Pumpkin flour qualities as affected by ultrasound and microwave pre-drying treatment

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ABSTRACT
The aim of this study was to assess the impact of innovative pre-drying treatments (ultrasound and microwave) on the physical, chemical, and functional properties pumpkin flour in comparison to untreated and freeze-dried (optimal control) flours. The impact was primarily related to the improvement of the flour’s nutritional, phytochemical, and functional properties. The pre-treated flours were superior to untreated flour and were nearer in quality to freeze-dried flour. The moisture, ash, crude fat, crude protein, crude fiber, and carbohydrate contents of flours ranged from 7.57 to 8.23%, 5.73 to 6.57%, 1.17 to 1.85%, 8.72 to 11.32%, 10.92 to 13.11%, and 61.47 to 64.23%, respectively. Among the pre-drying treatments, 20UM depicts the highest color change and retention of the bioactive component in pumpkin flour. The results also showed that the 30UM py-dried flour had higher water solubilities index (11.83%), water absorption (9.75 g/g), and oil absorption capacities (2.28 mL/g).

Introduction
Fruits and vegetables are consumed worldwide in both fresh and processed forms. Pumpkin is a traditional crop considered an excellent source of provitamin A carotenoids, which are very helpful in preventing vitamin A deficiency. Carotenoids are phytochemicals thought to reduce the risk of certain degenerative diseases and are responsible for the attractive color of many fruits and vegetables. Nevertheless, very little has been done on a pumpkin to overcome undernourishment, food poverty and income generation even in a favorable ecological conditions throughout East Africa. As such, it remains underutilized and less considered by many households. Due to the high moisture content (90–92%), pumpkins are bulky and difficult to handle and transport. Hence, most pumpkins produced are consumed in the area of production. Processing can transform pumpkins from perishable produce to stable foods with a long shelf-life, facilitating global transportation and distribution. For these reasons, freezing and drying may be suitable techniques for processing and extending pumpkins shelf life. Currently, powders are the core processed products of pumpkins.

Air drying is generally favored over freeze drying due to its low operating costs and shorter drying times. In conventional air drying, high temperatures are employed that adversely affect the texture, color, and nutritional value of the products in question. One way to produce high-quality dried products is to use pre-drying treatments, improving product quality. Hence, possible pre-drying treatment methods that reduce drying time while maintaining quality are imperative. Pre-drying treatment helps to reduce undesired changes such as antioxidant activity reduction, color, and textural changes. Additionally, it reduces drying time by relaxing tissue structure and producing a superior dry product. In this sense, recent advances in the use of emerging food processing
technologies have received considerable attention in reducing various adverse changes in final dried product properties. Among the so-called emerging technologies, ultrasound is a promising non-thermal pre-drying treatment commonly used before drying various agricultural products to improve mass transfer, drying time, reduce processing cost, and maintain quality properties.\[^8\] The application of ultrasound produces a sponge-like effect on the surface of the solid food samples, resulting in the formation of microspores in the food material.\[^9\] Also, ultrasonic pretreatment is a favorite because the process can be performed at low temperatures, which shrinks the probability of food degradation\[^10\] and permits the removal of moisture content from solids without producing a liquid phase change. The utilization of microwave energy has attracted much attention as it is said to improve the drying process by reducing processing time and operating costs.\[^7\] Applying microwave heating as a pre-drying treatment to moist material for drying operations results in internal moisture being heated up and migrating to the surface due to pressure differences for subsequent drying.\[^11\] Therefore, this study aimed to investigate the effect of combined ultrasound and microwave technologies as a pre-drying treatment on the physical, chemical and functional properties of pumpkin flour qualities.

**Materials and methods**

**Chemicals**

Standards and reagents used were gallic acids (97.5–102.5% sigma Aldrich, China), phytic acid sodium salt hydrate (Sigma Aldrich, Switzerland), (+-) Catechin hydrate (≥96.0%, Sigma Aldrich, China), Quercetin (≥98%, sigma Aldrich, Germany), Folin-Ciocalteu’s (2 N, Sigma Aldrich, USA), Vanillin (≥ 99.5%, UNI-CHEM, Roth, France), 2,2-Diphenyl-1-picrylhydrazyl (Sigma Aldrich, Germany), Aluminum Chloride (99% Loba chemic, India), Methanol (M.wt. = 34.02 g/mol, Biochem chemo-pharma, France), Nitric acid (69% Loba chemic, India) and Sodium carbonate(≥99.5% Carl Roth GmbH, Karlsruhe). All chemicals were of analytical grade.

**Material collection and preparation**

The fresh, healthy, and ripped pumpkin bought from a local market was washed properly in distilled water, peeled, and cleaned by discarding the seeds. Only the fleshy part was taken and sliced into (15 × 15 × 4) mm\(^3\) pieces. Slices were washed with distilled water, drained, and mopped with a paper towels. Untreated and freeze-dried (Lablyo plus, Germany) samples were used as a control sample to compare the effect of pre-drying methods; the remaining samples were treated with the following pre-drying methods before drying at 60°C in fluidized bed drier (TG 200, Germany). Dried slices were milled (Model BH24 1DY, Armfield, England) and then sieved through a standard 500-micron mesh sieve. The obtained flours were wrapped in aluminum foil bags and were kept at 4°C in a desiccator before use.

**Microwave pretreatment**

A programmable domestic microwave oven (Model-CE107BT, Samsung, Thailand) with a maximum output of 900 W at 2450 MHz was used. For processing, approximately 100 g of sample in slice form was spread evenly on the tissue paper and placed on the rotatable turntable to confirm the uniformity of microwave energy absorbed by each sample. The microwave oven was allowed to operate at a 300 W power level for 6 min,\[^12\] followed by cold water dipping to avoid the residual heating effect.

**Ultrasonic pretreatment**

A set of experimental pumpkin slice samples were immersed in distilled water and subjected to ultrasonic waves for 10, 20, and 30 min.\[^10,13\] Experiments were performed in an ultrasonic bath
(Model-EU-28, Akin Electronic, Turkey) at ambient water temperature (30°C) without mechanical agitation. The temperature raised during the experiments was lower than ±2°C at each treatment. Keep the water to fruit ratio at 4:1 by weight basis. Experiments were performed in isolated 250 mL Erlenmeyer flasks to keep away from interference among the samples and runs. For the combined treatment, other parts were further microwave blanched (Samsung, Model-CE107BT, Thailand) for six (6) min at 300 W power level as above after draining and wiping.

Drying methods

Freeze-drying

A pumpkin slices were dried using a laboratory-scale freeze dryer (Lablyo plus, Germany) as described by Marques, Prado, & Freire. One hundred grams of each sliced sample was put in the tray, frozen at −18°C for 3 hours, and then placed inside the freeze dryer chamber at 52 pa and −52°C. Thermocouple probes were used to regulate and adjust the product temperature on each tray during drying. The dried slices were milled (Model BH24 1DY, Armfield, England), sealed in aluminum foil bags and stored at 4°C until further tests were carried out.

Fluidized bed drying

The slices were drained well and spread for drying using a fluidized bed (TG 200, Germany) drier at 60°C with constant fluidizing drying air velocity set to 1.5 m/s to achieve fluidization. After being subjected to drying, weighing every interval of time until constant weight is achieved. The dried pumpkin slices were then grounded using a laboratory grinder (Model BH24 1DY, Armfield, England) and sieved through a 500-micron mesh sieve to remove coarse fiber. Pumpkin flours were sealed in aluminum foil bags and stored at 4°C for further analysis.

Physical property analysis

Color

Color analysis was carried out using Hunter Lab Colorimeter, Minolta. The color readings were presented as L*, a*, b* format where a* value ranges from −100 (greenness) to +100 (redness), the b* value varies from −100 (blueness) to +100 (yellowness), whereas the L* value, elucidate the degree of lightness, extend from 0 (black) to 100 (white). Siddiq et al. The black and white tile was used for instrument calibration before color measurement. The chroma factor (C*) was calculated by converting the Cartesian coordinates (b*, a*) based on the following formula:

\[
\text{Chroma}(C^*) = [(a^2 + b^2)^{1/2}] 
\]

Hue angle shows a relation between a* and b* and was calculated according to the formula:

\[
H^* = \tan^{-1}(b/a) 
\]

Color changes in comparison to the negative control sample are obtained from the following formula:

\[
DE = [(D_l)^2 + (D_a)^2 + (D_b)^2]^{1/2}
\]

Water activity, total soluble solids, and pH

The water activity of the pumpkin flours were measured by a water activity meter (HD-3A, NanBei, China). Total soluble solids (TSS) were determined for each sample according to the AOAC.
method using a digital refractometer (Model-A670, Hanon, China) at 25°C and expressed as °Brix. A 10 g of sample was balanced and dissolved in a beaker containing 25 mL of distilled water to form a slurry. It was placed for 10 min with constant stirring. The pH was then determined according to official methods AOAC\textsuperscript{[19]} with a pH meter (BANTE Multiparameter/China).

**Chemical properties**

Proximate values of the pumpkin flour samples were determined following the official methods AOAC\textsuperscript{[18]} Moisture content (MC) was determined by laboratory oven (Model 10-D1391/AD, SCA) set at 105°C. Total crude protein content (N × 6.25) was analyzed using the Kjeldahl method (K1160, Hanon, China) according to AOAC method No. 978.04. The total fat content was evaluated by using a soxhlet extractor following AOAC method No. 930.09. The total ash content was examined by using muffle furnace (MKF-07, natek, Turkey), following AOAC method No. 930.05. Crude fiber analysis was performed by neutralization method (BXB-06, Guangzhou, China), according to AOAC method No. 930.10. Carbohydrate was calculated by difference.

\[
\text{Carbohydrate} (\%) = 100 - (\text{Moisture} + \text{Crude fat} + \text{Ash} + \text{Crude protein}) \%
\]  

(4)

**Phytochemical analysis**

**Phytate content**

The phytate content of the samples was find out according to the method described in Deme \textit{et al.} \textsuperscript{[20]} One hundred milligrams of the sample were extracted with 10 mL of 0.2 N HCl in a mechanical shaker for one hour at room temperature. The extract was centrifuged at 3000 rpm for 30 min. Pure supernatant was used for phytate assessment. One milliliter of Wade reagent (containing 0.03% solution of FeCl\textsubscript{3} · 6H\textsubscript{2}O and 0.3% of sulfoisalicylic acid in water) was added to 3 mL of the sample solution (supernatant), and the mixture was vortexed for 5 s, and then read the absorption at 500 nm (Perkin Elmer Lamda 950 UV/Vis/NIR, UK) against a blank solution (3 mL extract solution mixed with 2 mL of 2.4% HCl). The sodium salt of phytic acid (5–36 mg/mL) was used as the standard to draw the calibration curve. Phytate concentration was estimated from a standard curve and the results were expressed as mg phytate per 100 g of dry matter.

**Tannin content**

tannin content was determined by the method of Gemed,\textsuperscript{[21]} modified from Burns (1971), using catechin as the tannin standard. About 1.0g of each sample was weighed in triplicates in a screw-cap test tube and extracted with 10 ml of 1% HCl in methanol for 24 hours at room temperature with mechanical shaking. After shaking for 24 hours, the solution was centrifuged at 1000 rpm for 5 minutes. Mix 1 mL of supernatant with 5 mL of vanillin-HCl reagent (mix equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol). D-catechin was used as a standard for tannin determination. A 0.01 g of D- catechin was measured and dissolved in 50 mL of 1% HCl in methanol, serving as a stock solution. A 0, 0.2, 0.4, 3, 0.6, 0.8, and 1 mL of stock solution were measured in a test tube and the volume of each test tube was regulated to 1 mL with 1% HCl in methanol. To each test tube, added 5 mL of vanillin-HCl reagent. Zero the spectrophotometer by using distilled water before evaluating the absorbance of sample solutions and the standard solution after 20 minutes at 500 nm (Perkin Elmer Lamda 950 UV/Vis/NIR, UK). The calibration curve was plotted from the sequence of standard solutions as absorbance versus concentration and the slope and intercept were used for calculation.
**Oxalate**

It was assessed by the AOAC\(^{[18]}\) method. One gram of the sample was taken in a 100-mL conical flask. Seventy-five milliliters of 3 mol/L H\(_2\)SO\(_4\) were poured and the solution was stirred occasionally with a magnetic stirrer for about 1 h and then clarified using Whatman No. 1 filter paper. The clarified samples (extract) (25 mL) were gathered and titrated against hot (80–90°C) 0.1 N KMnO\(_4\) solution to the point when a faint pink color was observed that persisted for at least 30 sec. Results were outlined as oxalate in mg per g of sample, and the concentration of oxalate in every sample was recorded from the calculation:

\[
1 \text{mL of } 0.1\text{N Permanganate } = 0.006303 \text{ g oxalate}
\] (5)

**Analysis of antioxidant activities**

**Sample extraction**

Samples were extracted according to previously defined procedures Ferreira et al.\(^{[22]}\). Extract 10 g of pumpkin flour with 100 mL of methanol for 24 h using a temperature shaker incubator (ZHWHY-103B) at 25°C and 150 rpm, then clarified through Whatman No. 1 paper. Again extract the residue with above two additional 100 mL portions of methanol. The combined methanolic extracts were then evaporated to dry using a Rota evaporator (R-300, Buchi, Switzerland) at 40°C, redissolved in methanol at a 50 mg/mL concentration, and stored at 4°C for further use.

**Total phenolic content**

Total phenolic content (TPC) was assessed using the Folin Ciocalteu assay and gallic acid (GA) as the standard, according to Xu & Chang.\(^{[23]}\) The mixture of the sample solution (50 \(\mu\)L), distilled water (3 mL), 250 \(\mu\)L of Folin-Ciocalteu’s reagents solution, and 7% NaCO\(_3\) (750 \(\mu\)L) was vortexed and incubated for 8 min at room temperature. Then, a dose of 950 \(\mu\)L of distilled water was added. The mixture was left at room temperature for two hours. Read the absorbance at 765 nm (Perkin Elmer Lamda 950 UV/Vis/NIR, UK) against distilled water as a blank. Gallic acid was used to construct the standard curve of 20–100 \(\mu\)g/mL \((r = 0.99)\). The results were mean ± standard deviation and indicated as mg of gallic acid equivalents/g of extract (GAEs). The total phenolic content of gallic acid equivalent (GAE) in flour extracts was determined by the following formula:

\[
C = \frac{cxV}{m}
\] (6)

where \(C\) is the total content of phenolic compounds, mg/g fresh material, in GAE; \(c\) is the concentration of gallic acid determined from the calibration curve; \(V\) is the volume of extract, L; \(m\) is the weight of extract, g.

**Total flavonoid content**

Total flavonoid content was estimated using the colorimetric method previously described by Xu & Chang.\(^{[23]}\) Briefly, mix 0.25 mL of the flour extract or quercetin standard solution with 1.25 mL of distilled water in a test tube, then add by 75 \(\mu\)L of 5% NaNO\(_2\) solution. After 6 min, add 150 \(\mu\)L of 10% AlCl\(_3\)\(_6\)H\(_2\)O solution, let stand for an additional 5 min, and then add 0.5 mL of 1 M NaOH. The mixture was accustomed to 2.5 mL with distilled water and stirred well. Absorbance was immediately assessed against the blank (the same mixture without the sample) at 510 nm using a UV-Visible Spectrophotometer (Lamda 950 UV/Vis/NIR, Perkin Elmer, UK). Results were evaluated and expressed as quercetin equivalents (mgQE/g sample) using the calibration curve of (+)-catechin.
The Linear range of the calibration curve was 25 to 200 μg/mL \( (r = 0.99) \). The extraction was conducted in triplicate.

**Free radical scavenging activity**

The influence of methanolic extracts on the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was assessed according to Woldegiorgis et al., \(^{24}\) A 0.004% DPPH radical solution in methanol was prepared, and then 4 mL of this solution was mixed with 1 mL of numerous concentrations (2–14 mg/mL) of the extracts in methanol. Finally, the samples were incubated for 30 min at room temperature in the dark. Scavenging ability was read spectrophotometrically (UV/Vis/NIR, Lambda 950 Perkin Elmer, UK) by checking the decline in absorbance at 517 nm. The absorption maxima were first settled by scanning freshly prepared DPPH from 200 to 800 nm by the scan way of the spectrophotometer. Ascorbic acid was used as a standard without extract and as the control. Inhibition of free radical DPPH in percent (I\%) was then calculated:

\[
\text{Radical scavenging activity} = \frac{A_o - A_1}{A_o} \times 100
\]

(7)

where \( A_o \) is the absorbance of the control and \( A_1 \) is the absorbance of the sample.

**Total carotene content**

It was estimated according to the method by de Carvalho et al. \(^{25}\) Approximately 1 g of the sample was taken in a mortar and crushed by adding 25 mL of acetone. This extract was filtered and collected in a volumetric flask. This extraction procedure was repeated until the sample became colorless. The extract was pooled together and transferred to a 500 mL separating funnel containing 40 mL of petroleum ether. Acetone was removed from the extract by the slow addition of distilled water. The addition of distilled water resulted in the separation of two-phases. Repeat the process by discarding the aqueous phase until no residual solvent remained. Then extract was transferred to a funnel containing 15 g of anhydrous sodium sulfate to a 50 mL volumetric flask. Read the sample absorbance at 450 nm after making up the volume to 50 mL by petroleum ether. The total carotenoid content was determined using the following formula:

\[
\text{Total carotenoid content} = \frac{A \times V \times 10^4}{P \times A_{1cm}^{1\%}}
\]

(8)

where \( A \) = absorbance; \( V \) = total extract volume (mL); \( P \) = Sample weight (g); \( A_{1cm}^{1\%} = 2592 \) (Beta carotene extinction coefficient in petroleum ether).

**Functional properties**

**Bulk density**

Bulk density (g/mL) was determined in a graduated cylinder by lightly adding 2 g of pumpkin flour into an empty 10 mL graduated cylinder and holding the cylinder on a vortex vibrator (XH-B, Hinotek, China) for 1 min. The volume was read and recorded. The measurements were made in triplicate. The ratio of the mass of the powder to the volume occupied in the cylinder decides the bulk density value in g/mL using Eq. (3) \(^{26}\)

\[
\text{Bulk density (g mL}^{-1}) = \frac{W}{V}
\]

(9)

where, \( W \) = grams of pumpkin powder; \( V \) = measuring volume
**Water absorption capacity and water solubility index**

It was assessed according to Que et al.\(^\text{[15]}\). Pumpkin flour (1 g) and water (10 mL) were vigorously mixed in a weighted 15 mL centrifuge tube, incubated in a 37°C water bath for 30 min, and then centrifuged (3000 \* g, 10 min) (TGL-16, Sichuan Shoke, China). The supernatant was collected in pre-weighed aluminum cans, and the residue was weighed after the water was evaporated at 105°C overnight.

\[
\text{Water absorption (g/g)} = \frac{\text{weight of centrifuged precipitate (g)}}{\text{weight of pumpkin flour (g)}}
\]

\[
\text{Water solubility (%)} = \frac{\text{weight of residue}}{\text{weight of pumpkin flour}} \times 100
\]

**Oil absorption capacity**

It was determined using the method of Que et al.\(^\text{[19]}\) with slight modifications. Specifically, it was done by mixing pumpkin powder (1 g) and 6 mL of corn oil in a centrifuge tube and stirring it for 30 s using a vortex mixer (XH-B, Hinotek, China), followed by centrifugation in a benchtop centrifuge (TGL-16, Sichuan Shoke, China) at 8000 rpm for 10 min. The volume of supernatant was recorded and an average was calculated from triplicate determinations.

\[
\text{The oil absorption capacity} = \frac{\text{mL of supernatant}}{\text{grams of pumpkin powder}}
\]

**Statistical analysis**

Statistical analysis was carried out using the software package SAS version 9.0 (SAS Institute, Inc., Cary, North Carolina, USA) using analysis of variance (ANOVA). Tukey’s HSD test at the significance level of 5% (P < .05) was used to determine significant differences among samples.

**Result and discussion**

The pumpkin flour quality responds differently to the application of ultrasound, microwave, and combined ultrasound followed by microwave pre-drying treatments due to a reduction in drying time through enhanced mass transfer.

**Physical properties**

The physical properties of pumpkin flour produced under different types of pre-drying treatment show a significant difference (p < .05) in all properties except pH and water activities values (Table 1 and 2). The data also depicts that water activity increased with ultrasound exposure time and microwave treatment, but it is not significantly (p < .05) different except for freeze-dried flour. In line with this finding, Szadzińska et al.\(^\text{[27]}\) reported that ultrasound application did not influence the dried potatoes’ water activity (aw).

Mothibe et al.\(^\text{[28]}\) also reported that microwave pre-drying treatment can reduce the water activity of dried apples more than ultrasound pretreatment. The results show that a water activity below 0.6, indicates it is microbiologically safe\(^\text{[29]}\). The pH value of the untreated pumpkin flour (6.46 \pm 0.16) was higher than that of pretreated and freeze-dried flours, but there was no significant difference among pretreated flours. This finding also showed that the total soluble solids (TSS) of untreated pumpkin flour (3.97 \pm 0.06) were lower than that of freeze-dried (4.53 \pm 0.21) and microwave pretreated (4.03 \pm 0.15) flours, but higher than that of ultrasound pretreated flours. As reported in previous study, the TSS of untreated pumpkin powder and salt pretreated were 7.5 and 8.3 °Brix,
Table 1. Effects of pretreatment methods on drying time, water activity, pH, and TSS content of Pumpkin flour.

| Sample code | Water activity | pH    | TSS      | Drying time (min) |
|-------------|----------------|-------|----------|------------------|
| FC          | 0.43 ± 0.02    | 6.46  | 3.97 ± 0.06 | 180              |
| FD          | 0.34 ± 0.02    | 6.21  | 4.53 ± 0.21 | 540              |
| FM          | 0.41 ± 0.00    | 6.36  | 4.03 ± 0.15 | 155              |
| 10 U        | 0.43 ± 0.03    | 6.38  | 3.77 ± 0.15 | 168              |
| 20 U        | 0.44 ± 0.00    | 6.24  | 3.43 ± 0.06 | 138              |
| 30 U        | 0.44 ± 0.00    | 6.23  | 3.2 ± 0.00  | 135              |
| 10 UM       | 0.43 ± 0.00    | 6.25  | 3.67 ± 0.06 | 148              |
| 20 UM       | 0.43 ± 0.02    | 6.22  | 3.67 ± 0.06 | 121              |
| 30 UM       | 0.44 ± 0.00    | 6.21  | 3.5 ± 0.17  | 119              |

Values are mean ± standard deviation. This means sharing the same letters in columns is not significantly different from each other (Tukey’s HSD test, p < .05). TSS, Total Soluble Solids; FC, untreated pumpkin flour; FD, Freeze-dried; FM, Microwaved pretreated; 10U, 10 min ultrasound pretreated; 20U, 20 min ultrasound pretreated; 30U, 30 min ultrasound pretreated; 10UM, 10 min ultrasound and 6 min microwaved pretreated; 20UM, 20 min ultrasound and 6 min microwaved pretreated; 30UM, 30 min ultrasound and 6 min microwaved pretreated pumpkin flour.

Table 2. Effects of pretreatment methods on color properties of Pumpkin flour.

| Sample code | L*     | a*    | b*    | C*   | H*    | DE    |
|-------------|--------|-------|-------|------|-------|-------|
| FC          | 79.80  | 5.82  | 6.29  | 41.05 | 81.64 | 0.00  |
| FD          | 71.35  | 23.33 | 48.72 | 54.07 | 64.46 | 21.54 |
| FM          | 77.00  | 6.29  | 41.05 | 41.53 | 81.28 | 3.15  |
| 10 U        | 78.62  | 5.95  | 40.16 | 40.60 | 81.58 | 1.46  |
| 20 U        | 75.82  | 5.47  | 42.34 | 42.69 | 82.65 | 4.94  |
| 30 U        | 74.33  | 5.64  | 42.11 | 42.49 | 82.37 | 6.07  |
| 10 UM       | 76.18  | 5.74  | 41.07 | 41.70 | 80.01 | 4.27  |
| 20 UM       | 72.58  | 5.74  | 44.58 | 45.23 | 80.28 | 9.06  |
| 30 UM       | 72.31  | 5.18  | 43.54 | 44.30 | 79.35 | 8.92  |

Values are mean ± standard deviation. This means sharing the same letters in columns is not significantly different from each other (Tukey’s HSD test, p < .05). L*, whiteness; a*, redness; b*, yellowness; C*, chroma; H*, hue angle; DE, color change; FC, untreated pumpkin flour; FD, Freeze-dried; FM, Microwaved pretreated; 10U, 10 min ultrasound pretreated; 20U, 20 min ultrasound pretreated; 30U, 30 min ultrasound pretreated; 10UM, 10 min ultrasound and 6 min microwaved pretreated; 20UM, 20 min ultrasound and 6 min microwaved pretreated; 30UM, 30 min ultrasound and 6 min microwaved pretreated pumpkin flour.

respectively,[30] which is higher than this finding. The reduction in TSS is reasonable because the longer samples are sonicated, the more soluble solutes are lost. The drying time for 20UM and 30UM pre-dried flour samples were lower than for the other flour samples. The possible reasons could be the faster the liquid flow rates through the food to the boundary where micro-channels are created during the ultrasonic application. A similar reduction in drying time was observed for pineapple,[31] melon[32] and mulberry[33] due to ultrasonic pre-drying treatment.

All color parameters for pretreated and untreated flours are shown in Table 2. Color is the first parameter customers use to judge the quality of dried products. The study revealed that the color parameters of pumpkin flours were affected by ultrasound, microwave, and combined pre-drying treatments, as shown in Table 2. Pumpkin is a good source of carotene, especially β-carotene, used as a coloring agent. [34] This study indicated that freeze-drying reduced discoloration and preserved the yellowness of pumpkin powders compared to other flours. L* values representing brightness were found to be lower in ultrasound-pretreated flours compared to those of microwave-pretreated flours. Microwaved and ultrasonic pretreatment had a lower L* value than untreated flour (79.80 ± 1.59) but higher than freeze-dried (71.35 ± 3.05) flour. For the ultrasound pretreated flour, the highest L* value was noted for 10U (78.62 ± 1.78), followed by 20U (75.82 ± 2.40) and 30U (74.33 ± 2.89), respectively. Samples pretreated with ultrasound for 20 min followed by 6 min microwave blanching had the highest b* value (44.58 ± 0.71), while samples pretreated at 30 min followed by 6 min microwave blanching had the highest a* value (8.18 ± 0.42). The chroma index for the untreated control flour is significantly different (p < .05) than other flour samples, with the FD (54.07 ± 1.22) recording the
highest and FC (40.10 ± 0.54) the lowest. The data reveals that while 20U and 30U show the highest hue angle, but FD shows the lowest, illustrating color purity on how an average person will recognize that color. The data reveals a significant difference between the color change of pretreated and untreated flours. Among the pretreated flours, the 20UM flour sample noticed the highest color change, the 10U (1.46 ± 0.23 was) samples showed the lowest as compared to the negative control flour, indicating that colors are not close to one another. The highest ΔE was likely due to high differences in the L* (lightness) and b* (yellowness) values. The possible reasons for these differences are the exposure of cellular-bound yellow pigmentation of the pumpkin slice to the cavitation effect of ultrasound. A similar finding was reported by Wang et al. on ultrasound pretreated carrot samples. Generally, untreated flour had significantly (p < 0.05) lower b* values than the pretreated ones, which means that the latter were less yellow (more orange) since the pretreating helped them to retain their color. This implies that the samples lost their pigment to become lighter. The results conform with previous investigations of longer drying times causing a change in food surface characteristics or producing more significant pigments losses (carotenoids and others), which lead to the color change. Microwave drying has been reported to prevent color damage during drying.

### Chemical properties

**Proximate**

The significant (p < 0.05) differences observed between the proximate parameters (moisture, protein, fat, fiber, ash, carbohydrate) of the pretreated and untreated flours were presented in Table 3. The low moisture content of the freeze-dried flours implied that it would have good storage qualities.

Why protein change No significant (p < 0.05) differences were observed between the crude fat and crude fiber values of the pretreated flours. Excluding moisture content, combined pretreatments improve the nutritional content of flour more than ultrasound and microwave pretreatment independently. Freeze-dried pumpkin flour depicted the highest value in all studied parameters except for moisture and carbohydrate content. While 20U and 30U flour samples were higher in nutritional content than FM, 10U flour was lower since it doesn’t have much difference from FC (untreated) flour. So longer (20, 30 min) ultrasound pretreatment time prevents more nutrient loss than microwave (for 6 min, at 300 W) pretreatment, which may be due to thermal effect. The result obtained indicates that 30 min ultrasound pretreated followed by 6 min microwaved blanched flour (30UM) had the highest protein (p < 0.05) (11.12 ± 0.62%) and crude fiber (12.98 ± 0.38%) (not significant) than others, while lower than freeze-dried flour. The highest two flours in carbohydrates content were 10UM (64.23 ± 0.23%) and 20UM (63.03 ± 1.86%), while the lowest two were FD (61.47 ± 1.03%) and FC (62.63 ± 0.95%). According to Toan et al. in ash, fat, protein, and crude fiber the content of pumpkin flour was 4.51, 0.85, 7.63, and 4.78, respectively lower than this finding for untreated flour.

### Table 3. Proximate composition of pumpkin flour as affected by pretreatment methods % (g/100 g dry weight basis).

| Sample code | Moisture % | Ash % | Crude fat % | Crude protein % | Crude fiber % | CHO % |
|-------------|------------|-------|-------------|-----------------|---------------|-------|
| FC          | 8.23 ± 0.21a | 5.73 ± 0.06d | 1.17 ± 0.29a | 8.72 ± 0.70d | 10.92 ± 1.31a | 62.63 ± 0.95ab |
| FD          | 5.88 ± 0.05b | 6.57 ± 0.06a | 1.85 ± 0.38b | 11.32 ± 0.16b | 13.11 ± 0.63a | 61.47 ± 1.03b |
| FM          | 8.17 ± 0.06c | 5.97 ± 0.06c | 1.25 ± 0.25c | 9.04 ± 0.25c | 11.34 ± 0.74a | 62.40 ± 1.05abc |
| 10 U        | 8.18 ± 0.17a | 5.80 ± 0.10g cd | 1.23 ± 0.25a | 8.93 ± 0.06a | 11.15 ± 0.83a | 63.84 ± 0.51ab |
| 20 U        | 7.99 ± 0.21a | 6.27 ± 0.06b | 1.45 ± 0.30a | 9.68 ± 0.35cd | 11.57 ± 0.06a | 63.05 ± 0.31ab |
| 30 U        | 7.78 ± 0.37a | 6.30 ± 0.10b | 1.50 ± 0.50a | 9.81 ± 0.63bcd | 12.10 ± 0.17a | 62.90 ± 0.48ab |
| 10 UM       | 8.03 ± 0.31a | 6.00 ± 0.10c | 1.25 ± 0.25c | 9.42 ± 0.16d | 11.45 ± 0.23a | 64.23 ± 0.23ab |
| 20 UM       | 7.64 ± 0.42a | 6.40 ± 0.10ab | 1.60 ± 0.17a | 10.88 ± 0.63abc | 12.40 ± 1.44a | 63.03 ± 1.86ab |
| 30 UM       | 7.57 ± 0.66a | 6.43 ± 0.06ab | 1.77 ± 0.25a | 11.12 ± 0.62ab | 12.98 ± 0.38a | 62.53 ± 0.76ab |

Values are mean ± standard deviation. This means sharing the same letters in columns is not significantly different from each other (Tukey’s HSD test, p < 0.05). FC, untreated pumpkin flour; FD, Freeze-dried; FM, Microwaved pretreated; 10U, 10 min ultrasound pretreated; 20U, 20 min ultrasound pretreated; 30U, 30 min ultrasound pretreated; 10UM, 10 min ultrasound and 6 min microwaved pretreated; 20UM, 20 min ultrasound and 6 min microwaved pretreated; 30UM, 30 min ultrasound and 6 min microwaved pretreated.
However, the carbohydrate content was higher, which could be attributed to the high fiber content of these flours. El-Deremy, [39] also reported a similar finding. Khandpur & Gogate, [40] reported that ultrasound pretreatment enhanced the nutritional quality of carrot, spinach, sweet lime, orange, and juices. The high nutrient retention in freeze-dried samples is not surprising as it has been reported to be one of the chosen drying methods to minimize the loss of nutrients and bioactive compounds. [41,42] Generally, the higher nutritional values observed in pumpkin flour in the present study were due to pretreatment methods which increased the loss of moisture and level of nutrients at any given weight. This was also in line with a study conducted by Morris et al., [14] who concluded that moisture removal might increase the nutrient content, which was the case in all the dried samples. Products with low moisture were generally stored longer [43] due to reduced microbial and chemical activity. In addition, the reason for an increase in protein content during the ultrasound treatment may be due to its mechanical effect that breaks up the food matrix into smaller particles that forms microspores within the food material, thus increasing the surface area, facilitating protein release, and increasing protein yield. [44]

**Phytochemical properties**

As shown in Table 4 phytate, tannins, oxylate, total phenols, total flavonoids, and total carotenoids were significantly influenced by pretreatment methods (P < .05). Anti-nutrients (phytate, tannin, and oxylate) in food materials have been reported to form complexes with several mineral elements, thereby making them biologically unavailable for human absorption and utilization. Significant differences (p < .05) were observed in the pretreated flours and controls phytate, tannin, and oxylate content. The lowest two in phytate content with no significant difference among them were 20UM (110.80 ± 8.12 mg/100 g) and 30UM (110.26 ± 8.66 mg/100 g), while freeze-dried and untreated flour samples had the highest (189.55 ± 0.00 and 142.36 ± 14.31 mg/100 g), respectively.

The finding indicated that microwave and ultrasound pretreatment reduces phytate, tannin, and oxylate content while combined pretreatment was more effective. With the increase of ultrasonic pre-drying treatment exposure time, phytate, tannins, oxylate content reduction in flour increased, but microwave pre-drying treatment was more effective in reducing phytate content (134.33 ± 11.12 mg/100 g). So probably due to the chemical degradation of phytate to lower inositol phosphates and inositol or cleavage of the phytate ring itself. [45] As shown in the study results, microwave and ultrasonic pretreatment reduced phytate, tannin, and oxylate content, while combined pretreatment was more effective. The high tannin (6.48 ± 0.40 mg/100 g) and phytate (189.55 ± 0.00 mg/100 g) content in the freeze-dried flour may indicate both susceptibility of both to hot temperatures. The content of total phenol (4.36 ± 155.36 mgGAE/g), total flavonoid (1.31 ± 160.61 mgQE/g), and total carotenoid (87.70 ± 4.56 µg/g) in microwave pretreatment flour was lower than those of flour.

**Table 4.** Phytochemical composition of pumpkin flour as affected by pre-drying treatment methods.

| Sample code | Phytate(mg/100 g) | Tannin (mg/100 g) | Oxylate mg | Total flavonoid (mgQE/g) | Total phenol (mgGAE/g) | Total carotenoid (µg/g) |
|-------------|------------------|------------------|------------|------------------------|-----------------------|------------------------|
| FC          | 142.36 ± 1.43a   | 3.55 ± 0.19a     | 3.63 ± 0.43a | 0.98 ± 0.05a           | 3.43 ± 0.19a          | 56.70 ± 0.50 a         |
| FD          | 189.55 ± 0.00a   | 6.48 ± 0.40a     | 4.29 ± 0.52a | 2.22 ± 0.22a           | 6.52 ± 0.16a          | 140.81 ± 1.53a         |
| FM          | 134.33 ± 1.11b   | 3.28 ± 0.24bc    | 2.21 ± 0.29d | 1.31 ± 0.16cde         | 4.36 ± 0.16cd         | 87.70 ± 4.56cde        |
| 10 U        | 139.37 ± 0.90a   | 3.45 ± 0.52bde   | 2.75 ± 0.10d | 1.28 ± 0.16de          | 4.44 ± 0.23bcd        | 81.29 ± 20.09de        |
| 20 U        | 138.98 ± 0.22cd  | 2.83 ± 0.30def   | 2.16 ± 0.25d | 1.63 ± 0.13bcd         | 5.58 ± 0.23abc        | 110.70 ± 7.79bcde      |
| 30 U        | 137.60 ± 0.97de  | 2.82 ± 0.08def   | 2.04 ± 0.16de | 1.52 ± 0.15bcd         | 5.06 ± 0.15abc        | 104.00 ± 4.51cde       |
| 10 UM       | 115.91 ± 0.95f   | 3.02 ± 0.27def   | 2.21 ± 0.06d | 1.36 ± 0.15cde         | 4.49 ± 0.20bcd        | 93.39 ± 1.88de         |
| 20 UM       | 110.80 ± 0.81g   | 2.71 ± 0.03ef    | 1.62 ± 0.04ef | 1.97 ± 0.14ab          | 6.31 ± 0.23ab         | 131.50 ± 9.84ab        |
| 30 UM       | 110.26 ± 0.87g   | 2.51 ± 0.05f     | 1.58 ± 0.06f | 1.73 ± 0.15bc          | 5.43 ± 0.48abc        | 122.51 ± 0.93abc       |

Values are mean ± standard deviation. This means sharing the same letters in columns is not significantly different from each other (Tukey’s HSD test, p < .05). FC, untreated pumpkin flour; FD, Freeze dried; FM, Microwaved pretreated; 10U, 10 min ultrasound pretreated; 20U,20 min ultrasound pretreated;30U,30 min ultrasound pretreated; 10 UM, 10 min ultrasound and 6 min microwaved pretreated; 20UM, 20 min ultrasound and 6 min microwave pretreated;30UM,30 min ultrasound and 6 min microwaved pretreated.
pretreated by ultrasonic for 20 min and 30 min, but higher than 10 min sonicated and untreated (FC) flour. A similar case was also reported by Kaseke, Opara, & Fawole, [46] who said microwave pretreatment enhanced total carotenoids, total phenolic content, and DPPH radical scavenging capacity of pomegranate seeds than the untreated one. Hayat et al., [47] also reported that microwave energy could increase the bioavailability of some phenolic compounds by liberating them from the food matrix. Among the pretreated flours, the 20UM flour samples had the highest total phenol, total flavonoid, and total carotenoid, which were 6.31 ± 229.99 mgGAE/g, 1.97 ± 135.22 mgQE/g, and 131.50 ± 9.84 µg/g, respectively. In contrast, the lowest 4.36 ± 155.36 mgGAE/g, 1.28 ± 155.23 mgQE/g, and 81.29 ± 4.56 µg/g were obtained for FM and 10 U flour samples, respectively. It implies that combined pretreatment resulted in higher retention of bioactive compounds than independent ultrasound and microwave pre-drying treatment alone and untreated flour. According to April et al., [48] and Hussain et al., [2] the total phenol and flavonoid content in pumpkin flour were 2.77 mgGAE/g and 2.46 mgQE/g, 1.35 ± 1.24 mgGAE/g, and 0.77 ± 0.63 mgQE/g, respectively, all lower than this finding. In line with these (Roongruangsri & Bronlund [49] also stated that pumpkin powder produced from oven dried at 50°C, 60°C, and 70°C contains about 25.99, 16.42, and 17.66 µg/g of total carotenoid content, respectively, lower than this finding. As depicted in Table 4, 30UM flour had lower TPC, TFC, and TCC than 20UM flour, it may be due to the extraction of phenolic compounds by water (phenolics losses) during extended ultrasound treatment time. [50] Opalic et al. [51] reported that prolonged sonication reduces dried apples’ the total phenolic, flavonoids content, and antioxidant capacity. As presented in Table 4, the content of total phenol, flavonoid, and carotenoids in freeze-dried flour was the highest, followed by 20UM and 30UM pretreated flours. Freeze-dried flour and 20UM flour had the highest total phenolic content, which was 47.46% and 45.72% higher than untreated flour, respectively, while microwave pretreated flour had the lowest total phenolic content (21.31%). The lowest two in total carotenoid retention were observed for 10 U (30.25%) and FM (35.35%) flour as compared to the best-case control sample (freeze-dried). Dini, Tenore, & Dini [52] reported that microwave treatment increased the bioactive compounds and antioxidants activity of pumpkin pulp and also Carpenter & Toftness [53] showed sonication pretreatment preserved it in dried onion slices. High carotenoid contents in flour show a more yellow color (20UM). Carotenoids are considered a significant source of vitamin A, which plays a crucial role in the human body by promoting eyesight, immune system, reproductive system, growth, and development, whereas deficiency of this vitamin is a common cause of infant mortality and blindness. [2]

Figure 1 illustrates the antioxidant scavenging activities of extract of pretreated and untreated pumpkin flour relative to ascorbic acid (control). The combined pre-drying treatment showed a stronger DPPH scavenging activity than single pretreatment alone. DPPH scavenging ability increases with the content of total phenol, total flavonoid, and carotenoid in flour, among which FD and 20UM flour were the highest, and 10 U and FC were the lowest. Because of their conjugated double bonds, carotenoids have a strong antioxidant capacity to scavenge free radicals. [54] Although the total flavonoid contents in pumpkin are lower than total phenolic s, even small concentrations of total flavonoid contents possess strong antioxidation potential. [55] This result is consistent with the findings of, [56] who reported that the ultrasonic pretreatment retained higher proportions of antioxidant properties, total phenolic and total flavonoid content compared to apple slices dried without ultrasound pretreatment. This study revealed that 20 minutes of ultrasound followed by 6 minutes of microwave (300 W) pre-drying treatment could be recommended as pre-drying conditions for obtaining the high-phytochemical composition of pumpkin flour. Titikan & Rungarun, [57] also reported that the composition of phenolics and carotenoids may also be affected by fruit and vegetables processing methods.

**Functional properties**

Table 4 exhibited that bulk density, water absorption capacity (WAC), water solubility index (WSI), and oil adsorption capacity (OAC) of pumpkin flour were affected by ultrasonic, microwave, and combined pre-drying treatment methods before drying. Bulk density was reduced with ultrasound and
microwave pretreatment, and the effect was more pronounced with an increase in the exposure time of ultrasound treatment. The impact of microwave pre-drying treatment on a bulk density was lower than that of ultrasonic and combined pre-drying treatment. Untreated flour had a higher bulk density (0.52 ± 0.01 g/cc), while freeze-dried flour had a lower bulk density (0.33 ± 0.01 g/cc). Lim et al.⁵⁸ indicated that the bulk density of pumpkin pretreated with Ca(OH)₂ and blanching then air-fried were 0.400 g/cc and 0.358 g/cc, respectively, comparable to this finding. According to Mirhosseini & Amid,⁵⁹ the low bulk density of freeze-dried flour might be due to increased volume rather than mass, although the solubility of freeze-dried flour may be significantly affected by the low bulk density.

As displayed in Table 5, ultrasound, microwave, and combined pretreatment of samples generally resulted in greater WAC, WSI, and OAC. In comparison, 30 UM flour had significantly (p < .05) the highest WAC (9.75 ± 0.24 g/g), WSI (11.83 ± 1.0%) and OAC (2.28 ± 0.11 mL/g), but the lowest content was observed for freeze-dried flour (6.13 ± 0.08 g/g, 5.95 ± 0.75%) expect for OAC on untreated flour (1.53 ± 0.06 mL/g), respectively. In addition, M. Asif-Ul-Alam et al.⁶⁰ pointed out that the water holding capacity of freeze-dried flour was lower than that of hot air-dried flour. High water absorption capacity improves yield and consistency, gives the body to food,⁶¹ and is an alternative emulsifier for food formulations.⁶² Higher water solubility was observed in 30 UM,

| Sample code | Bulk density (g/cc) | WAC (g/g) | WSI (g/g) | OAC (g/g) |
|-------------|---------------------|-----------|-----------|-----------|
| FC          | 0.52 ± 0.01ᵇ       | 7.03 ± 0.02ᶜ   | 6.05 ± 2.55ᶜ   | 1.53 ± 0.06ᶜ   |
| FD          | 0.33 ± 0.01ᵃ       | 6.13 ± 0.08ᶜ   | 5.95 ± 0.75ᶜ   | 1.90 ± 0.10ᵇ   |
| FM          | 0.49 ± 0.01ᵇ       | 9.11 ± 0.15ᵇ   | 7.05 ± 0.95ᶜ   | 1.93 ± 0.12ᵇ   |
| 10 U        | 0.51 ± 0.01ᵃᵇ     | 8.94 ± 0.32ᶜ   | 6.65 ± 1.15ᶜ   | 1.53 ± 0.06ᶜ   |
| 20 U        | 0.44 ± 0.01ᵇᶜ     | 9.47 ± 0.27ᵇᶜ  | 9.80 ± 1.00ᵇᶜ  | 2.00 ± 0.00ᵇ   |
| 30 U        | 0.42 ± 0.01ᵇᶜ     | 9.69 ± 0.29ᵇᶜ  | 10.05 ± 1.05ᵇᶜ | 2.00 ± 0.00ᵇ   |
| 10 UM       | 0.44 ± 0.01ᵇᶜ     | 9.33 ± 0.22ᵇᶜ  | 9.60 ± 1.00ᵇᶜ  | 1.97 ± 0.15ᵇ   |
| 20 UM       | 0.38 ± 0.01ᵇᶜ     | 9.71 ± 0.05ᵃ   | 11.17 ± 0.76ᵃ  | 2.03 ± 0.06ᵇ   |
| 30 UM       | 0.36 ± 0.01ᵇᶜ     | 9.75 ± 0.24ᵃ   | 11.83 ± 1.01ᵃ  | 2.28 ± 0.11ᵃ   |

Values are mean ± standard deviation. This means sharing the same letters in columns is not significantly different from each other (Tukey's HSD test, p < .05). WAC, water absorption capacity; WSI, Water solubility index; OAC, oil absorption capacity; FD, Freeze dried; FC, untreated pumpkin flour; FM, Microwaved pretreated; 10 U, 10 min ultrasound pretreated; 20 U, 20 min ultrasound pretreated; 30 U, 30 min ultrasound; 10 UM, 10 min ultrasound and 6 min microwaved pretreated; 20 UM, 20 min ultrasound and 6 min microwaved pretreated; 30 UM, 30 min ultrasound and 6 min microwaved pretreated.
indicating that more starch had been decomposed. The analysis showed that fluidized bed-dried pumpkin flour had higher water solubility and bulk density than freeze-dried flour, which is consistency with the findings of Que et al. [15]. According to Traynham et al.,[62] fruit and vegetable flours, which have high water absorption and oil adsorption capacities, can impart water-retention and fat-binding properties essential in bakery products and other select food applications. This study showed that with increase of ultrasound exposure time, the water solubility, water absorption capacity, and oil adsorption capacity of pumpkin flour also increased.

**Conclusion**

Ultrasound combined with microwave pre-drying treatment reduced the drying time than independent pretreatments alone, and the finding indicates that 20 UM treatments were more effective (32.78%) in reducing drying time. The present work describes the possibility of producing high-quality pumpkin flour in terms of the physical, chemical, and functional qualities by ultrasound, microwave, and combined pre-drying treatment. Pre-drying treatment decrease lightness due to the increase in the redness and yellowness of all pretreated samples. The high levels of crude protein and fiber found in pumpkin flour suggest that these raw materials are potential ingredients for developing nutritious foods. The analysis indicates that the flour moisture content and water activity values were within acceptable limits for safe storage. Untreated pumpkin flour showed greater bulk density, WAC, and WSI than freeze-dried flour, but lower than pretreated. Ultrasonication Pre-drying treatment for 20 min followed by microwave (300 W) blanching for 6 min before drying was the best process for maintaining color, total phenolic content, total carotenoids content, and DPPH activities during processing. This information is crucial for preparing pumpkin flour, which can be used as an ingredient for coloring and improving food’s nutritional and medicinal values.

**Abbreviation**

| 10U                  | 10min ultrasound pretreated |
|----------------------|-----------------------------|
| 20U                  | 20min ultrasound pretreated |
| 30U                  | 30min ultrasound pretreated |
| 10UM                 | 10min ultrasound followed by 6min microwaved pretreatment |
| 20UM                 | 20min ultrasound followed by 6min microwaved pretreatment |
| 30UM                 | 30min ultrasound followed by 6min microwaved pretreatment |
| AS                   | Ascorbic acid               |
| FC                   | untreated pumpkin flour    |
| FD                   | Freeze-dried                |
| FM                   | Microwaved pretreated       |

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