



## Physicochemical and Functional Properties of Pumpkin Seed Flour of *Cucurbita maxima* and *Cucurbita moschata* Species

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### ABSTRACT

Pumpkin seeds are rich source of nutrients and beneficial bioactive compounds. In Sri Lanka, pumpkin seeds are discarded without being used. Physicochemical characteristics of seeds and functional properties of pumpkin seed flour (PSF) of two varieties of *Cucurbita maxima* (Suprema and *Bingha F1*) and one variety of *Cucurbita moschata* (*Padma*) were determined. Seeds collected from each variety were subjected to different treatments before preparation of PSF: roast at 80-90 °C for 15 min, germinate and dry at 50-60 °C for 4h, and dry at 50-60 °C for 4h as the control, prior to prepare PSF. Antioxidant activity, total phenolic, total flavonoids and flour properties such as water holding capacity (WHC), swelling capacity (SC), oil holding capacity (OHC), emulsion stability (ES), organic molecule absorption capacity (OMAC)) of nine types of PSF were determined. A sensory evaluation (Hedonic scale) was conducted to determine the effect of 25, 50 and 75% substitution with PSF in biscuit production. The variety × treatment interaction effect was significant ( $p < 0.05$ ) for WRC, OHC, SC and OMAC but not for WHC and ES. Germinated and dried PSF of the Suprema variety possessed the highest WHC ( $6.31 \pm 0.16$  g/g), OHC ( $3.23 \pm 0.07$  g/g) and SC ( $6.00 \pm 0.28$  mL/g) while the highest antioxidant activity ( $90.06 \pm 0.24\%$ ) and flavonoid content ( $19.87 \pm 1.08$  mg QE/g) were observed in germinated dried seeds of *Bingha F1* variety PSF. In roasted PSF, the highest phenolic content ( $1.52 \pm 0.02$  mg GAE/g) was in *Bingha F1* variety. Substitution with 25% PSF was selected as the optimum level in formulation of biscuit with the acceptable sensory attributes. Hence, PSF possesses a high potential as a source of functional food ingredient in the food manufacturing industry.



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## INTRODUCTION

Pumpkin belonging to Cucurbitaceae family is a seasonal crop that consists of succulent mesocarp and many seeds (Jin *et al.*, 2013) and traditionally used as a food and animal feed. Pumpkin is native to Central America and approximately 26 species are reported in the world. Arjuna, ANK Ruhuna, Meemini, Samson, Lanka and Squash are the common varieties of pumpkins cultivated in Sri Lanka (Dissanayake *et al.*, 2018). Orange colour of the skin and flesh of the pumpkin is due to the presence of carotenoids (Azizah *et al.*, 2009). Although pumpkin seeds are generally considered as a by-product, seeds are rich source of bioactive compounds (Montesano *et al.*, 2018). Pumpkin seeds are often utilized directly for human consumption as a snack in many countries. Sri Lankan consumers show a low preference for pumpkin seeds and products made of pumpkin seeds. The excellent economical benefits can be achieved by valorization of this agro-waste into value-added food ingredients of products.

Pumpkin seeds have gained considerable attention due to the nutritional and health benefits over the recent years (Saraswathi *et al.*, 2018). According to the Kim *et al.* (2012), pumpkin seeds contain higher carbohydrates, protein, fat, fiber and ash content compared to flesh and peel. Pumpkin seeds consist of 4.06% of moisture, 3.8% of ash, 2.91% of crude fiber, 36.7% of lipids, and 34.56% of protein, 18.1% of soluble protein, 1.08% of sugar and 2.15% of starch (Habib *et al.*, 2015). *Cucurbita maxima* seeds consist of lipids (11-31%) with 73.1-80.5% of total unsaturated fatty acids and 15.9mg/100g of tocopherol (Apostol *et al.*, 2018). The research was conducted to determine the physicochemical and functional properties including total phenolics, total flavonoid and antioxidant activity of pumpkin seed flour (PSF)

and to investigate the applicability of PSF as an ingredient in bakery products.

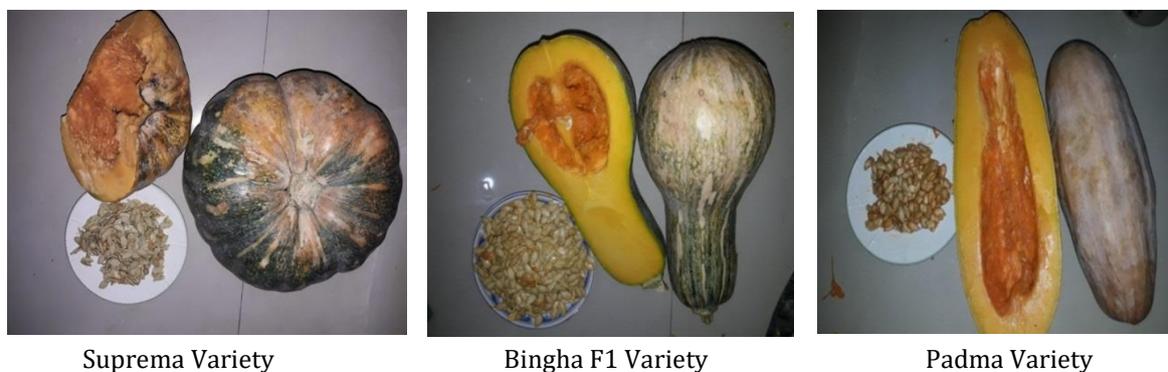
## METHODOLOGY

### Preparation of pumpkin seed flour

Pumpkin seeds from two varieties of *Cucurbita maxima* (Suprema and Bingha F1) and one variety of *Cucurbita moschata* (Padma) were collected from vegetable sellers in Kandy market, Sri Lanka (Figure 1). Seeds of the three varieties were manually cleaned and separately subjected to three types of drying prior to making flour. As the control treatment, seeds collected from the three varieties were dried at 50 - 60 °C for 4 hour using an air convection tray drier (DHG-9146A, China). In the second treatment, dried pumpkin seeds were subjected to roasting at 80-90 °C for 10-15 min in an air convection dryer (DHG-9146A, China). The dried or roasted seeds were allowed to cool and then ground with seed coat, sieved and packed for further experiments. In the third treatment, seed surfaces were sterilized by soaking in 75% of ethanol for 1 min and soaked in potable water for 12 hours at ambient temperature (25±2 °C) as a preliminary step. The soaked seeds were allowed to germinate within layers of cotton wool for 3 days with regular spraying of water. The germinated seeds were then dried at 50-60 °C for 4 hours in an air convection drier followed by cooling, grinding, sieving and packaging as described above.

### Measurement of physical properties of pumpkin seeds

Three axial dimensions, perpendicular to each other, length (L), width (W) and thickness (T) were measured in randomly selected 25 seeds



**Figure 1: Different *Cucurbita* varieties used for the study**

using micrometer with 0.01 mm accuracy (Devi *et al.*, 2018) and geometric mean diameter (GMD) and arithmetic mean diameter (AMD) were calculated using the following equations:

$$\text{GMD} = (L \times W \times T)^{1/3}$$

$$\text{AMD} = (L+W+T)/3$$

True and bulk densities and porosity of pumpkin seeds were measured using the methods described by Devi *et al.* (2018) with minor modifications. True density is defined as the ratio of mass of the sample to its volume. Fifty millilitres of water were placed in a 100 mL graduated measuring cylinder and 5 g of seeds were immersed in that water. The amount of displaced water was recorded from the graduated scale of the cylinder. The ratio of weight of seeds to the volume of displaced water was calculated as the true density.

The bulk density was measured as the mass per unit volume of the sample. Sample (5 g) was weighed into a 20 mL measuring cylinder. Volume of the seeds was measured. The porosity of bulk seeds was computed from the values of true density and bulk density using the relationship given by Mohsenin (1986).

$$\text{Porosity} = 1 - (\text{Bulk density} / \text{True density}) \times 100$$

### Analysis of chemical properties of pumpkin seed flour

Moisture, crude protein, crude fat, ash, total solids and crude fiber contents of PSF were determined using procedures described in AOAC (2020). pH value of PSF was determined using pH meter (ELE-511, Hong Kong).

The antioxidant activity of PSF was determined using DPPH radical scavenging method as described by Zarroug *et al.* (2016) with minor modifications. PSF extract was prepared by mixing 2 g PSF with 10 mL of 100% methanol using vortex. The same procedure was repeated for five times to complete the extraction. All the extracted samples were mixed and evaporated using a rotary evaporator at 40 °C until volume reduced to 10 mL. Different concentration of methanolic extracts ranging from 100ppm to 1000ppm was prepared. Each solution (0.75 mL) was mixed with 0.3mM DPPH in methanol and agitated and kept it in dark up to 30 min at room temperature. Free radical scavenging of DPPH was evaluated by measuring absorbance at 518 nm against the blank in a spectrophotometer. The antioxidant activity of each extract was expressed as (%) DPPH scavenging activity, accordingly to the equation:

$$\text{Antioxidant activity (\%)} = [(Ab - As) / Ab] \times 100$$

Where, Ab and As are absorbance of the blank and the sample, respectively.

The content of total polyphenols was analyzed using the Folin-Ciocalteu's colorimetric method as described by Nyam *et al.* (2009) with some modifications. Gallic acid stock solution (250ppm) was used to prepare the calibration curve. Dried PSF was extracted with 100% methanol. All extracts were combined and evaporated using rotary evaporator at 40 °C until volume reduced to 10 mL. Pumpkin seed extract (500µL) was mixed with 500 µL of distilled water and 2.5 mL of tenfold diluted Folin-Ciocalteu reagent. Four millilitres of 7.5% Na<sub>2</sub>CO<sub>3</sub> was added after 5 min. Vortexed mixture was kept in dark for 30 min and developed colour was measured at 765nm. Gallic acid (GA) standard curve (R<sup>2</sup>= 0.99) was used to determine the phenolic content and total phenolic content was expressed as mg GAE/g of dry weight of extract.

Aluminum chloride colorimetric assay, described by Dissanayake *et al.* (2018) with modifications was used to determine the total flavonoid content. A sample of PSF (500mg) was mixed with 10 mL of methanol and vortexed for 10 min followed by centrifugation for 5 min at 4000 rpm and supernatant was extracted. The extract (0.5 mL) was mixed with 2 mL of distilled water and 0.15 mL of 5% NaNO<sub>2</sub>. The mixture was kept in dark (5 min) and added 0.15 mL of 10% AlCl<sub>3</sub>. The mixture was kept in dark for 6 min and added 1 mL of NaOH (1moldm<sup>-3</sup>). Finally the volume was raised to 2.5 mL. The solution was mixed well and the absorbance was measured against the blank at 510nm. The standard curve (R<sup>2</sup>=0.99) was constructed using Quercetine (QE) and total flavonoid content was expressed as mg QE/g of PSF (dry basis).

### Analysis of physical properties of pumpkin seed flour

Colour value of the pumpkin seeds flour was measured using colorimeter (Chroma meter CR 410, Japan). For the determination of water holding capacity (WHC), 1g of PSF was mixed with 20 mL of distilled water and allowed to hydrate for 24 hours at 25±1 °C. The excess water was filtered off and weighed. The WHC was expressed as the quantity of water absorb per one gram of flour sample (Sangnark and Noomhorm, 2003).

Water retention capacity (WRC) was expressed as grams of water retained per gram of flour sample (Chantaro *et al.*, 2008). For that, 30 mL of distilled

water was added into 50 mL centrifuge tube in which 1 g of PSF and allowed to hydrate for 24 hours at  $25\pm 1$  °C. The rehydrated sample was centrifuged (3000 rpm, 20 min) and weighed the solid fraction.

The oil holding capacity (OHC) was determined using the method explained by Vazquez-Ovando *et al.* (2009) with minor modifications. One gram of PSF was transferred into a 50 mL centrifuge tube. Canola oil (20 mL) was added and placed in a cabinet for 24 hours at  $25\pm 1$  °C followed by centrifugation at 2200 rpm for 30 min. Weight of the sample was recorded and OHC expressed as gram of oil held per g of flour sample.

For the determination of swelling capacity (SC), 1g was mixed with 20 mL of distilled water and allowed to hydrate for 24 hours at  $25\pm 1$  °C. The volume of the sample was recorded after 24 hours. The SC was expressed as mL per gram of flour sample (Rosell *et al.*, 2009)

Organic molecule absorption capacity (OMAC) was determined as described by Vazquez-Ovando *et al.* (2009). Three grams of PSF was weighed into a 50 mL centrifuge tube and mixed with 10 mL of canola oil. The oil and PSF mixture was left in a cabinet at  $25\pm 1$  °C for 24 hours followed by centrifugation at 2000 rpm for 15 min. The precipitate was weighed and OMAC was expressed as the quantity of oil absorbed per gram of PSF.

Emulsifying activity (EA) and emulsion stability (ES) were determined using methods described by Vazquez-Ovando *et al.* (2009) with some modifications. Two grams of PSF was weighed and 100 mL of distilled water was added. The mixture was then homogenised for 2 min. 100 mL of Canola oil was added and homogenised for another 1 min. The emulsion was then transferred into a 50 mL centrifuge tube and centrifuged at 1200 rpm for 5 min. The emulsion volume was recorded. EA was expressed as volume of emulsion per 100 mL of the mixture. ES was determined by heating the prepared emulsion at 80 °C for 30 min. The emulsion was then cooled to room temperature and homogenised for 1 min. The emulsion was transferred into a 50 mL centrifuge tube and centrifuge at 1200 rpm for 5 min. The emulsion volume was recorded. ES was expressed as volume of the remaining emulsion per 100 mL of the original volume of the mixture.

## Preparation of pumpkin seed flour cookies

Wheat flour was substituted with 25%, 50% and 75% in formulation of cookies. Margarine (60 g), yeast (5 g), salt (5 g) and sugar (50 g) were mixed with different quantities (25%, 50% and 75%) of PSF and keeping the content of margarine, yeast, salt and sugar as constant in all treatments. All the ingredients were mixed and kneaded for 15 min to form a soft dough. A dough sheet was formed and cut into star shape with 3 cm diameter followed by baking at  $180\pm 2$  °C for 15 min. Unloaded cookies were allowed to cool and packed in airtight containers for further analysis.

## Sensory evaluation

The cookies made were evaluated on sensory attributes, colour, aroma, texture, sweetness, taste and overall acceptability by 34 untrained panellists using Hedonic scale (9 - like extremely, 1- dislike extremely).

## Data analysis

Statistical analyses were conducted using the statistical software program (Minitab17.0). The two-factor factorial experiment was conducted using a complete randomized design (CRD). Data were analysed according to a two-way ANOVA techniques and significant differences among varieties and PSF processing method were identified using the Tukey's test. Friedman test was used to analysis the sensory scores with a confidence level at ( $p<0.05$ ).

## RESULTS AND DISCUSSION

### Physical properties of pumpkin seeds

There was a significant difference ( $P<0.05$ ) in physical dimensions of three varieties of seeds. The highest length ( $15.37\pm 0.28$  mm), width ( $8.59\pm 0.13$  mm) and thickness ( $2.62\pm 0.07$  mm) measured in *Padma* variety while the highest true density ( $0.90\pm 0.04$  gcm<sup>-3</sup>) and porosity ( $63.47\pm 2.27\%$ ) were measured in *Suprema* variety (Table 1). Geometric mean diameter (GMD) and arithmetic mean diameter (AMD) were significantly different among the varieties ( $P<0.05$ ). The highest GMD and AMD were recorded from seeds obtained by *Padma* variety. There was significant difference in true density and bulk density ( $P<0.05$ ) among the varieties and the highest true density ( $0.90\pm 0.04$  g/cm<sup>3</sup>) and bulk density ( $0.33\pm 0.00$  g/cm<sup>3</sup>) shown

**Table 1. Mean±SD (n=3) of the physical properties of the seeds of the three pumpkin varieties**

Characteristics	<i>Bingha F1</i> ‡	<i>Padma</i> ‡	<i>Suprema</i> ‡
Length (mm)	13.46±0.25 <sup>b</sup>	15.37±0.28 <sup>a</sup>	13.50±0.17 <sup>b</sup>
Width (mm)	7.83±0.11 <sup>b</sup>	8.59±0.13 <sup>a</sup>	6.75±0.14 <sup>c</sup>
Thickness (mm)	1.97±0.08 <sup>c</sup>	2.62±0.07 <sup>a</sup>	2.29±0.06 <sup>b</sup>
True density (g/cm <sup>3</sup> )	0.50±0.02 <sup>b</sup>	0.61±0.01 <sup>b</sup>	0.90±0.04 <sup>a</sup>
Bulk density (g/cm <sup>3</sup> )	0.24±0.00 <sup>c</sup>	0.27±0.01 <sup>b</sup>	0.33±0.00 <sup>a</sup>
GMD(mm)	5.78±0.11 <sup>b</sup>	6.87±0.11 <sup>a</sup>	5.80±0.08 <sup>b</sup>
AMD (mm)	7.76±0.61 <sup>b</sup>	8.86±0.68 <sup>a</sup>	7.52±0.41 <sup>b</sup>
Porosity (%)	50.87±2.17 <sup>b</sup>	56.11±1.52 <sup>ab</sup>	63.47±2.27 <sup>a</sup>

‡Values with same letters within a row are not significantly different at  $p < 0.05$  based on the Turkey's test

by Suprema pumpkin seeds. Suprema variety seeds were smaller than the other two varieties. This would be reason for the highest bulk density and true density. The results were compatible with the previous finding of Devi *et al.* (2018), which indicate true density and bulk density as  $1.16 \pm 0.01 \text{ g/cm}^3$ ,  $0.39 \pm 0.01 \text{ g/cm}^3$  respectively. Porosity of seeds was significantly difference among the varieties ( $P < 0.05$ ). Suprema variety recorded the highest porosity value ( $63.47 \pm 2.27\%$ ) while Devi *et al.* (2018) reported  $65.42 \pm 0.47\%$  in *Cucurbita maxima* seeds. Porosity calculated as percentage ratio of the bulk density and true density. Suprema variety contains the highest true density and bulk density which ensure the highest porosity.

### Chemical properties of pumpkin seeds flour

The pH value in PSF was not significantly different among the varieties ( $p > 0.05$ ).

#### DPPH radical scavenging activity:

DPPH radical scavenging activity was significantly different within variety and treatment (T1 (roasted PSF), T2 (germinated and dried PSF) and control) ( $P < 0.05$ ). Germinated samples of *Bingha F1* variety recorded the highest DPPH radical scavenging activity,  $90.06 \pm 0.24\%$  ( $P < 0.05$ ). The similar results were reported by Dissanayake *et al.* (2018) as  $82.7 \pm 3.0\%$  in seeds of Sri Lankan pumpkin (*Hen wattakka*) variety. Results revealed that roasted pumpkin seeds contain higher radical scavenging activity than the other treatments. Similar results reported previously soy bean seeds (Woumbo *et al.*, 2017) and sesame seeds (Rizki *et al.*, 2015).

Total phenolic content was significantly different ( $P < 0.05$ ) among pumpkin varieties tested while total phenolic content was not significantly different ( $P > 0.05$ ) with T1 (roasted PSF), T2 (germinated and dried PSF) and control (Table 2).

The highest value  $1.52 \pm 0.02 \text{ mg GAE/g}$  was determined in roasted flour sample of *Bingha F1*. Results demonstrated lower value compared with the previous researchers, Sopan *et al.* (2014) and Dissanayake *et al.* (2018), which were  $6.5 \text{ mg GAE/mL}$  in *Cucurbita maxima* seeds and  $6.2 \pm 0.6 \text{ mg GAE/g}$  which was based on the Sri Lankan variety of *Cucurbita maxima* (*Hen wattakka*), respectively.

Thermal treatment has an effect on increasing total phenolic content in soya seeds and sesame seeds at  $120\text{-}150^\circ\text{C}$  up to 90 min (Woumbo *et al.*, 2017, Rizki *et al.*, 2015). Similar trend was observed in pumpkin seed in this experiment. Control samples of *Padma* variety showed very low total phenolic contents compared to control samples of Suprema variety while roasted samples of *Padma* variety contain highest total phenolic contents compared to roasted samples of Suprema variety. The reason for this change was due to formation of new compounds by Maillard reaction during roasting. Generally thermal treatment causes the availability of plant phenolic compounds in the food matrices by evaporation of intercellular water in foods, triggering the changes in lignocellulosic structure and promoting the denaturation of protein (Rizki *et al.*, 2015).

#### Flavonoid content:

The highest flavonoid content was recorded ( $19.87 \pm 1.08 \text{ mgQE/g}$ ) in germinated seeds of *Bingha F1* variety. The highest flavonoids content helps to improve the antioxidant activity, free radical scavenging activity and anti-bacterial activity of the body. Davi *et al.* (2018) and Dissanayake *et al.* (2018) reported higher content of flavonoid in *Cucurbita maxima* seeds of  $56.16 \pm 4.60 \text{ mgQE/g}$  and  $39 \pm 0.20 \text{ mgQE/g}$ , respectively. The reason would be the differences of the solvents used in the experiments. The present study shows a significant increment of flavonoid content in roasted seed flour and the

Table 2. Mean  $\pm$  SD (n=3) pH, total phenolic content, anti-oxidant activity, flavonoid content and proximate composition of PSF

Property <sup>†</sup>	<i>Bingha F1</i> <sup>‡</sup>			<i>Padma</i> <sup>‡</sup>			<i>Suprema</i> <sup>‡</sup>		
	Control	Roasted	Germinated	Control	Roasted	Germinated	Control	Roasted	Germinated
<b>pH</b>	6.07 $\pm$ 0.01 <sup>c</sup>	6.05 $\pm$ 0.03 <sup>c</sup>	6.15 $\pm$ 0.10 <sup>bc</sup>	6.33 $\pm$ 0.03 <sup>a</sup>	6.29 $\pm$ 0.03 <sup>ab</sup>	5.72 $\pm$ 0.01 <sup>d</sup>	6.04 $\pm$ 0.02 <sup>c</sup>	6.17 $\pm$ 0.01 <sup>bc</sup>	6.17 $\pm$ 0.01 <sup>bc</sup>
<b>TPC (mg GAE/mL)</b>	0.78 $\pm$ 0.02 <sup>bc</sup>	1.52 $\pm$ 0.02 <sup>a</sup>	0.69 $\pm$ 0.29 <sup>bc</sup>	0.42 $\pm$ 0.02 <sup>c</sup>	1.17 $\pm$ 0.05 <sup>ab</sup>	0.66 $\pm$ 0.03 <sup>bc</sup>	0.70 $\pm$ 0.08 <sup>bc</sup>	0.85 $\pm$ 0.06 <sup>bc</sup>	0.83 $\pm$ 0.06 <sup>bc</sup>
<b>AOX (%)</b>	89.4 $\pm$ 0.08 <sup>ab</sup>	89.1 $\pm$ 0.31 <sup>ab</sup>	90.1 $\pm$ 0.24 <sup>a</sup>	88.8 $\pm$ 0.43 <sup>ab</sup>	88.6 $\pm$ 0.31 <sup>ab</sup>	84.4 $\pm$ 1.16 <sup>c</sup>	87.6 $\pm$ 0.20 <sup>b</sup>	88.8 $\pm$ 0.29 <sup>ab</sup>	88.0 $\pm$ 0.39 <sup>ab</sup>
<b>FC (mg QE/mL)</b>	9.4 $\pm$ 1.93 <sup>a</sup>	18.9 $\pm$ 0.61 <sup>a</sup>	19.9 $\pm$ 1.08 <sup>a</sup>	15.1 $\pm$ 0.33 <sup>a</sup>	16.6 $\pm$ 0.50 <sup>a</sup>	9.00 $\pm$ 0.20 <sup>a</sup>	15.4 $\pm$ 0.63 <sup>a</sup>	17.2 $\pm$ 0.95 <sup>a</sup>	10.85 $\pm$ 0.14 <sup>a</sup>
<b>Moisture (%)</b>	5.25 $\pm$ 0.29 <sup>b</sup>	5.76 $\pm$ 0.10 <sup>a</sup>	6.05 $\pm$ 1.13 <sup>b</sup>	4.68 $\pm$ 0.82 <sup>b</sup>	4.96 $\pm$ 0.16 <sup>b</sup>	4.97 $\pm$ 0.80 <sup>b</sup>	4.52 $\pm$ 0.72 <sup>b</sup>	4.16 $\pm$ 0.42 <sup>b</sup>	6.33 $\pm$ 1.33 <sup>a</sup>
<b>TS (%)</b>	93.7 $\pm$ 0.28 <sup>a</sup>	94.2 $\pm$ 0.10 <sup>ab</sup>	92.9 $\pm$ 1.14 <sup>a</sup>	95.3 $\pm$ 0.82 <sup>a</sup>	95.0 $\pm$ 0.16 <sup>a</sup>	95.0 $\pm$ 0.80 <sup>a</sup>	95.5 $\pm$ 0.72 <sup>a</sup>	96.8 $\pm$ 1.00 <sup>a</sup>	89.5 $\pm$ 1.49 <sup>b</sup>
<b>Crude fat (%)</b>	27.7 $\pm$ 0.56 <sup>d</sup>	30.8 $\pm$ 0.94 <sup>cd</sup>	38.5 $\pm$ 1.08 <sup>ab</sup>	39.6 $\pm$ 2.19 <sup>ab</sup>	41.4 $\pm$ 1.81 <sup>a</sup>	33.6 $\pm$ 0.57 <sup>abc</sup>	36.8 $\pm$ 1.62 <sup>abc</sup>	35.5 $\pm$ 2.62 <sup>abc</sup>	33.6 $\pm$ 0.57 <sup>bcd</sup>
<b>Crude protein (%)</b>	18.6 $\pm$ 0.02 <sup>b</sup>	15.5 $\pm$ 0.00 <sup>g</sup>	19.8 $\pm$ 0.00 <sup>a</sup>	17.7 $\pm$ 0.00 <sup>d</sup>	16.0 $\pm$ 0.00 <sup>f</sup>	18.8 $\pm$ 0.00 <sup>b</sup>	17.4 $\pm$ 0.01 <sup>e</sup>	13.9 $\pm$ 0.07 <sup>h</sup>	18.2 $\pm$ 0.06 <sup>c</sup>
<b>Crude fiber (%)</b>	58.8 $\pm$ 0.73 <sup>d</sup>	66.0 $\pm$ 1.51 <sup>bc</sup>	77.7 $\pm$ 0.46 <sup>a</sup>	66.1 $\pm$ 3.36 <sup>bc</sup>	61.5 $\pm$ 0.88 <sup>cd</sup>	79.3 $\pm$ 1.03 <sup>a</sup>	70.5 $\pm$ 0.54 <sup>b</sup>	65.5 $\pm$ 0.29 <sup>bcd</sup>	77.7 $\pm$ 0.71 <sup>a</sup>
<b>Ash (%)</b>	4.52 $\pm$ 0.00 <sup>c</sup>	5.16 $\pm$ 0.40 <sup>ab</sup>	5.10 $\pm$ 0.31 <sup>ab</sup>	5.16 $\pm$ 0.12 <sup>ab</sup>	4.77 $\pm$ 0.16 <sup>c</sup>	5.96 $\pm$ 0.15 <sup>ab</sup>	4.96 $\pm$ 0.15 <sup>ab</sup>	5.66 $\pm$ 0.32 <sup>ab</sup>	6.33 $\pm$ 1.33 <sup>a</sup>

<sup>†</sup>TPC is Total Phenolic content; AOX is Antioxidant activity; FC is Flavonoid content

<sup>‡</sup>Values with same letters within a row are not significantly different at p< 0.05 based on the Turkey's test

**Table 3. Mean ± SD (n=3) physical properties of pumpkin seed flour**

Property <sup>†</sup>	<i>Bingha F1</i> <sup>‡</sup>			<i>Padma</i> <sup>‡</sup>			<i>Suprema</i> <sup>‡</sup>		
	Control	Roasted	Germinated	Control	Roasted	Germinated	Control	Roasted	Germinated
<b>Chroma value</b>	31.8±0.33 <sup>f</sup>	29.6±0.25 <sup>d</sup>	38.6±0.22 <sup>a</sup>	34.2±0.12 <sup>e</sup>	34.0±0.11 <sup>e</sup>	35.1±0.94 <sup>c</sup>	29.3±0.04 <sup>b</sup>	29.1±0.12 <sup>b</sup>	36.9±0.05 <sup>a</sup>
<b>Hue value</b>	-89.1±0.11 <sup>d</sup>	-88.0±0.06 <sup>e</sup>	89.5±0.12 <sup>a</sup>	-88.6±0.03 <sup>c</sup>	88.7±0.05 <sup>c</sup>	86.3±0.17 <sup>bc</sup>	87.5±0.17 <sup>e</sup>	87.96±0.05 <sup>e</sup>	89.1±0.06 <sup>ab</sup>
<b>WHC (g/g)</b>	5.16±0.05 <sup>ab</sup>	5.08±0.04 <sup>ab</sup>	6.08±0.05 <sup>a</sup>	4.86±0.11 <sup>ab</sup>	5.40±0.03 <sup>ab</sup>	6.13±0.03 <sup>a</sup>	3.31±0.45 <sup>b</sup>	5.55±0.05 <sup>ab</sup>	6.31±0.16 <sup>a</sup>
<b>WRC (g/g)</b>	4.34±0.04 <sup>a</sup>	4.00±0.31 <sup>a</sup>	2.71±0.00 <sup>e</sup>	3.20±0.07 <sup>cde</sup>	3.78±0.04 <sup>abc</sup>	3.80±0.03 <sup>ab</sup>	2.84±0.08 <sup>de</sup>	3.35±0.04 <sup>bcd</sup>	3.36±0.06 <sup>bcd</sup>
<b>SC (mL/g)</b>	4.83±0.03 <sup>bc</sup>	4.86±0.06 <sup>bc</sup>	5.00±0.00 <sup>b</sup>	4.40±0.05 <sup>c</sup>	4.40±0.10 <sup>c</sup>	5.33±0.12 <sup>b</sup>	5.00±0.00 <sup>b</sup>	5.00±0.00 <sup>b</sup>	6.00±0.28 <sup>a</sup>
<b>OHC (g/g)</b>	2.62±0.00 <sup>cd</sup>	3.20±0.12 <sup>a</sup>	2.94±0.05 <sup>abc</sup>	2.38±0.03 <sup>d</sup>	2.68±0.07 <sup>bcd</sup>	3.03±0.04 <sup>ab</sup>	2.38 ± 0.06 <sup>d</sup>	2.99±0.13 <sup>abc</sup>	3.23±0.07 <sup>a</sup>
<b>OMAC (g/g)</b>	1.88±0.01 <sup>d</sup>	2.29±0.14 <sup>a</sup>	2.21±0.09 <sup>ab</sup>	1.98±0.01 <sup>cd</sup>	1.95±0.03 <sup>cd</sup>	2.14±0.08 <sup>b</sup>	2.02±0.04 <sup>c</sup>	1.92±0.02 <sup>cd</sup>	2.24±0.01 <sup>ab</sup>
<b>EA (mL/100g)</b>	47±0.00 <sup>ab</sup>	44.7±0.33 <sup>c</sup>	47.7±0.33 <sup>a</sup>	44.7±0.66 <sup>c</sup>	44.0±0.00 <sup>c</sup>	45.7±0.33 <sup>bc</sup>	48.3±0.33 <sup>a</sup>	44.7±0.66 <sup>c</sup>	44.0±0.00 <sup>c</sup>
<b>ES (mL/100g)</b>	46.7±0.33 <sup>ab</sup>	45.0±0.00 <sup>ab</sup>	45.3±0.45 <sup>ab</sup>	45±0.00 <sup>ab</sup>	44.3±0.33 <sup>b</sup>	45.3±0.33 <sup>ab</sup>	46.0±0.00 <sup>ab</sup>	47.3±0.33 <sup>a</sup>	45.7±0.66 <sup>ab</sup>

<sup>†</sup> WHC is Water Holding Capacity, WRC is Water Retention Capacity, SC is Swelling Capacity, OHC is Oil Holding Capacity, OMAC is Organic Molecule Absorption Capacity, EA is Emulsifying Activity, and ES is Emulsion Stability.

<sup>‡</sup>Values with same letters within a row are not significantly different at p< 0.05 based on the Turkey's test

observation was similar to reported by Rizki *et al.* (2015). Thus, the roasted seed flour possessed higher potential of anti-oxidant activity such as scavenging of super peroxide radicals and hydroxyl radicals.

### Proximate composition

All the parameters in proximate composition were significantly different in variety-treatment interaction [T1 (roasted PSF), T2 (germinated and dried PSF) and control] ( $P < 0.05$ ). The highest moisture content ( $6.33 \pm 1.33\%$ ) and total solid content ( $89.47 \pm 1.49\%$ ) were determined in germinated samples of Suprema variety. However, moisture and total solid contents were significantly different ( $P < 0.05$ ) among the treatments, (T1 roasted PSF, T2 Germinate PSF). The lower moisture content ensures better shelf life and reduces the microbial spoilage of pumpkin seed flour (Ajayi *et al.*, 2006). The highest crude fat content of  $41.42 \pm 1.81\%$  was observed in roasted seeds samples of *Padma* variety. Crude fat content was significantly different among the treatments while it was not significantly different ( $P > 0.05$ ) among the varieties. Roasting increases fat content due to extraction of fat from the flour matrix. The loss of moisture during roasting concentrates the other macro molecules and higher fat content leading susceptible to rancidity (James *et al.*, 2015). Germinated *Bingha F1* demonstrates the highest crude fat content compared to other two varieties. Qualities of the flour can be differing mainly with the variety of the species. Non-conversion of free fatty acids to carbohydrates which may lead to increase in fat composition during germination (Afam-Anene and Onuoha, 2006).

Protein content of the samples was significantly different among the varieties and treatments ( $P < 0.05$ ). In germinated seed samples, the highest crude protein content  $19.75\%$  in *Bingha F1* variety and crude fiber content,  $79.25 \pm 1.03\%$  in *Padma* variety were determined. Thermal destruction of heat-treated samples leads to reduce the protein content. Kaushik *et al.* (2010) reported that total protein content increased after germination of seeds. Crude fiber content was significantly different within the variety and treatments ( $P < 0.05$ ). Further, germination increases the fiber in seed flour (Akintade *et al.*, 2019), dietary fiber and mineral bioavailability (Ghavidel and Prakash, 2007). Germinated seed samples of Suprema variety possessed the highest ash content ( $6.33 \pm 1.33\%$ ). Similar results were reported by Fouad and Rehab (2015) for lentil seeds. Ash content was significantly different within the treatments and the varieties ( $P < 0.05$ ). The

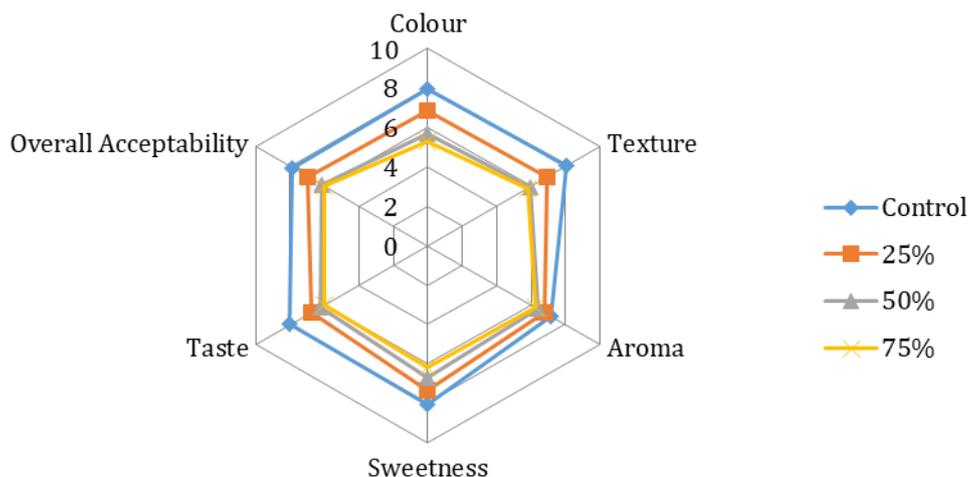
increment in ash content was due to the endogenous enzyme hydrolysis of complex organic compounds which release more nutrients leaving the anti-nutrients leach into the germination medium (Chikwendu, 2003) and phytase enzyme activity during germination which hydrolyze the bonds between protein and minerals become free. Therefore, increase the availability of minerals (Narsih *et al.*, 2012). The results of the present study was compatible the proximate composition values reported by Devi *et al.* (2018), where moisture  $5.53 \pm 0.26\%$ , crude protein  $28.90 \pm 1.36\%$ , crude fat  $31.75 \pm 0.45\%$ , crude fiber  $4.59 \pm 1.01\%$  and ash  $6.90 \pm 0.14\%$ , respectively and Saraswathi *et al.* (2018) reported that proximate composition of PSF as moisture  $4.8\%$ , protein  $15.9\%$ , fat  $38.0\%$  and ash content  $4.1\%$  in seed flour.

### Physical properties of pumpkin seeds flour

Colour values expressed as chroma (purity of the colour) and hue (lightness) values. Both chroma and hue values were significantly different among the varieties and the treatments ( $P < 0.05$ ). The highest hue value ( $89.5 \pm 0.1$ ) and chroma value ( $38.6 \pm 0.2$ ) were measured in *Bingha F1* variety (Table 3). There was a significant difference ( $P < 0.05$ ) in variety x treatment (T1-roasted PSF, T2- germinated and dried PSF and control) interaction in WRC, OHC, SC and OMAC while WHC and ES were not significantly difference ( $P < 0.05$ ). WHC was significantly different within the treatments ( $P < 0.05$ ). Higher the soluble dietary fiber content increases the WHC. The highest WHC,  $6.31 \pm 0.16\text{g}$  was recorded in germinated seeds of Suprema variety. Therefore, pumpkins seeds of *Bingha F1* variety contain a relatively higher content of soluble dietary fiber.

The highest WRC ( $4.34 \pm 0.04\text{g}$ ) was recorded in control samples of *Bingha F1* variety and significantly different within the varieties and treatments ( $P < 0.05$ ). Nyam *et al.* (2009) reported a lower WRC,  $2.58 \pm 0.14\text{g}$  in pumpkin.

Swelling Capacity (SC) of germinated seed samples of Suprema variety was  $6.00 \pm 0.28$  mL and significantly different among the varieties and the treatments ( $P < 0.05$ ). Comparably a lower value,  $3.25 \pm 0.50$  g was reported by Nyam *et al.* (2009). This might be due to the available fat content in pumpkin seeds. Previous researcher, Sowbhagya *et al.* (2007) reported the residual oil trapped inside the fiber matrix of seeds, water molecules absorption is restricted and leads to a lower swelling index. OHC in germinated seeds of Suprema variety was  $3.23 \pm 0.07$  g and significantly



**Figure 2.** Mean scores of the sensory attributes in 25, 50 and 75 % PSF substituted biscuits.

different with the variety and treatments ( $P < 0.05$ ). Pumpkin seeds were excellent in oil holding within the fiber matrix. A similar result,  $4.60 \pm 0.22$ g was reported by Nyam *et al.* (2009). The highest organic molecule absorption capacity (OMAC),  $2.29 \pm 0.14$ g was measured in roasted seed samples of *Bingha F1* variety and the values were significantly different among the varieties and the treatments ( $P < 0.05$ ). OMAC in *Bingha F1* variety was higher than value,  $1.31 \pm 0.13$  g reported by Nyam *et al.* (2009). The variation in insoluble dietary fiber in pumpkin seeds has a higher impact on OMAC. Effective and efficient higher OMAC value could be achieved with fat, bile acids, cholesterol, drugs and toxic compounds in the intestine (Vazquez-Ovando *et al.* 2009).

Emulsification Activity (EA) was significantly different among the variety and the treatments ( $P < 0.05$ ) while Emulsion Stability (ES) was significantly different among the variety ( $P < 0.05$ ) and significant differences were not seen among the treatment ( $P > 0.05$ ). PSF yielded from the control sample of Suprema variety shows the highest EA value ( $48.33 \pm 0.33$  mL) while the highest ES value,  $47.33 \pm 0.33$  mL in roasted seed flour. Nyam *et al.* (2009) reported similar value for EA  $46.25 \pm 4.33$  mL, and lower value ES  $38.75 \pm 1.44$  mL. Although pumpkin seeds had better EA, pumpkin rind flour possesses higher emulsion stability than the PSF because of higher oil content in pumpkin flour of the rind. Roasting inactivates the hydrolytic enzymes in seed flour and protects the oil fraction thus increase and maintaining the emulsion stability.

### Sensory properties

Mean scores of sensory attributes in cookies prepared by incorporating PSF, colour, taste,

texture, sweetness and overall acceptability of the treatments were significantly different at  $P < 0.05$  among the treatments while aroma was not significantly different ( $P > 0.05$ ) (Figure 2). The highest score for all sensory attributes were recorded at 25% substituted PSF in cookies compare to 50% and 75% substitution. Overall acceptability and sweetness were evaluated as like moderately while colour, aroma, texture and taste were evaluated as like slightly for 25% substituted cookies. Malkanthi *et al.* (2020) reported that 5% incorporation of PSF in string hoppers was scored the highest sensory attributes for appearance, colour, aroma, taste, texture and overall acceptability than 10% and 15% PSF incorporation. A gradual reduction of scores for all the sensory attributes was recorded with incorporation of more than 5% PSF in formulating.

### CONCLUSION

The highest bulk density, true density and porosity values were reported by pumpkin seeds of Suprema variety while the highest GMD and AMD were in *Padma* variety. Antioxidant activity and flavonoid content were the highest in germinated seed samples of *Bingha F1*. The maximum total phenolic content was reported in roasted seed flour sample of *Bingha F1* variety ( $1.52 \pm 0.02$  mgGAE/g). Germinated Suprema seeds had the highest moisture content and total solid content in roasted seed samples. Germinated samples of *Bingha F1* variety and roasted samples of *Padma* variety recorded the highest protein and crude fat content respectively. Germinated seed flour of Suprema variety has shown the best flour properties including the highest water holding capacity and swelling capacity. Therefore, seed

flour of Suprema variety has the highest potential for partially replacement of wheat flour in bakery products. Further pumpkin seeds can be used for oil extraction. The best level of substitution of PSF is 25% in formulation of biscuits with acceptable

sensory attributes. Formulation and development of value-added food products from PSF possesses higher potential to reduce the waste of pumpkin seeds and able to reduce the cost of production in bakery product with substantial health benefits

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