potential health benefits.[25,26] A number of flavonoids and isoflavonoids, including genistein, have been shown to inhibit α-glucosidase activity *in vitro*.[27-30] Therefore, phenolic-rich extracts of soybean may have potential as a source of anti-diabetic agents for control of post-prandial hyperglycemia and of anti-ACE agents for control of hypertension, a known complication of long-term diabetes and/ or hyperglycemia. Here, aqueous phenolic-optimized extracts of bioprocessed or sprouted soybean were assayed *in vitro* for inhibitory activity against porcine pancreatic α-amylase, yeast α-glucosidase, and rabbit lung ACE. The results were analyzed with respect for the antioxidant activities and total soluble phenolic contents of the extracts.

Materials and methods

Plant materials, chemicals, and microorganism

A glyphosate-tolerant soybean variety (Syngenta Seeds, Inc., Indianapolis,IN) was used in this investigation. Unless noted, all chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

R. oligosporus was grown on potato dextrose agar (PDA) in disposable plastic Petri plates (Fisher Scientific Co., Suwanee, GA) at room temperature (RT) for 5 d and maintained at 4°C with subculturing done every 3months. The fungus was activated by transferring onto PDA plate and cultured at RT for 20 d before use. *R. oligosporus* has long been used in the production of tempeh from soybean and is therefore considered to be food-grade.[31]

L. edodes was grown on PDA in disposable plastic Petri plates (Fisher) at RT for 14 d and maintained at 4°C with subculturing performed every 3 months. The fungus was activated by transferring onto PDA plate and cultured at RT for 20d before use. The fruit bodies of *L. edodes* are available in the food market as Shiitake mushroom and the strain is considered food-grade.

Sprouting of soybean and extract preparation

Soybean seed (10g) was primed overnight in 50mL of dH_2O with shaking (150rpm), and then sown between two slightly damp Kim-towels (Fisher) in plastic gardening flats covered with aluminum foil. The flats were incubated in dark cupboards for 0-10d. The rationale for soybean dark-germination was to drive the nutraceutical value of the soybean sprouts towards maximal phenolic content.[32]

Every 2d, each sprouted soybean set was collected and harvested *en masse* by homogenization in 100mL of dH_2O in a Waring laboratory blender set on 'HIGH.' The homogenate was clarified by centrifugation at 10,000 rpm at 4°C for 20 min. Extract supernatants were vacuum filtered through Whatman #1 filter paper, collected in conical 50 mL plastic centrifuge tubes (Fisher), and stored at 4°C. This liquid was used as the crude sprouted soybean extract. Protein content was determined using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA).

Solid-state bioprocessing and extract preparation

A 125mL Erlenmeyer flask containing soybean was used for solid-state bioprocessing. Soybean seed (10g) was soaked overnight with shaking (150rpm), rinsed once with dH_2O, drained, and then used for fungal bioprocessing. Prior to inoculation, the medium contained in flasks with cotton plugs was autoclaved at 121°C for 15 min.

For the *R. oligosporus* bioprocessing system, 10square plugs (1.27 cm^2) of activated *R. oligosporus* mycelium from PDA plates were inoculated into each flask. The flasks were incubated at RT for 10 d with harvesting every 2 d. For the *L. edodes* bioprocessing system, 10 square plugs (1.27 cm^2) of activated *L. edodes* mycelium from PDA plates were inoculated into each flask. The flasks were incubated at RT for 25 d with harvesting every 5 d.

After bioprocessing, 100mL of dH_2O was added into each fungus-soybean-containing flask and stirred with a spatula to dislodge the culture from the flask. Next, the culture was homogenized for 1min on 'HIGH' using a Waring blender, and the homogenate then centrifuged at 10,000rpm at 4°C for 20 min. The supernatant was vacuum-filtered through Whatman #1 filter paper. The resultant liquid was used as the crude bioprocessed soybean extract. Protein content was determined using the Bio-Rad Protein Assay Kit (Bio-Rad).

Treatment of α-amylase with soybean extracts

Treatment of amylase was performed as previously described, with some modifications.[18] Fifty mg of powdered porcine pancreatic amylase (Sigma) was added to 27mL of dH_2O. For each food extract, a volume equivalent to 400μg total phenolic content was added to the above solution, and the mixture adjusted to pH 6.9. After dilution to 30mL total volume, the amylase-food extract mixtures were incubated 1h at RT with stirring. The control mix used was 1mL of dH_2O in place of extract.

Characterization of α-amylase activity

α-Amylase activity was determined by the method of McCue and Shetty (2004), using starch as a substrate in a colorimetric reaction using 3,5-dinitrosalicylic acid.[18] A standard curve was generated for the splitting products (reducing groups) using D-(+)-maltose monohydrate. Activity was calculated as units/ mg protein, where 1 unit was defined as the amount of enzyme required to liberate 1μmol of maltose under assay conditions. Data was reported as amylase inhibition (AI) index values, defined herein as the ratio of the amylase activity of the control

(enzyme alone) to that of the enzyme/clonal extract mixture.[33] Values greater than 1 indicate amylase inhibition.

Treatment of α-glucosidase with soybean extracts

First, 7.5U of yeast α-glucosidase (Sigma) dissolved in 10 mM phosphate buffer (pH 6.3) was added to 27mL of 10 mM phosphate buffer (pH 6.3). To this was added, a volume of each soybean extract equivalent to 400μg of total phenolic content. Then, the mixture was diluted to 30mL with 10 mM phosphate buffer (pH 6.3) and incubated at RT for 1h with gentle stirring. The control mixture used was 1mL of buffer in place of extract.

Characterization of α-glucosidase activity

The inhibitory activity of the soybean extracts against yeast α-glucosidase was determined by measuring the formation of *p*-nitrophenol by α-glucosidase after reaction with *p*-nitrophenyl-α-D-glucopyranoside (PNP) substrate in the presence and absence of soybean extract. To a glass test tube was added, 0.2mL of 0.2 mM PNP substrate (dH_2O for control) and 0.2mL of the enzyme (α-glucosidase) -soybean extract mixture. This reaction tube was incubated 30min in a 30°C water bath. The reaction was stopped by addition of 0.6mL of 1M sodium carbonate. The reaction was decanted into a cuvette and absorbance at 405 nm determined by spectrophoto-meter. The molar extinction coefficient for *p*-nitrophenol used was 18,000 M^{-1} cm^{-1}.[34] Data was reported as α-glucosidase inhibition (aGI) index values, defined herein as the ratio of the α-glucosidase activity of the control (enzyme alone) to that of the enzyme/ soybean extract mixture.[33] Values greater than 1 indicate α-glucosidase inhibition.

Measurement of ACE inhibitory activity

ACE inhibition was assayed in the presence and absence of various concentrations of soybean phenolic extracts according to the method of Cheung and Cushman (1973) using hippuryl –L-histidyl-L-leucine as substrate.[35] Liberated hippuric acid was determined at 228 nm to evaluate ACE inhibitory activity. Percentage inhibition was calculated from the equation % inhibition = 100 [(C-B)-(SI-SB)]/(C-B), where C is the absorbance of the reaction mixture using the enzyme plus substrate, B is the absorbance of the reaction mixture using the substrate only, SI is the absorbance of the reaction mixture using the enzyme plus substrate plus soybean extract, and SB is the absorbance of the reaction mixture using the soybean extract only.

Determination of total soluble phenolic content

The total soluble phenolics content in each extract was determined using a previously described method.[36] A phenolic standard curve was established at 725nm with gallic acid (25-200 μg/ mL) in 95% EtOH.

Determination of antioxidant activity

The antioxidant activity of each food extract was determined as the ability of the extract to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radicals. A 0.1 mM DPPH radical solution in 95% ethanol was prepared. One mL of ethanolic DPPH solution was mixed with 1 mL of sample or 95% ethanol (as control), vortexed well, and then incubated for 30 min at RT. The samples were then centrifuged for 30 s at 13,500 rpm at RT. Absorbance of each sample at 517 nm was measured. This antioxidant activity was given as percentage (%) DPPH scavenging, calculated as [(control absorbance – extract absorbance)/ (control absorbance) x 100].

Protein structure analysis

RasMol (Windows Version 2.7.2.1; http://openrasmol.org/) Molecular Graphics Visualization Tool was used to visualize the published structure for Baker's yeast α-glucosidase (Protein Data Bank code: 1UOK) and human angiotensin I-converting enzyme (1O8A).[37]

Statistical analysis

Standard deviations of data are indicated in the figures. Data marked with different letters are significantly different by ANOVA at $P < 0.05$.

Results and Discussion

Effects of bioprocessing and sprouting on soybean anti-diabetic activity

Control of post-prandial hyperglycemia via modulation of pancreatic α-amylase or intestinal α-glucosidase to delay carbohydrate absorption by dietary anti-diabetic agents is an attractive strategy to control or prevent the onset of long-term complications of hyperglycemia and diabetes mellitus. Here, we investigated the effect of water-soluble extracts of soybean, phenolic-optimized via solid-state bioprocessing by dietary fungus (*R. oligosporus* or *L. edodes*) or sprouting via dark-germination, on the activity of PPA and yeast α-glucosidase.

All of the aqueous soybean extracts tested were found to possess significant α-amylase inhibitory activity (Fig. 1-3). Extracts of *R. oligosporus*-bioprocessed soybean had the strongest anti-amylase activity, specifically after 4-6 days of culture time (amylase inhibition (AI) index value = 1.99 ± 0.27 – 2.08 ± 0.19; Fig. 1). *L. edodes*-bioprocessing of soybean also improved extract antiamylase activity, though not as great as with *R. oligosporus*-bioprocessing (Fig. 2). *L. edodes*- bioprocessing most improved anti-amylase activity after 20d of culture time (AI index value = 1.48 ± 0.13; Fig. 2). Sprouting of soybean showed a similar ability to increase extract anti-amylase activity, which was highest after 4-6 d of germination time (AI index value = 1.31±0.14 - 1.33 ± 0.14; Fig. 3). Interestingly, extracts of autoclaved soybean (0d) showed a moderate anti-amylase activity (AI index value = 1.40 ± 0.2, Fig. 1; 1.39 ± 0.14, Fig. 2) which was higher than for non-autoclaved soybean extract (AI index value = 1.25 ± 0.06, Fig. 3).

In contrast to anti-amylase activity, the majority of the soybean extracts showed only slight inhibitory activity against α-glucosidase (Fig. 4-6). *R. oligosporus* bioprocessing of soybean increased anti-α-glucosidase activity after 4d of culture time (Fig. 4). The highest activity was observed after 6d of culture time (α-glucosidase inhibition (aGI) index value = 1.10 ± 0.02; Fig. 4). *L. edodes*-bioprocessing (Fig. 5) and sprouting (Fig. 6) of soybean had less ability to stimulate extract anti-α-

glucosidase activity than *R. oligosporus*-bioprocessing. *L. edodes* bioprocessing of soybean most improved extract anti-α-glucosidase activity after 5d of culture time (aGI index value = 1.035 ± 0.01; Fig. 5). Sprouting soybean most improved extract anti-α-glucosidase activity after 10d of germination (aGI index = 1.04 ± 0.0002; Fig. 6).

Relationship of extract antioxidant activity to anti-diabetic activity

As soybean extract anti-amylase activity was more pronounced than anti-α-glucosidase activity, we focused on the anti-amylase activity as representative of the soybean extract anti-diabetic activity for further comparison studies. Previous research with clonal herbal extracts reported an association between antioxidant activity and anti-amylase activity.[18] Therefore, we compared the anti-amylase activity of the soybean extracts to their antioxidant activity. For *R. oligosporus*-bioprocessed soybean, extract anti-amylase activity was strongly correlated to antioxidant activity (correlation coefficient = 0.85; Fig. 7). Extract anti-amylase activity for *L. edodes* bioprocessed soybean and for sprouted soybean was also

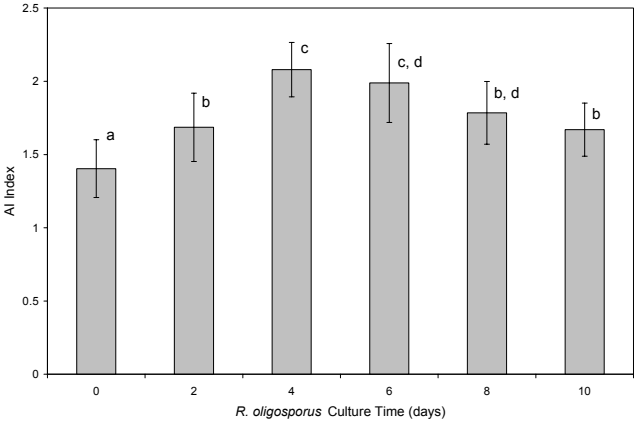

Figure 1. Amylase inhibition (AI) by extracts of soybean bioprocessed by *R. oligosporus*.

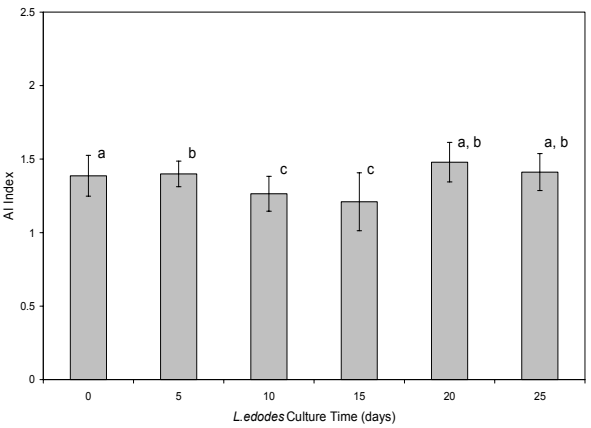

Figure 2. Amylase inhibition (AI) by extracts of soybean bioprocessed by *L. edodes*.

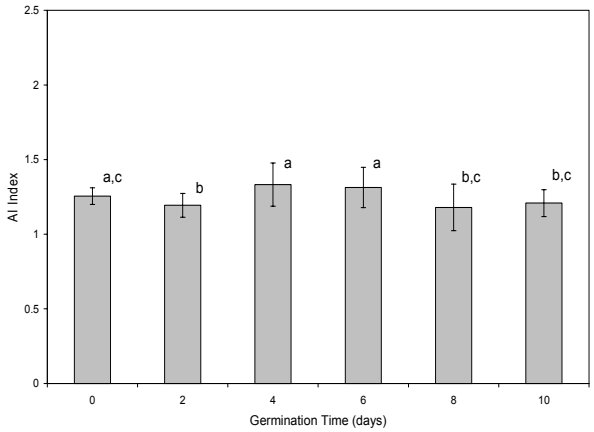

Figure 3. Amylase inhibition (AI) by extracts of sprouted soybean.

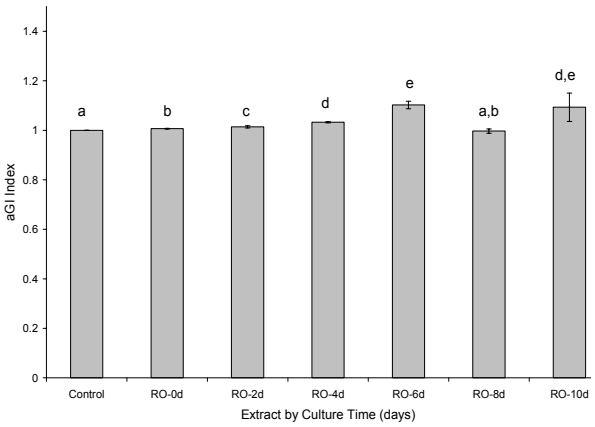

Figure 4. α-Glucosidase inhibition (aGI) by extracts of soybean bioprocessed by *R. oligosporus* (RO).

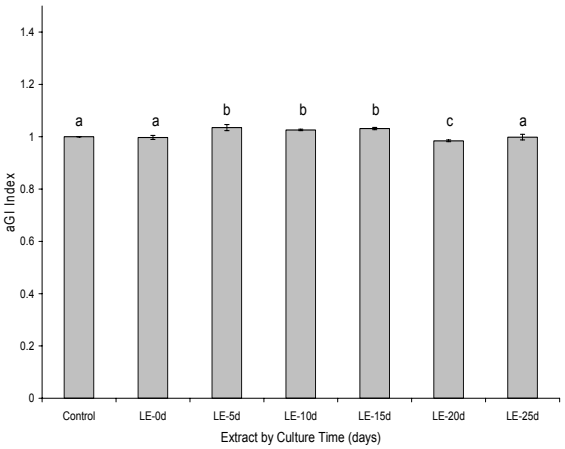

Figure 5. α-Glucosidase inhibition (aGI) by extracts of soybean bioprocessed by *L. edodes* (LE).

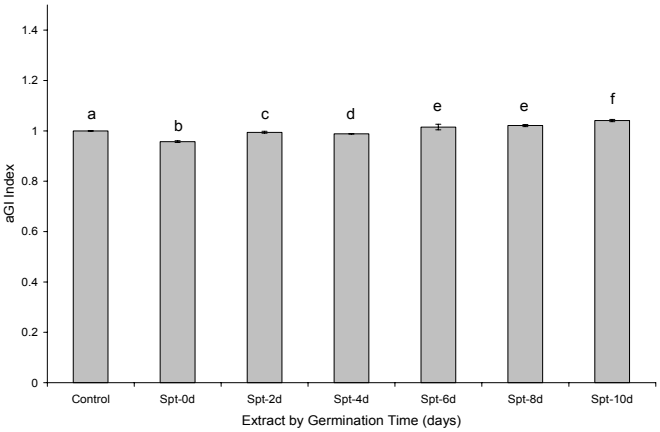

Figure 6. α-Glucosidase inhibition (aGI) by extracts of sprouted soybean (Spt)

moderately correlated to antioxidant activity (0.31, Fig. 8; 0.41, Fig. 9). These results suggest that antioxidant activity may play a role in the anti-amylase (e.g. anti-diabetic) activity of bioprocessed or sprouted soybean extracts.

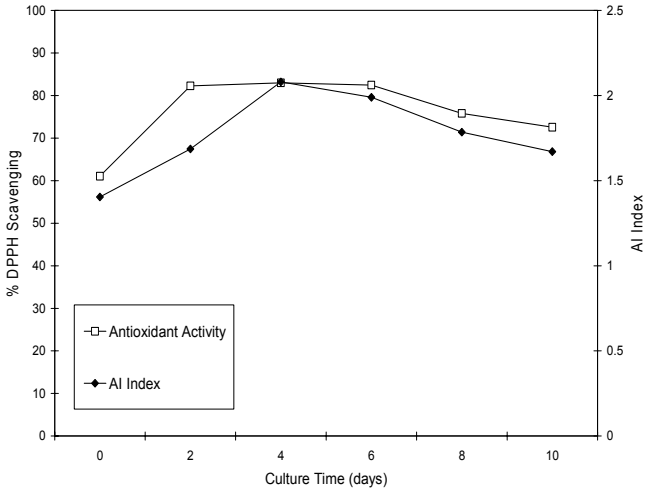

Figure 7. Amylase inhibition (AI) index versus radical-scavenging antioxidant activity of extracts of soybean bioprocessed by *R. oligosporus*.

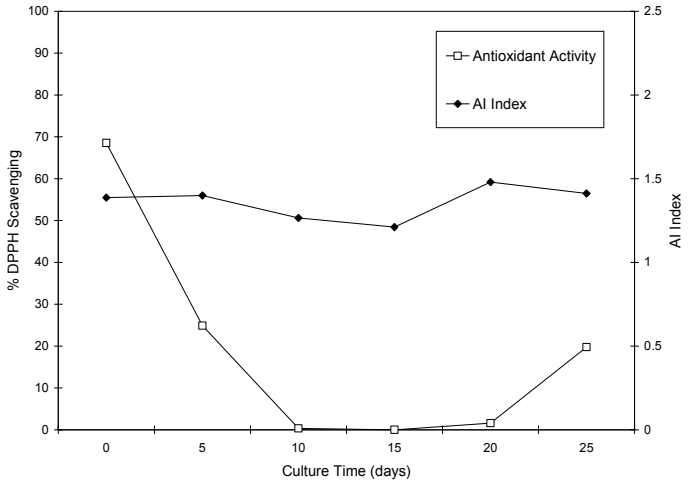

Figure 8. Amylase inhibition (AI) index versus radical-scavenging antioxidant activity of extracts of soybean bioprocessed by *L. edodes*.

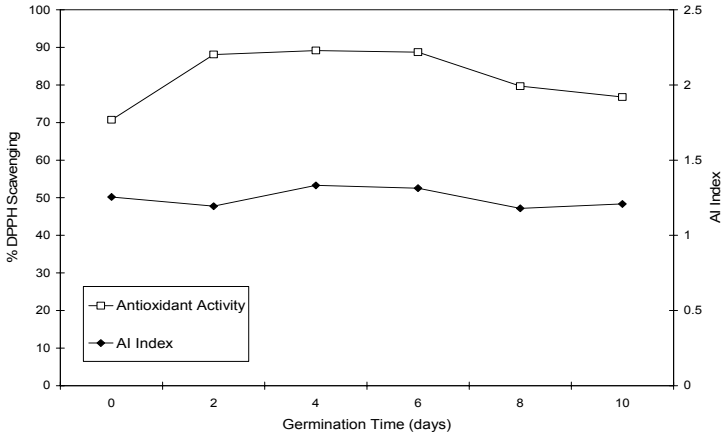

Figure 9. Amylase inhibition (AI) index versus radical-scavenging antioxidant activity of extracts of sprouted soybean.

Relationship of extract total soluble phenolic content to anti-diabetic activity

Previous research has shown that plant phenolic extracts and purified phenolic compounds can inhibit the *in vitro* activity of α-amylase.[17,18,28] Therefore, anti-amylase activity was compared to total soluble phenolic content in each of the soybean extracts. For extracts of *R oligosporus* bioprocessed soybean, anti-amylase activity was slightly correlated to total soluble phenolic content over 10d of culture time (correlation coefficient = 0.25; Fig. 10). Over 8d of culture time, the correlation was much stronger (coefficient = 0.59). For extracts of *L. edodes* - bioprocessed soybean, anti-amylase activity was slightly negatively correlated to total soluble phenolic content (coefficient = -0.34; Fig. 11). Anti-amylase activity was most strongly correlated to total soluble phenolic content for extracts of sprouted soybean (coefficient = 0.81; Fig. 12). These results suggest that phenolic mobilization may also play a role in the anti-amylase (e.g. anti-diabetic) activity of bioprocessed or sprouted soybean extracts, perhaps in relation to the mobilization of specific types of phenolics during bio-processing or sprouting.

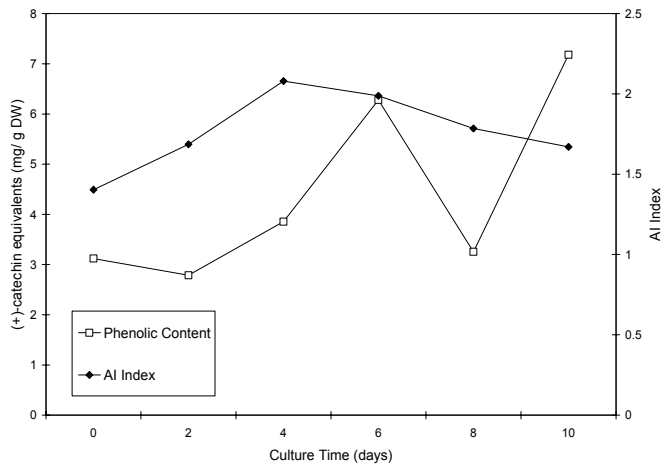

Figure 10. Amylase inhibition (AI) index versus total soluble phenolic content in extracts of soybean bioprocessed by *R. oligosporus*.

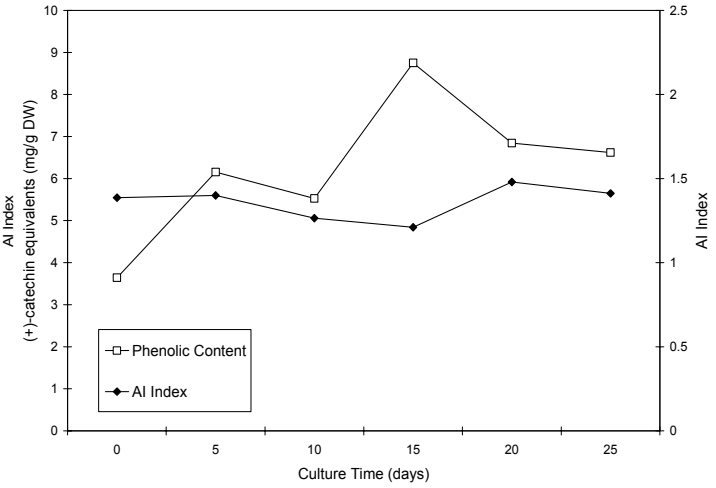

Figure 11. Amylase inhibition (AI) index versus total soluble phenolic content in extracts of soybean bioprocessed by *L. edodes*.

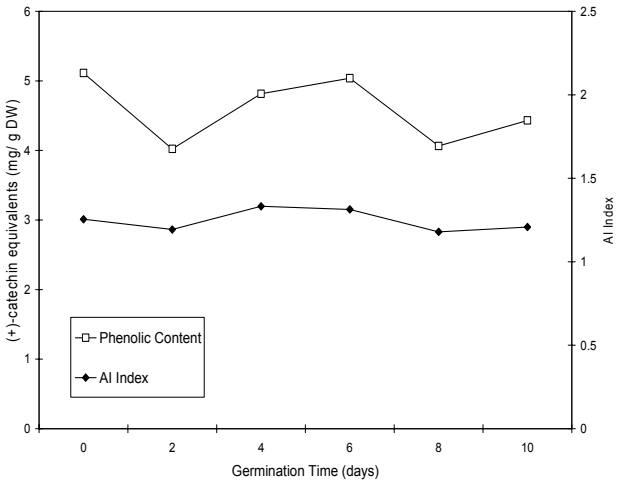

Figure 12. Amylase inhibition (AI) index versus total soluble phenolic content in extracts of sprouted soybean.

Effects of bioprocessing and sprouting on soybean anti-hypertensive activity

Control of hypertension via modulation of angiotensin I-converting enzyme (ACE) by dietary anti-hypertensive agents is an attractive strategy to control a problematic complication of long-term type 2 diabetes mellitus. Here, we investigated the effect of water-soluble extracts of soybean, phenolic-optimized via solid-state bioprocessing by dietary fungus (*R. oligosporus* or *L. edodes*) or sprouting via dark-germination, on the activity of rabbit lung ACE.

All of the aqueous soybean extracts tested possessed significant ACE inhibitory activity (Fig. 13). Bioprocessing of soybean by *R. oligosporus* had no effect on further improving or reducing ACE inhibition (0d extract: 94.96% ± 1.71; 6d extract: 95.55% ± 1.69). Bioprocessing of soybean by *L. edodes* negatively affected (i.e decreased) ACE inhibition (0d extract: 95.33% ± 1.68; 20d extract: 44.10% ± 2.00). Soybean bioprocessed by *L. edodes* for 20d was found to have much reduced antioxidant activity (Fig. 8), which may potentially be involved in the reduced enzyme inhibition. ACE inhibitory activity of soybean extracts was only increased by sprouting (0d extract: 90.29% ± 2.06; 6d extract: 93.50% ± 1.84).

Towards an understanding of soybean extract anti-diabetic activity

Previous research with clonal herbal extracts reported an association between antioxidant activity and anti-amylase activity, further suggesting that the antioxidant activity of the herbal extracts may affect the 5 disulfide bridges located on the external surface of amylase and therein induce mild inhibition by affecting slight changes in the structure of the enzyme.[18] Here, we see a similar possibility with extracts of *R. oligosporus*-bioprocessed or sprouted soybean extracts (Fig. 7,9). However, *L. edodes* bioprocessed extracts that lose antioxidant activity during middle stages of bioprocessing, yet maintain some degree of anti-amylase activity (Fig.8). Previously, we speculated that the loss of antioxidant activity during bioprocessing may be due to a sensitivity of *L. edodes* to soybean phenolics as antimicrobials which stimulates fungal detoxification activities that decrease phenolic antioxidant activity.[38] Later, we reported that bioprocessed soybean extract antimicrobial activity was associated with laccase activity, which is known to involve the production of quinones via oxidation of phenolics.[39] As the antioxidant activity of phenolics is believed to involve the transient formation of quinones, the anti-amylase activity of antioxidant-deficient *L. edodes*-bioprocessed soybean extracts may potentially involve the interaction of oxidized phenolics, quinones or semi-quinones formed by the fungal phenolic detoxification.[40]

If we are to assume that the inhibition of amylase by the soybean extracts involves an interaction of the extract antioxidants with the disulfide bridges that affects structural integrity and thereby causes a mild inhibition, the apparent inability of the soybean extracts to inhibit α-glucosidase may follow a similar logic. An analysis of the structure of Baker's yeast α-glucosidase for disulfide bridges reveals that there are none, especially not on the surface of the molecule, where they would have to be in order to interact with extract antioxidants (Fig. 14). Therefore, by the above logic, the inability of the soybean extracts to inhibit the activity of yeast α-glucosidase may be due to the absence of disulfide bridges in the outer surface of the enzyme with which antioxidants could interact.

Investigation of the physical structure of human angiotensin I-converting enzyme (homologous to that of rabbit ACE), revealed the presence of four cysteine groups in the protein, three of which are close to or exist on the surface of the molecule and which possess disulfide bridges (Fig. 15). As soybean phenolic antioxidant extracts were able to inhibit ACE activity, this inhibition may potentially have resulted from interactions between the soybean phenolic antioxidants and the disulfide bridges (oxidized cysteines) that reside on the surface of the ACE enzyme, therein affecting slight changes in the structure of the enzyme that could possibly translate into enzyme inhibition, as was hypothesized for clonal oregano phenolic extracts and α-amylase inhibition.[18] It should be noted that ACE inhibitory activity by soybean extract was reduced when the extract antioxidant activity was also reduced (*L. edodes*-bioprocessed soybean extracts 0d vs. 20d; Fig. 8, Fig. 13).

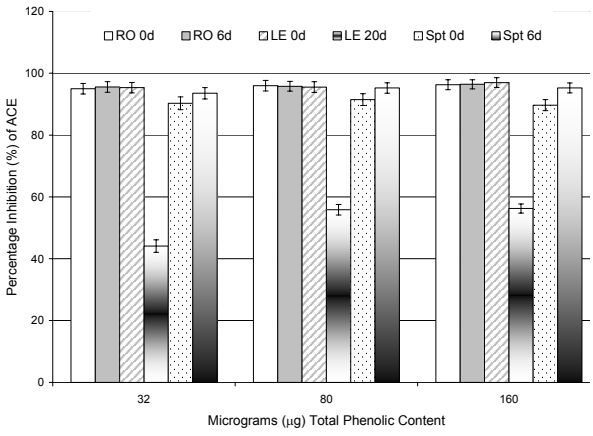

Figure 13. Inhibition of angiotensin I-converting enzyme (ACE) by selected bioprocessed or sprouted soybean extracts. RO, *Rhizopus oligosporus*; LE, *Lentinus edodes*; Spt, sprouted soybean.

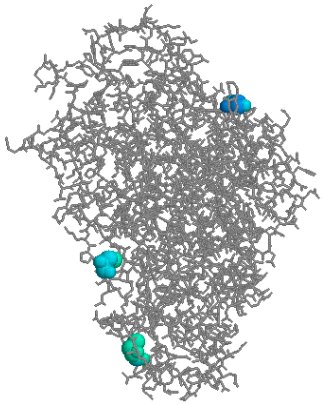

Figure 14. Structure of Baker's yeast □-glucosidase. The protein backbone is shown in wireframe design; cysteine molecules are shown in space-fill design.

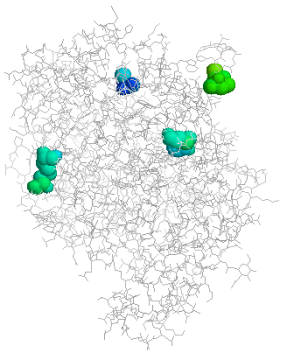

Figure 15. Structure of human angiotensin I-converting enzyme. The protein backbone is shown in wireframe design; cysteine molecules are shown in space-fill design; arrows identify cysteine groups with disulfide bridges.

Conclusion

Natural α-amylase and α-glucosidase inhibitors from food-grade plant sources offer an attractive therapeutic approach to the treatment of post-prandial hyperglycemia by decreasing glucose release from starch and delaying carbohydrate absorption in the small intestine and may have potential for use in the treatment of diabetes mellitus and obesity.[41,42] Powerful synthetic α-glucosidase inhibitors (ie voglibose) are available, but can cause hepatic disorders and/ or various negative gastrointestinal symptoms (as carbohydrates blocked from absorption in the small intestine are fermented by bacteria in the colon) at high doses.[42] Food-grade phenolic α-amylase inhibitors from dietary plant extracts are potentially safer, and therefore may be a preferred alternative for modulation of carbohydrate digestion and control of glycemic index of food products. Here, we report that soybean extracts possess potential anti-diabetic activity existent as a significant ability to inhibit α-amylase, and that this activity can be improved by thermal-processing (autoclave) of the soybean substrate, as well as via solid-state bioprocessing by dietary fungus (*R. oligosporus* or *L. edodes*) and by dark-germination sprouting. Further, we report that soy-bean extracts possess potential anti-hypertensive activity existent as a significant ability to inhibit rabbit lung ACE. The anti-amylase activity of soybean extracts was associated with extract antioxidant activity and to phenolic mobilization during bioprocessing or sprouting. The strong anti-ACE activity of soybean extracts was associated with extract antioxidant activity, but was not dose-dependent at the concentrations tested.

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