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Nutritional, biochemical and phytochemical characterization of seeds and seed oil of pumpkins (*Cucurbita maxima*) grown in Malawi

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Pumpkin is a significant part of dish when in season in Malawi. In this study, the proximate composition, physicochemical and phytochemical properties were determined from pumpkin seed and oil. The *Cucurbita maxima* seed proximate compositions (%) were dry matter (97.58±0.26), crude protein (32.47±0.15), ash (7.38±0.05), crude fat (46.39±0.06), crude fiber (12.29±0.17) and carbohydrate (4.18±0.21), respectively. The physicochemical composition showed that saponification and ester values, in mg KOH/g oil, were the highest, registering 161.705±12.281 and 161.022±12.018 values followed by peroxide value (32.213±0.677 meq O_2/kg), iodine value (17.769±0.147 g I_2/g), free fatty acid as oleic acid (1.964±0.025 mg/100 g), refractive index (1.446±0.000), acid value (0.987±0.0125 mg KOH/g) and specific gravity (0.8985±0.00370). Results in seed flour on phytochemical properties, in mg/100 g, were phytate (119.07±6.65), oxalate (1462.77±97.518), alkaloids (90.997±1.574) and flavonoids (61.7605±7.6035). The oil phytochemical content, in mg/100 g, were highest in alkaloids (826.08±0.71), followed by phytate (309.7±16.4), flavonoids (235.36±8.97) and oxalate (87.272±0.612). In seed flour, the pH value was 6.9±0.00, titratable acidity (0.03±0.00 mg/g), vitamin C and phosphorus, in mg/100 g were 4.9876±0.0075 and 329.92±52.04, respectively. Findings from this study showed that pumpkin seed is a good source of essential nutrients for human nutrition and vegetable oil for industrial uses.

Key words: Pumpkin (*Cucurbita maxima*), vegetable oil, proximate composition, physicochemical, phytochemicals, saponification value, vitamin C, minerals.

INTRODUCTION

The demand for vegetable oils and fats in emerging countries, stimulated by population and income growth, is estimated to increase for better nutrition and use in fuel and industrial purposes like biofuel (Fry and Fitton, 2010; OECD/FAO, 2021). Vegetable oil consumption in sub-Saharan Africa (SSA) is growing at 2.8% arising

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from both per capita demand and population growth by 2030 (OECD/FAO, 2021). It is reported that in the midst of COVID-19 challenges, in SSA, vegetable oil imports increased compared to meat and sugar, vegetable oil imports volumes are projected to exceed production in next 10 years (OECD/FAO, 2021).

Vegetable oils have vital importance in meeting global nutritional demands through its uses as food and other uses in industries (Kumar et al., 2016; El-Hamidi and Zaher, 2018). The world vegetable oil consumption is mainly based on soybean (*Glycine max*), palm, rapeseed and sunflower (*Helianthus annuus*) oils with 31600, 30500, 15500 and 8600 million kilograms annually (Stevenson et al., 2007). However, these traditional vegetable oil sources fail to satisfy the increasing domestic and industrial demands (Kumar et al., 2016).

Pumpkins, locally known as maungu in Malawi, belong to the genus *Cucurbita* and Cucurbitaceae family and are grown in the tropics and sub-tropical countries (See et al., 2007). Pumpkin belongs to the three main categories which are *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata* (Dotto and Chacha, 2019). Pumpkins fruits are consumed as either vegetables or desert (pie) and the seeds are also edible. The seeds are roasted and eaten as snacks and sometimes oil is extracted (Kulczyński et al., 2020). Other authors have previously reported that pumpkin seeds have high levels of minerals like potassium, zinc, magnesium, manganese and phosphorus (Elinge et al., 2012; Devi et al., 2018). The seeds are used in fortification of some foods (Elechi et al., 2020). Pumpkin seeds have high levels of functional compounds and therefore are conventionally employed in herbal, diseases treatment and other medicinal uses (Stevenson et al., 2007). Previous studies indicate that consumptions of pumpkin seeds, rich in zinc, improve sexual health, stimulate ejaculatory latency and decrease lead contaminants side effects (Abd El-Ghany et al., 2010; Gundidza et al., 2009). Pumpkin seeds though considered as food, are usually considered as agricultural and agro-industrial wastes despite being excellent sources of bioactive compounds with significant nutraceutical properties (Devi et al., 2018). The seeds have positive pharmacological effects and are considered as anti-diabetic, antifungal, antibacterial, anti-inflammatory and antioxidant (Syed et al., 2019).

Pumpkin seed oil is used for frying in other countries in Africa and the Middle East but in south Austria, Slovenia and Hungary the oil is used as salad oil (Wenzl et al., 2002). The oil has strong antioxidant properties with vast healthy benefits like delaying of hypertension development, lessening bladder and urethral pressure as well as colon and rectum cancer levels (Stevenson et al., 2007). Consumption of pumpkin seed oil prevents growth of prostate besides reducing the size of prostate, lung, gastric and breast cancer (Stevenson et al., 2007). The oil composition in the seeds is estimated at 47.99±0.01, 38.0% (Saad et al., 2021; Elinge et al., 2012) and 33.12±2.20%, 47.03% with some differences depending on type and genetic diversity (Ike et al., 2020; Younis et al., 2000). Pumpkin seeds have high quality oil and are reported to contain high quality proteins in the range of 30.23% (Syed et al., 2019). The oil is either dark green (Badr et al., 2011), dark green or dark brown in color (Stevenson et al., 2007). The oil contains a high amount of free fatty acids (FFAs) like oleic (43.8%), linoleic (33.1%), palmitic (13.4%) and stearic (7.8%) as fractions of the total FFAs (Badr et al., 2011).

In Malawi, pumpkins are mostly grown for consumption of the flower, fruits and leaves as fruits and vegetables. The seeds are usually thrown away and not used despite the high quality of oil from pumpkin seeds. This could obviously be attributed to either limited or lack of information on the nutritive composition of the pumpkin seeds grown in Malawi. Therefore, the aim of the current study was to evaluate the crude oil, phyto-chemical and physicochemical content of seed and oil from pumpkins grown in Malawi with the expectation of obtaining nutritional information on pumpkin seeds grown in Malawi as opposed to get information on pumpkin seeds grown in other parts of the world.

MATERIALS AND METHODS

Sample collection and preparation

Ripe pumpkins (*C. maxima*) fruits were randomly harvested from the garden in 2017 growing season in Lilongwe district, Malawi. The fruits were crushed to remove the seeds which were cleaned in distilled water and the seeds were subsequently sun-dried. The dried seeds were ground using a 1 mm sieve in a Thomas-WILEY model 4 Laboratory Mill to analyze the physicochemical and phytochemical of the seeds.

Nutritional composition

Nutritional composition like dry matter (DM), ash, crude protein (CP), crude fat and crude fiber (CF) were analyzed from the ground samples using AOAC (2016) methods. Chemical analyses like titratable acidity as oleic acid, pH and phosphorus (P) were also analyzed from the same samples.

Dry matter using oven method

Dry matter was analyzed by following AOAC, 2016 method with minor modifications. The crucibles were thoroughly washed, dried in the oven, cooled in a desiccator and weighed. 2.5 g of the sample was put in the crucible of known weight and dried to constant weight at 105°C for 5 h. The sample DM was calculated as the fraction of the original dry weight and was expressed as a percentage by being multiplied by 100.

Ash content determination using muffle furnace

Dry sample (2.5 g) was weighed in the porcelain crucibles, placed in the muffle furnace and burnt to ashes at 550°C for 5 h. The ash

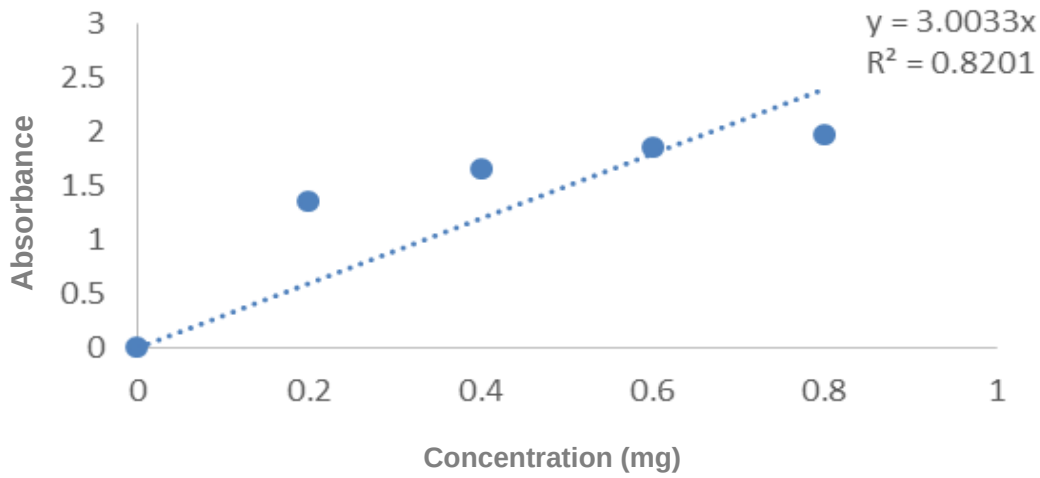

Figure 1. Standard graph fpr carbohydrates.
Source: Authors 2022

content as a percentage was calculated using Equation 1 (AOAC, 2016).

$$\% \, Ash = \left[\frac{W3-W2}{W1-W2}\right] x \, 100 \quad (1)$$

where W_1 is mass, in grams, of crucible and sample before igniting the sample, W_3 is mass of crucible and ash after igniting the sample and W_2 is mass of crucible only.

Crude protein (CP) using micro-Kjeldahl method

The amount of nitrogen (N) in the samples was determined by using micro-Kjeldahl method and was converted to crude protein by multiplying by a factor of 6.25. In this method, the samples were digested in concentrated (98%) sulphuric acid and the digests were distilled into weak 4% boric acid. The distillates were finally titrated with 0.1 M hydrochloric (HCl) acid using mixed indicator which contains methyl red and bromocresol green (AOAC, 2016).

Determination of crude fat

Finely ground sample (2.5 g) was placed in porous extraction thimbles to extract the crude oil using petroleum ether as a solvent in a Soxhlet apparatus for 16 h. The Soxhlet apparatus had a water-cooled condenser fitted above the 250 ml flat bottomed flask which contained petroleum ether. The petroleum ether was heated at 40 to 60°C for 16 h continuously extracting the fat. After 16 h of extraction, the rotary evaporator was then used to separate crude fat from petroleum ether. The 250 ml flat bottomed flask with crude oil was dried in the drying oven to constant weight at 105°C to remove moisture. The amount of crude fat, expressed as a percentage of the dry weight sample, (AOAC, 2016) was calculated using Equation 2.

$$\text{Crude fat } \% = \left[\frac{A-B}{W}\right] x \, 100 \quad (2)$$

where A= weight of flask and oil after extraction, B = weight of flask only, and W = weight of sample in grams.

Determination of crude fiber from pumpkin seeds

In determining crude fiber, 2 g of the samples was boiled in 200 ml of weak sulphuric acid (1.25%) and sodium hydroxide (1.25%) for 30 min, respectively after adding a few drops of anti-foaming agents. The mixture was filtered and the residues were washed for three times with hot water. After washing with hot water, the residues were then washed with 95% ethanol and dried at 105°C for 5 h to constant weight. The dried residues were placed in a muffle furnace and ignited at 550°C for 5 h. The crude fiber was calculated as the difference between the weight of the residues and ash and expressed as a fraction of the sample weight in percentages (AOAC, 2016).

Determination of carbohydrates composition

The carbohydrate content in pumpkin seed flour was determined by Anthrone method as described by Harintha and Jayadev (2017) with minor modifications. 100 mg of the sample was hydrolysed by boiling in 100 ml of HCl for 10 min. The mixture was filtered, cooled and diluted to 100 ml. 1 ml of the filtrate was pipetted into a Pyrex test tube and 4 ml of a freshly prepared 0.2% Anthrone reagent (0.2 g Anthrone in 500 ml concentrated sulphuric acid) was added. The mixture was let to stand in an ice water bath to cool and for colour development for 10 min. A stock solution of glucose solution of 1 mg/ml was prepared and standard solutions of 0 to 0.8 mg were prepared. Absorbance of both samples and standards was measured at 620 nm using uv spectrophotometer. Total carbohydrates / sugar content was calculated using standard curve equation y=3.0033x as shown in Figure 1.

Oil extraction procedure

A Soxhlet extractor was used to extract the oil using petroleum ether (bp 40 to 60°C) as a solvent. 20 g of the finely ground sample was placed into a porous thimble in a Soxhlet apparatus connected to a weighed 250 ml flat bottomed quick fit flask containing 200 ml petroleum ether which was continuously boiled at 40 to 60°C. After 16 h of extraction the petroleum ether was separated from the oil by using a rotary evaporator. The crude oil in the flask was then dried at 105°C in the oven for 2 h to constant

weight. The crude oil was kept in a tight closed container and refrigerated with no any further treatment waiting for some analysis (FAO/WHO, 1999).

Physicochemical analysis of oils

The physicochemical properties of the oils were analyzed by following Association of Official Analytical Chemists (AOAC), 2016 methods with minor modifications as described subsequently.

Determination of saponification value (SV)

Oil (1 g) was weighed in a conical flask followed by the addition of 50 ml ethanolic potassium hydroxide (KOH, 1 M). The mixture in the flask was refluxed until it became clear for 1 h. A blank sample was prepared with only 50 ml ethanolic KOH and was refluxed as the samples. After refluxing the mixture was cooled, a drop of phenolphthalein indicator was added and then titrated to a faint pink color end point using 1.0 M hydrochloric acid (HCl). The following equation was used to compute saponification value (SV).

$$\mathrm{SV(mg\ KOH\ g^{-1}\ oil)} = \frac{\mathrm{(A-B)\ x\ N\ x\ 56.02}}{\mathrm{W}} \quad (4)$$

In this equation A= Blank ethanolic HCl volume in ml, B=sample ethanolic HCl volume in ml, N= normality of HCl, and W=weight of sample/oil in grams.

Determination of acid value (AV) composition in the oil

This was determined by mixing 1 g of the oil with a solution of 25 ml of absolute ethanol and diethyl ether (1:1) in a 250 ml conical flask. The mixture was then warmed at 40°C in a water bath for 5 min. Three drops of phenolphthalein indicator were added and the mixture was titrated using 0.1 M potassium hydroxide (KOH, 0.1 M) to a faint pink color that lasted for 30 s. The following equation was used to calculate acid value of the samples as described by Negash et al. (2019):

$$Acid\ Value\ (mg\ KOH\ g^{-1}\ oil) = \frac{ml\ (KOH)\ x\ N\ x\ 56.1}{W} \quad (5)$$

In this case N = concentration of KOH in normality, W (g) = sample weight in grams.

Determination of free fatty acids (FFAs)

FFAs values were determined by titrating 1 g of oil against 1.0 M potassium hydroxide, after adding phenolphthalein indicator, to a faint pink color. FFAs are calculated as oleic acid equivalent where 0.1 M KOH = 28.2 g oleic acid. The following equation was used to calculate FFAs:

$$FFA\ (mg\ 100\ g^{-1}\ oil\ as\ oleic\ acid) = \frac{Titre\ volume\ (ml)\ of\ KOH\ x\ 0.1N\ x\ 28.2}{W} \quad (6)$$

where N = Concentration of ethanolic KOH in Normality and W (g) = sample weight in grams.

Determination of peroxide value (PV)

Peroxide value was determined by the method described by Negash et al. (2019) with minor modifications. 1 g of oil was mixed with 20 ml of glacial acetic acid: chloroform solvent (3:2 v/v) in a 250 ml conical flask, 1 ml of potassium hydroxide (saturated) was then added to the mixture and was kept in the dark for 1 min. Then the mixture was titrated against 0.1 M sodium thiosulphate ($Na_2S_2O_3$) solution using 5 ml of starch as an indicator after adding 30 ml of distilled water. A blank sample was prepared and treated as the samples. Peroxide value was calculated as meq oxygen per kilogram oil using the equation:

$$PV\ (meq\ O_2\ Kg^{-1}\ oil) = \frac{\left((V_2 - V_1)\ x\ M\right)\ x\ 100}{W} \quad (7)$$

where V_1= 0.1 M Na_2S2O_3 titre volume in ml for blank, V_2 = sample titre volume in ml, and W (g) = sample in grams.

Determination of iodine value (IV)

Iodine value was determined by following the methods described by the Association of Official Analytical Chemists (AOAC, 2016) and Choudhary and Pande (2000) methods with some modification in replacing carbon tetrachloride with cyclohexane.
Oil (0.5 g) of 20 ml of cyclohexane: glacial acetic acid (1:1 V/V) solution and 10 ml of Wijs reagent were mixed in a 250 ml conical flask, thoroughly mixed and kept in the dark for 1 h. The mixture was titrated with 0.1 M $Na_2S_2O_3$ solution, to colorless end point using starch as an indicator, after adding 15 and 100 ml of 15% potassium iodide (KI) and distilled water. Equation 8 was used to calculate IV:

$$\mathrm{IV\ (g\ I_2\ g^{-100}\ oil)} = \frac{\mathrm{(B-S)\ x\ M\ x\ 126.9\ x\ 100}}{\mathrm{W\ x\ 1000}} \quad (8)$$

In this equation B= ml of sodium thiosulphate (0.1 M) used in titrating the blank, S= ml of sodium thiosulphate (0.1 M) used in titrating the sample, 126.9= iodine molar mass, M= molarity of sodium thiosulphate, and W= weight in grams of the sample.

Calculation of ester value (EV)

EV was computed as the difference between the saponification value (SV) and acid value (AV) using the following equation:

$$\mathrm{EV\ (mg\ KOH\ g^{-1})} = \mathrm{SV - AV} \quad (9)$$

Determination of oil refractive index

Bellingham and Stanley No. A83304 refractometer was used to measure the refractive index of the pumpkin seed crude oil. The oil was spread on the lower prism of the instrument, the box was closed and water was let to flow around the refractometer jacket at 25°C. The refractometer light was adjusted and the compensator knob was turned to get a dark borderline on the cross wires which was viewed through the refraction view piece. The reading was taken from the scale view using the eyepiece.

Determination of specific gravity

Specific gravity of the oil was determined by following the method described by AOAC (2016) with minor modification. A 25 ml density bottle (pycnometer) was accurately weighed W_a to constant weight and filled with distilled water and was weighed again W_b. The

density bottle was dried and filled with the pumpkin oil and was weighed W_c and specific gravity was calculated as follows:

$$\text{specific gravity} = \frac{Wb-Wa}{Wc-Wa} \quad (10)$$

Titratable acidity of C. maxima seed flour

The sample (2 g) was dissolved in 100 ml of distilled water which was titrated with 0.1 M NaOH using phenolphthalein as an indicator and TA was expressed per 100 g of the sample. The calculated TA was converted to oleic acid by multiplying by the molecular mass of 0.282 g (AOAC, 2016).

$$TA\ (g\ g^{-100}\ oil) = \left(\frac{(V_{NaOH}\ x\ M_{NaOH}\ x\ 0.282)}{W}\right) x\ 100 \quad (11)$$

where V_{NaoH} = titre volume of sodium hydroxide, M_{NaoH} = molarity of sodium hydroxide.

Determination of vitamin C of Cucurbita maxima seed flour

The method described by Raghu et al. (2007) was used to analyze for vitamin C with some modifications. 2 g of the sample and 20 ml of distilled water were mixed in the 250 ml conical flask and shaken for 10 min. After shaking the mixture was titrated with 2, 6 dichlorophenolindol solution. A standard solution of ascorbic acid (0.2 mg/100 ml) was prepared as reference. Vitamin C was expressed in mg/100 g of dry matter.

pH using pH meter

This was measured by dissolving 2 g of the sample in 100 ml of distilled water using GLP pH meter at 25°C (AOAC, 2016).

Phosphorus determination using UV-Spectrophotometer

Phosphorus was determined by following AOAC (2016) method with minor modifications. The weighed samples (1 g) in porcelain crucibles were ignited in a muffle furnace at 550°C to constant weight. The ash was mixed with 3 ml of 3 M hydrochloric (HCl) acid and filtered using cotton wool. The filtrate was diluted to 100 ml in a volumetric flask. The diluted digested samples, 0.75 ml, were pipetted into 20 to 25 glass vials and diluted with 9 ml of distilled water. Standard samples were prepared by pipetting 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 ml of 1 mg/100 g into 20 to 25 ml vials and diluted with 9 ml of distilled water. Phospho-vanado-molybdate/Molybdate reagent (solution, 2 ml) was added in each vial and after 1 h of color development, absorbance was measured at 860 nm wavelength using a DR 5000 WAGTECH projects ultra-violet visible spectrophotometer.

Phytochemical analysis of oils

Determination of phytic acid content

Phytic acid was analyzed by Davis and Reid method as described by Abulude (2007) with minor modification. 2 g of the sample was dissolved in 30 ml of 2% hydrochloric acid for 3 h with continuous shaking. The mixture was filtered and 25 ml of the filtrate was mixed with 5 ml of ammonium thiocyanate solution which was titrated against ferric chloride solution containing 1.95 mg Fe/ml to brownish yellow color that lasted for 5 min. Phytate content was calculated as follows:

Phytate phosphorus = iron equivalent × 1.95 mg of titre

Phytate = phytate phosphorus × 3.65 g (12)

Determination of alkaloids content

Alkaloids content was determined by dissolving 5 g of the sample in 10 ml of acetic acid in ethanol (1:5 v/v) and the solution was left to stand for 4 h. The filtered solution was evaporated to a quarter of the original solution and concentrate ammonium hydroxide solution was added dropwise till precipitation was complete. The precipitate was filtered and dried in the drying oven to constant weight (Obadoni and Ochuko, 2001).

Determination of oxalate content

Oxalate content of the samples was analyzed by dissolving 1.0 g of the sample in 75 ml of 1.5 M sulphuric acids, stirred for 1 h and filtered. The filtrate (25 ml) was titrated while hot with 0.05 M potassium permanganate to a faint pink color that persisted for 30 s. Oxalate composition in the samples was calculated using the following equation (Mishra et al., 2017):

1 ml of 0.05 M $KMnO_4$ = 2.2 mg Oxalate (13)

Determination of flavonoids content

The flavonoids composition in the samples was determined by the method of Boham and Kocipai-Abyazan (1994) as described by Kwada and Tella (2009). Flavonoid was extracted from 10 g of the sample with 300 ml of methanol: water (80:20 v/v) solution at room temperature for 1 h. The solution was filtered through a 125 mm Whatman filter paper and the filtrate was dried in the oven in a weighed porcelain crucible to constant weight.

Statistical analysis

The samples were chemically analyzed in triplicates in the laboratory and the mean and standard error value of each chemical parameter was calculated using descriptive statistics in Microsoft excel.

RESULTS AND DISCUSSION

Nutritional composition of *C. maxima* seed flour

Results on nutritional composition of *C. maxima* L. seeds are presented in Table 1. It can be observed that crude protein, ash, crude fat and crude fiber expressed on dry matter basis were generally high compared to the values obtained in previous studies (Elinge et al., 2012; Habib et al., 2015). The crude protein value of 32.47±0.15% was comparable to 34.56% (Habib et al., 2015) for *C. maxima* but higher compared to the value of 27.48% for *C. pepo* L. (Elinge et al., 2012). The crude protein content was comparable to that of sunflower (*H. annuus*) seeds

Table 1. Nutritional composition of pumpkin (*Cucurbita maxima L.*) seeds.

Nutrient	Composition% (mean ± SD)
DM	97.58±0.26
Crude protein	32.47±0.15
Ash	7.38±0.05
Crude fat	46.39±0.06
Crude fiber	12.29±0.17
Carbohydrates	4.18±0.21

Mean ± SD; DM= Dry matter.
Source: Authors 2022

Table 2. Physicochemical properties of pumpkin seed oil.

Parameter	Value (mean ± SD)
Saponification value (mg KOH/kg)	161.705±12.281
Unsaponifiable matter%	56.0±7.75
Iodine Value (g I_2/g)	17.769±0.147
Peroxide Value (meq O_2/kg)	32.213±0.677
Acid Value (mg KOH/g)	0.987±0.0125
Refractive Index at 25^oC	1.446±0.000
Specific gravity at 25^oC	0.8985±0.0037
Ester Value (mg KOH/g)	161.022±12.018
Free Fatty Acids (mg/100 g as oleic acid)	1.964±0.025

Source: Authors 2022.

(27.53±0.00%), *Arachis hypogaea* seeds (26.43±1.15%) and soybean (*G. max*) seeds (36.6±0.7%) (Saad et al., 2021; Shibli et al., 2019; Siulapwa and Mwambungu, 2014). The high protein content in the pumpkin seeds makes them suitable for food fortification aimed at improving protein content of foods. The seed ash value of 7.38±0.05% was higher compared to values of 5.92±0.81% (Ike et al., 2020) and 3.8% (Habib et al., 2015) obtained by other researchers for *C. pepo L.* and *C. maxima* L., respectively. The pumpkin seed ash content was also higher compared to the value of 3.66±0.00% reported in previous studies (Saad et al., 2021) and 2.00±0.11 to 2.17±0.05% (Shibli et al., 2019) for *H. annuus* and various cultivars of *A. hypogaea*, respectively. The high amount of ash content denotes that *Cucurbita* seeds have high concentration of minerals that could assist in speeding up metabolic processes, growth and development improvement (Jacob et al., 2015). The crude fat content of 46.39±0.06% was higher compared to values of 36.7% (Habib et al., 2015) and 33.12±2.20 (Ike et al., 2020) for *C. maxima* L. and *C. pepo* L. but was comparable to 47.99±0.01% for pumpkin seed flour reported in Libya (Saad et al., 2021). Other authors have previously reported crude fat values of 49.80±3.53% (Shibli et al., 2019) for raw *A. hypogaea* and 50.80±0.01 and 48.68±2.82% for *H. annuus* seeds (Saad et al., 2021) in studies conducted in Pakistan and Libya, respectively which were comparable to the pumpkin seed value from this study. Fats are important because they provide the body with maximum energy compared to other nutrients like proteins and carbohydrates (Oluyemi et al., 2006). The seeds had a crude fiber value of 12.29±0.17% which was higher compared to the value of 7.24±0.64% for pumpkin seed flour reported in Nigeria (Ike et al., 2020). Carbohydrate/Sugar content was lower compared to the value of 19.75±2.47 for *C. maxima* and *C. pepo* L. seed flour (Ike et al., 2020). However, the carbohydrate (sugar) content was comparable to the value of 4.24±0.03% for *A. hypogaea* seeds (Mustapha et al., 2015). The variations between the proximate compositions from this study and that of previous studies on pumpkin seed flour could be attributed to pumpkin variety, maturity stage, and environmental stress as well method of analysis employed.

Physicochemical composition of *C. maxima* seed oil

Results on physicochemical properties of *C. maxima* seed oil are presented in Table 2. The physicochemical properties are significant properties for defining the

quality of oils for their suitability for consumption (Mengistie et al., 2018). Saponification value measures the molecular weight of oils (Mengistie et al., 2018) and type of fatty acids present in oils (Tsado et al., 2018; Adejumo et al., 2013). Results from this study have revealed that crude pumpkin seed oils have high number of carbon atoms as evidenced by the obtained saponification value, in mg KOH/g oil, of 161.705±12.281 which was lower compared to 184.20±5.37 for crude pumpkin seed oil (Bwade et al., 2013). The saponification value of crude oil obtained in this study was however, below the recommended values of 180-199 (FAO/WHO, 1999) for edible oil, 187-196 and 189-195 for *A. hypogaea* and *G. max* oils, respectively (FAO/WHO, 1999).

Iodine value defines the extent of oil unsaturation which is the number of double bonds of the oil (Syed et al., 2012; Tsado et al., 2018) and oils with high values have more double bonds meaning that the oils could easily undergo oxidation and rancidification reaction (Tsado et al., 2018). The iodine value of 17.769±0.147 g I_2/g oil was lower as compared to the value of 111.74 g I_2/g oil for pumpkin whole seed oils (Agustina et al., 2019) but comparably similar to the value of 16.00±0.96 for seed oil from Nigeria (Bwade et al., 2013). The extracted oils low iodine values mean that the oils are saturated and have long shelf life because they cannot easily undergo oxidation and rancid reaction (Tsado et al., 2018).

Peroxide and free fatty acid values are the two most significant factors used to measure the characteristics of edible vegetable oils. These two factors are needed in crude oil refining processes and the suitability of the oil for consumption purposes declines with the increasing amount of free fatty acids in the oils (Hasan et al., 2019). Vegetable oil peroxidation and adulteration are defined by peroxide value (Tsado et al., 2018) which may be used to define oil quality and stability during shelf life (Adejumo et al., 2013; Hasan et al., 2019; Negash et al., 2019). The extracted crude oil had peroxide value of 32.213±0.677 meq O_2/kg oil which was higher compared to the value of 8.66±0.21 meq O_2/kg oil for extracted crude pumpkin (*C. pepo*) seed oils (Bardaa et al., 2016). The peroxide value obtained from this study was above the required value for health consumption of 5.0 and 10.0 meq O_2/kg oil (FAO/WHO, 1999) for edible fat/oils and virgin and cold pressed fat and oils, respectively.

The free fatty acid value (1.964±0.025 mg/100 g oil) obtained in this study was lower compared to the value of 270 mg/100 g oil reported by other researchers for *C. maxima* in Macedonia (Alfawaz, 2004). Other authors in Egypt reported comparable free fatty acid value of 2.27±0.42 mg/100 g oil for pumpkin (*Cucurbita moschata*) seed oil (Abd El-Aziz and Abd El-Kalek, 2011).

Acid value defines the degree of oil spoilage, basing on free fatty acids (FFAs), from enzymatic activity (Hasan et al., 2019). The acid value obtained in this study was lower compared to the values of 13.5±0.6 and 11.6±0.6 (Amin et al., 2019b) for native and hybrid pumpkin (*C. maxima*) seed oils but was higher compared to the value of 0.53±0.25 mg KOH/g oil (Alfawaz, 2004) for *C. maxima* seed oils. The obtained low acid value indicated that pumpkin seed oil is suitable for consumption and has long shelf life (Hasan et al., 2019). However, the acid value obtained in this study was below the recommended values of 10 mg KOH/g oil for virgin palm oil and 4 mg/g oil for coconut oil (FAO/WHO, 1999). Refractive index defines either the degree of unsaturation of the oil or the length of chain of fatty acids in the triglycerides (Mengistie et al., 2018). The measured refractive index (1.446±0.000) was comparable to 1.4±0.1 and 1.5±0.1 (Amin et al., 2019b) for native and hybrid *C. maxima* seed oil observed in Bangladesh Saudi Arabia. The observed refractive index value was comparable to the recommended value of 1.460-1.465 (FAO/WHO, 1999) for *A. hypogaea* seed oil. The variations in the physicochemical compositions from this study with respect to the previous values could be attributed to variety and origin of the pumpkin seeds as well chemical analytical procedures used.

Phytochemical composition of *C. maxima* seed flour and oil

Results in Table 3 present the phytochemical and chemical properties of *C. maxima* L. seed flour and oil. Phytochemicals are naturally occurring bioactive anti-nutritive/non-nutritive compounds from plants which protect both plants and humans from various diseases (Sivakumar et al., 2018). The phytate content in crude oil was higher compared to the value of 0.00023 mg/g for *C. pepo* L. (Elinge et al., 2012) but comparable to 4.18 mg/g for *A. hypogaea* oil (Inuwa et al., 2011). Alkaloid content for the extracted crude oil was higher compared to the value of 330 mg/100 g for *Glycine max* flour whereas in seed flour, the alkaloid content was lower compared to the value of 250 mg/100 g for *A. hypogaea* flour (Mbagwu et al., 2011). Flavonoids are polyphenolic compounds fractions in foods and are anti-oxidant and anti-cancer (Tiwari and Husain, 2017). The flavonoids content in seed flour was higher compared to the value of 25 mg QE/100 g for Pusha Vishwas variety of pumpkin (*Moschata*) seed flour (Singh and Kumar, 2022).

Vitamin C content in pumpkin seed flour was 4.988±0.006 mg/100 g which was lower compared to the values of 15.00±0.58 and 10.750±0.29 mg/100 g for indigenous and hybrid pumpkin seed flour in a study conducted in Bangladesh (Amin et al., 2019a). Other authors have previously reported vitamin C content of 0.272 mg/100 g DM pumpkin seed flour (Syed et al., 2019). Phosphorus content in pumpkin seed flour was

Table 3. Phytochemical and chemical content of seed oil and flour.

Parameter	Crude oil (mean ± SD)	Flour (mean ± SD)
Phytate (mg/g)	3.097±0.164[a]	119.070±6.650[e]
Oxalate (mg/100 g)	87.272±0.612[b]	1462.770±97.518[f]
Alkaloids (mg/100 g)	826.080±0.710[c]	90.997±1.574[g]
Flavonoids (mg/100 g)	235.360±8.970[d]	61.761±7.604[h]
pH	ND	6.9±0.00
Titratable acidity as oleic acid (g/100 g)	ND	0.030±0.00
Vitamin C (mg/100 g)	ND	4.988±0.006
Phosphorus (mg/100 g)	ND	329.920±52.040

ND= Not determined, SD=standard deviation. For each parameter, means with different superscript in the same column were significantly different (P≥0.05).
Source: Authors 2022.

329.920±52.040 mg/100 g which was higher compared to values of 0.740±0.01 and 0.680±0.01 for native and hybrid *C. maxima* L. reported in Bangladesh (Amin et al., 2019a) and 47.68±0.04 mg/100 g in a related study done in Nigeria (Elinge et al., 2012). Interestingly, other researchers have reported a phosphorus value of 1232 mg/100 g which was higher compared to the value obtained from this study (Syed et al., 2019). Phosphorus is found in blood and cells and most of the non-skeletal phosphorus is inorganic which exist as nucleic acid, phosphor-lipids, ATP and sugar phosphate. Phosphates act as buffers that prevent changes in the acidity of body fluids because phosphates combine with hydrogen ions (Serna and Bergwitz, 2020). The differences in the phytochemical levels as compared to values obtained in previous related studies could be attributed to the origin of the pumpkin seeds and methods used to analyze the chemical compositions.

Conclusion

Results obtained from this study have shown that pumpkin seeds are high in essential nutrients such as crude protein (32.47±0.15%), crude fat (46.39±0.06%) and fiber but low in carbohydrate (sugar content). The high oil yield of the seeds and the close resemblance of its properties to the characteristics of other oil seeds and their oils like *A. hypogaea*, *H. annuus* and *G. max* in terms of proximate composition, physicochemical and phytochemical properties make them potential sources of vegetable oil. The high protein and oil content of the seeds present pumpkin seeds as a suitable ingredient for other food applications such as food fortification. Therefore, pumpkin seeds and oil might be a cheap alternative for conventional food fortifiers and vegetable oils in developing countries to mitigate the effects of rising vegetable oil prices.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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