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Nutritional Profiling of Underutilised *Citrullus lanatus mucosospermus* Seed Flour

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Abstract: The seed of *Citrullus lanatus mucosospermus*, known as egusi, is versatile and explored for its oil and flour functionality. Raw flour can be used as a raw material in a nutritional program due to its oil-rich, remarkably high protein content, and richness in omega-6 fatty acids. There is a need to explore eco-friendly defatting methods using the supercritical CO_2 extraction method ($SFECO_2$) to preserve this seed's generic richness and to control the flour–oil ratio in processing formulations. The supercritical fluid extraction method uses temperature, pressure, and CO_2 flow rate to determine the best yield and extraction parameters. Defatted egusi flour (DEF) was extracted using three runs. Firstly, at 60 °C, 30 g/h, and 450 bar (DEF1); secondly, at 55 °C, 30 g/h, and 600 bar (DEF2); and thirdly, extraction was performed at 75 °C, 30 g/h and 600 bar (DEF3). Trace and major elements were analysed using Agilent 7700 quadruple ICP-MS (Agilent Technologies Network, Palo Alto, CA, USA) and Thermo Cap 6200 ICP-AES (Thermo Scientific, Waltham, MA, USA), respectively. The sugar was separated on a gas chromatograph coupled to a Mass Selective Detector (MSD). The fundamental pasting property measurements were performed using a Rapid Visco Analyser RVA 4500 Perten instrument Sin 214 31208-45 Australia. Data analysis was conducted using IBM SPSS version 29 software (v. 2022). The protein content of defatted egusi flour ranged from 48.4 for DEF2 to 60.4% *w*/*w* for DEF1 and differed significantly, with a rich amino acid high in glutamine ranging from 9.8 to 12.9 g/100 g). DEF2 (512.0 cP) showed the highest peak viscosity and was the most viscous among the samples. Defatted flour with lower temperature and lower pressure (60 °C and 450 bar) offered the best nutritional properties, proffering defatted egusi flour from $SFECO_2$, a novel flour for dietary programs.

Keywords: defatted flour; nutritional; Egusi; amino acid; supercritical CO_2 extraction; minerals

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1. Introduction

Egusi seed, originating from the Egusi melon (*Citrullus lanatus mucosospermus*), holds substantial promise as a staple food crop in tropical regions [1]. Indigenous to tropical Africa and Asia, the Egusi melon thrives in warm climates, making it well-suited for cultivation in these areas. The seed, commonly used in traditional cuisines across these regions, presents numerous nutritional and economic opportunities [1].

In tropical areas where food security is often a concern, Egusi seeds are a valuable food source due to its rich nutrient profile [2]. The seeds are renowned for their high protein content, essential fatty acids, vitamins, and minerals, making them a significant component of local diets. Egusi seeds offer versatility in culinary applications, utilised as a primary ingredient in soups, stews, and various traditional dishes [3].

Moreover, Egusi cultivation holds economic potential for tropical farmers. The demand for Egusi seeds transcends local markets, with the potential for export to international markets and a growing interest in exotic and nutritious food products [1]. Furthermore, the Egusi melon plant exhibits resilience to specific environmental conditions prevalent in tropical regions, making it a viable crop choice for small-scale farmers [4].

Egusi melon (*Citrullus lanatus mucosospermus*) seeds, known for their bitter pulp, are principally used to extract oil, which is widely used in Africa and Asia [5]. This oil, high in omega-6 fatty acids like linoleic acid, has the potential for use as a biodiesel source. Conventional defatting processes, such as heat pressing and chemical extraction, frequently produce flour with undesired properties such as discolouration, off-flavours, and lower nutritional value [6]. Furthermore, using harmful solvents in these processes risks customers' health. To solve these restrictions, this work used supercritical fluid extraction (SFE), which elevates carbon dioxide (CO_2) above its critical temperature and pressure, converting it into a supercritical fluid state. This change increases CO_2's solvating potency, making it an excellent medium for defatting processes [7]. Defatted egusi flour is a versatile and healthy alternative to traditional egusi flour, with potential uses in commercial food manufacturing and nutritional interventions. Continued research and development in processing techniques and product innovation can help to extend its use and advantages in the food business and public health programmes.

Supercritical CO_2 extraction, which was developed in the 1980s, has been used successfully in a variety of seed defatting procedures, including grape seed, rapeseed, and coriander [8]. This approach is versatile in seed processing, with applications including green coffee, black tea, decaffeination, hop extract, and essential oil extraction [9]. The resulting defatted meal from SFE shows potential as a functional ingredient, owing to its beneficial amino acid profile, which makes it suitable for inclusion into malnutrition programmes and promoting diversification in the food processing industry [10].

Despite their underutilisation when compared to other oilseeds, egusi seeds have inherent nutritional benefits, particularly protein content, which improves food system functionality [11]. Previous research has shown that protein isolates from Egusi flour may be prepared using water and NaOH extractions, with the ensuing functional qualities including water- and oil-holding capabilities. Furthermore, the effect of NaCl concentrations on heat stability highlights the potential of Egusi flour as an essential material in combating malnutrition [12].

This study assesses the nutritional and technological aspects of Egusi flour derived via supercritical CO_2 extraction, shining light on its potential as a helpful resource in nutritional programmes and the food processing industry.

2. Materials and Methods

2.1. Source of Egusi Seed, Chemicals, and Reagents

Dehulled egusi was purchased from a local Cape Town and South Africa seed store. All chemical reagents were obtained from Merck Pty South Africa, and distilled water was used for the study. The supercritical fluid extraction equipment (Swiss Nova) used in this study was located at the Process Engineering Department of Northwest University, Potchefstroom Campus, South Africa.

2.2. Production of Defatted Egusi Flour Using Supercritical CO_2 Extraction

After supercritical fluid extraction, three experimental iterations were used to obtain defatted egusi flour [13]. These experiments, labelled DEF1, DEF2, and DEF3, were carried out by the criteria set by Olubi et al. [7]. Each run entailed loading around 2 kg of raw, dehulled egusi seed into the extractor. The system was pressurised in increments ranging from 400 to 600 bar until attaining the goal pressures of 450 and 600 bar, respectively. The column temperature and pressure were then stabilised, and contact with the egusi seed was maintained for at least 15 min at a steady supercritical CO_2 flow rate of 30 g/h.

A thermoregulatory apparatus adjusted the extractor outlet temperature to 55 °C, 60 °C, and 75 °C. This modulation was accomplished by regulating the separation of the extract from the solvent during depressurisation. Defatted egusi flour (DEF) was collected and measured in a glass container. The following analysis evaluated the nutritional and technological properties of DEF.

2.3. Macro and Micronutrient Composition of Defatted Egusi Flour

2.3.1. Proximate Analysis of Defatted Egusi Flour

The moisture, crude protein, fibre, and ash were determined using the Association of Official Analytical Chemists Method 934.01 (AOAC, 2005). 20. Crude protein (N × 6.25) was determined using the Nitrogen analyser (Leco Truspe N-630-100-200-230 V Amps 12 A, Saint Joseph, MI, USA). The ash content was analysed gravimetrically after incineration in a muffle furnace (Laboratory 1200C 64L Heating Electric Muffle Furnace with K Type Thermocouple, Fuzhou, China) for 2 h at 600 °C based on AOAC Method 942.05 (AOAC, Rockville, MD, USA). A moisture analyser determined moisture content (Denver instrument IR-30 ISO 9001, Laboratory Instrument Specialists, Inc., Glendale, CA, USA). Finally, carbohydrate content was obtained by subtracting the flour's protein, fat, ash, moisture, and fibre content from 100 [100 − (moisture + ash + protein + fat)] [14].

2.3.2. Amino Acid Profiling of Defatted Egusi Flour

Reagent Preparation

Eluent A and B (Cas 186003839) were used as received in the AccQ Tag Ultra Derivatization Kit, Water kit (Waters Corporation, Milford, MA, USA) (186003836). Eluent A (50 mL) was prepared by mixing with 950 mL of deionised water, while Eluent B was supplied [14]. A weak wash solvent was made by adding 5% acetonitrile in water, while a strong wash solvent was used at 95% acetonitrile (Cas 75-05-8/100030). The preparation of 6-Aminoq, quinoline N-succinimidyl ester (AQC) with the catalogue number [Cas 148757-94-2] was carried out by drying in acetonitrile of 1 mL. AQC was added to the reagent in a vial containing 3 mg of AQC [15]. This vial was then heated, vortexed, and sonicated to dissolve the reagent completely. A derivatising agent of 20 µL was used for each sample, allowing a 1 mL sample for each reaction. The derivatising agent was prone to hydrolysis and was stored in a desiccator to maintain its stability for approximately one week.

Internal Standard (L-Norvaline) [Cas 6600-40-4/841505] ISO 90012] [16] was prepared by weighing 10 mg of L-Norvaline into a 15 mL centrifuge tube and making up to 10 mL with MilliQ water. This 1000 ppm L-Norvaline stock solution was diluted five times to produce a 200 ppm solution used during sample preparation. The derivatisation reaction was also sped up by heating the vials at 55 °C for 10 min before analysis. The amino acids were measured as free amino acids or after hydrolysis of proteins using standard 6 M hydrochloric acids (HCl) [Cas 7647-01-0] acid digestion as an 800 µL sample + 200 µL of Norvaline stock solution. A dilution of 10 µL was used during derivatisation. The standards were prepared using an 80 µL std solution + 20 µL of Norvaline stock solution with no dilution factor [15].

Derivatisation Procedure of Amino Acids

Borate buffer (Cas 101645) of 70 µL was poured into a 200 µL glass and inserted into a 2 mL glass vial. A diluted egusi flour/standard solution of 10 µL was added. AQC reagent of 20 µL was added, and the sample was capped and vortexed well to mix. The mixture was transferred into vials previously heated in an oven/heating mantle at 55 °C for 10 min. After 10 min at 55 °C, the vials were ready and loaded into the autosampler tray for analysis [17].

Chromatographic Analysis of Amino Acids

A chromatographic analysis used Waters Acquity Ultra Performance Liquid Chromatography (UPLC) with a fitted photodiode array (PDA) detector to separate the amino acids. A sample/standard solution of 1 µL was injected into the mobile phase, conveying the derivatised amino acids onto the Ultra Tag C_{18} column (2.1 × 50 mm × 1.7 µm) held at 60 °C. The column's elution of analytes was carried out by running a gradient [18].

A PDA detector detected the analytes eluting off the column, with each amino acid coming off the column at a single retention time. Mass Lynx software 4.2.1 performed the instrument control and data acquisition by integrating the peaks at the defined retention

times and plotting calibration curves for each amino acid based on the peak response (peak area/internal standard peak area) against concentration.

2.3.3. The Mineral Content of Defatted Egusi Flour

Sample Digestion

The acid-extractable elemental content of the defatted egusi flour was digested at elevated temperature and pressure, and this was performed on a MARS microwave digester using ultra-pure HNO_3 (Cas 101645). The extractant was made up to 50 mL after cooling with distilled water, subsequently identified by ICP-AES and ICP-MS for the selected analytes [19].

Trace Element Analysis

Agilent 7700 quadruple ICP-MS was used to analyse the trace elements. Samples were weighed using a 0.4 mL/min micro-mist nebuliser and sprayed into a Peltier-cooled spray chamber at a temperature of 2 °C with a carrier gas flow of 1.05 L/min. Under He-collision mode, elements V, Cr, Mn, Fe, Co, Ni, Cu, and Zn were analysed to remove polyatomic interferences. NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) calibrated the instrument for traceable standards and quantified selected elements. Where samples have undergone a digestion step, the results were corrected for the dilution factor resulting from the digestion procedure [20].

Major Element Analysis

A Thermo Cap 6200 ICP-AES (Thermo Scientific, Waltham, MA, USA) was used to analyse the major elements (Na, K, Ca, Mg, P, and Si). The instrument was calibrated using NIST traceable standards to quantify selected elements. In addition, NIST-traceable quality control standards from De Bruyn Spectroscopic Solutions, Bryanston, South Africa, were used to verify the calibration's accuracy before sample analysis as well as throughout the calculations to monitor errors [21].

2.3.4. Sugars in Defatted Egusi Flour

Approximately 10 mg of the egusi flour was extracted with 1000 µL of 70% (Cas 67-56-1/106012) methanol/water (*v*/*v*). The sample mix was briefly vortexed for 2 min and incubated for 180 min at 60 °C. The set sample was centrifuged, and the supernatant was transferred into clean 1000 µL centrifuge tubes. Ribitol (Cas 488-81-3) was added as an internal standard. The supernatant of 500 µL was then dried in a speed vac. Methoxamine hydrochloride (2.5%) of 100 µL in pyridine (Cas 593-56-6/814911) was used to reconstitute the dry samples and was incubated for 120 min in an oven maintained at a temperature of 40 °C. Subsequently, specimens were trimethylsilylated with 50 µL N-Bis (trimethylsilyl) trifluoroacetamide (BSTFA + TMCS, 99:1) [Cas 25561-30.2/110255] and further incubated for 30 min at 60 °C [22].

A gas chromatograph (6890N, Agilent Technologies Network, Palo Alto, CA, USA) coupled to Agilent technology inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent Technologies Inc., Palo Alto, CA, USA) was used for the separation. The helium carrier gas operates at a 1 mL/min flow rate, with the injector temperature constant set at 250 °C. The sample (1 µL) was injected into a split ratio of 5:1. The oven temperature was set as 80 °C for 1 min and finally increased to 300 °C at a rate of 7 °C/min for 2 min. The MSD was operated in a full scan mode, and the source and quad temperatures were kept at 230 °C and 150 °C, respectively. The transfer line temperature was maintained at 280 °C. The mass spectrometer was operated under electron impact mode at an ionisation energy of 70 eV, scanning from 35 to 500 m/z [19].

2.4. Functional Properties of Defatted Egusi Flour

2.4.1. Water Absorption and Solubility Index of Defatted Egusi Flour

The water absorbance index (WAI) of defatted egusi flour was determined using Kumar et al.'s method [23] with slight modifications. The WAI was determined using 2.5 g of extracted defatted egusi flour. The sample was suspended in 30 mL of distilled water at 30 °C in a pre-weighed 50 mL centrifuge tube. The mixture was stirred at intervals of 30 min twice and centrifuged at 3500 rpm/10 min. The supernatant obtained was poured into a tared evaporating dish, weighed, and dried. The W was defined as gel per gram solids in Equation (1).

$$(\text{WAI}) = \frac{\text{weight of sediment}}{\text{weight of dry solid}} \times 100 \quad (1)$$

The water solubility index (WSI) is the water-soluble fraction in any sample extract [24]. It was obtained from the number of dried solids recovered after evaporating the WAI test's supernatant. Equation (2) was used to measure the WSI.

$$\text{WSI } (\%\ w/w) = \frac{\text{Weight of dissolved supernatant}}{\text{Weight of dry sample}} \times 100 \quad (2)$$

2.4.2. Pasting Properties of Defatted Egusi Flour

The pasting measurements were performed using a rapid visco analyser RVA 4500 Perten instrument Sin 214 31208-45 Australia. Defatted egusi flour (3.5 g) was prepared as a slurry by manually adding water and flour for 3 min. The stirring paddle further stirred the mixture at 960 rpm for 10 s, and the stirring continued at 160 rpm for the remaining 13 times of the test. The system's temperature was kept at 50 °C for 1 min, with constant water flowing to cool the system [23]. The values of peak viscosity, breakdown viscosity, holding strength, pasting temperature, and final thickness were evaluated [25]. Each measurement was carried out in triplicate.

2.5. Statistical Data Analysis

All data were collected in triplicate. To establish mean differences between treatments, they were subjected to multivariate analysis of variance. Duncan's multiple range tests were used to separate means where variation existed. IBM (New York, NY, USA) SPSS (Chicago, IL, USA), 2016, was used to carry out all data analyses.

3. Results and Discussion

3.1. Effect of Supercritical CO_2 Extraction on the Proximate Composition of Egusi Flour

The proximate composition of defatted egusi flour in Table 1 shows a low moisture content, which differed significantly ($p \leq 0.05$), with DEF3 (75 °C and 600 bar) having the lowest moisture content. The recommended water intake of egusi flour can vary depending on its specific application and the desired consistency of the final product. Generally, when using egusi flour as a thickening agent in soups or stews, the amount of water added should be adjusted based on personal preference and the desired thickness of the dish. The protein content was 48.4% for DEF2 (55 °C and 600 bar) and 60.4% w/w for DEF1 (60 °C and 450 bar), with a p-value of <0.05, indicating a significant difference. The protein difference could be attributed to the effects caused by the high pressure (600 bar) and lower temperature (55 and 60 °C) during extraction. Protein is structurally modified at high pressure, while low temperature reduces the defatted flour extractible components [26]. The protein content differences between egusi flour obtained via supercritical fluid extraction (SFE) and conventional heat and solvent oil extraction methods can be attributed to the preservation of protein integrity, minimal solvent interaction, selective fat removal, and reduction in oxidative degradation provided by SFE. These scientific elements work together to explain why egusi flour prepared using SFE has a higher protein content than standard extraction methods [27]. The protein content of raw and defatted egusi flour was higher than that of cereals (7.5–12 g/100 g) [28] and eggs (12.8 g/100 g) [29]. The protein in egusi flour is high

compared to defatted sunflower seed, soy flour, and flaxseed flour, which are 8.20, 44.7, and 20.6 g/100 g, respectively [30]. Protein is a significant body component, essential to every cell [31]. Repairing and building muscles require substantial amounts of protein; a lack of protein would also negatively affect nails and hair [28,32]. The colour of egusi flour is a creamy white colour, as shown in Figure 1, which offers a viable high-protein alternative flour to vegetarians and those who cannot afford high-protein meat and meat products. It could also be a significant raw material for ready-to-use therapeutic food (RUTF).

Table 1. Proximate composition of raw and defatted egusi flour.

		Defatted Egusi Flour [1,2]		
Proximate (%)	**Raw Egusi**	**DEF1**	**DEF2**	**DEF3**
Moisture	8.1 ± 0.0^{a}	10.1 ± 0.0^{a}	6.6 ± 0.5^{b}	5.3 ± 0.0^{c}
Crude protein	28.4 ± 0.0^{a}	60.4 ± 0.1^{a}	48.4 ± 0.4^{b}	60.1 ± 0.6^{a}
Crude fat	52.0 ± 0.0^{a}	0.6 ± 0.0^{b}	0.4 ± 0.0^{b}	0.7 ± 0.0^{b}
Carbohydrate	5.2 ± 0.0^{a}	19.5 ± 1.3^{b}	34.9 ± 1.7^{c}	23.4 ± 2.3^{c}
Fibre	2.7 ± 0.1^{a}	4.4 ± 0.3^{a}	3.4 ± 0.3^{b}	4.4 ± 0.4^{a}
Ash	3.6 ± 0.0^{a}	5.0 ± 1.3^{a}	6.3 ± 1.9^{a}	6.1 ± 2.5^{a}

[1] Values are mean ± standard deviation of 5 replicates. Means with a different superscript in each row differ significantly ($p \leq 0.05$). [2] DEF1 = flour extracted at 60 °C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extracted at 55 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar; DEF3 = flour extracted at 75 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar.

Figure 1. Defatted egusi flour DEF1 = flour extracted at 60 °C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extracted at 55 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar; DEF3 = flour extracted at 75 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar).

The ash content ranged from 5.3% for DEF1 to 6.8 w/w for DEF3, with no significant difference. The defatted egusi flour was also a good carbohydrate source with values ranging from 19.5% for DEF1 to 34.9% w/w to DEF3, with a significant ($p \leq 0.05$) difference between the samples. DEF2 was significantly higher in carbohydrates at low temperatures (55 °C) and high pressure (600 bar). Starch granules are not destroyed at low temperatures during a supercritical extraction procedure [33].

The dietary fibre ranged between 3.4 and 4.6% w/w, with DEF2 significantly lower than DEF1 and DEF2. Fibre is essential for promoting digestibility, reducing blood cholesterol,

and reducing the risk of colon cancer [34]. The high fibre content of defatted egusi flour makes it a functional ingredient in therapeutic food formulation.

3.2. Effect of Supercritical CO_2 Defatting on the Amino Acid Composition of Defatted Egusi Flour

The amino acid composition indicated in Table 2 shows the presence of 14 amino acids, with glutamine being the highest. Glutamine is found chiefly in muscles, consisting of 19% nitrogen, making it the primary carrier of nitrogen in muscle cells. Another glutamine attribute is its flavour-enhancing role in food [35]. In defatted egusi flour, glutamine was as high as 12.9 g/100 g. The concentration of sulphur-containing amino acids (methionine) decreased with increased pressure and temperature to 600 bar and 75 °C. The decrease in sulphur-containing amino acids reflects the instability of amino acids when subjected to heat treatment and high-pressure [36]. Methionine considerably contributes to body cells' functionality by influencing cellular redox state and helping with detoxification [24].

Table 2. Amino acid content of defatted egusi flour.

	Defatted Egusi Flour [1,2]		
Amino Acids (g/100 g)	**DEF1**	**DEF2**	**DEF3**
Essential			
Histamine	1.5 ± 0.0^{a}	1.4 ± 0.1^{b}	1.1 ± 0.0^{c}
Methionine	1.9 ± 0.3^{a}	1.8 ± 0.1^{ab}	2.3 ± 0.1^{c}
Valine	2.9 ± 0.1^{a}	2.7 ± 0.1^{b}	7.64 ± 0.3^{b}
Isoleucine	2.2 ± 0.2^{a}	2.1 ± 0.1^{b}	2.8 ± 0.2^{c}
Threonine	1.5 ± 1.4^{a}	2.0 ± 0.2^{a}	4.4 ± 0.2^{c}
Leucine	4.31 ± 0.1^{a}	4.21 ± 0.0^{b}	1.1 ± 1.0^{a}
Phenylalanine	3.09 ± 0.3^{a}	2.95 ± 0.1^{b}	2.4 ± 0.1^{a}
Non-Essential			
Glutamic	12.9 ± 1.0^{a}	11.8 ± 1.0^{c}	2.1 ± 0.2^{a}
Proline	2.4 ± 0.2^{a}	2.2 ± 0.1^{b}	1.4 ± 0.1^{a}

[1] Values are mean ± standard deviation of 9 replicate. Means with a different superscript in each row differ significantly ($p \leq 0.05$). [2] DEF1 = flour extracted at 60 °C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extract at 55 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar; DEF3 = flour extracted at 75 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar.

According to Akande, 2011, in sunflower seeds, the amino acid content is high enough, with lysine and methionine ranging from 0.6 to 0.7 g/100 g and 0.3 to 0.5 g/100 g, respectively [37]. Lysine is not produced in animals or humans, making it an essential amino acid that must be ingested in food with high lysine protein [38]. The recent FAO pattern for the amino acid advised consuming 15–22 mg/kg/day of methionine and 30 mg/kg/day of lysine [28]. Defatted egusi flour was a source of lysine, ranging between 2.4 g/100 g for DEF1 and 2.6 g/100 g for DEF2, with no significant differences.

The high glutamic and aspartic acid contents in defatted egusi flour were similar to those in seaweeds (26). These compositions are high in egusi flour, as the essential amino acid present is similar to the recommended value. Arginine in egusi flour ranges between 7.6 and 9.9 g/100 g for DEF1, DEF2, and DEF3, respectively, with no significant difference between the three flour samples. Arginine is a conditional amino acid depending on individuals' health status and age. Infants cannot synthesise or create arginine internally, making the amino acid nutritionally essential for such a class of individuals. More so, a healthy body synthesises arginine independently, making it conditionally necessary for those with health-related issues [39]. Egusi flours showed a significant difference in their amino acid content depending on the pressure and temperature of extraction. There was an increase in the nutritional properties of defatted egusi flour at low temperatures and pressure because the solid matrix had minimal pressure and temperature.

3.3. Mineral Composition of Defatted Egusi Flour

Sixteen trace minerals and five major elements were detected in defatted egusi flour obtained after supercritical extraction in Table 3. The principal element (Table 3), phosphorus, ranged from 1698.9 to 2046.4 mg/100 g, with DEF3 being the highest, with a significant ($p \leq 0.05$) difference in the samples, followed by K, Mg, Ca, and Fe.

Table 3. Trace and major mineral composition of defatted egusi flour.

	Defatted Egusi Flour [1,2]		
Mineral (mg/100 g)	**DEF1**	**DEF2**	**DEF3**
B	2.7 ± 0.11 [a]	3.3 ± 0.045 [b]	3.6 ± 0.22 [b]
Al	17.1 ± 0.29 [a]	8.6 ± 0.07 [c]	11.0 ± 0.02 [b]
Ti	0.7 ± 0.01 [a]	0.5 ± 0.01 [b]	0.6 ± 0.02 [c]
Cr	0.3 ± 0.00 [a]	0.1 ± 0.00 [b]	0.1 ± 0.00 [a]
Mn	7.7 ± 0.12 [a]	7.8 ± 0.05 [b]	8.5 ± 0.14 [c]
Fe	44.4 ± 0.54 [a]	26.7 ± 0.11 [c]	33.0 ± 0.56 [b]
Ni	0.5 ± 0.01 [a]	0.4 ± 0.01 [a]	0.5 ± 0.01 [b]
Cu	3.7 ± 0.07 [a]	3.9 ± 0.06 [b]	4.1 ± 0.08 [c]
Zn	9.6 ± 0.15 [a]	12.0 ± 0.16 [b]	12.8 ± 0.21 [c]
Sr	0.9 ± 0.00 [a]	1.1 ± 0.01 [b]	1.2 ± 0.02 [c]
Mo	0.2 ± 0.00 [a]	0.3 ± 0.01 [b]	0.4 ± 0.01 [c]
Ba	0.8 ± 0.07 [a]	0.8 ± 0.05 [a]	0.8 ± 0.02 [a]
Si	14.0 ± 0.11 [a]	10.7 ± 0.04 [b]	12.7 ± 0.12 [c]
Ca	166.8 ± 0.79 [a]	186.1 ± 0.96 [b]	201.27 ± 1.26 [c]
K	1208.1 ± 17.34 [a]	1298.6 ± 15.34 [b]	1413.3 ± 18.58 [c]
Mg	807.5 ± 2.16 [a]	880.1 ± 2.03 [b]	957.8 ±13.52 [c]
Na	3.4 ±10.11 [a]	8.5 ± 0.15 [b]	9.4 ± 0.11 [c]
P	1698.9 ± 8.52 [a]	1877. 8 ± 8.21 [b]	2046.4 ± 5.95 [c]

[1] Values are mean ± standard deviation of 18 replicates. Means with a different superscript in each row differ significantly ($p \leq 0.05$). [2] DEF1 = flour extracted at 60 °C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extracted at 55 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar; DEF3 = flour extracted at 75 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar.

Elements in the defatted flours differed significantly in concentration, influenced by the pressure and temperature used during extraction. The mineral content was significantly increased with an increase in temperature ($p \leq 0.05$) and high in defatted flour DEF3 (75 °C 600 bar) except for trace elements (Fe, Al, Ba, Hg, Pb, and Si), where the highest concentration was found in DEF1 (60 °C and 450 bar). The high mineral content could be due to the affiliation of elements to low-temperature processing, causing minimal disruption in its constituents [22]. The effect of high temperature and pressure on the extracted flour during a supercritical extraction ensures minimum contact between solvent and solute, as solvent extraction CO_2 was released at pulse speed. This steady release of CO_2 separated the liquid from a solid base; thus, more nutrients were observed in the residue [29].

Phosphorus, the highest in egusi flour, is necessary for human life. High phosphorus offers an additional functional food to be incorporated into daily diets. Calcium is an essential structural component of bone, vital in forming a healthy body. Egusi contains a high calcium concentration, making it an alternative calcium food source. Egusi is also high in magnesium, with the highest DEF3 being 957.8 mg/100 g, with a significant ($p \leq 0.05$) difference between the three samples. Muscle and nerves' proper functionality can be aided with magnesium and the heart's steady rhythmic flow [40].

Calcium, potassium, and magnesium play a significant role in repairing worn-out body cells. In appreciable amounts, DEF1, DEF2, and DEF3 contain trace elements, such as zinc, manganese, and iron. These elements are essential for enzyme metabolism and the proper functioning of individual cells in the body. The high phosphorus, magnesium, potassium, and calcium content in egusi flour make it a good supplement for pregnant and

lactating women, children, and older adults [41]. The mineral content was significantly high in the defatted flour extracted at high temperatures and pressures.

3.4. Sugars of Defatted Egusi Flour

There were nine sugars present in defatted egusi flours, as seen in Table 4. Sucrose in defatted egusi flour ranged from 86.5 to 109.4 mg/100 g and differed significantly ($p \leq 0.05$), with the lowest sucrose found in DEF3. When sugar is exposed to heat, it dissolves into a thick syrup depending on the quantity; caramelisation only occurs when a rise in temperature leads to a deep brown syrup [42]. Therefore, as the temperature increased, the sugar concentration of defatted egusi flour decreased significantly. This partial caramelisation was responsible for the pale white colour observed in the defatted flour, slightly differing in the three flour samples. Overall, the controlled operating conditions, reduced oxygen exposure, shorter extraction time, and selective extraction of lipids offered by SFE prevent complete caramelisation in defatted flour. This results in a high-quality product with preserved colour, flavour, and nutritional properties. Sucrose is a common carbohydrate found in many plants and plant parts. Sucrose is a disaccharide, combining two monosaccharides, glucose and fructose, with the formula $C_{12}H_{22}O_{11}$. It is broken down in humans to form monosaccharides (glucose and fructose) by sucrase enzymes [43].

Table 4. Suga metabolite in defatted egusi flour.

	Defatted Egusi Flour [1,2]		
Sugars	**DEF1**	**DEF2**	**DEF3**
D–fructose	2.2 ± 0.21^{a}	2.4 ± 0.37^{b}	1.4 ± 0.12^{ab}
D–galactose	0.8 ± 0.00^{a}	0.9 ± 0.12^{a}	0.4 ± 0.05^{b}
Mannose	0.1 ± 0.05^{a}	0.2 ± 0.10^{b}	0.7 ± 0.17^{c}
Glucose	0.8 ± 0.14^{a}	1.0 ± 0.19^{a}	0.8 ± 0.11^{a}
Mannitol	8.0 ± 0.37^{a}	9.0 ± 1.45^{a}	0.8 ± 0.13^{b}
Sorbitol	8.2 ± 0.41^{a}	9.2 ± 1.53^{a}	4.0 ± 0.54^{b}
Sucrose	$106.\ 4 \pm 75.00^{a}$	109.4 ± 97.54^{a}	86.5 ± 13.32^{b}
Trehalose	2.2 ± 0.75^{a}	2.0 ± 0.81^{a}	1.9 ± 0.29^{a}

[1] Values are mean ± standard deviation. Of 8 replicates. Means with a different superscript in each row differ significantly ($p \leq 0.05$). [2] DEF1 = flour extracted at 60 °C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extract at 55 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar; DEF3 = flour extracted at 75 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar.

Sucrose can be easily absorbed into the body but has a relatively low glycaemic index due to its fructose content, which minimises blood glucose [44]. Fructose, as a monosaccharide, has a minimal impact on blood glucose levels compared to other carbohydrates. This unique characteristic of sucrose contributes to the moderation of blood glucose concentration during metabolic processes [45].

Understanding the interplay between sucrose, fructose, and sorbitol in defatted egusi flour provides valuable insights into the potential impact on human metabolism. Further research and exploration of these components contribute to a detailed understanding of the nutritional properties of defatted egusi flour and its possible applications in dietary management and health-conscious food choices [46].

3.5. Effect of Extraction Condition on Functional Properties of Defatted Egusi Flour

The water absorption index (WAI) and water solubility index (WSI) of defatted egusi flour in Table 5 showed WAI ranging from 52.5 to 57.6% w/w. There was a significant increase in the water absorption index of defatted egusi flour. The increased water absorption could be due to the starch structure in the defatted flour, expanding its crystalline structure for easy water absorption [47].

Table 5. Water absorbance and water solubility index of defatted egusi flour.

Parameters (% *w*/*w*)	Defatted Egusi Flour [1,2]		
	DEF1	**DEF2**	**DEF3**
WAI	56.3 ± 2.4 [a]	52.5 ± 3.7 [b]	57.6 ± 3.2 [a]
WSI	68.0 ± 4.0 [a]	65. 3 ± 6.1 [b]	73.3 ± 6.1 [c]

[1] Values are the mean ± standard deviation of 2 replicates. Means with a different superscript in each row differ significantly ($p \leq 0.05$). [2] DEF1 = flour extracted at 60 °C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extract at 55 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar; DEF3 = flour extracted at 75 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar.

The WSI shows an increase, which was significantly ($p \leq 0.05$) different in the samples. The water solubility index of defatted egusi flour ranged from 65—3 to 73.3% *w*/*w*. The increase in the water solubility index has proven that the supercritical CO_2 extraction method improved the flour's functionality [47]. The WAI and the water solubility index (WSI) of the three defatted flours were similar at temperatures below 60 °C. WAI and WSI show the magnitude of the interaction within the starch chains for both the amorphous and crystalline states [34]. The different pressures and temperatures used during extraction could lead to varying functionalities [48].

3.6. Effect of Extraction Condition on Pasting Properties of Defatted Egusi Flour

The pasting properties of DEF1, DEF2, and DEF3 in Table 6 showed a significant difference in viscosity. The peak viscosity of defatted egusi flours ranged from 386.0 to 512.0 (cP), which was significantly ($p \leq 0.05$) different across the three samples. DEF2 (512.0 cP) showed the highest peak viscosity and was the most viscous. This high viscosity could be due to the low temperature during extraction (55 °C, 600 bar). Low-temperature extraction prevents starch breakdown, encouraging gelatinisation and enabling a viscous substance [49,50]. These results indicated that low-temperature extraction significantly affected the pasting viscosities under the experimental conditions, and defatted egusi flour would behave differently during heat processing. Meanwhile, the pasting temperature and final viscosity did not differ significantly.

Table 6. Pasting properties of defatted egusi flours.

Parameter (cP)	Defatted Egusi Flour [1,2]		
	DEF1	**DEF2**	**DEF3**
Peak viscosity	386.0 ± 51.80 [a]	512.0 ± 13.00 [b]	398.3 ± 9.61 [c]
Breakdown viscosity	306.0 ± 45.21 [a]	373.0 ± 36.72 [b]	191.3 ± 10.97 [c]
Holding strength	53.3 ± 4.92 [a]	57.7 ± 3.51 [b]	49.0 ± 2.00 [c]
Pasting temperature (°C)	92.7 ± 1.31 [a]	93.0 ± 0.50 [a]	93.8 ± 0.25 [a]
Final viscosity	126.7 ± 2.30 [a]	123.7 ± 2.08 [a]	126.3 ± 1.15 [a]

[1] Values are mean ± standard deviation of 5 replicates. Means with a different superscript in each row differ significantly ($p \leq 0.05$). [2] DEF1 = flour extracted at 60 °C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extract at 55 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar; DEF3 = flour extracted at 75 °C, carbon dioxide flow rate 30 g/h, and pressure 600 bar.

The holding strength and breakdown viscosity were low in the sample extracted at high temperature and pressure (75 °C, 600 bar) [DEF3]. The low holding strength and breakdown viscosity could be attributed to high pressure and temperature on the starch structure, causing thinness in the starch and reducing water absorption and retention [51]. This holding period is commonly associated with a viscosity breakdown [52]. The defatted egusi flour breakdown viscosity showed flour with stable thickness for DEF1 and DEF2. Based on egusi flour qualities, low temperature and pressure are the ideal process parameters for producing optimal egusi flour, as indicated by the low temperatures of 55 and 60 °C, respectively.

3.7. Conclusions

The nutritionally dense flour obtained from the supercritical CO_2 extraction of egusi oil has a creamy white colour, with a protein level of about 60% *w*/*w* and a carbohydrate content of about 30%. Out of all the processing parameters that were investigated, the flour obtained at low pressure and low temperature (60 °C and 450 bar) was the best option because it had better physicochemical and nutritional qualities. The defatted egusi flour's high protein content makes it an excellent choice for supplementing flours with less nutritional value. It offers a chance to improve the nutritional profile of different food products via its possible use in composite flours in the food sector. Defatted egusi flour is versatile and should be used with other ingredients as a potential thickening agent and binder.

The results underscore the effectiveness of supercritical CO_2 extraction in generating defatted egusi flour and emphasise the significance of particular processing settings in shaping the nutritional and functional characteristics of the end product. Fortified and texturally enhanced food industry needs can be met using defatted egusi flour, a nutrient-dense and sustainable ingredient, to solve nutritional shortages in different food compositions. This particular flour can be used in various food systems to exert an even more significant effect, given more investigation and study into its uses and processing condition optimisation.

3.8. Significance Statement

This study emphasises the importance of defatted egusi flour made by supercritical CO_2 oil extraction and stresses its nutrient density. This study offers important information about the defatting procedures for this oilseed and similar species, including ideal extraction pressure and temperature ranges of 450 bar and 60 °C. Defatted egusi flour is more than just a raw material; it can be used to make processed, precooked foods, offering novel approaches to health problems, including obesity and malnutrition. According to this study, defatted egusi flour is a flexible component with significant nutritional and public health implications.

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