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Exploring the Potential of Watermelon Seed as a Novel Dietary Ingredient: Effects on Lipid Profile and Hematological Parameters in Rats

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ABSTRACT

Background and Objective: Watermelon (*Citrullus lanatus*) is a globally cultivated fruit with its seed often disposed of as waste only to be replanted in the next season, however, these seeds are rich in nutrients. Despite their potential nutritional value, watermelon seeds remain an underutilized seed. Watermelon seed, an underutilized African crop, was analyzed for its biochemical and hematological effects in rats after different processing methods. **Materials and Methods:** Watermelon seeds were obtained from a farm market in Nigeria, cleaned of stones and divided into four and subjected to different processing methods (germination, soaking, and boiling) the fourth part was left unprocessed. They were thoroughly dried and milled into flours. Proximate composition was determined using standard methods and analytical procedures. **Results:** The proximate composition revealed that the protein, content ranges from 14.00-18.77%, oxalate, 0.14-0.23/100 g;, tannin 18.92-33.68/100 g and phytate, 0.11-0.36/100 g, WAC 33.33-53.85%, OAC 33.33-42.86%, BD 0.48-0.67%, FS 30.37-66.66% and ES 16.66-46.15%, the results showed that the processing significantly increased ($p<0.05$) the amino acid content of water melon seed. Significant ($p<0.05$) elevation was also observed in some hematological parameters of rats fed with supplemented diets of the processed seed flours. **Conclusion:** These results also showed that the processed flours can be used for improvement of nutrient bioavailability and nutrient density hence may be useful for confectionery products, aerated foods and high nutrient density weaning foods.

KEYWORDS

Lipid profile, hematological indices, watermelon, watermelon seeds, watermelon seed flour, soaking, boiling, germination, bioavailability, nutrient density

INTRODUCTION

Globally, malnutrition is a very critical public health problem and is responsible for morbidity and mortality in children below 5 years of age in developing countries[1], in Nigeria, the high rate of malnutrition recorded among children is are result of an increase in population, poverty, fall in agricultural practices, scarcity, shortage and high cost of animal protein and ignorance have been identified as some of the causes of malnutrition[1] and these are said to have severe consequences on human well-being by aggravating poverty, irreversible mental damages, poor growth among children[2]. However, nutrition improvement is

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the main prerequisite for the reduction of high infant and under-five mortality rates, the assurance of physical growth, social and mental development of children as well as academic achievement since the amount and nutritional quality of protein intake in children is important because of their essential functions in physical and mental development[3]. Given prevalent food shortages, food scarcity, and high prices of protein-rich foods, this research work, focused on the exploitation of underutilized and unconventional plant resources. Some of the underutilized plant seeds may fit well into subsistence agriculture as alternative protein sources. There are many parts of plants available, that are rich in nutrients, one of which is plant seeds.

Watermelon (*Citrullus lanatus*) is a fruit crop, a herbaceous creeping plant belonging to the family Cucurbitaceae. It is a vine-like flowering plant. It is a berry having a thick rind (exocarp) and fleshy center (mesocarp and endocarp), and it is mainly propagated by seeds and thrives best in warm areas. It is a tropical plant and requires a lot of sunshine and a high temperature of over 25°C for optimum growth[2]. It contains about 93% water, hence named watermelon. The fruit is known to be a good source of lycopene and carotenoids. It helps quench the free radicals that contribute to disease conditions like asthma, atherosclerosis, diabetes, colon cancer, and arthritis. It is also high in fiber and citrulline; an amino acid the body uses to make arginine[3].

Previous studies showed that protease inhibitors were reduced by 70%, lectin by 79%, and tannin by 69% in pigeon peas when boiled for 80 min, but phytate is heat-resistant and not as easily degraded with boiling[4,5]. Soaking has been used to process foods and reduce levels of antinutrients. During germination, water diffuses through the seed coats into the embryo, which has been almost completely dry during the period of dormancy, causing a swelling of the seed; the swelling is often so great that the seed coat is ruptured. Thus with the absorption of oxygen by the seed, energy is made available for growth[6]. This study aims to provide insights into the potential health benefits of processed and unprocessed watermelon seed as a dietary ingredient.

MATERIALS AND METHODS

Seed preparation: The study was conducted between May to August, 2023. As 6 kg watermelon seeds were purchased from a fruit market in Madalla, Niger State, Nigeria. The seeds were cleaned of stones, sand, and other particles, each of the seeds was divided into four portions (raw seeds, soaked seeds, germinated seeds, and boiled seeds) and each portion was processed differently.

The methods described by Ahamefule *et al.*[7] were used to prepare the boiled samples. The 1 kg of the seed sample was subjected to boiling at 100°C for 15 min at the rate of 1 kg of the seed to 5 L of water using a kerosene stove after which water was drained off using 10 mm sieve and the boiled seeds were spread on hessian sacks for 48 to 72 hrs under sunlight until dry. After drying, the seeds were milled and kept in plastic containers at room temperature for further analysis.

Saulawa *et al.*[8] method was used to prepare the soaked samples, 1 kg of the seed sample was weighed into 5 L plastic containers, which were then filled with 5 L cold water for 24 to 48 hrs at room temperature. Water was drained from the soaked seed using a sieve with a mesh size of 10 mm and the seed was air-dried under the sun for 48 to 72 hrs. After drying, the seeds were milled and kept at room temperature in plastic containers for further analysis.

Germination was achieved as described by Kayembe and Jansen[9], by soaking the seeds for 24 hrs. Afterward, they were spread indoors on hessian sacks on the floor, covered with aluminum foil to exclude light, and were allowed to germinate for three days. Water was applied once daily to provide moisture during sprouting. Thereafter, the germinating seed was dried for 48 to 72 hrs under sunlight, and ground, and then kept at room temperature pending further analysis.

Determination of proximate composition

Determination of moisture content: Horwitz[10] method was used in determining the moisture content of the sample. After cooling in a desiccator, the weight of a dry sterile evaporating dish was taken. The 2 g of the sample were weighed into a pre-weighed dry evaporating dish. The dish and the sample were reweighed before placing them in a hot air oven at a temperature of 105°C for 3 hrs. After drying, the dish and the sample were allowed to cool in a desiccator containing silica gel. The dish and the dry samples were reweighed, this process was repeated three times until a constant weight was obtained. The moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{\text{Weight of original sample - Weight of dry sample}}{\text{Weight of original sample}} \times 100$$

Determination of ash content: Horwitz[10] method was used to determine ash content analysis, in a pre-weighed porcelain crucible, 2 g of the sample were weighed and incinerated in a muffle furnace for 4 hrs at 600°C. The furnace was allowed to cool down to approximately 250°C. The crucibles were removed and placed in a desiccator for 30 min and weighed. The ash content of the sample was calculated as follows:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Sample weight}} \times 100$$

$$= \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where:

W_1 = Weight of empty crucible
W_2 = Weight of crucible+sample before drying and/or ashing and
W_3 = Weight of crucible+Ash

Determination of crude fibre: Guoyao[11] method was used for crude fibre determination of this sample. The 2 g of each sample were weighed into a filter crucible and placed on the hot extraction units of the Tecator fibretec system. The extraction was carried out with 30 mL of 98 % sulfuric acid (H_2SO_4) for 14 min, by boiling. The H_2SO_4 was removed by switching on the vacuum pump and washed out (three times) with warm distilled water. Sequential to the H_2SO_4 removal, 100 mL of sodium hydroxide (NaOH) was added and boiled for 14 min before removing it. The residues in the crucibles were dried at 100°C overnight, after which they were cooled in a desiccator for 30 min and weighed, they were ashed in a muffle furnace at 600°C for 3 hrs after weighing. The furnace was allowed to cool to at least 250°C, and then the crucibles were cooled in a desiccator for 30 min and weighed.

Percentage CF was calculated as follows:

$$\text{CF (\%)} = \frac{W_1\,(g) - W_2\,(g)}{W_3\,(g)} \times 100$$

Where:

W_1 = Dry mass of sample after extraction
W_2 = Mass of ash
W_3 = Weight of sample

Determination of crude protein: Macro Kjeldahl method[10] was used to determine the nitrogen (N) concentration of the sample. The 2 g of the sample were weighed into a digestion flask. A digestion mixture of 10 g sodium sulfate (Na_2SO_4) and 0.4 g elemental selenium was added together with 25 mL of concentrated (98%) sulphuric acid (H_2SO_4) was also added into the same digestion flask with the sample.

The flasks were put on a block digester until the solution was clear 45 min for the digestion of the sample. After cooling the solution, 35 mL of boric acid solution (40 g solution of H_2BO_3 in 10 mL methyl red and 25 mL methyl blue made up to 1000 mL) was added. Distilled water (350 mL), zinc granules, and 100 mL NaOH (45%) were added as well. Then the solution was allowed to boil for about 10 min until about 200 mL of the distillate was remaining for the distillation. The distillate will be titrated with 0.1M HCL. The values were calculated by the titration of a blank sample.

The percentage of N in a sample was calculated as follows:

$$\text{N (\%)} = \frac{\text{F} \times (\text{Titrration} - \text{Blank})}{\text{Sample mass}} \times 100$$

Where:

F = Factor is associated with the strength of the H_2SO_4

Percentage CP will be calculated as follows:

$$\text{CP (\%)} = \text{N (\%)} \times 6.25$$

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Determination of crude lipid: The crude lipid content of each seed sample was determined using the Soxhlet method[12]. The 5 g of each sample was measured into a thimble of known weight (W_1), they together weigh w_2. The thimble with the sample was placed inside a Soxhlet extractor. As 300 mL of petroleum ether was poured into a 500 mL round bottom ground joint flask, which was set in an electrically connected heating mantle. The heating and extraction process was continued for 24 hrs, after which the thimble with content was removed, dried in an oven at 50°C for 24 hrs, cooled in a desiccator and weighed (W_3). The percentage lipid content of each sample was calculated:

$$\text{Lipid (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where:

W_1 = Weight of the thimble

W_2 = Initial weight of the sample and thimble

W_3 = Final weight of the sample and the thimble

Carbohydrate content determination: Ihotu and Etim[13] were used in total carbohydrate content determination. The sum total amount of crude protein, crude fat, moisture, and ash of each of the samples was added and subtracted from 100. The percentage of carbohydrate was the value obtained:

$$\text{Carbohydrate (\%)} = 100\text{-(Moisture (\%)+Ash (\%)+Protein (\%)+Fat (\%))}$$

Determination of antinutritional factors

Determination of cyanogenic glycosides: The alkaline picrate method as described by Ihotu and Etim[13] was used in the determination of cyanogenic glycoside content. The 50 mL of distilled water was used in a conical flask to dissolve 5 g of the seed sample, which was allowed to stay overnight to extract cyanide. The extract was filtered, and the filtrate was used for cyanide determination. Sample filtrate (1 mL), and alkaline picrate (4 mL) were added and allowed to incubate in a water bath for 5 min. After reddish brown color development, the absorbance was read at 490 nm. The cyanide content was extrapolated from a cyanide standard curve.

Determination of phytates: Horwitz[10] was used in phytate determination. The 10 g of sample were weighed into the conical flasks and then extracted with 50 mL of 3% TCA for 45 min with occasional swirling by hand. The phytate was precipitated as ferric phytate with the solution of iron (III) chloride. The precipitate was converted to sodium phytate with a 3% solution of NAOH before digesting with an acid mixture of an equal portion of concentrated H_2SO_4 and 65% ClO_4. The liberated phosphorus was quantified calorimetrically at 620 nm after color development with ammonium molybdate to which sodium sulfate and hydroquinone solutions were added.

Calculation: 1 cm^3 of 0.02 m $Fecl_3$ = 0.60L

$$\text{Conectration of phytate phosphorus} = \frac{\text{Titer value} \times 0.0601\,\text{phytate}}{\text{Weight of sample}} \times 100$$

Determination of tannins: The Folin Denis Spectrophotometric method was employed to determine the tannin content of the sample. The sample 1 g was dissolved in distilled water (10 mL) and shaken. This mixture was allowed to stand for 30 min at room temperature. After 30 min, the mixture was centrifuged and the extract was obtained. Supernatant (2.5 mL) was transferred into a 50 mL volumetric flask. Similarly, standard tannic acid solution (2.5 mL) was transferred into a separate 50 mL flask. Folin-Denis reagent (1 mL) was measured into each flask, followed by 2.5 mL of saturated Na_2CO_3 solution. The mixture was diluted to mark in the flask (50 mL) and incubated for 90 min at room temperature. The absorbance was measured at 250 nm using a Spectrophotometer. The tannin content was calculated as follows Horwitz[10]:

$$\text{Tannin (\%)} = \frac{At}{As} \times C\frac{100}{W} \times Vf$$

Where:

At = Absorbance of the test sample
As = Absorbance of standard solution
C = Concentration of standard solution
W = Weight of sample
Vf = Total volume of extract

Determination of oxalates: The method described by Horwitz[10] was used to determine the oxalate content of seed samples. Sample (2 g) was weighed into a 250 mL beaker; distilled water (190 mL) and 6 molar HCl (10 mL) were added to the beaker and allowed to stand for 5 min while mixing it at 30 sec intervals. The volume was made up of distilled water (250 mL) after which 50 mL was measured out and titrated using a few drops of methyl red indicator while adding drop by drop concentrated ammonium until there is a color change of faint yellow. It was then heated using a steam water bath to boil, removed, and allowed to cool before filtering and heating again to boil. The 5% $CaCl_2$ (10 mL) was added while constantly stirring and another $CaCl_2$ (5 mL) was added to give more precipitate of oxalate from the sample. This was removed and allowed to stand overnight, after which it was filtered, the precipitate was washed into a beaker using 1:4 H_2SO_4 acids and then rinsed with 5 mL of hot distilled water. The solution was heated and titrated against 0.05 N $KMnO_4$. Titre value of the blank was subtracted from that of the sample and multiplied by 50 to get the result in mg/00 g of the sample.

Calculation: 1 m of 0.05 N $KMnO_4$ = 3.0 mg Oxalate.

Determination of saponins: The saponins content in the sample was determined using the standard method described by Horwitz[10]. Finely ground sample (1 g) was weighed into a 250 mL beaker and the methyl alcohol (100 mL) was added. The mixture was shaken on a shaker for 5 hrs to ensure uniform mixing. Thereafter, the mixture was filtered using filter paper into a beaker and a 40% saturated solution

of magnesium carbonate (20 mL) was added. The mixture obtained with saturated $MgCO_3$ was again filtered through a filter paper to obtain a clear colorless solution. Colorless solution (1 mL) was pipetted into 50 mL volumetric flask and 5% $FeCl_3$ solutions (2 mL) were added and made up to mark with distilled water. The reaction was stopped for 30 min to allow the development of blood red color. The 0-10 ppm standard saponin was prepared from saponin stock solution. The standard solutions were treated similarly with 2 mL of 5% $FeCl_3$ solution as done for the 1 mL sample above. The absorbance of the saponin content in the sample as well as the standard solution was read after color development on a spectrophotometer at a wavelength of 380 nm:

$$\text{Saponin (\%)} = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of sample}} \times 100$$

Determination of functional properties of seeds flour

Determination of water and oil absorption capacity: The method of Sosulski *et al.*[14] was used to determine the water absorption capacity of the flours. Sample (1 g) was dissolved in distilled water (10 mL) and allowed to stand for 30 min at ambient temperature (30±2°C), and centrifuged for 30 min at 3000 rpm.

Sosulski *et al.*[14] method was used in the determination of oil absorption. It was examined as percent oil bound per gram flour. Sample (1 g) was mixed with 10 mL soybean oil (Sp. Gravity 0.9092) and allowed to stand for 30 min at ambient temperature (30±2°C), then centrifuged for 30 min at 300 rpm. Water absorption was examined as the percent water bound per gram flour.

Determination of emulsion activity and stability: The method described by Yasumatsu *et al.*[15] was used to determine emulsion activity and stability. For emulsion (1 g sample, 10 mL distilled water, and 10 mL soybean oil) was prepared in a calibrated centrifuged tube. The emulsion was centrifuged for 5 min at 300 rpm. The ratio of the height of the emulsion layer to the total height of the mixture was calculated as emulsion activity in percentage. The emulsion stability was estimated after heating the emulsion contained in the calibrated centrifuged tube at 80°C for 30 min in a water bath, cooling for 15 min under running tap water and centrifuging at 2000 g for 15 min. The emulsion stability expressed as a percentage was calculated as the ratio of the height of the emulsified layer to the total height of the mixture.

$$\text{Emulsifying activity} = \frac{\text{Height of emulsified layer}}{\text{Height of total contents in the tube}} \times 100$$

Determination of bulk density: The method described by Singh *et al.*[16] was used to determine bulk density, which says the volume of 100 g of the flour was measured in a 250 mL measuring cylinder, and the apparent (bulk) density was calculated after tapping the cylinder on a wooden plank until no visible decrease in volume was noticed, and based on the weight and volume.

Determination of swelling capacity: The method described by Okaka and Potter[17] was used to determine swelling capacity where a 100 mL graduated cylinder was filled with the sample to the 10 mL mark. Distilled water was added to give a total volume of 50 mL. The top of the graduated cylinder was tightly covered and mixed by inverting the cylinder. The suspension was inverted again after 2 min and was left to stand for a further 8 min, and the volume occupied by the sample was taken after the 8 min.

Determination of foam capacity (FC) and foam stability (FS): Narayana and Narasinga[18] method with slight modification was used to determine the foam capacity (FC) and foam stability (FS). A flour sample (1 g) was added to distilled water (50 mL) in a graduated cylinder at 30±2°C. The suspension was made to foam after being mixed and shaken for 5 min. The volume of foam at 30 sec after whipping was expressed as foam capacity using the formula:

$$\text{Foam capacityt (\%)} = \frac{\text{Volume of foam (AW)} - \text{Volume of foam (BW)}}{\text{Volume of foam (BW)}} \times 100$$

Where:
AW = After whipping
BW = Before whipping

The volume of foam was recorded 1 hr after whipping to determine foam stability as a percent of initial foam volume.

Animal studies: Thirty weanling albino rats weighing 70 to 100 g were used in this study. The animals were acclimatized for one week before the start of the experiment. They were allowed to be housed in the Biochemistry Animal House, Federal University of Technology, Minna, and as well receive normal rat chows and water *ad libitum*. After a week, they were divided into five groups of six animals each.

The study involved five distinct groups, each receiving a specific diet to evaluate the effects of different processing methods on watermelon seed flour consumption.

- **Group 1 (Control):** This group was fed a standard diet without the inclusion of watermelon seed flour. It serves as a baseline for comparison against the experimental groups
- **Group 2 (Unprocessed watermelon seed flour-based diets):** Animals in this group received diets formulated with raw, unprocessed watermelon seed flour to assess its direct nutritional and physiological effects
- **Group 3 (Boiled watermelon seed flour-based diets):** This group was fed a diet containing watermelon seed flour that had been boiled prior to incorporation, allowing the evaluation of changes in nutrient composition and bioavailability due to thermal processing
- **Group 4 (Germinated watermelon seed flour-based diets):** The diet for this group included watermelon seed flour obtained from germinated seeds, enabling an assessment of potential enzymatic modifications and improvements in digestibility and nutrient availability
- **Group 5 (Soaked watermelon seed flour-based diets):** Animals in this group were provided with a diet containing watermelon seed flour derived from seeds soaked in water before drying and processing. This treatment helps determine the impact of soaking on antinutritional factors and nutrient release

This classification allows for a comparative evaluation of the effects of different processing techniques on the nutritional quality and health outcomes of experimental animals.

The experimental diet and water were offered *ad libitum* to the rats for 28 days. The rats were weighed to obtain their initial weights so that their weight changes can be determined at the end of experimental feeding. The experimental diet was formulated based on the American Institute of Nutrition (AIN) method as described by Reeves *et al*.[19].

Collection of blood samples: At the end of the 28 day feeding experiment, the rats were weighed to obtain their final weights. Each rat was anesthetized under diethyl ether in a desiccator and euthanized.

Blood samples were collected in EDTA sample bottles for hematological analysis and plain bottles for lipid profile analysis. Blood samples collected for biochemical parameters were pinned using a bench-top centrifuge at 3000 rpm for 10 min to separate the serum from the plasma.

Assays for hematological parameter: Blood samples were collected into sample bottles containing a speck of Tetra Acetic Ethylene Diamine Acid (EDTA) powder. The following hematological parameters were determined, packed cell volume (PCV), white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb) and mean corpuscular hemoglobin concentration (MCHC) were determined by using automated hematology analyzer following the manufactures instruction (Mindray 3000 plus).

Determination of lipid profile: Serum cholesterol level was measured using the method described by Allain *et al*.[20]. High-density lipoprotein content was determined as described by Albers *et al*.[21]. Low density lipoprotein content was determined using the method of Assmann *et al*.[22]. Triglyceride was determined using glycerol-phosphate oxidase method described by Albers *et al*.[21].

Data analysis: Statistical analyses were performed using Analysis of Variance (ANOVA) followed by Duncan's multiple range tests using SPSS program 20.0. The $p \leq 0.05$ were considered to be significant and all values were expressed as Mean±SEM.

RESULTS

The proximate composition of unprocessed and processed *Citrullus lanatus* (watermelon) seed flour varied significantly among samples. Moisture content was highest in WMR (3.56%) and lowest in WMS (0.04%). Ash content ranged from 1.70% (WMR) to 4.93% (WMB). Carbohydrate content was highest in WMS (40.41%) and lowest in WMR (33.20%). Protein levels varied, with WMB having the highest (18.77%) and WMG the lowest (13.25%). Fibre content was highest in WMR (21.09%) and lowest in WMS (9.40%). Fat content was highest in WMS (29.05%) and lowest in WMG (22.64%). Energy values ranged from 490.38 kcal/100g (WMG) to 528.33 kcal/100 g (WMS), indicating differences in nutrient composition among the samples in Table 1.

The antinutrient composition of *Citrullus lanatus* (watermelon) seed flour varied among the samples. Tannin content was highest in WMR (33.68 mg/100 g) and lowest in WMS (18.92 mg/100 g). Phytate levels ranged from 0.11 mg/100 g (WMB) to 0.36 mg/100 g (WMR), while oxalate was highest in WMR (0.23 mg/100 g) and lowest in WMG (0.14 mg/100 g). Saponin content was significantly higher in WMR (3.46/100 mg) and lowest in WMB (0.64/100 mg). Trypsin inhibitor (TI) content remained relatively low across samples, ranging from 0.05 mg/100 g (WMB) to 0.07 mg/100 g (WMR). Cyanogenic glycoside (CG) was highest in WMR (1.99 mg/100 g) and lowest in WMG and WMB (0.71 mg/100 g), indicating variations in the antinutrient profile due to processing in Table 2.

The functional properties of *Citrullus lanatus* seed flour varied across samples. Bulk density ranged from 0.48 g/cm^3 (WMR) to 0.67 g/cm^3 (WMB). The WMR had the highest water absorption (53.85%) and foaming capacity (6.67%), while WMB showed the highest oil absorption (42.86%) and foaming stability (66.66%). Emulsion capacity was highest in WMS (44.00%) and lowest in WMR (26.66%), while emulsion stability peaked in WMG (46.15%). These variations indicate the influence of processing on the flour's functional properties in Table 3.

The amino acid composition of *Citrullus lanatus* seed flour varied across samples. Arginine had the highest concentration (10.16-10.83/100 g), while tryptophan had the lowest (0.93-1.23/100 g). Essential amino acids such as leucine (5.60-6.10/100 g) and lysine (3.92-4.75/100 g) were present in notable amounts. Glutamic acid (15.29-16.65/100 g) and aspartic acid (8.00-8.99/100 g) were the most abundant non-essential amino acids. Compared to Guoyao[11], watermelon seed flour exhibited higher levels of arginine and phenylalanine but lower tyrosine and isoleucine, indicating its potential as a protein source Table 4.

Table 1: Proximate composition of unprocessed and processed *citrullus lanatus* (water melon) seed flour

Sample	WMS	WMG	WMB	WMR
Moisture	0.04±0.28^{a}	1.26±0.01^{b}	0.31±0.02^{a}	3.56±0.03^{c}
Ash	3.83±0.09^{b}	4.45±0.02^{c}	4.93±0.23^{d}	1.70±0.15^{a}
Carbohydrate	40.41±0.77^{a}	38.87±1.55^{c}	36.92±0.82^{a}	33.20±1.11^{b}
Protein	16.90±0.60^{b}	13.25±1.70^{a}	18.77±0.63^{c}	14.00±1.01^{a}
Fibre	9.40±0.58^{a}	19.36±0.21^{c}	12.25±0.25^{b}	21.09±0.67^{d}
Fat	29.05±0.07^{d}	22.64±0.03^{a}	26.82±0.02^{c}	26.45±0.03^{b}
Energy (Kcal/100 g)	528.33±1.04^{d}	490.38±0.22^{a}	513.10±0.96^{c}	511.20±0.83^{b}

Values are expressed as means of triplicate determinations±SEM, Values along rows with different superscript are significantly different ($p<0.05$), WMS: Watermelon soaked, WMG: Watermelon germinated, WMB: Watermelon boiled and WMR: Watermelon unprocessed

Table 2: Antinutrient composition of unprocessed and processed *citrullus lanatus* (Water melon) seed flour (mg/100 g)

Sample (mg/100 g)	WMS	WMG	WMB	WMR
Tannins	18.92±0.13^{a}	31.40±0.13^{c}	27.92±0.11^{b}	33.68±0.32^{d}
Phytate	0.12±0.01^{b}	0.29±0.01^{c}	0.11±0.01^{a}	0.36±0.01^{d}
Oxalate	0.18±0.01^{c}	0.14±0.01^{a}	0.17±0.00^{b}	0.23±0.01^{d}
Saponins (g/100 mg)	2.45±0.03^{b}	2.32±0.04^{b}	0.64±0.01^{a}	3.46±0.03^{c}
T I	0.06±0.2^{b}	0.06±0.01^{b}	0.05±0.02^{a}	0.07±0.01^{c}
CG	1.37±0.01^{b}	0.71±0.32^{a}	0.71±0.06^{a}	1.99±0.01^{c}

TI: Trypsin Inhibitor, CG: Cyanogenic Glycoside, Values are means of triplicate determinations±SEM, Values along rows with different superscript are significantly different ($p<0.05$), WMS: Watermelon soaked, WMG: Watermelon germinated,WMB: Watermelon boiled and WMR: Watermelon unprocessed

Table 3: Functional properties of selected processed and unprocessed *citrullus lanatus* (watermelon) seed flour

Sample	WMS	WMG	WMB	WMR
BD (g/cm^{3})	0.63±0.21^{b}	0.59±0.14^{b}	0.67±0.21^{a}	0.48±0.21^{a}
WAC (%)	33.33±0.82^{a}	33.33±0.75^{a}	33.33±0.78^{b}	53.85±0.82^{b}
OAC (%)	33.33±0.05^{a}	33.33±0.13^{a}	42.86±0.03^{b}	33.33±0.05^{a}
FC (%)	1.96±0.39^{b}	1.96±0.18^{a}	2.86±0.36^{c}	6.67±0.32^{d}
FS (%)	50.37±0.35^{b}	50.37±0.01^{a}	66.66±0.07^{d}	57.14±0.04^{c}
EC (%)	44.00±0.65^{c}	43.33±0.47^{c}	33.33±0.35^{b}	26.66±0.67^{a}
ES (%)	16.66±0.49^{a}	46.15±0.48^{d}	40.21±0.32^{c}	30.05±0.49^{b}

WAC: Water absorption capacity, OAC: Oil absorption capacity, BD: Bulk density, EC: Emulsion capacity, FC: Foaming capacity, FS: Foaming stability, ES: Emulsion stability, Values are expressed as means of triplicate determinations±SEM, Values along rows with different superscript are significantly different ($p<0.05$), WMS: Watermelon soaked, WMG: Watermelon germinated, WMB: Watermelon boiled and WMR: Watermelon unprocessed

Table 4: Amino acids levels of unprocessed and processed *citrullus lanatus* (watermelon) seed flours

Amino acids (g/100 g)	WMS	WMG	WMB	WMR	Guoyao[11]
Histidine	2.24 ± 0.01^{d}	1.85±0.03^{a}	2.00±0.01^{b}	2.17±0.02^{c}	2.4
Isoleucine	3.21±0.12^{b}	2.70±0.17^{a}	2.81±0.06^{a}	3.01±0.01^{b}	4.2
Leucine	6.10±0.06^{b}	5.60±0.17^{a}	5.81±0.12^{b}	5.84±0.06^{b}	4.9
Lysine	4.75±0.12^{c}	4.03±0.02^{a}	3.92±0.02^{a}	4.45±0.03^{b}	4.2
Methionine	2.20±0.12^{a}	2.00±0.29^{a}	2.30±0.17^{a}	2.13±0.02^{a}	2.2
Phenylalanine	4.00±0.29^{c}	3.37±0.06^{a}	3.10±0.06^{a}	3.72±0.02^{b}	2.8
Threonine	4.22±0.01^{c}	3.83±0.02^{b}	3.61±0.06^{a}	3.55±0.03^{a}	4.0
Tryptophan	0.93±0.02^{a}	1.23±0.05^{c}	1.05±0.03^{b}	1.07±0.02^{b}	
Valine	4.62±0.01^{b}	4.10±0.17^{a}	3.75±0.19^{a}	71±0.06^{a}	4.2
Arginine	10.83±0.06^{a}	10.16±0.58^{a}	10.41±0.01^{a}	0.20±0.15^{a}	2.0
Cysteine	2.42±0.01^{c}	2.36±0.06^{c}	2.24±0.02^{b}	1.94±0.02^{a}	
Glycine	5.37±0.02^{d}	4.82±0.01^{c}	3.66±0.06^{a}	3.85±0.03^{b}	
Proline	4.90±0.23^{a}	5.00±0.12^{a}	4.50±0.12^{a}	4.60±0.23^{a}	
Serine	3.70±0.12^{a}	3.35±0.06^{a}	3.20±0.12^{a}	4.00±0.58^{a}	
Tyrosine	2.41±0.02^{b}	2.06±0.01^{a}	2.41±0.01^{b}	2.10±0.06^{a}	4.1
Alanine	3.37±0.06^{a}	3.56±0.01^{a}	3.87±0.06^{b}	4.52±0.15^{c}	
Glutamic acid	15.61±0.01^{b}	15.29±0.06^{a}	16.65±0.03^{d}	15.75±0.03^{c}	
Aspartic acid	8.00±1.15^{a}	8.68±0.01^{a}	8.99±0.01^{a}	8.75±0.03^{a}	4.0

Values are means of triplicate determinations±SEM, Values along rows with different superscript are significantly different ($p<0.05$), WMS: Watermelon soaked, WMG: Watermelon germinated, WMB: Watermelon boiled and WMR: Watermelon unprocessed

Table 5: Composition of *citrullus lanatus* (watermelon) seed flours based diet (per kg diet)

Ingredient	Control diet	Diet containing unprocessed seed (g)	Diet containing boiled seed (g)	Diet containing soaked seed (g)	Diet containing germinated seed (g)
Seed sample	-	680	490	610	620
G.nut cake	130	-	-	-	-
Cornflour	670	120	310	190	180
G.nut oil	5	5	5	5	5
Maize bran	100	100	100	100	100
Bone meal	25	25	25	25	25
Glucose	43	43	43	43	43
Premix	5	5	5	5	5
Salt	17	17	17	17	17
Methionine	3	2	3	2	3
Lysine	2	3	2	3	2

Premix composition (1.25 kg), Vitamin D3 (1,7000,000 IU), Vitamin A (8,000,000 IU), Vitamin E (5,000 mg), Vitamin K3 (1,500 mg), Folic acid (200 mg) Niacin (15,000 mg), Vitamin B2 (3,000 mg), Vitamin B12 (5 mg), Vitamin B1 (1000 mg), Vitamin B6 (1000 mg), Biotin, Antioxidant (125,000 mg) Calpan (5,000 mg).Cobalt (100 mg), Selenium (100 mg), Iodine (1,000 mg), Iron 25,000 mg, Manganese (45,000 mg), Copper (3,000 mg), Zinc (35,000 mg) and Choline Chloride (100,000 mg). Source: Abdel-Shafy *et al*.[29]

Table 6: Hematological parameters of rats fed unprocessed and processed *citrullus lanatus* supplemented diets

Hematological parameter	Control	Uwmsf	Swmsf	Gwmsf	Bwmsf
HB (g/dL)	16.2±0.15^{e}	9.9±0.5^{a}	10.3±0.33^{b}	11.8±0.08^{c}	12.1±0.4^{d}
PCV (%)	48.0±1.2^{d}	29.0±0.8^{a}	31.0±0.4^{b}	35.0±1.0^{c}	36.0±0.9^{c}
MCV (Fl)	87.0±0.04^{d}	77.0±0.01^{c}	76.0±0.03^{b}	71.0±0.13^{a}	7.01±0.1^{a}
MCH (pg)	22.0±0.23^{b}	22.0±0.18^{b}	20.0±0.81^{a}	24.0±0.62^{c}	24.0±0.59^{c}
MCHC (g/dL)	33.0±0.56^{b}	31.0±0.22^{a}	33.0±.55^{b}	34.0±0.72^{c}	34.0±.70^{c}
RBC (×10^{9}/L)	4.9±0.03^{c}	4.5±0.12^{a}	4.7±0.04^{b}	4.9±0.05^{c}	5.1±0.01^{d}
WBC (×10^{9}/L)	5.2±0.31^{a}	7.9±0.21^{e}	5.8±0.29^{b}	6.7±0.1^{d}	6.4±0.09^{c}
PLC (×10^{9}/L)	278.0±45.21^{e}	267.0±15.86^{d}	223.0±20.05^{a}	249.0±11.3^{b}	261.0±35.34^{c}
L	58.0±5.25^{c}	49.0±2.98^{b}	60.0± 3.91^{c}	38.0±3.21^{a}	38.0±4.0^{a}
NEU	42.0±6.13^{b}	33.0±0.18^{a}	34.0±0.20^{a}	52.0±6.87^{c}	59.0±7.52^{d}
BASO	1.0±0.17^{b}	3.0±0.09^{c}	00.0±0.00^{a}	1.0±0.11^{b}	00.0±0.00^{a}
ECO	3.0±0.07^{b}	5.0±0.3^{c}	3.0±0.09^{b}	3.0±0.11^{b}	2.0±0.02^{a}
MONO	6.0±0.09^{c}	10.0±0.11^{d}	3.0±0.04^{b}	6.0±0.08^{c}	1.0±0.02^{a}

Values are expressed as means of triplicate determinations±SEM, Values along rows with different superscript are significantly different ($p<0.05$), Pack Cell Volume (PCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Lymphocytes (L), Monocytes (MONO), Eosinophils (ECO), Neutrophils (NEU), Basophils (BASO) and Hemoglobin Concentration (Hb) and SWMSF: Soaked Watermelon seed flour, GWMSF: Germinated Watermelon seed flour, BWMSF: Boiled Watermelon Seed Flour and UWMSF: Unprocessed Watermelon seed flour

The composition of *Citrullus lanatus* (watermelon) seed flour-based diets per kg varied across processing methods. Unprocessed seeds were used in the highest quantity (680 g/kg), while boiled (490 g/kg), soaked (610 g/kg), and germinated (620 g/kg) seeds had lower proportions. The control diet contained groundnut cake (130 g) and a higher amount of cornflour (670 g), which was reduced in seed-based diets. Other ingredients, including maize bran (100 g), bone meal (25 g), glucose (43 g), premix (5 g), salt (17 g), methionine (3 g), and lysine (2 g), remained consistent across all formulations Table 5.

Hematological parameters varied across diets. The HB and PCV were highest in the control group (16.2±0.15 g/dL, 48.0±1.2%) and lowest in UWMSF (9.9±0.5 g/dL, 29.0±0.8%). RBC was highest in BWMSF (5.1±0.01×10^{9}/L) and lowest in UWMSF (4.5±0.12×10^{9}/L). WBC peaked in UWMSF (7.9±0.21×10^{9}/L), while the control had the lowest (5.2±0.31×10^{9}/L). The PLC was highest in the control (278.0±45.21×10^{9}/L) and lowest in SWMSF (223.0±20.05×10^{9}/L). The BWMSF and GWMSF showed better hematological responses, suggesting processing improves dietary effects (Table 6).

Figure 1 shows the swelling capacity of unprocessed and processed watermelon seed flour. Swelling capacity of flour refers to its ability to absorb water and swell when heated, Fig. 1 showed that soaked

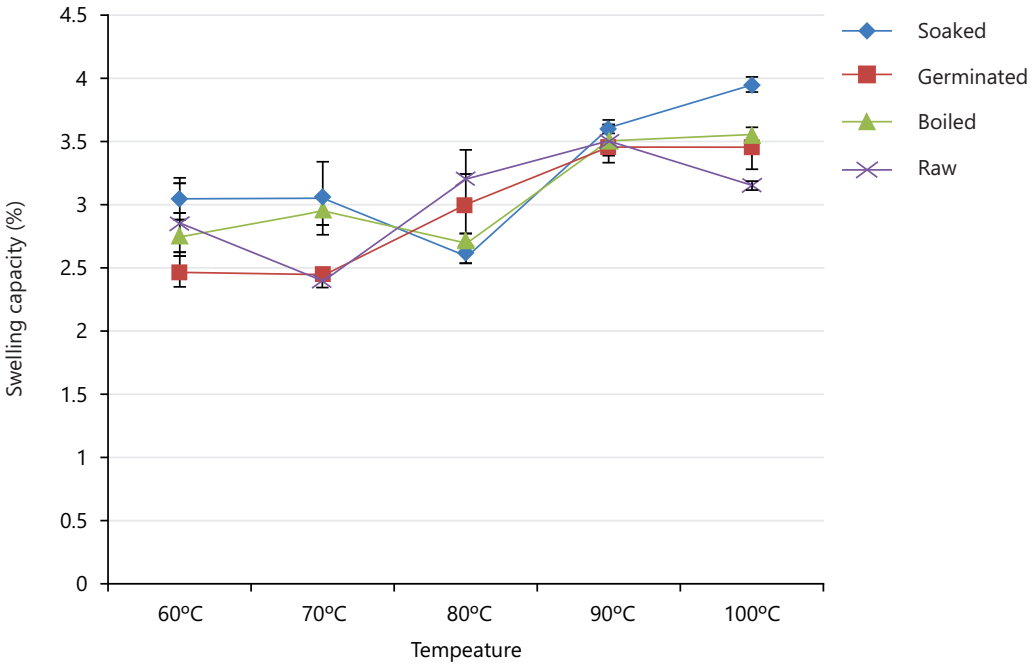

Fig. 1: Swelling capacity of unprocessed and processed watermelon seed flour

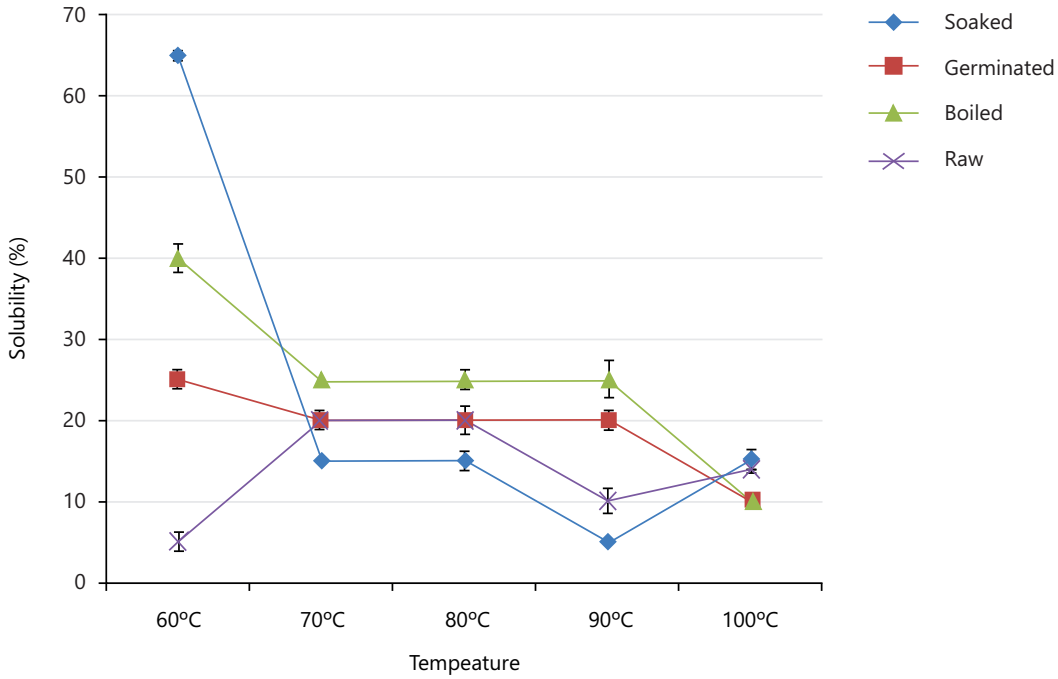

Fig. 2: Solubility properties of unprocessed and processed watermelon seed flour

watermelon seed flour gives the highest swelling capacity of 3.5% at 100°C. This implies moderate starch gelatinization, the flour may produce a balanced texture in baked goods and may be suitable for bread making, cakes, and pastry production, and general purpose baking.

Figure 2 showed that soaked seed flour of watermelon has the highest level of solubility at 60°C and 100°C when compared to the raw seed flour, the solubility rate in boiled and germinated watermelon seed flour was observed to be constant at 70, 80, and 90°C. This suggests that processed watermelon seed flours can help stabilize emulsion, increase enzyme activity, and process higher start breakdown due to its higher solubility capacity, which in turn is often needed in baked goods, sauce, dressing, and instant food.

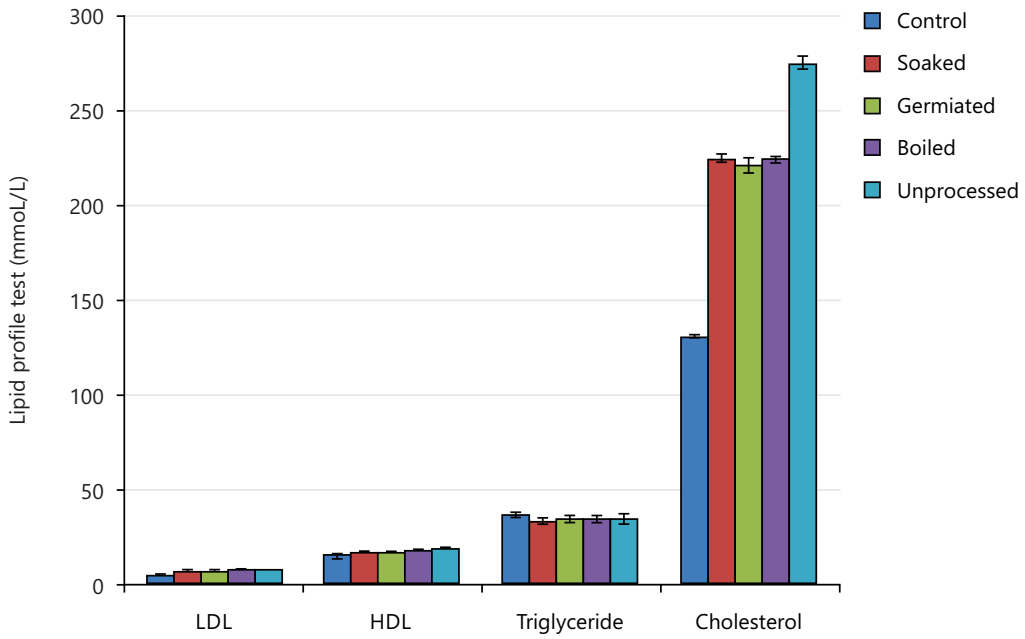

Fig. 3: Serum lipid profile parameters of rats fed with processed and unprocessed *citrullus lanatus* seed flour supplemented diets

Figure 3 showed the serum lipid profile parameters of rats fed with processed and unprocessed *citrullus lanatus* seed flour supplemented diets. The highest value obtained was observed in unprocessed watermelon seed flour. Consumption of processed watermelon seed flour has been shown to reduce serum lipid profile parameters compared to the unprocessed flour. This can be attributed to the enhanced bioavailability and efficacy of bioactive compounds present in the flour.

DISCUSSION

The shelf life of food products is important and is predicted by moisture content, higher moisture content naturally encourages organisms present in flour to grow, producing off odor and flavors. Results from this study (Table 1), showed a decrease in the moisture contents of all the seed flours in their processed state than in the unprocessed state when compared. This could be due to the drying technique efficiency with a prolonged drying period especially for the soaked and boiled seeds, as for the germinated seeds, the lower moisture content might be as a result of water utilization for metabolic activities initiated by soaking. The lower moisture content obtained in this study suggests higher dry matter yield[23] which indicate the presence and quality of nutrients in a food sample and these could enhance storage stability.

The proximate composition of this research work showed that the different seed flours (processed and unprocessed) are good sources of protein. This is higher when compared to the protein content of some commonly consumed seeds in Nigeria, namely *Citrullus colocynthis,* rapeseed, and sunflower[24], but lower when compared to that of groundnut cake 50-55%.

In this study, germination, boiling, and soaking tend to lower antinutrient content in all the seed flours when compared to the unprocessed seed flour (Table 2), this reduction in antinutrient content concerning processing methods was also reported by Adebayo[25] which could be as a result of leaching of the antinutrient in water especially phytate since is water soluble. Tannins reduction during germination might be a result of the formation of a hydrophobic association of tannins with seed proteins and enzymes, while a decrease in phytate content during germination could be a result of phytase activity, as reported for other germinated cereals[26].

Water absorption capacity (WAC), serves as an index for the maximum amount of water a product can absorb and retain, and is also important in softening andincreasing digestibility[26] while oil absorption capacity (OAC) reveals if a product will be suitable in facilitating enhancement in flavor and mouth feel when used in food preparation[27]. In this study, these values varied, they were either higher or lower than the reports from previous study[28,29], for instance, the bulk density of watermelon was higher when compared to that of soybean flour (0.38 g/cm^3)[28], but comparable to that of Bambara groundnut which ranges from 0.60 to 0.75 g/cm^3)[29]. The bulk density (BD) of flour samples influences the amount and strength of packaging material; mouth feel, energy density, and texture[30]. For WAC (Table 3), the value significantly increased ($p<0.05$) in all the processed seed flours compared with soybeans (1.12/100 g; Alfaro *et al*.[31].

The emulsion capacity and stability (EC and ES) are important parameters in the production of pastries, coffee whiteners, and frozen desserts[29]. The EC of all the seed flours ranges from 16.61 to 44.00% while ES ranges from 16.66 to 83.33%, this suggests that the flour blend may be a good emulsifying agent. This may be due to both the soluble and insoluble protein (this is possible because the protein can emulsify and stabilize the emulsion by decreasing the surface tension thereby providing electrostatic repulsion on the system) as well as another component such as polysaccharides (polysaccharides can also help in stabilizing the emulsion by increasing the viscosity of the system)[29].

Swelling power (SP) and solubility (S) provide evidence of the magnitude of interaction between the starch chain within the amorphous and crystalline domain. The SP and S of flours have been used to provide evidence for the association of binding forces within granules[32]. When an aqueous suspension of starch granules is heated, they become hydrated and swelling takes place.

Appiah *et al*.[33] classified starch to be highly swelling (30% or higher in 95°C), moderately restricted swelling (16 to 20% in 95°C) or highly restricted swelling (below 16% in 95°C). From this study, all the seed flours are in the highly restricted swelling category (below 16% at 95°C) and this result was similar to that of Oke *et al*.[32] on yam flour. This low SC (Fig. 1) and S (Fig. 2) reported in this study would be desirable for the manufacture of value-added products such as noodles and composite blends with cereals and it suggests that all the seed flours could be good sources of nutrients to its consumers and bring about food security in developing countries. The low value of SP and S of this seed flour might be due to protein-amylose complex formation.

Essential amino acids are amino acids that cannot be synthesized by the body, they can be gotten through diet, however, the quantity of a dietary protein is a measure of its essential amino acids, which are required for growth and maintenance[34]. Non-essential amino acids are equally important as they also help in growth and development. Soaking significantly increased the essential and non-essential amino acids except for tryptophan where a decrease was seen generally across the seed flours (Table 4).

The increment seen in soaked seed flours of *Citrullus lanatus* might be a result of the hydrolytic breakdown of nutrient components during soaking, this report is similar to the one reported by Bujang and Nurul[35] in soaking of groundnuts, soybean, and garbanzo. Leucine is the most abundant essential amino acid in all the seed flours while glutamic acid is the most abundant non-essential amino acid.

Results obtained showed that the value of HB, PCV, MCHC, and RBC, increases significantly (p 0.05) when compared to that of the unprocessed seed flour for *Citrullus lanatus.* This increment is an indication that the processing methods significantly improved the quality of the *Citrullus lanatus* seed flour. The improvement may be due to, among other factors, inactivation of the antinutrient factor present in the seed flour and transformation of some of the component nutrients to non-toxic, more readily digestible, absorbable form[36].

The result obtained in this study (Fig. 3), revealed that serum TG and LDL of all the processed groups of *Citrullus lanatus* decreased when compared to the unprocessed seed, citrulline is present in *Citrullus lanatus* seed[37], which may be the possible reason for improving the lipid profile parameter in this group. Previous reports had it that L-citrulline can improve endothelium-dependent vasorelaxation and relax arterial smooth muscle by an effect on cyclic GMP. On a general note, this study revealed that these seed flours are a rich source of amino acids. This suggests that these seed flours are nutritionally high-quality foods.

CONCLUSION

The consumption of watermelon seed flour could improve the lipid and hematological components of the blood, The chemical composition showed that soaking and boiling can be used to improve the nutritional composition of watermelon seeds and hence the flour. This study also revealed that watermelon seeds contain some antinutrients which could be reduced by boiling and germination. This can be incorporated into diets since the seed possesses good nutritional properties. It can therefore be said that watermelon seed flour can be used as a nutritional tool to improve and sustain the health of the consumers.

SIGNIFICANCE STATEMENT

The purpose of this study was to determine the impact of the processing techniques (soaking, germinating, and boiling) of the flour prepared from *Citrullus lanatus* (watermelon) seeds on the lipid profile and hematological characteristics of rats. The findings revealed that processed watermelon seed flour had positive effects on lipid profile and some blood parameters, signifying a nutrition value added. This work adds knowledge to the knowledge base of processing methods that affect the nutritional quality and healthy benefits of watermelon seeds, propounding them as functional food applications.

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