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Original research

Evaluation of antidiabetic and antioxidant properties of selected soybean varieties and their suitability to incorporate into wheat bread

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ABSTRACT

This study evaluates the antioxidant and antidiabetic activities of selected soybean varieties, Sri Lankan P.B 1 and Indian MACS-330. The methanolic extracts of soybean were *in-vitro* evaluated for their antioxidant, α–amylase, and amyloglucosidase inhibitory activities. Mixed flours with different proportions of soy flour (3%, 5%, and 8%), in wheat flour were investigated for their bread quality. Sensory properties of the bread were evaluated by trained panelists and the proximate composition of bread was assessed according to AOAC procedures. The research results show that the inhibition activity of α-amylase and amyloglucosidase did not differ significantly ($P<0.05$) from each variety. However, the total phenolic and flavonoid content of the Sri Lankan P.B 1 variety was not significantly difference ($p > 0.05$) than the Indian MACS-330 variety. The methanolic extracts from soybean may inhibit key-enzymes associated with type 2 diabetes, and thus may explain part of the mechanism by which soybeans exerts this health-promoting effect. As soy flour content increased, all the macro-nutrient parameters increased except carbohydrate content. The highest overall sensory score was the bread with 5% added soybean from Sri Lankan P.B 1. In conclusion, the functional and nutritional properties of the bread can be improved by the addition of soy flour.

Keywords: Soybean; α- amylase activity; Amyloglucosidase activity; Anti-oxidant activity; Type 2 diabetic

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1. Introduction

Diabetic mellitus is one of the most prominent metabolic syndromes characterized by hyperglycemia, resulting in insufficient or inefficient activity of insulin secretion, with alteration in macronutrient metabolism activity (Barber et al., 2021). Type 1 diabetes is generated from the altering of the genes and type 2 diabetes is a condition that happens due to an unhealthy lifestyle. During the lifetime, type 1 diabetes cannot be controlled and type 2 diabetes can be controlled through the management of the post-prandial blood glucose level (Janghorbani et al., 2007).

The practical approach to the management of post-prandial glucose is the prevention of glucose absorption after consuming glucose-enriched foods. While digestion of the complex polysaccharides, the intestinal α-amylase is converted into oligosaccharides and then produces simple monosaccharides when the presence of the intestinal amyloglucosidase (Malenčić et al., 2008). When the presence of α-amylase and amyloglucosidase inhibitors, helps to reduce simple glucose absorption and manage the glucose intake into the blood vessels. Most prominent synthetic drugs such as acarbose, voglibose, and miglitol are widely used as anti-diabetic drugs due to the inhibitory activity of the key enzyme linked to type 2 diabetes (Saito et al., 1998).

A long-term complication of type 2 diabetes condition helps to promote non-communicable diseases like Persistent hypertension. Phytochemicals such as phenolic with strong antioxidant properties have been reported to be good inhibitors of these key enzymes linked to type 2 diabetic enzymes thus rendering a holistic way to control hyperglycemia and other diabetic complications arising from oxidative stress (Epstein & Sowers, 1992). Hypertension and type-2

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diabetes are interrelated metabolic disorders. *In-vitro* and *in-vivo* clinical studies have indicated that specific phenolic phytochemicals are directly involved in the management of hypertension and hyperglycemia condition (Bakris et al., 2000).

Apart from chemical synthetic medications, natural medicines from plant sources have been obtained and are free of side effects to control diabetes (Prabakaran et al., 2018). In Sri Lanka, there are plenty of underutilized food crops importantly cereals, yams, and tuber crops which are not systematically exploited regarding anti-diabetes activity (Chandrasekara & Joseph Kumar, 2016).

Among the natural plant sources, Soybean (*Glycine max (L.) Merrill*) is one of the most important crops, categorized under the legume, which is the prominent source of protein and other active plant constituents. Due to the presence of a higher amount of the major protein and other health protection plant constituents currently, most of the researchers give attention to investigating its role of the prevention of several chronic degenerative diseases (Mesa et al., 2008).

Soybean is a rich and relatively unique source of polyphenolic compounds, which have been subjected to various *in-vitro* and *in-vivo* antioxidant capacity analyses (Ramdath et al., 2017). Although existing studies significantly improved, our knowledge related to key enzymes that inhibit the activity of type 2 diabetes. However, the research gap remains including the key enzyme inhibition activity of Sri Lankan P.B 1 soybean variety against the imported selected varieties and the incorporation of soy flour into wheat bread to reduce carbohydrate quantity (Chandrasekara & Joseph Kumar, 2016).

This study will reflect the effect of *in-vitro* α-amylase and amyloglucosidase enzyme inhibition action with the aids of controlling diabetes mellitus and antioxidant properties of selected Sri Lankan P.B 1 soybean variety and its suitability to incorporate into wheat bread with compared import Indian MACS-330 variety. And also, Sri Lanka has the possibility and the opportunity to expand soybean food utilization. Hence, it is better to initiate awareness programs leading to expanding soy food consumption. Therefore, these research findings may be important to expand soya bean production and formulation into the products.

2. Material and Methods

2.1. Sample Collection & Preparation

Soybean, *Glycine max (L.) Merrill*, P.B 1 Sri Lankan variety was collected from the Madawachchiya, Anuradhapura Sri Lanka. *Glycine max* MACS-330 Indian variety was collected from the Indian Maharashtra state, India. Two types of collected samples were washed and dried using a laboratory hot air oven (MEMMERT NLE 500, Germany) at 45 ºC for 48 –72 hours up to 14% moisture. The dried seeds were grounded using a laboratory-scale grinder (Philips HL772, Thailand) and sifted through a 250 µm sieve. Flour samples were packed and stored at -20 ºC for further analysis.

2.2. Chemicals

Enzymes of Porcine pancreatic α amylase and amyloglucosidase were purchased from Sigma- Aldrich (Sigma Co., St. Louis, MO, USA). Chemicals of 2,2-diphenyl- 1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, Gallic acid, Rutin, Sodium Hydroxide, Sodium Potassium Tartrate, dinitro salicylic acid were purchased from Sigma- Aldrich (Sigma Co., St. Louis, MO, USA). Ethanol, methanol, ether, and other chemicals were analytical grade.

2.3. Preparation of hydro-methanolic extract

Sample extraction was performed according to a slightly modified method of Hettiarachchi et al. (2021). Approximately 1.0 g of the soybean flour samples were weighed and mixed with 20 ml methanol/water (80:20 v/v). The samples were vortexed at high speed for 5 min and then, centrifuged at 3200 ×*g* for 15 min in an EBA-20 centrifuge (Hettich, Tuttlingen, Germany). The centrifuged samples were kept for 24 hours to complete the extraction. The extracted solution was filtered through Whatman filter paper No.42 (Whatman, Maidstone, United Kingdom). The extracted solution was stored below –18 °C research was completed.

2.4. Physiochemical Properties

2.4.1. Proximate composition

Proximate composition of the samples was evaluated following the standards method of AOAC 2000 including moisture, crude fat, and ash content. Crude fat content was determined using the Kjeldhal method and crude fat content was determined using the Soxhlet method.

2.5. Anti-diabetic Properties

2.5.1. In-vitro α amylase inhibitory assay

In-vitro α-amylase activity of both selected Soy flour verities was evaluated using a slightly modified method of Chiranthika et al., 2021. Briefly, the amount of 1.00 mg of P.B 1 Sri Lankan soy flour and MACS-330 Indian Soybean flour samples were weighed into test tubes, and freshly prepared 100 µL of α- amylase enzyme was added into each test tube. The enzyme mixture was prepared using 27.5 mg of enzyme, dissolved in 100 mL of 20 mmol sodium phosphate buffer with 6.7 mmol of sodium chloride to maintain pH at 6.9. Freshly prepared 100 µL of 1% of starch solution (w/w) was added to the reaction mixture. After the reaction, the mixture was incubated at 37 °C for 10 min using a heating bath. End of the incubation period, the reaction was terminated by placing it into a boiling water bath for 5min before adding 200 µL of dinitro salicylic acid solution for color development. Then the reaction system was diluted with the addition of 2.20 ml distilled water and absorbance was read using a UV/Visible spectrophotometer (Thermo Scientific 201, United States) at 540 nm. The experiments were conducted in triplicates and the absorbance of blank, control was measured. The α-amylase inhibitory activity was expressed as a percentage of inhibition.

2.5.2. In vitro amyloglucosidase inhibition assay

This analysis was done according to the method described by Chiranthika et al., 2021. Briefly, 1.00 mg of samples were weighed into test tubes, and freshly diluted 6.5 U/ml amyloglucosidase and 0.1 M sodium phosphate buffer were added into each test tube and followed by incubated in the water bath at 37 °C for 20 min. The amount of 20 µL of 1% starch solution (w/w) was added into the reaction mixture and further incubated for 10min at 37 °C. Then, freshly prepared 100 µL of dinitro salicylic acid was introduced as a color development reagent. The reaction mixture was placed into a

boiling water bath at 100 °C for 5 min to terminate the reaction. After terminating the reaction, the mixtures were diluted by adding 2.00 ml of distilled water. The blank was prepared by replacing amyloglucosidase and 1% starch solution with sodium phosphate buffer and replacing the amyloglucosidase enzyme with sodium phosphate buffer, control was prepared. The absorbances of the samples were measured using the UV/Visible spectrophotometer (Thermo Scientific 201, United States) at 540 nm.

2.6. Total Dietary fiber (DF) analysis

Enzymatic–gravimetric AOAC method (AOAC. 1995) was carried out to determine the DF content of soybean flour using heat-stable α-amylase (terminal), protease (pH 7.5, 60 ºC, 30 min) and amyloglucosidase with required modifications. The amount of 1.0 g of each flour sample was weighed into 600 ml analytical beakers and 50.0 ml of freshly prepared 0.1 M phosphate buffer was added with maintaining pH at 6.0. After adding 100 µL of heat-stable α-amylase, each beaker was heated at 95 ºC for 15 minutes followed by the introduction of 5.0 mg of protease for 30 minutes at 60 ºC for digestion. Four volumes of 95% ethanol (w/w) were added to the reaction mixture and held overnight to precipitate dietary fiber. The solution part was filtered out through a crucible and the precipitate was washed out two times using a 20 ml portion of 78% ethanol, 95% ethanol, and acetone. Then collected precipitates were dried at 105 ºC using a hot air oven (MEMMERT NLE 500, Germany), and weight was recorded. Finally, the precipitates were subjected to determine the protein and ash content.

2.7. Resistant starch

The resistant starch content of the samples was investigated using the modification method of (Ragaee, & Abdel-Aal, 2006). An amount of 100 mg of flour samples were weighed into the test tubes and freshly prepared 4.0 ml of pancreas α-amylase (10 mg/mL) and 3U/ml of amyloglucosidase were added into flour samples and then the mixture was placed at 37 ◦C for 16 hours incubation period. After, 99% ethanol 4 mL was added to the reaction mixture and then centrifuged at 1500 g for 15 min. The liquid portion was discarded and 2 M potassium Hydroxyl was added. The reaction mixtures were put into an ice bath for 15 min and finally, incubated by introducing 8.0 ml of sodium acetate buffer (1.2 M, pH 3.8). An amount of 0.1 ml of amyloglucosidase enzyme was added to the reaction mixture and incubated at 50 ◦C for 30 min. The 0.1 mL of aliquot was transferred into glass test tubes and again incubation was completed by introducing of GOPOD reagent at 50 ◦C for 30 min. The absorbance was recorded using the UV/Visible spectrophotometer (Thermo Scientific 201, United State) at 510 nm.

2.8. Anti-oxidant properties

2.8.1. Total polyphenol content

The methanolic extraction of soybean samples was subjected to determine the total polyphenolic content by following the modification method of Hettiarachchi et al., 2021. The amount of 0.2 ml of Folin-Ciocalteu (0.5 $moll^{-1}$) was added into 1.0 ml of the test sample and incubated in a dark place for 15 min. Then, 5 ml of 7.5% Na_2CO_3 was added to the reaction mixture and again incubated for 2 hours at room temperature. Finally, the absorbance was measured using UV/Visible spectrophotometer 840-210800 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 760nm. Total polyphenol content was expressed as milligrams of Gallic acid equivalents (GAE).

2.8.2. Total flavonoid content

The total flavonoid content of the samples was analyzed using the Spectroscopic method described by Yusnawan, 2018. The amount of 1.5 ml methanolic extracted solution was diluted using 3.5 ml of distilled water and then 0.3 ml of 5% $NaNO_2$ (w/w) was added to the dilute mixture and incubated for 5 min at room temperature.

Approximately 0.3 ml of 10 % $AlCl_3$ was added and kept at room temperature for 6min. Then, 2 ml of 1.0 M NaOH was added to the solution mixture and the solutions were immediately made up to 10 ml by adding distilled water. The absorbance was measured using the UV/Visible spectrophotometer (Thermo Scientific 201, United States) at 510 nm. Total flavonoid content was expressed as grams of Rutin equivalents (RE).

2.8.3. DPPH radical scavenging ability

This was done according to Hettiarachchi et al., 2021. To assess the stability of free radicals, freshly prepared methanolic DPPH solution (0.1mmol•l^{-1}, 3.9 ml) 0.1ml was added and vortexed for 15 s. Then, the reaction mixture was incubated at room temperature for 30 min in a dark place, absorbance was measured at 517 nm and methanolic DPPH was used as the control. Radical Scavenging activity (RSA) expressed as equation (1),

$$\text{RSA} = \frac{\text{A0}-\text{A}}{\text{A0}} \times 100\% \quad (1)$$

A_0 and A are the absorbances of the control and sample respectively.

2.9. Functional Properties of Soybean Flour

For functional properties, the water absorption index (WAI) and oil absorption index (OAI) were determined according to the modified method by Juliant et al., 2017. One gram of each sample was suspended in 5 ml of water (WAI) or soybean oil (for OAI) in centrifuge tubes. The slurry was shaken for 1 min and centrifuged at 3000 rpm for 15 min. Centrifuged samples were allowed to stand at room temperature for 2 hours, and the supernatant was decanted and discarded. WAI and OAI were expressed as the weight of sediment/initial weight of flour samples (g/g). The solubility index of the flour samples was determined according to the method of Kisambira et al., (2015). The bulk density of the soy flour was measured by the method described Muttakin et al., 2015. The bulk density was measured by pouring the flour samples into a 100 ml top-fitted graduated cylinder. The weight of the mass cylinder filled with 100 ml of powder was measured. Bulk density was defined as powder weight (g) divided by powder volume (100 ml). Water solubility was determined by the method described by Chiranthika et al., 2021. The 0.5 g samples were heated in 10 ml distilled water bath at 60°C for 30 minutes without mixing. The samples were centrifuged at 1600 rpm for 10 minutes.

The supernatant was separated (5 mL), dried, weighed and calculated using below equation (2).

$$Water\ Solubility = \frac{Weight\ of\ the\ soluble\ starch(g) \times 100}{Weight\ of\ the\ initial\ sample(dry\ basis)\ (g)} \quad (2)$$

2.10. Evaluation of the levels of incorporation into wheat Bread

2.10.1. Bread Preparation and Evaluation

The loaf was prepared using the straight dough method with reference to the AACC International 2000 standard. Mixed flour (3%, 5%, and 8% wheat flour with soy added) was used for the bread-making process. The amount of 15 g instant yeast, 30 g of refined sugar, 15 g of refined salt, 20 g of RBD Palm olein, 2 g of carboxymethyl cellulose and 2 g of sodium stearoyl lactylate were added to 1000 g of the flour mixture. All ingredients were dry mixed for 2 minutes and wet mixed for 12 minutes using the "HOBART" mixer (Model A-200) until the gluten network was developed. After the well-developed dough was divided into 600 g and put into the mold and placed inside the proofer at 37°C temperature and 75% relative humidity (RH) for 2 hours. Baking was done at 220 °C for 24 minutes using a deck oven electric Sottoriva, Italy.

The moisture, protein, ash, and crude fiber content were determined using the standard method AOAC 2000. The loaf volume of the bread was determined by the modification method of AOAC 2000 instead of rapeseeds, using mustard seeds, and loaf specific volume (LSV) was calculated using the below equation (3).

$$\text{LSV} = \text{loaf Volume (ml)} / \text{Loaf Weight (g)} \quad (3)$$

The color of the bread samples was determined by using a chroma meter (Minolta Type CR-300, Japan) and considering the parameters L*, a* and b*. The L* scale ranges from 0 black to 100 scale extends from a negative value (green hue) to a positive value (red hue), and the b* scale ranges from negative blue to positive yellow.

A sensory evaluation was conducted to evaluate the aroma, taste, texture, and crumb color of the bread sample. The bread samples were sliced into pieces with uniform thickness and served with water. Fifteen trained panelists were randomly selected from "Prima Company" to perform the evaluation. Panelists evaluated bread samples 5-pointing hedonic scale quality analysis with 5 =liked very much, 4= liked, 3 = neither liked nor disliked, 2= disliked, and 1= disliked very much.

2.11. Statistical Analysis

The results of triplicate experiments were pooled and expressed as mean ± standard deviation (SD) using the SPSS software version 26. Statistical significance was determined by independent t-test and significance was accepted at $P \leq 0.05$.

3. Results and Discussion

3.1. Proximate composition

The proximate composition of the soybean varieties is shown in Table 1. The available moisture percentage of the P.B. 1 Soybean variety was significantly higher than the Indian MACS-330 variety ($P<0.05$). The storage ability of the flour mainly depends on the moisture percentage and if the flour has more than 10% moisture, it shows the lowest storage ability and higher fungal attack (Huang, Wu, & Yen, 2006). The ash content of the Sri Lankan variety is significantly higher than the Indian MACS -330 ($p<0.05$). The highest quantity of ash is caused to reduce the whiteness of the bread crumb.

The fat, protein, and crude fiber percentages are not significantly different for both varieties ($P<0.05$). Normally soybeans are considered as a protein-rich plant source and it used to treat protein energy malnutrition in the world (Stobaugh et al., 2016).

Table 1. The independent t-test results on proximate composition of Soybean varieties. P.B 1 variety and Indian MACS – 330 variety values represent mean ± standard deviation ($P<0.05$) of triplicate readings.

Content (g/100g)	Sri Lankan P.B 1 Variety	Indian MACS-330
Moisture	11.66 ± 0.98	4.27 ± 0.89
Protein	35.35 ±3.69	42.33 ± 2.52
Fat	16.37 ± 0.42	17.60 ±0.85
fiber	6.34 ± 0.67	3.04 ± 0.19
Ash	8.85 ± 0.67	5.98 ± 0.25

3.2. Functional Properties

The functional properties of the samples are presented in Table 2. Under the functional properties, oil holding capacity, water holding capacity, solubility, and bulk density of the flour were tested. Functional properties mainly depended on the protein content of the flour. WHC and OHC are dependent on the protein content and protein has both hydrophilic and hydrophobic properties that can bind with water and oil (Chiranthika et al 2022).

The intrinsic factors of the molecular arrangement, the composition, and the surface polarity of the amino acid chain are affected for the functional properties of the Soybean flour. If the flour has higher water absorption qualities, it makes desirable qualities to the baked products also. Foodstuff has higher WAC and OAC capacity, which indicates that, protein can prevent fluid losses inside the food matrix. Bulk density is mainly dependent on the interrelated factors of the molecules including attraction of intensity of the inter-particle forces, particle size, and the number of contact points (Ma et al., 2022).

Table 2. The independent t-test results on functional properties of Soybeans. P.B 1 variety and Indian MACS – 330 variety values represent mean ± standard deviation ($P<0.05$) of triplicate readings.

Property	Sri Lankan P.B 1 Variety	Indian Macs-330
WHC (g/g)	3.00 ± 0.25	3.92 ± 0.09
OHC (g/g)	2.53 ±0.20	3.14 ± 0.14
Swelling Index (%)	2.06 ±0.08	3.63 ±0.34
Solubility (%)	28.33±0.82	42.77±1.34
Bulk Density(g/g)	0.75 ± 0.09	0.52 ± 0.04

3.3. Antioxidant Properties

Soybean flour has antioxidants like isoflavonoids and their derivate, phospholipids, tocopherol, amino acids, and peptidase which helps to enhance the desirable characteristics of the products as well as gives more health benefits. Antioxidants help to reduce cancer properties and oxidative stress through the scavenging of the body cells. Most of the flavonoids and the antioxidant properties are destroyed due to the preparation methods and temperature (Rice-Evans et al., 1996).

Investigation of the antioxidant activity, the polyphenolic, and total flavonoid distribution in the selected varieties agreed with earlier research reports. Total flavonoid content, polyphenol content, and

the DPPH scavenging activity are shown in Table 3. According to the represented data in Table 3, the total flavonoid content and the polyphenolic content of the Sri Lankan P.B 1 variety are not significantly different than the Indian imported MACS–330 variety (P>0.05).

The polyphenolic content of the Sri Lankan P.B 1 variety showed 2.46 ± 0.62 mg/g against 8.04 ± 1.02 mg/g and of Indian MACS-330 soy flour. The total flavonoid content of the P.B 1 variety is 1.02 ± 0.51 mg/g against the 3.33 ± 0.51 mg/g of Indian MACS – 330 varieties. Plant derivatives can act as free radical receptors which help to cut down the incidence of certain chronic diseases, diabetes, cancers, and cardiovascular diseases, through the management of oxidative stress (Prakash et al., 2007).

In the recent research, 30 species of soybean samples which are grown in India were subjected to evaluate the antioxidant properties and, those showed wide variation from 6.4 to 81.7 mg GAE/g of total polyphenolic content and the 3.2 to 44.6 mg QE/g of total flavonoid content. (Prakash, Upadhyay, Singh, & Singh, 2007). The DPPH inhibition activity of the Sri Lankan P.B 1 variety showed 12.66 ± 1.16% against 15.66 ± 2.06% of Indian MACS – 330 varieties which are not significantly different from each other (P<0.05). The antioxidant properties are highly dependent on the variety, geography, and each other physicochemical process parameters (Georgetti et al., 2006). Quercetin and catechin are the most prominent phenolic compounds in soybean and it reported to inhibit the pro-inflammatory effect in human monocytes (Mugabi et al., 2022). However, recent research investigates the flavonoid of the soybean that helps to manage hypertension the diabetes and nondiabetic people.

Table 3. The antioxidant properties of the soybean P.B 1 variety and Indian MACS - 330 varieties. Values represent mean ± standard deviation (P<0.05) of triplicate readings.

Property	Sri Lankan P.B 1 Variety	Indian Macs-330
Total Polyphenolic acid (mg/ GAE)	2.46 ± 0.62	8.04 ± 1.02
Total Flavonoid content (g/RE)	1.02 ± 0.51	3.33 ± 0.52
DPPH Inhibition Activity (%)	12.66 ±1.16	15.66 ± 2.06

3.4. Antidiabetic properties

α-amylase enzyme promotes the hydrolyze 1-4 linkages in starch and amyloglucosidase is leveraged to convert high molecular weight starch into simple, more absorbable compounds such as glucose, maltose, and maltotriose (Chiranthika et al., 2022).

Control of post-prandial hyperglycemia is the control of pancreatic α-amylase or intestinal α-glucosidase activity to delay carbohydrate absorption. At the experimental results α – amylase activity of the Sri Lankan P.B 1 soybean variety has 1.79 ± 0.59 % and the Indian MACS-330 variety has 2.17 ± 0.93 % and samples were not significantly different in each sample (p <0.05). If the food is rich in soybean products, which can inhibit α -amylase, α -glucosidase activities and intestinal α-glucosidase inhibitors help to avoid hyperglycemia and maintain normal blood sugar levels (Ademiluyi, & Oboh, 2013).

The sprouting time is also strongly interrelated to the control key enzymes related to diabetes. The strong anti-amylase activity was observed for 4-6 days of culture time and higher amylase inhibit index (Ademiluyi, & Oboh, 2013). This research also showed as in Table 4 that the anti-amylase activity of both soybean varieties is higher than the amyloglucosidase activity.

Table 4. The independent t-test results on In-vitro alpha-amylase and amyloglucosidase activity of soybean. P.B 1 variety and Indian MACS- 330 variety values represent mean ± standard deviation (P<0.05) of triplicate readings.

Variety	α-amylase activity (%)	Amyloglucosidase activity (%)
Sri Lankan P.B 1	1.76 ±0.14	0.98 ± 0.06
Indian MACS-330	2.10 ±0.17	0.89 ±0.03

The resistant starch content of the Sri Lankan variety (P. B 1) was 6.63 ± 0.09 % and the Indian MACS- 330 variety was 6.11 ± 0.13 % respectively and the resistant starch content of the samples was significantly different (P< 0.05). Recent research revealed that the total dietary fiber content of the Sri Lankan Soybean P.B 1 variety was 18.51 ± 0.67 % against 3.33 ± 0.52 mg/g of the MISB1 variety (Yu et al., 2021). The dietary fiber quantity of the Sri Lankan and Indian samples were shown in Table 5.

Table 5. The test results on Dietary fiber, resistant starch quantity of soybean.P.B 1 variety and Indian MACS- 330 variety values represent mean ± standard deviation (P<0.05) of triplicate readings.

Variety	Dietary Fiber (%)	Resistant Starch (%)
P.B 1	17.79±0.59	6.63± 0.09
Indian MACS-330	21.67±0.93	6.11± 0.13

3.5. Physical and sensory properties of bread samples

The proximate composition of the prepared breads was evaluated with the AOAC standards and breads with different incorporation levels (3%, 5%, and 8%) were compared with the control prepared without adding soya flour. The proximate compositions of the breads are presented in Table 6. When increasing soy flour quantity, all the parameters were increased except carbohydrate. This might be happened due to soy flour containing higher amount of solid matter with high emulsifying properties which help to bond molecules (Taghdir et al., 2017).

When increasing the protein quantity, alternatively it helps to enhance the energy of the food. With the increase of soybean flour, it gives an additional amount of ash that causes to reduce the whiteness of the bread crumb. The same as other studies, the ash content increased with increasing levels of soy flour in the bread samples (Farzana & Mohajan, 2015). Bread with 8% P.B 1 Soy flour has significantly ($p < 0.05$) higher proximate composition than other incorporation levels.

The crumb and the crust of the different incorporation levels of the bread are presented in Figure 1 and 2 and Table 7 shows a, b and L values of the crumb and crust. With the increment of soybean incorporated levels, the L value, and bread lightness were decreased and the yellowish color was increased. The lightness value of all six samples was significant difference (P< 0.05) compared to the P.B 1 variety and the Indian MACS-330 variety. The Sri Lankan P.B. 1 variety has the lowest L value than the Indian MACS-330.

Table 6. The independent t-test results on the proximate composition of different levels of Soybean flour incorporated Wheat bread. Values represent the mean ± standard deviation of triplicate readings. Values with the same superscript on the same row are not significantly different (P > 0.05).

Result	Control	Sri Lankan Soybean flour Variety			Indian Soybean Flour		
		3%	5%	8%	3%	5%	8%%
Moisture (g)	38.06^{a}± 0.33	35.58^{cd}±0.40	36.30^{bc}±0.38	38.32^{a}±0.04	34.62^{d}±0.05	36.09^{c}±0.4	37.38^{ab}±0.15
Protein (g)	8.54^{d}±0.22	9.35^{c}±0.17	10.35^{b}±0.03	13.15^{a}±0.03	9.23^{c}±0.29	10.7^{b}±0.38	12.79^{a}±0.22
Fat (g)	2.82^{f} ±0.21	3.37^{e}±0.24	4.63^{c}±0.36	6.37^{a}±0.18	3.03^{f}±0.03	4.05^{d}±0.40	5.47^{b}±0.32
Crude Fiber (g)	1.50^{e}±0.68	2.64^{d}±0.56	3.10^{c}±0.83	3.66^{b}±0.28	4.09^{a}±0.16	3.00^{e}±0.35	3.22^{c}±0.23
Ash (g)	2.09^{c}±0.48	2.29^{b}±0.33	3.04^{b}±0.05	3.64^{a}±0.33	2.04^{c}±0.55	2.53^{bc}±0.12	2.97^{b}±0.13
Carbohydrate(g)	50.04^{a}±0.63	48.00^{bc}±1.95	43.17^{d}±1.30	36.28^{e}±2.03	47.03^{c}±0.20	44.17^{d}±1.23	39.06^{e}±0.51

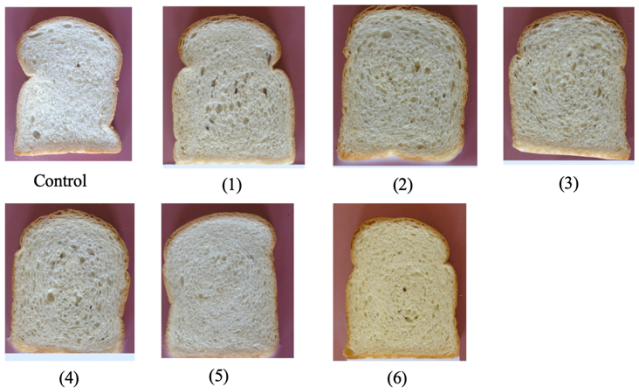

Fig. 1. Different incorporation levels of P.B 1 variety soybean incorporated bread and the Indian Macs-330. **Picture 1:** 3% 0f P.B. 1 soybean incorporated bread, **Picture 2:** 5% P.B.1 soybean flour incorporated bread, **picture 3:** 8% P.B. 1 soybean incorporated bread, **Picture 4:** 3% Indian Macs-330 soybean flour incorporated bread, **Picture 5:** 5% Indian Macs-330 incorporated bread, **Picture 6:** 8% Indian Macs-330 soybean flour incorporated bread the 0% of the wheat flour presented bread is used as the control.

The color of the soybean flour added to bread samples was improved from creamy to brown. The darker color of the bread samples with soy flour may be due to the presence of yellow pigment in soy flour and the Maillard reaction that happened during the processing steps. When increasing levels of soy flour, the volume of the bread is significantly reduced because of the increase of the solid content, which affects the yeast fermentation and CO_2 retention ability inside the bread crumb (Taghdir et al., 2017). The physical properties of the loaf especially, the specific volume and the baking losses of the different levels of the soybean flour added samples were presented in Table 8. The baking losses of the bread are significant differences in each sample ($p<0.05$). With the increment of the soy flour, the solid content of bread was increased and it cause to a reduction in baking losses (Liu et al. 2005). Recent research reveals that the weight of the bread increased with an increase in the soy flour percentage and it showed remarkable decreases in the specific volume of the bread (Shittu et al., 2007).

The loaf volume is affected by the quality of the bread. Loaf weight is determined by the quantity of dough baked and the amount of moisture and carbon dioxide diffused out of the loaf during baking (Shittu et al., 2007). The gas production of the bread is higher for the lower incorporation levels of soybean flour and the higher loaf weight of composite bread samples is a result of less retention of carbon dioxide gas in the blended dough, hence providing a dense bread texture (Rao & Hemamalini, 1991). The dough's extensibility is reduced with an increased substitution level of non-wheat flour protein (Chauhan et al.,1992). This research also showed that the increment of the soybean flour decreased the loaf volume and increased the bread loaf weight.

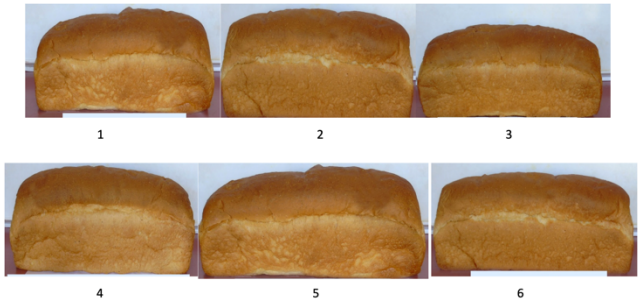

Fig.2. Different incorporation levels of P.B 1 variety soybean incorporated bread and the Indian MACS-330. **Picture 1:** 3% 0f P.B. 1 soybean incorporated bread, **Picture 2:** 5% P.B.1 soybean flour incorporated bread, **picture 3:** 8% P.B. 1 soybean incorporated bread, **Picture 4:** 3% Indian MACS-330 soybean flour incorporated bread, **Picture 5:** % Indian MACS-330 incorporated bread, **Picture 6:** 8% Indian MACS-330 soybean flour incorporated bread.

The sensory test was conducted using 15 of participants and according to the sensory results, more than 55% selected 5% Sri Lankan soybean-incorporated bread as their first preference. According to the panel results, when increasing levels of the soybean flour physical parameters (Bread volume) and the color is significantly different from the control sample, and bitterness was increased with the increment of the soybean level.

4. Conclusion

This research focused on evaluating the properties of both local and Indian varieties because of the production volume of the local variety as the replacement of Indian varieties. Research findings were reported that the soybean P.B1 variety and MACS -330 can inhibit α-amylase and the amyloglucosidase activities *in-vitro*. As well as both varieties showed antioxidant properties that help to cure certain diseases. With the increment of the soybean flour, the protein content was also significantly increased. Due to the presence of a higher amount of protein alternatively, it helps to treat malnutrition. Soybean consumption in Sri Lanka is very low and this article's data revealed that if the bread was incorporated with soya bean flour, which alternatively enhances the nutrient content and helps to treat to some extent to control the blood glucose level than consumption of whole wheat bread. We also expect to improve the processing methods to overcome undesirable flavors, such as the beany flavor in the development of soy-based foods, and increase the incorporation levels for the food formulations.

Table 7. The independent t- test results on color changes of different levels of incorporated Soybean flour for Wheat Bread. Values represent the mean ± standard deviation of triplicate readings. Values with the same superscript on the same row are not significantly different (P > 0.05). *L value represents lightness a* value indicates redness b* indicates yellowness.

Variety	Crumb color			Crust Color		
	a*	b*	L*	a*	b*	L*
Control	64.27^{a}±1.05	0.35^{f}±0.2	0.21d±0.55	46.55^{a}±0.26	5.86^{d}±0.45	0.44^{e}±0.12
S. L variety 3%	56.40^{bc}±1.54	1.02^{d}±0.35	0.32^{cd}±0.12	43.54^{b}±1.57	8.86^{b}±0.61	0.37^{e}±0.14
S. L variety 5%	47.95^{e}±0.55	1.82^{b}±0.15	0.34^{c}±0.38	42.06^{b}±0.70	9.35^{b}±0.28	0.42^{e}±0.65
S. L variety 8%	48.06^{f}±0.81	2.66^{a}±0.19	0.51^{a}±0.15	38.10^{c}±1.64	12.50^{a}±0.16	0.67^{cd}±0.86
Indian variety 3%	56.20^{e}±0.65	0.57^{e}±0.95	0.16^{e}±0.25	45.49^{ab}±1.07	7.35^{c}±0.15	0.55^{d}±0.25
Indian variety 5%	54.70^{cd}±0.52	1.00^{d}±0.49	0.36^{bc}±0.17	43.40^{b}±0.97	9.25^{b}±0.15	0.96^{a}±0.01
Indian variety 8%	52.17^{e}±0.72	1.44^{c}±0.15	0.48^{b}±0.13	47.10^{a}±0.68	12.99^{a}±0.44	0.85^{bc}±0.02

Table 8. The independent t-test results on baking losses (%) and the specific volume (g/cm^3) of the different incorporation levels of the bread. Values represent the mean ± standard deviation of triplicate readings. Values with the same superscript on the same row are not significantly different (P > 0.05).

Result	Control	Sri Lankan Soybean flour Variety			Indian Soybean Flour		
		3%	5%	8%	3%	5%	8%%
Baking Loses (%)	15.12^{a}±0.12	14.2^{a}±0.05	14.1^{a}±0.08	12.8^{a}±0.04	13.2^{a}±0.05	13.5^{a}±0.02	12.7^{a}±0.07
Specific volume(g/cm^3)	2.70^{a}±0.13	2.64^{a}±0.66	2.50^{ab}±0.06	1.06^{d}±0.10	2.56^{a}±0.11	2.24^{b}±0.09	1.72^{c}±0.23

Conflict of interest

The authors declare that there is no conflict of interest.

References

AOAC, 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 54-21 and 10-10B. AACC International, St. Paul, M.N.

AOAC, 1995. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 54-21 and 10-10B. AACC International, St. Paul, M.N.

Ademiluyi, A.O. and Oboh, G., 2013. Soybean phenolic-rich extracts inhibit key enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. Experimental and toxicologic pathology, 65(3), pp.305-309. https://doi.org/10.1016/j.etp.2011.09.005

Bakris, G.L., Williams, M., Dworkin, L., Elliott, W.J., Epstein, M., Toto, R., Tuttle, K., Douglas, J., Hsueh, W. and Sowers, J., 2000. Preserving renal function in adults with hypertension and diabetes: a consensus approach. American Journal of kidney diseases, 36(3), pp.646-661. https://doi.org/10.1053/ajkd.2000.16225

Barber, E., Houghton, M.J. and Williamson, G., 2021. Flavonoids as human intestinal α-glucosidase inhibitors. Foods, 10(8), p.1939. https://doi.org/10.3390/foods10081939

Chandrasekara, A. and Joseph Kumar, T., 2016. Roots and tuber crops as functional foods: a review on phytochemical constituents and their potential health benefits. International Journal of food science, 2016. https://doi.org/10.1155/2016/3631647

Chauhan, G.S., Zillman, R.R., Eskin, N.A.M., 1992. Dough mixing and breadmaking properties of quinoa-wheat flour blends. Int. J. Food. Sci. Tech. 27, 701–705. https://doi.org/10.1111/j.1365-2621.1992.tb01241.x

Chiranthika, N.N.G., Gunathilake, K.D.P.P. and Chandrasekara, A., 2020. Potential Applications of Cereals and Yams as Functional Foods to Reduce the Risk of Chronic Non-Communicable Diseases. Asian Journal of Research in Biochemistry, 7(4), pp.53-69. DOI:10.9734/AJRB/2020/v7i430148

Chiranthika, N.N.G., Chandrasekara, A. and Gunathilake, K.D.P.P., 2021. In vitro α amylase and amyloglucosidase inhibitory activities of selected underutilized cereals, yams, and root crops. Journal of Medicinal Plants, 9(3), pp.105-110. https://doi.org/10.22271/plants.2021.v9.i3b.1271

Chiranthika, N.N.G., Chandrasekara, A. and Gunathilake, K.D.P.P., 2022. Physicochemical characterization of flours and starches derived from selected underutilized roots and tuber crops grown in Sri Lanka. Food Hydrocolloids, 124, p.107272. https://doi.org/10.1016/j.foodhyd.2021.107272

Epstein, M. and Sowers, J.R., 1992. Diabetes mellitus and hypertension. Hypertension, 19(5), pp.403-418. https://doi.org/10.1161/01.HYP.19.5.403

Farzana, T. and Mohajan, S., 2015. Effect of incorporation of soy flour to wheat flour on nutritional and sensory quality of biscuits fortified with mushroom. Food science & nutrition, 3(5), pp.363-369. https://doi.org/10.1002/fsn3.228

Georgetti, S.R., Casagrande, R., Vicentini, F.T.M.D.C., Verri Jr, W.A. and Fonseca, M.J.V., 2006. Evaluation of the antioxidant activity of soybean extract by different in vitro methods and investigation of this activity after its incorporation in topical formulations. European Journal of Pharmaceutics and Biopharmaceutics, 64(1), pp.99-106. https://doi.org/10.1016/j.ejpb.2006.04.003

Hettiarachchi, H. A. C. O., Gunathilake, K. D. P. P., & Jayatilake, S. (2021). Effect of In-vitro Gastrointestinal Digestion and Dialysis Process on Phenolic Compounds and Antioxidant Capacity of Selected Underutilized Fruits in Sri Lanka. Tropical Agricultural Research, 32(2), 212. https://doi.org/10.4038/tar.v32i2.8468

Huang, S.M., Wu, C.H. and Yen, G.C., 2006. Effects of flavonoids on the expression of the pro-inflammatory response in human monocytes induced by ligation of the receptor for AGEs. Molecular nutrition & food research, 50(12), pp.1129-1139. https://doi.org/10.1002/mnfr.200600075

Janghorbani, M., Van Dam, R.M., Willett, W.C. and Hu, F.B., 2007. A systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. American Journal of Epidemiology, 166(5), pp.495-505. https://doi.org/10.1093/aje/kwm106

Julianti, E., Rusmarilin, H. and Yusraini, E., 2017. Functional and rheological properties of composite flour from sweet potato, maize, soybean and xanthan gum. Journal of the Saudi Society of Agricultural Sciences, 16(2), pp.171-177. https://doi.org/10.1016/j.jssas.2015.05.005

Kisambira, A., Muyonga, J.H., Byaruhanga, Y.B., Tukamuhabwa, P., Tumwegamire, S. and Gruneberg, W.J., 2015. Composition and functional properties of yam bean (Pachyrhizus spp.) seed flour. https://dx.doi.org/10.4236/fns.2015.68076

Liu, J., Chang, S.K. and Wiesenborn, D., 2005. Antioxidant properties of soybean isoflavone extract and tofu in vitro and in vivo. Journal of Agricultural and Food Chemistry, 53(6), pp.2333-2340. https://doi.org/10.1021/jf048552e

Malenčić, D., Maksimović, Z., Popović, M. and Miladinović, J., 2008. Polyphenol contents and antioxidant activity of soybean seed extracts. Bioresource Technology, 99(14), pp.6688-6691. https://doi.org/10.1016/j.biortech.2007.11.040

Ma, K.K., Greis, M., Lu, J., Nolden, A.A., McClements, D.J. and Kinchla, A.J., 2022. Functional performance of plant proteins. Foods, 11(4), p.594. https://doi.org/10.3390/foods11040594

Mesa, M.D., Silván, J.M., Olza, J., Gil, Á. and del Castillo, M.D., 2008. Antioxidant properties of soy protein–fructooligosaccharide glycation systems and its hydrolyzates. Food research international, 41(6), pp.606-615. https://doi.org/10.1016/j.foodres.2008.03.010

Mugabi, R., Byakika, S. and Mukisa, I.M., 2022. Effects of Feed Moisture Content, Soybean Ratio, and Barrel Temperature on Physical and Functional Properties of Extruded Maize-Soybean Flour Blends. Tanzania Journal of Science, 48(2), pp.447-459. DOI: 10.4314/tjs.v48i2.19

Muttakin, S., Kim, M.S. and Lee, D.U., 2015. Tailoring physicochemical and sensorial properties of defatted soybean flour using jet-milling technology. Food Chemistry, 187, pp.106-111. DOIhttps://doi.org/10.1016/j.foodchem.2015.04.104

Prabakaran, M., Lee, K.J., An, Y., Kwon, C., Kim, S., Yang, Y., Ahmad, A., Kim, S.H. and Chung, I.M., 2018. Changes in soybean (Glycine max L.) flour fatty-acid content based on storage temperature and duration. Molecules, 23(10), p.2713. https://doi.org/10.3390/molecules23102713

Prakash D., Upadhyay, G., Singh, B.N. and Singh, H.B., 2007. Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (Glycine max). Food Chemistry, 104(2), pp.783-790. https://doi.org/10.1016/j.foodchem.2006.12.029

Ragaee, S., Abdel-Aal, E.S.M., 2006. Pasting properties of starch and protein in selected cereals and quality of their products. Food Chem. 95, 9–18. https://doi.org/10.1016/j.foodchem.2004.12.012

Ramdath, D.D., Padhi, E.M., Sarfaraz, S., Renwick, S. and Duncan, A.M., 2017. Beyond the cholesterol-lowering effect of soy protein: a review of the effects of dietary soy and its constituents on risk factors for cardiovascular disease. Nutrients, 9(4), p.324. https://doi.org/10.3390/nu9040324

Rao, H.P., Hemamalini, R., 1991. Effect of incorporating wheat bran on rheological characteristics and bread making the quality of the flour. J. Food Sci. Tech. 28, 92–97.http://ir.cftri.res.in/id/eprint/8008.

Rice-Evans, C.A., Miller, N.J. and Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free radical biology and medicine, 20(7), pp.933-956. https://doi.org/10.1016/S0891-5849(98)00315-3

Saito, N., Sakai, H., Suzuki, S., Sekihara, H. and Yajima, Y., 1998. Effect of an α-glucosidase inhibitor (voglibose), in combination with sulphonylureas, on glycaemic control in type 2 diabetes patients. Journal of International Medical Research, 26(5), pp.219-232. https://doi.org/10.1177/030006059802600501

Shittu, T.A., Raji, A.O., Sanni, A.O., 2007. Effect of baking time on some physical properties of bread loaf. Food Res Int. 40 (2), 280–290. https://doi.org/10.1016/j.foodres.2006.10.012

Shobana, S., Sreerama, Y.N. and Malleshi, N.G., 2009. Composition and enzyme inhibitory properties of finger millet (Eleusine coracana L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. Food Chemistry, 115(4), pp.1268-1273. https://doi.org/10.1016/j.foodchem.2009.01.042

Stobaugh, H.C., Ryan, K.N., Kennedy, J.A., Grise, J.B., Crocker, A.H., Thakwalakwa, C., Litkowski, P.E., Maleta, K.M., Manary, M.J. and Trehan, I., 2016. Including whey protein and whey permeate in ready-to-use supplementary food improves recovery rates in children with moderate acute malnutrition: a randomized, double-blind clinical trial. The American Journal of clinical nutrition, 103(3), pp.926-933. https://doi.org/10.3945/ajcn.115.124636

Taghdir, M., Mazloomi, S.M., Honar, N., Sepandi, M., Ashourpour, M. and Salehi, M., 2017. Effect of soy flour on nutritional, physicochemical, and sensory characteristics of gluten-free bread. Food Science & Nutrition, 5(3), pp.439-445 https://doi.org/10.1002/fsn3.411

Yu, X., Yang, T., Qi, Q., Du, Y., Shi, J., Liu, X., Liu, Y., Zhang, H., Zhang, Z. and Yan, N., 2021. Comparison of the contents of phenolic compounds including flavonoids and antioxidant activity of rice (Oryza sativa) and Chinese wild rice (Zizania latifolia). Food Chemistry, 344, p.128600. https://doi.org/10.1016/j.foodchem.2020.128600

Yusnawan, E., 2018, January. Effects of different extraction methods on total phenolic content and antioxidant activity in soybean cultivars. In IOP Conference Series: Earth and Environmental Science (Vol. 102, p. 012039). IOP Publishing. DOI 10.1088/1755-1315/102/1/012039