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Impact of germination on the physicochemical and rheological properties of flours and starch granular morphology of *Panicum miliaceum* (Proso millet), *Paspalum scrobiculatum* (Kodo millet), and *Setaria italica* (Foxtail millet)

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A B S T R A C T

Germination is an inexpensive and effective technique that can increase the nutritional quality of flours and change their physicochemical and rheological properties. In this study, three underutilized millet species, namely, *Panicum miliaceum*, *Paspalum scrobiculatum*, and *Setaria italica* were germinated and subsequent changes in their carbohydrate composition, functional characteristics, rheological properties, antinutritional factors and starch granular morphology were analyzed and compared. Germination resulted in significant ($p < 0.05$) decreases of starch, amylose, amylopectin, and resistant starch contents while increasing the dietary fiber content of the studied millet flours. Water holding capacity and oil holding capacity significantly ($p < 0.05$) increased; meanwhile the swelling power and water solubility significantly ($p < 0.05$) decreased in all studied millet flours. Starch granule morphologies were proved by the changes in starch degradation upon germination process. Antinutrients such as phytate and oxalate were significantly lower in germinated millet flours. These findings impress the application of germination in developing nutritionally rich flours with altered functional and rheological properties to incorporate into functional food formulations.

Keywords: Germination; Millet; Physicochemical properties; *Panicum miliaceum*; *Paspalum scrobiculatum*; *Setaria italica*

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1. Introduction

The utilization of cereals in the food industry is rising due to their phytochemicals and dietary fibers which can promote human health and well-being (Owheruo & Ifesan, 2018). *Panicum miliaceum* (Proso millet), *Paspalum scrobiculatum* (Kodo millet) and *Setaria italica* (Foxtail millet) are three types of millets belonging to the family Poaceae. Evidences reflect that these selected millet varieties have medicinal values such as antidiabetic, anti-hypertensive, anti-oxidant, anti-proliferative and anti-atherogenic (Chiranthika et al., 2020). The utilization of millet in functional food formulations is high in developing countries. However, except finger millets above-mentioned millet species are still found to be underutilized in Sri Lanka. It has been reported that millets can compete and replace conventional cereals such as wheat, rye, oats, and barley while delivering more nutrients and additional health benefits to consumers who are in any age category (Satish et al., 2017). *S. italica*, *P. scrobiculatum*, and *P. miliaceum* are prominently consisted of starch while dietary fibers are available including resistant starch in considerable levels as functional food ingredients. Dietary fibers are digested slowly in the human large intestine and consumers are satisfied with low calories due to its bulkiness. Resistant starch (RS) is a type of starch that resistant to digest in human small intestine and cereals contain RS type 1. Intake of cereal dietary fibers and RS can reduce the risk of non-communicable diseases such as type II diabetes mellitus, cardiovascular diseases, and certain types of cancers.

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In relation to cereal-based functional food development, more attention is on improving the existing nutritional and physicochemical qualities of flours. There are some pre-processing techniques which can improve the nutritional, physicochemical, and functional properties of cereals for the betterment of consumers. Germination is an inexpensive and effective technique that can improve the nutritional, physicochemical, and functional properties of cereals. Due to the germination effect, enzymatic hydrolysis of starch, protein, and fat is occurred and converts them to simple and more absorbable sugars, peptides, and fatty acids. Furthermore, bioactive compounds such as dietary fiber content are also increased. Besides, elevating the nutritional value, germination can effect on physicochemical and rheological properties, enhancing the quality of final product (Yang et al., 2021). Hence, there is more attention to germinated grains rather than consuming them in native form. Hence, it impresses the importance of studying about those unconventional millet species to evaluate the capability of using them in functional food product formulations with potential health benefits. Therefore, the objective of this study was to evaluate the impact of germination on the physicochemical and rheological properties of flours and the morphology of starch granules of *P. miliaceum*, *P. scrobiculatum* and *S. italica* which are available in Sri Lanka. Findings of this study may increase the utilization of locally available underutilized millet species in the development of functional food products and that the information could be useful even for other countries which cultivated these millets.

2. Material and Methods

2.1. Materials

P. miliaceum, *P. scrobiculatum*, and *S. italica* were purchased from local market, Sri Lanka. Potato starch, amylose (standard for amylose determination), sodium hydroxide, hydrochloric acid, acetone, ethanol, phosphoric acid (analytical grade), amylase, pancreatin, amyloglucoidase and all other chemicals were purchased from Sigma (Sigma Aldrich Co., St. Louis, MO, USA).

2.2. Sample preparation

2.2.1. Germination of millet grains

The germination procedure in Yang et al. (2021) was slightly modified and followed. Grains of the selected three millet species were thoroughly washed with distilled water to remove dust and other external materials before being soaked them for 24 hrs. After soaking, the soaked water was drained out and the seeds were layered on tissues and kept at room temperature in dark condition. After the seeds got germinated, the root hairs were removed and dried them in a hot air oven (MEMMERT NLE 500, Germany) at 45°C until becoming to a constant weight.

2.2.2. Flour preparation from P. miliaceum, P. scrobiculatum, and S. italica

All three native and germinated grains were grounded using a laboratory scale grinder (Philips HL772, Thailand) followed by sifting through a 250 µm sieve. Flour samples were packed and stored at -20℃ for starch isolation and further analysis. Moisture contents of the flour samples were at 14%.

2.3. Isolation of starch

Starch was isolated from both native and germinated millet grains by alkali extraction described in (Sodhi & Singh, 2003). Flour samples were steeped in 5 volumes of 0.2% sodium hydroxide solution for 24 h to facilitate starch isolation. The steeped liquor was removed and then the slurry was diluted by adding the prepared sodium hydroxide solution. Then the mixture was stirred using a magnetic stirrer for 10 minutes and it was kept to settle overnight. The supernatant (cloudy part) was removed, and the remaining was again diluted to the original volume by adding sodium hydroxide solution. The previous step was repeated until the supernatant observed as clear and formed a negative result to the biuret test. Then the starch was suspended in distilled water and washed 2-3 times with distilled water until the supernatant showed no any pink colour to phenolphthalein. Finally, the starch was collected and dried in a hot air oven (MEMMERT NLE 500, Germany) at 45℃.

2.4. Chemical composition of carbohydrate

2.4.1. Total starch content

The total starch contents of native and germinated flours were determined following the procedure described by Bjorck et al. (1985). The flour samples (100 mg) were weighed in to test tubes. Then 0.2 mL of 80% ethanol was added to each sample and the solution was mixed using a vortex mixture for 15 seconds. Cold sodium hydroxide (2.0 mL, 1.7 M) was added and the mixture was stirred for 15 minutes using a magnetic stirrer in an ice water bath. Then 8.0 mL of sodium acetate buffer (600 mM, pH 3.8) was added and stirred the mixture. Then 0.1 mL of thermo-stable α amylase was added immediately and followed by 0.1 mL of amyloglucosidase was added. The mixture was kept for incubation at 50℃ for 30 minutes. Then the tubes were removed and allowed to cool up to room temperature. An aliquot of 2.0 mL from each sample was transferred to microcentrifuge tubes and they were centrifuged at 12,000 rpm for 5 min. The amount of 1.0 mL of the supernatant solution was transferred to a test tube containing 4mL of 100 mM sodium acetate buffer (100 mM, pH 5.0) plus calcium chloride (5 mM). Then 0.1 mL of aliquot from each sample was transferred to test tubes and 3.0 mL of GOPOD reagent was added. The mixtures were incubated at 50℃ for 20 minutes and absorbance readings were taken at 510 nm by UV/ Visible spectrophotometer (Thermo Scientific 201, United State). Finally, the total starch content was calculated.

2.4.2. Amylose and amylopectin

Amylose content of native and germinated flour samples was determined following the modified method in Chrastil (1987). Lipid extraction was done prior to the quantification of amylose. Flour samples (100 mg) from each were measured into centrifuge tubes and 10.0 mL of petroleum ether was added to extract the fat from the flour samples. Samples were incubated for 30 min at 60°C and followed by centrifuged at 3000rpm for 15 minutes and the supernatant solution was discarded. Lipid extraction of flour

samples was done for three times. After lipid extraction, 09 mL of 1M sodium hydroxide and 1 mL of ethanol were added in to each lipid free sample contained in 100 mL volumetric flasks. The mixtures were heated at 95℃ for 15 min in a water bath and followed by allowing cooling before making up to 100 mL with distilled water. Aliquot of 5 mL was pipetted out to another 100 mL volumetric flask and followed by the addition of 1 mL of 1N acetic acid and 2 mL of iodide solution and made up to 100 mL with distilled water. The blue color was read at 620 nm after 30 min at 25℃ by UV/ Visible spectrophotometer (Thermo Scientific 201, United State). Potato amylose was used as the standard and the amylose contents of the samples were analyzed regarding the standard curve. Amylopectin content was expressed as a percentage as a deduction of amylose content of the sample from the total starch content of the same sample.

2.4.3. Total dietary fiber

Total dietary fiber content was determined using the modified AOAC enzymatic-gravimetric procedure (Prosky, 1979). One gram from each flour sample was weighed and 50 mL of phosphate buffer (pH 6.0) was added to each weighed sample. Then the samples were heated with 0.1 mL of termamyl alpha-amylase at 95℃ for 15 minutes and then digested with 5.0 mg of protease for 30 minutes at 60℃ followed by incubated with 0.3 mL of amyloglucosidase at 60℃ for 30 minutes. Ethanol (95%, four volumes) was added to precipitate dietary fiber and kept for overnight. Then the solution part was filtered out and the precipitate was washed off using 78% ethanol, 95% ethanol, and acetone. The precipitate was then oven-dried at 105^{o}C for overnight in a hot air oven (MEMMERT NLE 500, Germany) and then weighed. Values obtained by the enzymatic method were then corrected by analyzing protein and ash in the samples.

2.4.4. Resistant starch

Resistant starch contents of the isolated starches were determined using the Megazyme's assay kit (Megazyme International Ltd, Bray, Ireland). Initially, 100 mg of sample was weighed and 4.0 mL (10 mg/mL) of α amylase from the pancreas containing amyloglucosidase (3 U/mL) was added. The mixture was incubated at 37℃ exactly for 16 h and subsequently 4.0 mL of 99% ethanol was added with vigorous stirring and centrifuged (1500g for 10 minutes). The supernatant was poured out and again suspended with 2.0 mL portion of 2 M KOH in an ice water bath. The mix was incubated with 8.0 mL of sodium acetate buffer (1.2 M, pH 3.8) and 0.1 mL portion of amyloglucosidase enzyme at 50℃ for 30 minutes. The 0.1 mL of aliquot was transferred in to a glass test tube and followed by incubation was done by adding GOPOD reagent at 50℃ for 30 minutes. The absorbance values were measured at 510 nm.

2.5. Functional properties

2.5.1. Water holding capacity (WHC) and oil holding capacity (OHC)

Water and oil holding capacity of millet flour samples were analyzed by a centrifugal procedure as described by Traynham et al. (2007) with slight modifications. 0.5 g of flour samples were suspended with 5 g water or oil in a centrifuge tube. The slurry was mixed in a vortex mixture (ZX3, Thomas Scientific, United States) for 1 min at room temperature and centrifuged at 3000g for 10 min (Spectrafuge™, Corning, Inc, United States). The supernatant was poured carefully into a tared evaporating dish. Then the water holding capacity and oil holding capacity were calculated as in equation (1), respectively.

$$WHC\ or\ OHC\ (g/g) = \frac{V_1 - V_2}{V_3} \quad (1)$$

where V_1 was water or oil added to the sample (g), V_2 was water or oil removed from the sample (g) and V_3 was mass of flour sample (g).

2.5.2. Swelling power and water solubility

Swelling power was analyzed by following the method described in Kumoro (2019) with slight modifications. The flour samples (0.1 g) were heated with 10.0 mL of distilled water in a heating water bath at 95°C for 30 minutes with gentle mixing. The flour samples were centrifuged at 1600 rpm for 15 minutes. The weight of the precipitate was measured and calculated using the following equation (2).

$$Swelling\ poer\ (g/g) = \frac{Weight\ of\ sedimental\ paste}{Weight\ of\ the\ sample \times \left[1 - \frac{Water\ solubility}{100}\right]} \quad (2)$$

Water solubility was determined by the method describer in Kumoro (2019) with slight modifications. The 0.5 g samples of the tested millet flours were heated in a 10 ml distilled water bath at 95°C for 30 minutes without mixing. Each sample centrifuged at 1600 rpm for 10 minutes. The supernatant was collected (5.0 ml), dried, weighed, and calculated using the equation (3).

$$Water\ solubility\ (\%) = \frac{Weight\ of\ soluble\ sample}{Initial\ weight\ of\ sample} \times 100 \quad (3)$$

2.6. Differential scanning colorimetry

The thermal characteristics of native and germinated starches of three millet species were measured using a differential scanning colorimeter (DSC Q200, TA Instruments, India). The samples (about 10 mg) and distilled water were placed in a DSC hermetic aluminium pan. The sample was sealed and heated in the machine from 20°C to 85°C (10 °C/min). An empty aluminium pan was used as the reference. The temperatures were reported as onset (To), peak/melting (Tm), and conclusion (Tc).

2.7. Visco amylograph

Gelatinization characteristics, which reflect the rheological properties of flours, were determined using Brabender Visco-amylograph (BRBENDER OHS, DUISBURG, Germany) according to the standard operational instructions of the particular instrument (AACC 22-10). Eighty grams of sieved flour were

weighed and mixed firmly in a beaker with 450 mL water to smooth the slurry without lumps. This slurry was poured into the central bowl. The probe was placed carefully in the center of the bowl. The instrument was allowed to continue working until the temperature reaches 95°C. When a temperature of 95°C was reached, the slurry was held at 95°C for 30 min, followed by cooling from 95°C to 50°C. It was again held for 30 min at 50°C before ending the process.

Table 1. Chemical composition of carbohydrate of native and germinated *P. miliaceum*, *P. scrobiculatum* and *S. italica* millet species.

Crop	Treatment	Total starch %	Amylose %	Amylopectin %	Total Dietary fiber %	Resistant starch %
P. miliaceum	Native	70.9±1.3^{f}	32.5±0.35^{f}	38.40±0.35^{e}	8.70±0.12^{b}	3.80±0.01^{d}
	Germinated	60.8±0.67^{c}	25.8±0.12^{c}	35.00±0.12^{a}	19.01±0.40^{e}	2.20±0.23^{b}
P. scrobiculatum	Native	63.5±0.7^{e}	26.0±0.84^{e}	37.50±0.84^{c}	8.11±0.88^{a}	2.63±0.14^{c}
	Germinated	56.2±1.2^{b}	20.0±0.45^{b}	36.20±0.45^{b}	17.94±0.22^{d}	1.85±1.01^{a}
S. italica	Native	61.7±1.2^{d}	23.8±1.1^{d}	37.90±1.1^{d}	14.18±1.32^{c}	7.10±0.32^{f}
	Germinated	53.4±1.6^{a}	18.6±0.47^{a}	34.80±0.47^{a}	32.93±0.85^{f}	4.70±0.26^{e}

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

Table 2. Functional properties of native and germinated flours obtained from *P. miliaceum*, *P. scrobiculatum* and *S. italica* millets.

Crop	Treatment	Water holding capacity (g/g)	Oil holding capacity (g/g)	Swelling power (g/g)	Water solubility %	Water holding capacity (g/g)
P. miliaceum	Native	2.77±0.08^{b}	1.51±0.24^{f}	15.01±0.011^{f}	5.44±0.01^{f}	2.77±0.08^{b}
	Germinated	3.88±1.02^{e}	1.96±0.15^{c}	7.33±0.01^{a}	1.39±0.57^{b}	3.88±1.02^{e}
P. scrobiculatum	Native	2.08±0.11^{a}	1.47±0.12^{e}	12.91±0.071^{e}	4.62±0.32^{e}	2.08±0.11^{a}
	Germinated	3.50±0.70^{d}	1.77±0.13^{b}	10.25±0.017^{d}	2.02±0.12^{c}	3.50±0.70^{d}
S. italica	Native	3.25±0.72^{c}	1.38±0.85^{d}	9.51±0.18^{c}	4.22±0.18^{d}	3.25±0.72^{c}
	Germinated	5.87±1.01^{f}	1.56±0.33^{a}	8.08±1.24^{b}	1.24±0.08^{a}	5.87±1.01^{f}

Results are expressed as mean values ± standard deviations. Different letter superscripts express significant differences between values of the same column ($p < 0.05$).

Table 3. Effect of germination on antinutritional factors in *P. miliaceum*, *P. scrobiculatum* and *S. italica*.

Crop	Treatment	Phytic acid (mg/g)	Oxalate (mg/g)
P. miliaceum	Native	8.8±0.02^{f}	5.8±0.21^{f}
	Germinated	7.2±0.01^{d}	4.3±0.14^{c}
P. scrobiculatum	Native	7.4±0.01^{e}	5.3±0.12^{e}
	Germinated	6.2±0.10^{c}	2.8±0.01^{a}
S. italica	Native	5.6±0.01^{b}	4.6±0.01^{d}
	Germinated	3.5±0.12^{a}	3.1±0.11^{b}

Results are expressed as mean values ± standard deviations. Different letter superscripts express significant differences between values of the same column ($p < 0.05$).

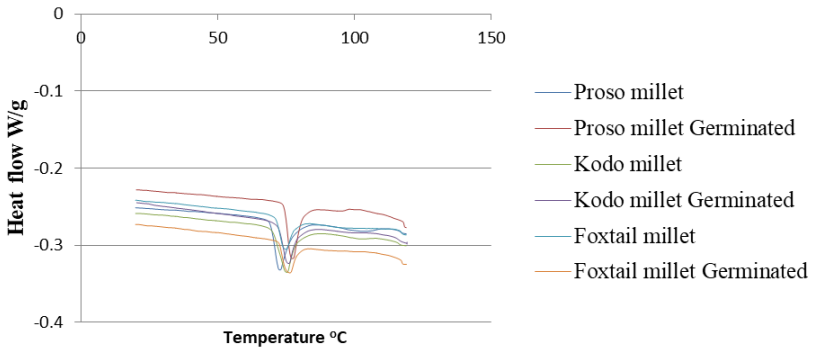

Fig. 1. Thermograms of native and germinated millet starches.

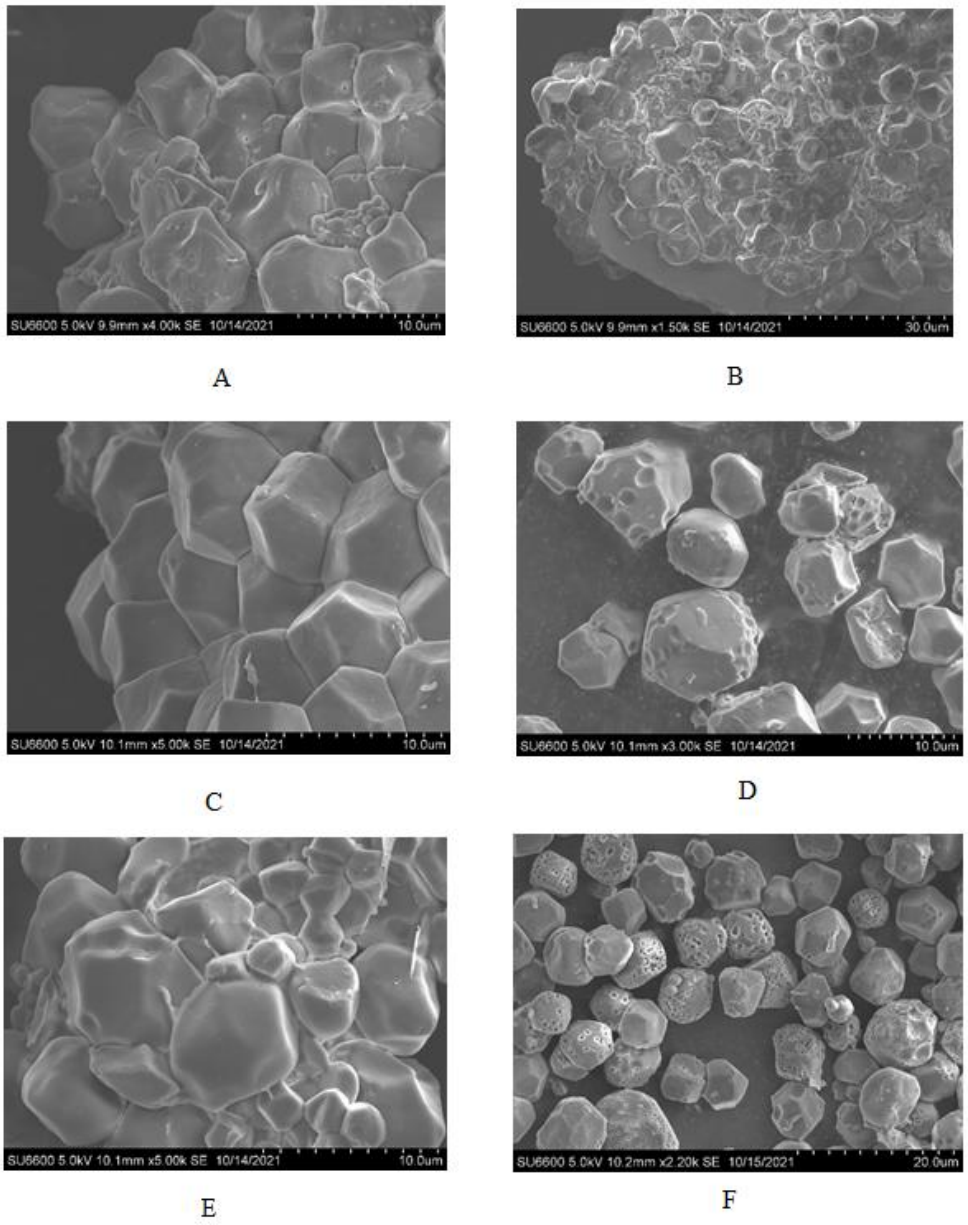

Fig. 2. Scanning electron microscopic images of starches isolated from (A) native *S. italica*, (B) germinated *S. italica*, (C) native *P. scrobiculatum*, (D) germinated *P. scrobiculatum*, (E) native *P. miliaceum* and (F) germinated *P. millaceum*.

2.8. Scanning electron microscopy

Starch granular characteristics of starch obtained from native and germinated millet flour samples were observed. Samples were mounted on aluminium stubs with sticky conductive carbon tape to adhere the samples to the stubs and the stubs were vacuum metalized with gold by Ion Sputter (HITACHI E 1010). Microscopic images were observed using a scanning electron microscope (HITACHI SU2200, Japan) at 5 kV accelerating voltage, a magnification of 1000×and a distance of 9.9 mm to 10.2 mm.

2.9. Antinutritional factors

2.9.1. Phytic acid content

Phytic acid was analyzed with slight modification to the method described in Reddy (1978). Two grams of the flour sample was measured into a beaker and soaked in 100 ml of 2% HCl. After soaking for 5h, it was filtered. Then 25 ml of the filtrate was transferred into a conical flask followed by 5 ml of 0.3% potassium thiocyanate solution was added. The mixture was then titrated with

standard ferric chloride ($FeCl_3$) solution. Presence of brownish-yellow colour for 5 min is indicating the end point. Phytic acid content was calculated as in equation (4) by assuming that it contains 20% phosphorus by weight.

$$Phytic\ acid\ concentration = \frac{Titrate\ volume \times 0.064}{100 \times Sample\ weight} \quad (4)$$

2.9.2. Oxalate content

Titrametric method was used to analyze oxalate content in native and germinated millet flours. Sample of 1 g was measured into a 100 mL conical flask and 75 mL of 3M H_2SO_4 solution was added. The mixture was stirred for 1h using a magnetic stirrer. Then the mixture was filtered through Whatman No 1 filter paper. Twenty five milliliters of filtrate was taken and titrated with hot 0.1N $KMnO_4$ solution. Presence of faint pink colour for 30 s was taken as the end point. It was then filtered using Whatman No.1 filter paper. From the filtrate, 25 ml was taken and titrated against hot (80-90°C) 0.1N KMnO4 solution up to a faint pink color persisted for at least 30 s (Nissar et al., 2017).

2.10. Statistical analysis

All 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 the Tukey's test in SPSS 16.0 software.

3. Results and Discussion

3.1. Effect of germination on the chemical composition of carbohydrates of selected millet species

Chemical compositions of carbohydrates in native and germinated millet flours of *P. miliaceum, P. scrobiculatum,* and *S. italica* are shown in Table 1. The total starch, amylose, amylopectin, dietary fiber, and resistant starch contents were significantly difference ($p < 0.05$) in three millet species and native flour of a particular millet species was significantly different from germinated flour's carbohydrate composition. Starch content of a particular food crop vary depend on some factors such as genetic structure, geographic area, and maturity stage. The total starch contents in native flours were ranged from 61.7±1.2 to 69.9±1.3%. The highest significant value of total starch content in native flours was observed in *P. miliaceum* while the lowest starch content was in *S. italica* species. A significant decrease ($p < 0.05$) in the total starch content was found after germination of all three millet species. Starch content of *P. miliaceum* decreased from 69.9 to 60.8% and the starch contents of *P. scrobiculatum* and *S. italica* decreased from 63.5 to 56.2% and 61.7 to 53.4% respectively. The approximate reduction percentages of starch contents in *P. miliaceum, P. scrobiculatum,* and *S. italica* were as 13%, 11.5%, and 13.5% respectively. Amylose and amylopectin are the major biopolymers of starch. Unique physicochemical and functional characteristics of a particular starch mainly are depending on the individual proportions of amylose and/or amylose and amylopectin ratio. Native *P. miliaceum, P. scrobiculatum, and S. italica* grains consisted with 35.5, 26.0 and 23.8% of amylose contents, respectively. The data in Table 1 indicate the significant reduction ($p < 0.05$) of amylose content after germination. Amylose contents of native and germinated three millet flours ranged from 32.5 to 25.8%, 26.0 to 20.0%, and 23.8 to 18.6% in *P. miliaceum, P. scrobiculatum and S. italica* respectively. Previous studies have also shown similar results that reflect the impact of germination on decreasing starch and amylose contents in *P. miliaceum* (Yang et al., 2021) and using *S. italica* (Nazni & Devi, 2016). Amylopectin content also decreased after germination of the studied millet flours. Furthermore, as a range, they were reduced from 38.4 to 35.00%, 37.50 to 36.20%, and 37.90 to 34.80% in *P. miliaceum, P. scrobiculatum and S. italica*. The reduction of total starch, amylose, and amylopectin contents in germinated millet grains occurring compared to native flour samples may be due to the increasing of α amylase enzymatic hydrolysis of starch to form simple and more absorbable sugars in respiration metabolism to provide energy for germinating seedlings (Li et al., 2020).

When considering the total dietary fiber contents of the studied native flours, *S. italica* showed the significantly ($p < 0.05$) the highest dietary fiber content (14.18%), meanwhile *P. scrobiculatum* and *P. miliaceum* showed 8.70% and 8.11% of total dietary fiber contents respectively. After the germination process, there was a significant increasing pattern ($p < 0.05$) of dietary fiber contents in all three millet flour samples. The total dietary fiber contents were increased in *P. miliaceum* from 8.70 to 19.01%, in *P. scrobiculatum* from 8.11 to 17.94%, and the highest increment was observed in *S. italica* from 14.18 to 32.93%. Similar results was obtained by (Sharma et al., 2017) by evaluating the germination effect of *P. scrobiculatum.* During the germination process, the structure of the polysaccharides in the cell wall matrix gets modified to induce a new primary cell wall and interruption of protein-carbohydrate complex also occurred. These changes may result the formation of new dietary fibers for cell wall biosynthesis and increase the total dietary fiber content (Sharma et al., 2017). Since evidences show that dietary fiber can provide several health benefits against non-communicable diseases such as type II diabetes mellitus, cardio vascular diseases, and cancers, germinated flour can be incorporated into food products to obtain more nutritional value.

Cereals contain resistant starch type I (RS I), which has been underlined as a functional food ingredient providing therapeutic effects against the risk of non-communicable diseases (Aigster et al., 2011). Results in Table 1 reflect the RS contents in native millet starch and the changing of RS content with the effect of germination. The highest significant ($p < 0.05$) level of RS content was in native *S. italica* species (7.10%), while native *P. miliaceum* and *P. scrobiculatum* contained 3.80% and 2.63 % of RS respectively. However, there was a reduction of RS content in all three germinated millet starches after germination. RS is a type of starch resistant digest in human small intestine and fermented in human colon by the activity of gut microflora and produce beneficial short chain fatty acids. During germination, the starch molecules become loose and smooth and susceptible to degradation by amylolytic enzymes. Due to the enzyme activity, pores are made on starch granule surfaces and give a chance to enzymes to penetrate and act on starch degradation. Although RS is resistant to digest, some proportions could be undergone for degradation and that may be the reason for reducing RS content in germinated starch compared to native starch in the present study. This result is linear with the previous studies conducted by (You et al., 2016).

3.2. *Effect of germination on the functional properties of selected millet grain flours*

Functional properties of native and germinated millet flours of *P. miliaceum*, *P. scrobiculatum*, and *S. italica* are shown in Table 2. Water holding capacity (WHC), oil holding capacity (OHC), swelling power, and water solubility were significantly difference ($p < 0.05$) among three millet species and these parameters were significantly differences ($p < 0.05$) among native and germinated flours of each studied millet species.

Water holding capacity (WHC) of a particular flour reflects as the ability to hold its own or/and added water under processing conditions such as pressing, centrifuging, or heating (Traynham, 2007). In native flour samples, the highest significant ($p < 0.05$) level of WHC was observed in *S. italica* while the lowest WHC was shown by *P. scrobiculatum*. After germination, the results exhibited the significant increasing ($p < 0.05$) of WHC in all three millet species. The increment was ranged as 2.77 g/g to 3.88 g/g for *P. miliaceum*, 2.08 g/g to 3.50 g/g for *P. scrobiculatum* and 3.25 g/g to 5.87 g/g for *S. italica*. Dietary fiber content is one of the major factors that affects on the WHC of a particular flour (Al-Sheraji et al., 2011). Since dietary fiber can hold and retain more water, it has a positive correlation with WHC. Increasing of dietary fiber contents in germinated *P. miliaceum*, *P. scrobiculatum*, and *S. italica* flours could be the possible reason for increasing WHC in germinated flour compared to native flour and increasing the binding sites due to the breakdown of polysaccharides through germination could be resulted the higher WHC level. Furthermore, it is reported that germination can attribute the increasing of protein content and changing the protein qualities (Li et al., 2020). Since amorphous protein can absorb water, the increasing of protein upon germination may also be a possible reason for higher WHC levels in germinated flours. The flours which are having high WHC can be used in bakery food product preparation.

Oil holding capacity (OHC) is defined as the ability of flours to retain its inner or added oil while applying a force (Traynham, 2007). Regarding OHC of the studied three millet flours, a significant difference was observed in the native and germinated samples. Germination has attributed the increasing of OHC compared to native flour samples. There are some factors affected on OHC such as hydrophobic amino acids, nonpolar sites in polysaccharides (dietary fibers), and amylose that have hydrophobic cavities for fat molecule binding. The impact of germination on amylose and dietary fiber contents were analyzed in the present study. When considering the correlation between the obtained results of amylose content and OHC, there was no positive correlation. Although amylose content became reduced with germination, the OHC levels were high in the germinated samples. However, there was a positive relationship between dietary fiber content and OHC, since both factors were higher in germinated flours in all three species. Regarding the ability of hydrophobic proteins to bind fat molecules, it is reported that the germinations induces the exposing of hydrophobic sites in polypeptides by unfolding them and lipid molecules can physically bind to the sites while increasing OHC (Handa et al., 2017). OHC is essential in flavour retention of a product since fat/oil can bind and retain flavor compounds. Importantly, OHC of germinated *P. miliaceum*, *P. scrobiculatum*, and *S. italica* flours were found in this study are higher than OHC of wheat flour (1.26 g/g) (Joshi et al., 2015). Furthermore, these flours could be incorporated into bakery foods which required high OHC such as fried savoury snacks.

Swelling power and water solubility of starch in particular flour reflect the hydration ability which is important in food processing. The swelling power and water solubility are having a positive relationship with amylopectin fraction in starch since amylopectin is having three-dimensional branched structure, thus providing more space to hold water molecules. Related to the obtained results, starch and amylopectin contents became low after germination and similarly there was a significant reduction of swelling power and water solubility of the studied millet flours after germination. This result is linear with those reported previously (Azeez et al., 2022) by studying the germination effect on brown finger millet. However, some studies reported opposite results to the present findings that as swelling power and water solubility increased due to germination of waxy and non-waxy proso millets. The reason has been explained as the increasing of water soluble compounds, more and changes in protein and polysaccharides attributed to the increment of swelling power and water solubility of the studied proso millet flour in the previous studies.

3.3. *Starch gelatinization parameters analyzed by differential scanning colorimeter (DSC)*

Thermograms of the starch isolated from native and germinated millets are shown in Fig. 1. An endothermic peak around 60 to 90℃ was observed for all isolated starches from germinated and non-germinated millet grains in this study. When considering non-germinated grains, the highest peak gelatinization temperature was observed in *S. italica* while it contained significantly lower levels of amylose compared to the other two studied species. It has been explained as the gelatinization temperature and amylose content of starch have negative correlation, thus, higher amylose content consists of more amorphous and less crystalline regions, resulting as lower gelatinization temperature. The gelatinization temperatures as to, T_p and T_c were increased in all isolated starch samples after germination. The reason for that increasing gelatinization temperature after germination may be due to the reduction of available water (water activity) to facilitate the starch gelatinization process. In detail, various types of enzymes such as proteases and amylases are activated during germination and hydrolytic end products are produced such as sugars, amino acids, peptides, and non-starch polysaccharides that may compete with starch for available water, resulting reduction of water activity and consequent increasing of gelatinization temperatures (Chung et al., 2012). The increasing of gelatinization temperatures of germinated starches indicated that it would be required more energy to gelatinize the starch granules (AL-Ansi et al., 2021). The changes in gelatinization temperature could be attributed to the difference in shape and size of the granules, amylose content, relative crystallinity, and amylopectin chain length.

3.4. *Scanning electron microscopic images of starch granular morphologies of native and germinated millet species*

The scanning electron microscopic (SEM) technology is an important tool for observing the microstructures of various constituents contained in food crops. Fig. 2 presents the starch granular morphologies of native and germinated millet species. The results revealed evident structural differences between the two samples. The native starch granules (Fig 2 A, C, and E) of study on

millet species showed a polygonal shape with smooth surfaces and continuous structure. Germination causes starch degradation mainly by the enzymatic action of amylase and α-glucosidases. Due to the enzyme hydrolysis, the continuous structure has been destroyed and pins and pores were formed on the surface of starch granules making a rough surface. The pins and pores can be observed in starch granule specimens of germinated *S. italica*, *P. scrobiculatum* and *P. miliaceum* as show in Fig 2(B, D and F). The enzymes can penetrate through the pores and continue the starch degradation from inside the granule. The changes occurred in the surface and the increase in pores and pins, which are evidenced in the microscopic images of the millet species after germination, may be related to the enzymatic hydrolysis and starch degradation.

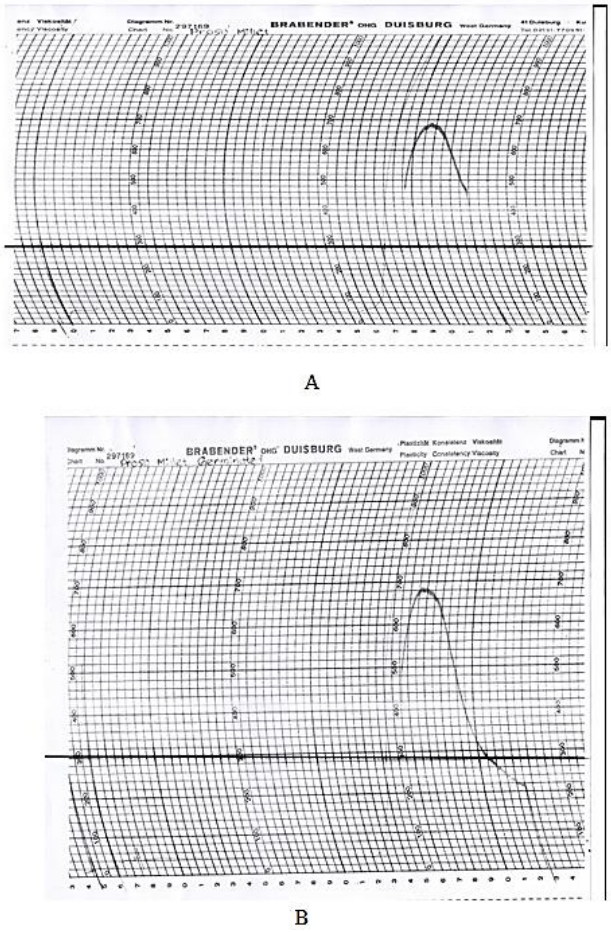

Fig. 3. Visco amylograms of (A) native and (B) germinated *P. miliaceum* flours.

3.5. *Visco-amylographs*

Pattern of the standard amylogram curve is unique to a material whose major component is starch. Presence of components other than starch in the sample might have an impact to deviate the curve from the standard amylogram. Except native and germinated flour samples of *P. miliaceum*, other samples of native and germinated *P. scrobiculatum* and *S. italica* deviated the standard amylogram curves without exhibiting any peak during the heating stage of the standard amylograph procedure. Pasting temperatures of native and germinated *P. millaceum* flours were 76.5 and 78℃, respectively. Peak temperature (92.25℃) was the same in both native and germinated *P. miliaceum* flour. Peak viscosity was higher in germinated *P. miliaceum* flour (1140 BU) compared to native *P. miliaceum* flour (1160 BU). It may assume that some component other than the starch in the sample, which can contribute to the friction during the starch gelatinization in the heating step of the standard amylograph procedure, have caused this deviation in the amylogrms curves of *P. scrobiculatum* and *S. italica* flours. Compositional study showed *P. miliaceum* contained a significantly higher proportion of starch compared to the other two millet species which were studied. Since starch is the subject which undergoes gelatinization, the low level of starch and increasing other components such as dietary fiber in *P. scrobiculatum* and *S. italica* may cause such non-detectable pattern of amylogram. Flours of the studied millet species were analyzed by visco amylograph without using isolated starches in them, since to determine flour characteristics with the purpose of applying flours directly to the food formulations. Fig. 3 shows the visco amylograms of native and germinated *P. miliaceum* flours.

3.6. *Antinutritional factors*

Phytic acid and oxalate contents were determined in native and germinated flours. Changes in antinutritional factors on the germination of the selected three millet species were shown in Table 3.

Phytic acid and oxalate are considered as antinutrients in cereals. However, in the safe range, they induce some beneficial effects. Phytic acid and oxalate are considered as antinutrients due to interference with mineral bioavailability. Obtained results showed germination has been significantly reduced phytate and oxalate contents in all studied millet species. Higher proportions of these antinutrients condense in the bran of whole grain cereals (Suma & Urooj, 2014) and they may remove by milling process. Since consumption of whole grain cereals is more beneficial than consuming milled grains, germination can be applied as a technique to reduce the antinutritional factors in cereals and consume them as whole grains.

4. Conclusion

Germination is a natural processing technique that can be used to change the composition, rheological properties, functional characteristics, starch granular morphologies, and antinutritional factors of flours and starch without chemical treatment. The findings of this study indicated that germination improved the nutritional richness of flours, such as increasing dietary fiber and decreasing antinutrients of the flours. The functional properties of flours were greatly changed by germination. The current study advances the current understanding of the effects of germination on the carbohydrate composition and different physicochemical and rheological parameters of *P. miliaceum*, *P. scrobiculatum* and *S. italica* flours with clear applications to improve the utilization of these minor cereals available in Sri Lanka.

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Conflict of interest

The authors declare that they do not have any conflict of interest.

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