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Physicochemical, in vitro antidiabetic and sensory characteristics of leavened functional bread made with *Lasia spinosa* and *Nelumbo nucifera* rhizome flours composited with wheat flour

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Abstract

Bread is considered one of the most popular bakery products consumed in several world regions including Sri Lanka. The potential of utilization of two rhizome flours (*Lasia spinosa* and *Nelumbo nucifera*) was evaluated in bread formulation with four different substitution levels (20, 40, 60, and 80%). After the incorporation of *L. spinosa* and *N. nucifera* flours, the alterations in compositional, functional, in vitro anti diabetic activity, in vitro starch digestibility and sensory attributes were observed. The results revealed that the total dietary fiber, resistant starch and ash contents were higher while protein, fat and starch contents were lower in the rhizome flour incorporated breads. The functional properties showed that the water absorption index and water solubility increased with increasing of rhizome or proportion. The results showed that 20% rhizome flour incorporated bread had the highest sensory score compared to other percentage ratios and contained a higher level of dietary fiber, resistant starch, antidiabetic activity, and low rate of starch digestibility compared to control bread. The mixture of *Lasia spinosa* and *Nelumbo nucifera* rhizome flours could be incorporated up to 20% substitution level with wheat flour to formulate functional bread with more nutritious and functional along with sensory acceptability.

Keywords Antidiabetic, Bread, Functional, *Lasia spinosa*, *Nelumbo nucifera*

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Graphical Abstract

Lasia spinosa

Nelumbo nucifera

All-purpose wheat flour
Salt
Yeast
Sugar
Fat source
Water

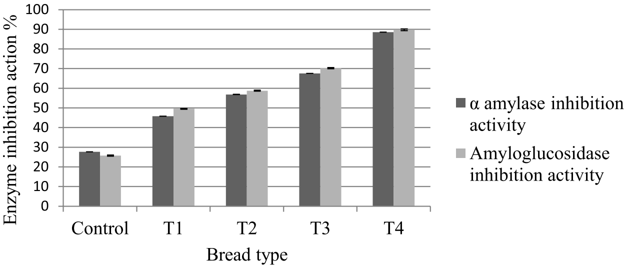
100% Wheat flour - Control

20% root flour – T_1

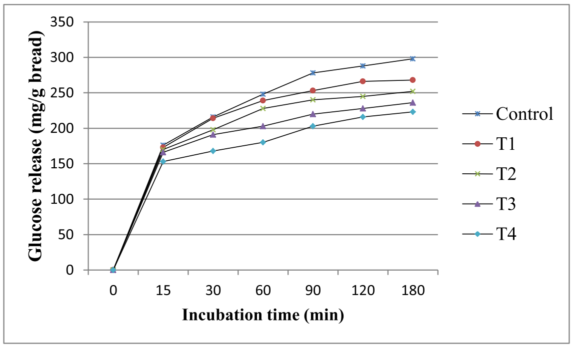
40% root flour – T_2

60% root flour – T_3

80% root flour – T_4



α amylase and amyloglucosidase inhibitory activity



Digestograms of breads from different flour blends

Introduction

There is an apparent increase in the consumption of bakery food items due to lifestyle changes and dietary patterns of people. Bread is one of the highly available bakery products consumed in various types, in many regions of the world including Sri Lanka (Lau et al. 2015). The consumption of bread has increased may be due to its better nature of availability, accessibility and affordability. Wheat is the main food crop that is used in making bakery products including breads. However, wheat is not a tropical crop in many countries including Sri Lanka. Sri Lanka imports large quantities of wheat flour annually, and has to pay a huge cost, facing economic challenges. More importantly, customers are concerned about the nutritional values and health benefits of the foods they consumed. Hence, wheat bread is generally in the high glycemic index (GI) food category and the regular consumption of high GI foods may be a risk factor for chronic diseases such as type II diabetes and cardiovascular disease (Ranawana & Henry 2013). Furthermore, in bread processing, the added water and high temperature used in baking result in the gelatinization of starch in the bread. Digestion of gelatinized starch tends to be rapid in the human small intestine, and this causes elevation of postprandial blood glucose and insulin concentrations thus.

Increasing the risk of diabetes (Borczak et al. 2015). Gelatinized starch tends to be rapidly in human small intestine, and it causes elevation of postprandial blood glucose and insulin concentrations thus increasing the risk of diabetes (Borczak et al. 2015). Therefore, customer demand is highly gained by functional foods. Functional food means, a food or a part of a food which can give health benefits to the consumer other than fulfilling the conventional nutrient requirement (Chiranthika et al. 2020). The researchers have been interestingly working on converting conventional wheat bread into a functional food product and intensifying it as good medicine. There are plenty of functional bioactive compounds in natural food sources which can give therapeutic effects or health benefits to consumers. Dietary fiber and resistant starch (RS) have been recognized as vital functional food ingredients which promised to give many health benefits by incorporating them to a food product. Dietary fiber is included with polysaccharide and lignin and are resistant to digestion the in human digestive tract by digestive enzymes. They can provide many health related benefits such as reducing blood glucose level, blood cholesterol levels, increasing healthy cholesterol (High density lipoprotein), reducing blood pressure and weight management which are important in controlling non-communicable diseases (NCDs) (Anderson et al. 2009). Resistant starch (RS) is also a part of starch that is not readily digested in the human small intestine but gets fermented in the colon by colorectal bacteria and produces short-chain fatty acids, and also supports to control of blood glucose and serum cholesterol levels and acts as prebiotics for healthy probiotic bacteria (Chiranthika et al. 2020). There has been an expanded definition for dietary fiber which included RS also, however RS showed some characteristics such as better appearance, texture, d mouth feel compared to traditional dietary fiber which is important in product development (Charalampopoulos et al. 2002). American Dietetic Association (ADA) and the Institute of Medicine have been recommended to intake of 14 g of dietary fiber per 1000 kcal, or 25 g/day for adult women and 38 g/day for adult men (Pandey et al. 2013). Hence, these dietary fiber and RS are valuable functional ingredients. The incorporation of natural food sources containing these dietary fibers in a novel bread formulation as composite flour may provide several nutritional and healthy benefits compared to traditional wheat bread. Discovering and introducing uncommon or non-conventional food sources into the food industry is very important to maintain food security and it will be an emerging research interest in the food and nutrition research area. Therefore, in this study, two underutilized crops in Sri Lanka; *Lasia spinosa* and *Nelumbo nucifera* flours are incorporated with wheat flour at a significant level to prepare novel bread formulation as a functional food. Previous research has shown that there are significant levels of dietary fiber in *Lasia spinosa* and *Nelumbo nucifera* rhizome flours along with other bioactive compounds and comparatively lower content of starch which may provide health benefits to the consumers (Chandrasekara & Kumar 2016; Ham et al. 2017). It has been found that *Lasia spinosa* rhizome can act as an anti-diabetic, antioxidant, anti-hyperlipidemic, anti-bacterial, anti-inflammatory, and anti-tumor agent (Kankanamge & Amarathunga 2017) while *Nelumbo nucifera* rhizome can provide health benefits such as hypoglycemic, antidiarrheals, antimicrobial, diuretic, anti-inflammatory and anti-obesity (You et al. 2014). Besides nutritional value and health benefits, the processability of above mentioned flour is very important in the context of bread formulation. Therefore, dietary fibers are considered an important functional ingredients in dough formation in bread systems because of their ability to bind and hold large quantities of water (Koletta et al. 2014). However, with the increase proportion of rhizome flours in the blend, the gluten strength of wheat flour will not be enough for a proper gluten network. Therefore, an additional quantity of gluten will be added to the formulation to compensate for the defect.

There is no proper literature that shows the utilization of these flours in leavened bread formulation as a blend. The main objective of the present research study was to evaluate the effect of 20, 40, 60 and 80% incorporation levels of *Lasia spinosa* and *Nelumbo nucifera* rhizome flours on rheological and physicochemical properties of the composited flour as well as on the sensory attributes of the formulated breads, prepared to target consumers who wish to have a healthy diet. The current research was also intended to observe the fate of functional ingredients such as dietary fiber and resistant starch of the raw flour to the d bread.

Materials and methods

Amyloglucosidase, pancreatin, potato starch amylose, acetone, phosphoric acid, hydrocholic acid, sodium hydroxide, and amylase were purchased from Sigma Co., St. Louis, MO, USA. The *L. spinosa* and *N. nucifera* rhizomes were procured from the local market in Pannala, Sri Lanka. Wheat flour was purchased from a local supermarket, in Sri Lanka under the brand name “Prima”.

All the purchased chemicals were of analytical grade.

Preparation of *L. spinosa,* and *N. nucifera* flour

Different proportions of wheat flour and *L. spinosa,* and *N. nucifera* flour were used in this study to prepare breads. Rhizomes were washed, peeled, and removed defective parts. Cleaned rhizomes were cut into thin slices (thickness ~ 3 mm) and immediately soaked in citric acid solution (0.25%) for 20 min to avoid a browning reaction (Hettiarachci et al. 2020) and dried using the hot air oven (MEMMERT NLE 500, Germany) at 45 °C up to 14% moisture. The dried rhizome slices were grounded using a grinder (Philips HL772, Thailand) and sifted through a 250 µm sieve. A hundred percent wheat flour was used as the control.

Preparation of breads

Table 1 shows different substitution levels of wheat flour with *L. spinosa,* and *N. nucifera* rhizome flours and other ingredients used for bread formulations.

All ingredients were mixed manually to form the dough for 20 min and kept for fermentation for 1 h. Then the dough was punched to release excess gas and make into the shape of bread and put into bread molds. After 30 min of second fermentation they were baked in a preheated bakery oven for 30 min at 200 °C. Baked breads were immediately removed from molds and allowed to cool before packing.

Methodology

Water hydration properties of different blends of flour mixtures

The effect of incorporating *L. spinosa* and *N. nucifera* flours in different substitution levels on the water hydration properties of all-purpose wheat flour was evaluated. Sample (0.25 g) from each blend was mixed with 10 mL of distilled water and it was kept for 30 min. Then the mixture was centrifuged at 718 g for 30 min. The supernatant solution was oven dried at 105 °C until a constant weight was obtained (Heo et al. 2014). Water absorption index (WAI), water solubility (WS), and swelling power (SP) were calculated using the following equations;

$$\text{Water absorption index (WAI)} = \frac{\text{Weight of wet sediment}}{\text{Weight of dry sample}}$$

$$\text{Water solubility \% (WS)} = \frac{\text{Weight of dry supernatant}}{\text{Weight of dry sample}} \times 100$$

$$\text{Swelling power (SP)} = \frac{\text{Weight of wet sediment}}{\text{Weight of dry sample}} \times \left[\frac{1 - \text{WS}}{100}\right] (\%)$$

Table 1 Different substitution levels of wheat flour with *L. spinosa,* and *N. nucifera* rhizome flours and other ingredients used for bread formulation

Ingredients	Control	20% (T_1)	40% (T_2)	60% (T_3)	80% (T_4)
Wheat flour (g)	100.0	80.0	60.0	40.0	20.0
L. spinosa (g)	0	10.0	20.0	30.0	40.0
N. nucifera (g)	0	10.0	20.0	30.0	40.0
Instant dry yeast (g)	1.25	1.25	1.25	1.25	1.25
Sugar (g)	1.25	1.25	1.25	1.25	1.25
Fat (g)	1.00	1.00	1.00	1.00	1.00
Salt (g)	0.50	0.50	0.50	0.50	0.50
Water (mL)	110.0	120.0	123.0	135.0	140.0
Wheat gluten (g)	0	7.50	12.50	17.50	25.00

Proximate composition of formulated breads

Analyses were conducted following AOAC analytical standards methods. Crude fat content was analyzed by the soxhlet extraction method (963.15), crude protein content was analyzed following the Kjeldahl method and ash analysis was conducted according to (923.03) (Asimah et al. 2016). The total starch content of formulated breads was determined following the method described by Bjorck et al. (1985). Total dietary fiber content was analyzed using the modified AOAC enzymatic-gravimetric procedure (Prosky 1986) and resistant starch content was determined using Megazyme's resistant starch assay kit (Megazyme International Ltd, Bray, Ireland).

Colour and textural properties of formulated breads

The color properties (L*, a*, b*) were determined using a CIELAB 1976 L*,a* and b* color scale. L* values represented lightness, a* values represented redness to greenness, and b* values represented yellowness to blueness. The bread samples were evaluated for both crust and crumb colors in triplicate and mean values were calculated. Brownness Index (B.I.) was calculated as reported by Eduardo et al. (2013).

$$\text{BI} = 100 \times \left(\frac{\text{X} - 0.31}{0.17}\right)$$

$$\text{X} = \frac{(\text{a} + 1.75\text{L})\text{a}}{(5.645\text{L} + \text{a} - 3.012\text{b})}$$

The firmness of bread crumbs was determined on slices of the center of each bread loaf using a texture analyzer TA-XT plus (Stable Micro Systems, Gudaiming, Surrey, UK) followed by AACC Method 74-09 (Koletta et al. 2014). Each bread slice was cut into a thin slice thickness of 25 mm. For each test, two slices from the center of each bread loaf were used. Measurements from three bread loaves were taken for each formulation and mean values were calculated.

Physical parameters of bread

Loaf volume, specific volume and loaf weight were determined using the procedures described by Shittu et al. (2007). The weight of the breads was measured after allowing them to cool. Loaf volumes were calculated following the rapeseed displacement method and specific volume was calculated as follows;

$$\text{Specific volume} = \frac{\text{Loaf volume (mL)}}{\text{Loaf weight (g)}}$$

In vitro antidiabetic analysis

The enzymes α amylase and amyloglucosidase inhibition assay were performed for in vitro antidiabetic activity of prepared breads following the method described in Chiranthika et al. (2021).

In vitro starch digestibility analysis

In vitro, starch digestibility of breads was determined following the method described in (Jang et al. 2015). Bread samples were grounded and sieved through a 100 um siever. One gram of grounded bread powder was weighed into a 500 mL beaker. Then 20 mL of distilled water was added and the mixture was incubated at 37 °C. Pancreatin (2.50 mL) and bile salt solution (prepared by adding 0.05 g pancreatin and 0.3 g of bile extract in 35 mL of 0.1 M NaHCO3) were added with amyloglucosidase (taken as 0.2 mL per gram of starch contained in sample). The pH was maintained at 7.5 with 0.1 N NaHCO3. Aliquots (0.1 mL) were taken at 30, 60, 90,120, and 180 min during the digestion process and the aliquots were mixed with 1.40 mL of ethanol. These mixtures were centrifuged at 600 g for 5 min. The released glucose content of the supernatants was measured using the GOPOD kit at 510 nm.

Sensory evaluation

Screening and training of the sensory panel The duo-trio discriminative test was used to screen capable panelists for saltiness and sweetness (Gunathilake et al. 2013). Based on the obtained results, selected panelists were selected for training sessions. The panelists were trained to do an unstructured descriptive test, using a horizontal scale of 15 cm long with anchor points of 1.5 cm from each end.

Evaluation of sensory properties of formulated breads The bread samples were arranged in a balanced and randomized order. Each sample had a three-digit code to minimize the expected error of the panelist. Trained panelists evaluated the bread samples for taste, crust and crumb color, crust and crumb texture, aroma and overall acceptability on a horizontal 15 cm long scale. The relative placements of the scores on the 15 cm line were recorded.

Statistical analysis

All the performed analyses were conducted in triplicate and the data were expressed as mean ± standard deviation. The sample means were compared at the 95% confidence level ($p < 0.05$) using Tukey's test in SPSS 16.0 software.

Results and discussion

Water hydration properties of different blends of flour mixtures

Water hydration properties such as water absorption index, water solubility and swelling power of different flour blends were analyzed and obtained results are shown in Table 2.

The water absorption index is defined as the volume acquired by the starch granule and whole matrix after swelling in excess water. The results show WAI was significantly ($p < 0.05$) increase with increasing the level of rhizome flour incorporation. The significantly highest ($p < 0.05$) WAI was observed in the 80% substituted blend. Though starch content was decreased with increasing substitution, more dietary fibers in rhizome flours may attribute to increasing WAI in high substitution levels. The effect of incorporating *N. nucifera* and *L. spinosa* rhizome flours together into a bread formulation has been not revealed yet. However, according to the available literature, *N. nucifera* rhizome flour has been used to make bread sticks and they reported the water absorption that the amount of water required for dough development was increased with the incorporation of *N. nucifera* rhizome flour (Thanushree et al. 2017). Bread made by incorporating soy flour has also shown an increase in water absorption capacity when increasing substitution levels due to the higher soluble protein content of soy flour (Menon et al. 2015). Water solubility was also significantly ($p < 0.05$) increased with the level of substitution and it might have been affected by different constituents in added flours. However, Swelling power was significantly ($p < 0.05$) decreased with increasing the rhizome flour incorporation. Starch is the major component subjected to swelling and with the incorporation of the substituted flours the dietary fiber content was increased while starch content decreased with increased percentage substitution. Dietary fiber may interrupt the swelling of starch granules owing to an inhibition of the starch gelatinization process. Hence, the swelling ability

Table 2 Water hydration properties of different flour blends for bread formulation

Sample	Water absorption index (WAI)	Water solubility (WS, %)	Swelling power (SP)
Control	2.75 ± 0.01^{a}	1.50 ± 0.21^{a}	3.81 ± 0.01^{e}
T1	2.82 ± 0.12^{b}	1.75 ± 0.12^{b}	2.87 ± 0.02^{d}
T2	2.97 ± 0.02^{c}	2.42 ± 0.01^{c}	2.42 ± 0.21^{c}
T3	3.25 ± 0.14^{d}	2.50 ± 0.11^{d}	2.29 ± 0.01^{b}
T4	3.55 ± 0.02^{e}	3.75 ± 0.12^{e}	2.15 ± 0.14^{a}

Values expressed as means ± SD ($n = 3$). Different letter superscripts express significant differences between values of the same column ($p < 0.05$)

of starch granules may reduce and subsequent reduction of SP may occur. Further, Protein content, as well as the amylase/amylopectin ratio of the starch also affects swelling power (Emmanuel et al. 2010). A similar trend of results was observed in wheat, moringa leaf powder and millet flour incorporated with composite flour. The swelling power decreased with the incorporation of moringa leaves and millet flour into wheat flour (Chandra 2022).

Proximate composition and physicochemical characteristics of control and formulated breads

The proximate composition and physicochemical characteristics of formulated breads are shown in Table 3.

The proximate composition of formulated breads prepared by incorporating *L. spinosa* and *N. nucifera* flours at 20, 40, 60, and 80% indicates that it contains high levels of total dietary fiber, resistant starch and minerals and low level of fat, protein and total starch contents compared to control bread. The protein content of the breads ranged from 8.56 to 15.10%. The highest significant ($p < 0.05$) protein content was in control bread and protein content was decreased with increasing percentage incorporation of rhizome flours since those flour contains a lower amount of protein. A similar pattern of decreasing protein content in cassava flour incorporated bread has been reported by (Akintayo et al. 2020). Fat contents of the breads were significantly ($p < 0.05$) different among substitution levels and it was decreased when the substitution level is high. Only 1% fat was added in the formulation and rhizome flours contain a minor amount of fat. Decreasing fat content with increasing rhizome flour incorporation is improving the functional value of the formulated breads. Total ash content is a general measure of quality and it reflects the mineral content in food products. The ash contents of breads were significantly ($p < 0.05$) increased with the level of substitution level of rhizome flours. This indicates that rhizome flour incorporated in breads may contain high amounts of minerals. Total starch content was significantly ($p < 0.05$) decreased when the substitution level is higher. The lower level of starch content is important when these bread formulations are introduced as functional food products. Dietary fiber provides several health benefits such as both soluble and insoluble dietary fiber. Insoluble fiber reduces the bowel transit time, could increase fecal bulk, and makes feces softer. Soluble dietary fiber could delay gastric emptying, reduce the rate of glucose absorption, reduce serum cholesterol level and further helps to reduce the risk of non-communicable diseases such as type II diabetes and cardiovascular disease (Shobha et al 2015). The highest dietary fiber content (43.65%) was in 80% rhizome flour incorporated bread and resistant starch content was also high in 80% substituted bread with rhizome flour. Control bread showed 29.55% of moisture content while moisture content slightly increased from 20 to 80% substitution level of rhizome flour which ranged from 30.25 to 33.22%. Water activity is an important factor that affects the quality and shelf life of bread. If the water activity is too high, the bread may become too moist and prone to microbial spoilage. If the water activity is too low, the bread may become dry and stale. According to the results of the present study, there was no significant difference ($p < 0.05$) in water activity values of wheat and all new bread formulations. As in the literature, the water activity of wheat bread was around 0.9 (Schmidt and Fontana, 2020) and it was not similar to the water activity value of prepared wheat bread in the present study. However, the water activity of bread may vary due to the incorporation of different flour sources other than wheat flour as well as processing conditions and other ingredients used in bread formulation may also affect the water activity of the product.

Color and textural properties of breads

The colour phase (L*, a*, b*) was determined, where L* represented the lightness, a* represented redness to greenness, and b* represented yellowness to blueness. The hardness of the bread was analyzed for textural properties. The results of color parameters and hardness of formulated breads are shown in Table 4.

The L* (lightness) values of breads decreased from 58.04 to 34.55, the a* (redness) values increased from 13.04 to 15.14 and the b* (yellowness) values of breads

Table 3 Physicochemical characteristics and proximate composition (%, dry basis) of breads

Sample	Protein	Fat	Ash	Total starch	Total dietary fiber	Resistant starch	Moisture	a_w
Control	15.10 ± 0.02^e	2.35 ± 0.24^e	1.95 ± 0.20^a	50.05 ± 0.03^e	2.05 ± 0.11^a	0.85 ± 0.11^a	29.55 ± 0.01^a	0.72 ± 0.01^a
T1	14.52 ± 0.01^d	1.90 ± 0.01^d	2.65 ± 0.02^b	38.85 ± 0.02^d	12.46 ± 0.10^b	2.80 ± 0.14^b	30.25 ± 0.02^b	0.69 ± 0.15^a
T2	13.45 ± 0.14^c	1.75 ± 0.05^c	2.82 ± 0.02^c	26.45 ± 0.21^c	22.53 ± 0.03^c	5.85 ± 0.21^c	32.14 ± 0.01^c	0.67 ± 0.01^a
T3	10.50 ± 0.12^b	1.65 ± 0.12^b	3.04 ± 0.01^d	23.72 ± 0.03^b	33.45 ± 0.20^d	6.25 ± 0.03^d	32.37 ± 0.03^d	0.65 ± 0.02^a
T4	8.56 ± 0.05^a	1.35 ± 0.22^a	3.28 ± 0.01^e	12.50 ± 0.02^a	43.65 ± 0.15^e	9.58 ± 0.15^e	33.22 ± 0.10^e	0.66 ± 0.01^a

Values expressed as means ± SD ($n = 3$). Different letter superscripts express significant differences between values of the same column ($p < 0.05$)

Table 4 Crust and crumb color and firmness of breads prepared from different flour blends

Bread type	**Crust**			**Crumb**			**Brownness Index (Crust)**	**Crumb hardness (N)**
	L*	**a ***	**b ***	**L***	**a ***	**b ***		
Control	58.04 ± 0.01^{e}	12.53 ± 0.01^{a}	29.02 ± 0.12^{e}	70.44 ± 0.13^{e}	1.44 ± 0.01^{a}	21.67 ± 0.02^{e}	42.51 ± 0.02^{a}	15.56 ± 0.01^{a}
T1	55.82 ± 0.02^{d}	13.04 ± 0.02^{b}	28.53 ± 0.11^{d}	60.41 ± 0.12^{d}	4.18 ± 0.02^{c}	25.67 ± 0.03^{d}	45.43 ± 0.01^{b}	18.45 ± 0.02^{b}
T2	49.27 ± 0.03^{c}	13.16 ± 0.02^{c}	27.44 ± 0.02^{c}	59.19 ± 0.03^{c}	3.71 ± 0.02^{b}	22.77 ± 0.03^{c}	54.67 ± 0.12^{c}	19.25 ± 0.01^{c}
T3	42.03 ± 0.01^{b}	14.59 ± 0.03^{d}	23.74 ± 0.03^{b}	56.16 ± 0.01^{b}	5.17 ± 0.01^{e}	22.23 ± 0.02^{b}	68.32 ± 0.12^{d}	25.50 ± 0.01^{d}
T4	34.55 ± 0.03^{a}	15.14 ± 0.02^{e}	16.86 ± 0.03^{a}	53.75 ± 0.02^{a}	4.92 ± 0.03^{d}	20.91 ± 0.01^{a}	88.43 ± 0.11^{e}	35.65 ± 0.01^{e}

Values expressed as means ± SD ($n = 3$). Different letter superscripts express significant differences between values of the same column ($p < 0.05$)

decreased from 29.02 to 16.86 with increasing amounts of rhizome flour in breads, indicating that the color of the breads became increasingly reddish-black. The Brownness index of the bread crust was increased with increasing levels of rhizome flour addition. The value of b^* indicates yellowness in the bread, which may be given by wheat flour portion but the b^* value gradually decreased for rhizome flour incorporated breads which can be explained because of the red hue of the *N. nucifera* and *L. spinosa* rhizome flours. A similar pattern in changing L^*, a^*, and b^* color values in kidney bean and malted finger millet incorporated breads (Bhol & Bosco 2014).

The hardness of bread is defined as the maximum force required to compress the bread. The hardness of the bread crust was significantly high ($p < 0.05$) with an increasing incorporated amount of rhizome flour into the breads. The hardness of breads increased with the addition of rhizome flour containing a higher amount of dietary fiber may be due to the inhibition of proper gluten network formation and the reduction of the capacity of gas retention of the dough by constituents in rhizome flour. Increasing the hardness of breads when incorporating non-wheat sources such as red kidney bean flour (Bhol & Bosco 2014), and finger millet flour (Patil et al. 2016) was reported in line with the results of the present study.

Physical parameters of breads

The loaf volume, loaf weight and specific volume were determined in formulated breads, and the results are shown in Table 5.

The weight of bread loaves was significantly ($p < 0.05$) increased when the level of rhizome flour substitution increased. The weight of the bread loaf is determined by the quantity of dough baked and the amount of carbon dioxide and moisture released out of the loaf during baking. The reduction of carbon dioxide retention ability in composite flour dough results in a subsequent increase in loaf weight. In line with the present study, Menon et al. (2015) reported an increase in loaf volume in high protein soy flour, sprouted mung bean flour and mango kernel flour incorporated breads compared to 100% wheat bread. Results of loaf volume and specific volume showed that control bread and 20 and 40% levels of rhizome flour incorporated breads had no significant ($p < 0.05$) difference. However, 60 and 80% rhizome flour incorporated breads showed lower levels of loaf volume and specific volume. Gluten activity is responsible for proper dough development and is reflected by a high volume. The reduced volume observed in a high percentage of rhizome flour incorporated breads may be due to the dilution of gluten though gluten powder was added to the formulation. The high dietary fiber content in both rhizome flours may interfere with protein hydration thus, the gluten network may dilute in bread dough. In line, with this observation, Penella et al. (2013) reported a 3.55% decrease in a specific volume of bread prepared with 30% amaranth flour incorporation.

Table 5 Effect of substitution ratio on loaf weight, loaf volume and specific volume of *L. spinosa* and *N. nucifera* flour incorporated breads

Samples	**Loaf weight (g)**	**Loaf volume (cm^3)**	**Specific volume (cm^3)**
Control	233.25 ± 1.77^{a}	561.50 ± 1.50^{c}	2.46 ± 0.08^{c}
T1	232.67 ± 2.24^{a}	560.00 ± 0.01^{c}	2.40 ± 0.02^{c}
T2	230.22 ± 2.64^{a}	558.50 ± 1.50^{c}	2.39 ± 0.01^{c}
T3	236.84 ± 2.72^{c}	550.00 ± 0.05^{b}	2.11 ± 0.03^{b}
T4	236.07 ± 2.92^{c}	548.60 ± 0.01^{a}	1.96 ± 0.02^{a}

Values expressed as means ± SD ($n = 3$). Different letter superscripts express significant differences between values of the same column ($p < 0.05$)

In vitro antidiabetic activity of formulated breads

Diabetes mellitus is considered one of the most common non-communicable diseases characterized by elevated fasting blood glucose levels (≥ 125 mg/dl). Reducing postprandial blood glucose levels by inhibiting carbohydrate hydrolyzing enzymes has been recognized as an effective treatment against diabetes. In this study, in vitro α-amylase and amyloglucosidase inhibitory activities of control and formulated breads were analyzed and results showed in Fig. 1.

The α amylase and amyloglucosidase inhibition action ranged from 27.65–88.55% and 25.75–89.75% in control to 80% substitution level. The highest α amylase and amyloglucosidase inhibition actions were observed in 80%, the maximum substitution level. Since the incorporation of rhizome flours, the bread contains more functional food substances such as dietary fiber, resistant starch and other bioactive compounds which may contribute to enzyme inhibitory action. Since the formulated breads showed an anti-diabetic effect, they may be considered as functional bread with further bioassays for different health benefits. Research conducted by Olagunju et al. (2021) reported as multigrain bread prepared to incorporate amaranth acha grain flours showed significantly higher α amylase and α glucosidase inhibitory activities proving it has better anti-diabetic properties compared to control wheat bread.

In vitro starch digestibility analysis

Figure 2 shows the effect of bread with incorporated rhizome flours on starch digestibility in comparison with 100% wheat bread (control).

Glucose release was measured as the reducing sugars degraded from starch by digestive enzymes. Generally, starch hydrolysis sharply increased up to 15 min, and then gradually increased at a rhizome flour compared to control bread. The lowest rate of starch digestibility pattern was observed in 80% of substituted bread. High dietary fiber in rhizome flours acts as a physical barrier between digestive enzymes and starch which slows down the starch digestibility. Previous research reported that breads prepared by substitution of banana pseudo stem flour at a 10% level and added with some hydrocolloids

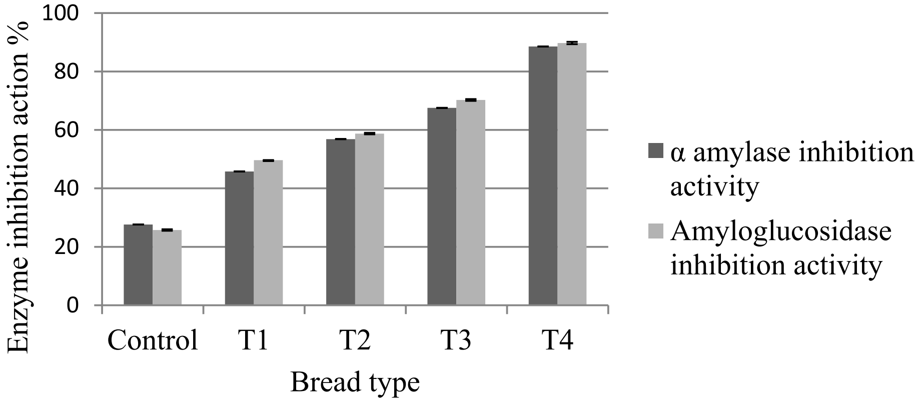


Fig. 1 α amylase and amyloglucosidase inhibitory activity of breads from different flour blends

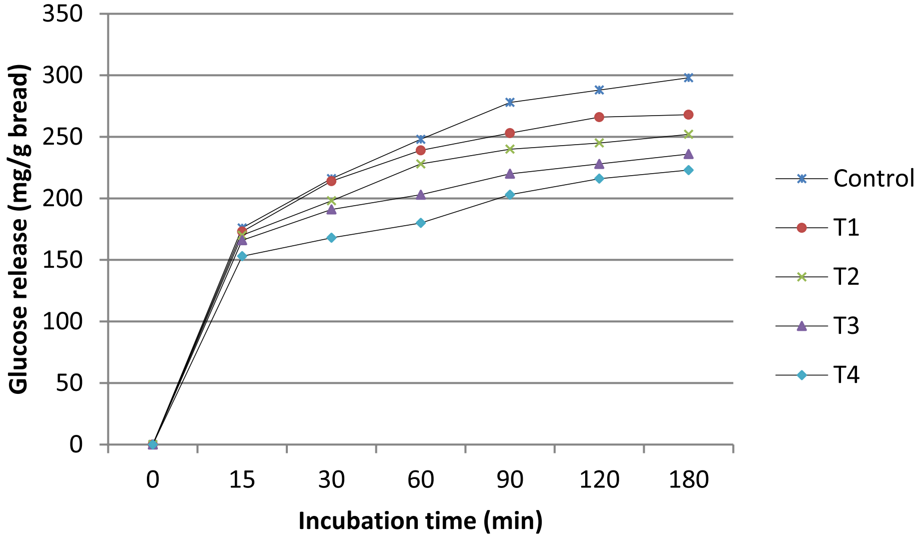


Fig. 2 Digestograms of breads from different flour blends

Table 6 Sensory properties of formulated breads

Bread type	Taste	Crumb color	Crust color	Crumb texture	Crust texture	Aroma	Overall acceptability
T1	10.16 ± 1.12^{c}	6.01 ± 2.5^{a}	6.57 ± 2.7^{a}	8.59 ± 1.0^{b}	7.43 ± 3.6^{a}	9.03 ± 2.0^{c}	9.71 ± 1.1^{d}
T2	8.56 ± 1.1^{b}	7.07 ± 3.5^{a}	7.08 ± 3.1^{a}	5.84 ± 2.4^{a}	8.28 ± 3.0^{a}	$8.68 \pm 2.9^{b,c}$	8.14 ± 1.2^{c}
T3	5.85 ± 3.0^{a}	9.81 ± 2.9^{a}	9.34 ± 3.1^{a}	5.39 ± 2.3^{a}	7.03 ± 2.4^{a}	6.70 ± 2.3^{b}	5.41 ± 2.7^{b}
T4	4.11 ± 2.4^{a}	7.66 ± 3.6^{a}	7.83 ± 3.5^{a}	5.34 ± 2.7^{a}	5.83 ± 2.7^{a}	4.00 ± 1.0^{a}	3.52 ± 1.4^{a}

Values are presented as mean ± SD, $n = 15$

resulted in a slower enzymatic hydrolysis rate than the control white bread (Ho et al. 2015).

Sensory evaluation of formulated breads

The sensory properties of formulated breads were evaluated by 15 trained panelists and descriptive analysis results were shown in Table 6.

Sensory analysis was conducted to compare four different bread formulations with 20, 40, 60 and 80% rhizome flour incorporation. The higher mean value is more preference on taste/ crumb color/ crust color/ crumb texture/ crust texture/ aroma and overall acceptance. Descriptors for blends with different superscripts in each column are significantly different from each other ($p < 0.05$).

It was found that significantly higher ($p < 0.05$) values for taste, crumb texture and aroma were obtained by T_1. However, there were no significant ($p < 0.05$) differences in crumb color, crust color and crust texture of formulated breads though the substitution levels of rhizome flours varied. Overall acceptability on formulated breads had a significant difference ($p < 0.05$) among each bread sample. The highest value for overall sensory acceptability was observed on $T_{1.}$ The results showed 20% rhizome flour incorporated bread was given the highest sensory acceptability compared to 40, 60 and 80% incorporation.

Conclusion

This research was focused on formulating functional food products and recognizing those nonwheat flour sources that could be used in the food industry thereby saving foreign exchange by reducing the quantity of wheat importation to the country. Also, the increase in the use of composite flour is improving local agro-business and it would encourage farmers to grow more of those kinds of underutilized food crops. Therefore, leavened breads were made with different incorporation levels of *Lasia spinosa* and *Nelumbo nucifera* and the bread made by substituting *Lasia spinosa* and *Nelumbo nucifera* rhizome flours for wheat flour at a 20% level (*Lasia spinosa*: *Nelumbo nucifera* = 10%: 10%) was selected as the best formulation with the significantly highest ($p < 0.05$) sensory acceptance. Dietary fiber content, resistant starch content and antidiabetic activity increased and starch digestibility rate decreased with increased substitution levels of rhizome flours. However, breads became less acceptable in their physical parameters like loaf volume, specific volume and hardness when increasing substitution levels though, they showed higher nutritional and functional properties. These two rhizome flours can be used in preparing other bakery products even, as a substitution for wheat flour.

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Authors' contributions
NNGC conceptualized the study, designed experiments, KDPPG supervised data collection and the study, NNGC analyzed data, prepared the draft manuscript, KDPPG and AC read and edited the manuscript. All authors read and approved the manuscript.

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Availability of data and materials
All data supporting this study are included in this manuscript. Further details are available upon request from the corresponding author.

Declarations

Ethics approval and consent to participate
Informed written consent was obtained from each subject participating in the sensory evaluation. Ethical approval was obtained from the Ethics Review Committee of Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka (202110HI05).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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