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Chemical and Functional Study on Bottle Gourd Seed

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ABSTRACT:

Whole and hulled bottle gourd seeds were analyzed. Physical tests of the seeds showed that the average weight of 100 seeds was 26.86 g, and the volume of the seeds was about 29 ml. The percentage of husks was 55.74%, a higher percentage than the cotyledons, which gave 44.26%. The apparent density was 0.263 g/ml and the true density was 0.905 g / liter and the porosity rate was 28.88%. The whole seed has a length and width of 17.17 ml and 9.7 ml, respectively, and the hulled seeds are about 15.71 ml in length and 4.78 ml in width. Chemical composition results: Moisture and total ash increased from the whole seed from 3.4% and 4.05%, respectively, to 5.5% and 7.5% in the hulled seed sample. Whole seeds were characterized by a high percentage of crude fiber, which was 15%, while it was about 1% in hulled seeds. The fat and protein of whole seeds were about 38.33% and 24.03%, respectively, and hulled seeds were rich in fat and protein, which were 40.87%, and 26.43%, and when the fat was removed, it reached the protein in the hulled seeds was 67.12% and the fat was 0.83%. The percentage of carbohydrates in the sample of defatted hulled seeds was about 17.74% and the whole seeds were 14.86%. The hulled seed sample was characterized by a high level of potassium, followed by phosphorus, and their percentages reached 1397 and 1229 mg/100 g, respectively. The seeds were distinguished by their content of amino acids, especially the non-essential amino acid glutamic acid. Its percentage in hulled seeds and whole seeds was 13.93 and 11.09 g/100 g of protein, respectively. The percentage of the essential amino acid lysine in hulled seeds was 7.55 and in whole seeds was 4.55 g/100 g of protein.

Keywords: Bottle gourd; *lagenaria siceraria*; Chemical and Functional Properties.

1. Introduction

Bottle gourd (*lagenaria siceraria*) is one of the ancient crops that was grown since the pre-Columbian era in both the Old and New World. Its seeds are viable, and this helped in its wide geographical distribution. The shape and size of the fruits differ according to gender and type. The squash can have a narrow neck that does not contain seeds, or it can have a wide neck that contains seeds. The wide part of the squash fruit, which mainly contains the seeds, varies from cylindrical or flat to circular or long and narrow (Figure 1). The appearance varies in terms of whether the color is dark green or light green and can be

striped, spotted, or solid, and the length of the fruit ranges from 150 to 1000 mm, depending on the type and gender of the fruit (Gajera, 2017).



Figure (1): The external appearance (a) and longitudinal section (b) of bottle gourd fruits

The color of bottle gourd seeds varies from yellowish white to dark brown, and they have a smooth or leathery surface. Some species are characterized by wavy margins in the presence or absence of prominent lines on the seeds (Mladenovic *et al.*, 2012). Bottle gourd seeds contain a high percentage of oil (35%-46%), protein (24%-38%) and many phytochemicals such as vitamins B and C and minerals (potassium, iron, sulfur, magnesium, calcium and are rich in Specific copper) and amino acids with saponins and natural biologically active compounds that are produced secondary, including phenolic compounds, glycosides, flavonoids, and alkaloids (Rahaman, 2003), and the oil extracted from the seeds is rich in essential fatty acids, especially of the unsaturated type, such as linoleic acid and sterolic acids, as it has been compared Oil obtained from seeds such as sunflower oil and grape seed oil (Axtell & Fairman, 2013). Hulled seeds contain a protein percentage of about 35% and an oil percentage of about 39.22%. Whole seeds are deficient in all essential amino acids except for the amino acid valine, and hulled seeds contain the highest amount of essential amino acids compared to whole seeds (Hassan *et al.*, 2008), and the nutritional value contained in the seeds has been compared with the seeds of other plants, such as sunflower seeds and soybean seeds, and the comparison was positive (Wicken *et al.*, 1984). Bottle gourd is generally considered one of the neglected plant crops that are not well exploited for food, as it was used in the past as eating and drinking utensils, and its seeds are a by-product that is disposed of as waste or used as animal feed, even though they contain many useful nutritional components, and it is possible to use these resources Available in the manufacture and development of new and useful food products, therefore, the chemical and functional properties of these seeds and the flour resulting from

them were studied. This research aimed to identify the physical properties of the seeds, extract oil from the seeds, identify the chemical composition of the seeds and seed flour, and estimate the amino acids in the hulled seeds.

2. Materials and methods

2.1. Materials

Bottle gourd fruits were obtained from farms located in the Wadi Al-Shati region in southwestern Libya. The fruits were cut, the seeds were separated from the filamentous network inside the fruits, and the seeds were taken from them (Figure 2). The seeds were washed and dried in the sun for two days, after which some of the seeds were removed and the outer shell was removed. The hulled seeds were taken to extract the oil to obtain crude oil and seed flour.



Figure 2: Whole seeds (a) and hulled seeds (b) of bottle gourd

2.2. Methods

2.2.1. Average weight and volume of 100 seeds

The average seed weight and volume (100 seeds) were estimated according to the method mentioned by (Williams *et al.*, 1983), where one hundred seeds were weighed randomly in three replicates and the average was taken to determine the weight of the hundred seeds. As for the volume, it was known by transferring the hundred seeds. A 250 ml graduated cylinder contains 100 ml distilled water, and the volume of one hundred seeds was calculated by increasing the volume of displaced water as follows:

$$\text{Volume of 100 seeds (ml)} = \text{total volume of seeds and water in the graduated cylinder} - 100$$

2.2.2. Percentage of cotyledons and husks

then filtered from the oil and solvent and left to dry to get rid of traces of the solvent (Figure 2). The crushed seeds were ground passed through a sieve and stored in bags.



Figure (3): Pictures showing whole seed flour, hulled seed flour, and oil

2.2.7. Chemical composition

2.2.7.1. Moisture content

A weight of 5 g of the sample was taken in a moisture estimation dish, and the moisture was determined using a drying oven at a temperature of 105 °C. The sample was weighed until the weight was constant, and the percentage of moisture was calculated as stated in the AOAC method (2005).

2.2.7.2. Crude protein

The Kjeldahl method was used to estimate total nitrogen by wet digestion with concentrated sulfuric acid with digestion discs, distillation, and titration with hydrochloric acid of a known standard. The percentage of nitrogen in the sample was calculated using a conversion factor of 6.25 according to what was stated in the AOAC method (2005).

2.2.7.3. Crude oil

The crude oil content of seeds was determined using chloroform as an organic solvent, and extraction was carried out by the Soxhlet method (AOAC, 2005).

2.2.7.4. Total ash

Total ash in seed flour was estimated by burning a 3-g sample in a muffle furnace at 550°C for 3 hours, and after ignition maintaining a temperature of 550 °C, the weight of the ash was taken accurately and the percentage of total ash was calculated as stated in the AOAC method (2005).

2.2.7.5. Crude fibre



Fibers in seed flour were determined by digestion with dilute sulfuric acid and then with dilute alkali (NaOH). After filtration and washing, the precipitate was dried in a drying oven at a temperature of 110°C until the weight was constant, and the precipitate was burned in an incineration oven at a temperature of 550°C for half an hour to calculate the percentage of crude fibers as stated in the AOAC method (2005).

2.2.7.5. Estimation of mineral elements

After burning of the samples, the resulting ash was dissolved in 10 ml of 6N hydrochloric acid (HCl), transferred quantitatively and the volume was completed to 25 ml in a standard flask after filtering on ash-free filter paper, and the resulting solution was used to estimate the various mineral elements after making dilutions. Iron, calcium, magnesium, manganese, zinc, and copper were determined in the previously prepared ash solution using an atomic absorption spectrophotometer (AOAC, 2005). Both sodium and potassium were determined by Flame Photometer as reported by the method of Black *et al.* (1965), phosphorus, was determined colorimetrically by the ammonium molybdate method, using a spectrophotometer at a wavelength of 650 nm in the presence of a standard curve, as stated in AOAC (2005).

2.2.7.6. Estimation of carbohydrates

Total carbohydrates were calculated by subtracting the total crude protein, crude fat, ash, and crude fiber from the dry weight of the sample as stated in AOAC (2005).

2.2.7.7. Determination of amino acids

A sample of seed flour was prepared by weighing 50 milligrams in a 50 ml tube with a tight cap, and 5 ml of pyroformic acid was added to it. The tube was closed tightly and placed in an ice bath for 16 hours, then 0.25 ml of sodium bisulfate solution and 5 ml of 6N hydrochloric acid were added, the tube was tightly closed and placed in the oven at a temperature of 110 °C for 24 hours, then it was cooled and filtered into a standard flask with a capacity of 25 ml, and 5 ml of the filtrate was taken in a beaker with a capacity of 100 ml, and dried in a glass desiccator under vacuum at The presence of concentrated sulfuric acid and potassium hydroxide. The results of dry hydrolysis were taken and dissolved in 2 ml of sodium citrate buffer solution, pH 2.2. The amino acids were determined, except for the amino acid tryptophan, by injecting the previously prepared hydrolysis product into a High-Performance Amino Acid Analyzer Beckman 7300 (Duranti and Cerletti, 1979).

3. Results and discussion

3.1. Physical tests of seeds

It is noteworthy that the natural properties of seeds vary according to the type and gender of the fruits and their moisture content. Table 1 shows the natural properties of bottled gourd seeds. In this study, the weight of 100 seeds was 26.86 grams, which is a close result to what was mentioned by Yetisir (2008) in a study on several types of *L.siceraria* fruits from several regions in Turkey, his results for the weight of 100 seeds ranged from 11.0 to 28.8 g, while the result recorded by (Buthelezi *et al.*, 2019) was far from it, as he stated that the average weight of 100 Seeds for his samples were 15.6 g, as shown in Table (1). The true density of bottle gourd seeds in this study was 0.905 g/ml, and this is close to what was recorded by Mohammed *et al.*, (2017), so the results of the true density that he estimated for the samples The genus (*L. cylindrica*), according to its moisture content, ranging from 0.98 to 1.27 g/cm³. The result of the bulk density of seeds, as shown in Table 1, was 0.263 g/cm³, which is less than the result reached by (Mohammed *et al.*, 2017) for the genus (*L. cylindrica*), which was 0.373 to 0.6053 g/cm. cm³ due to the increase in moisture content in its samples.

The results of the research showed that the percentage of cotyledons and husks was 44.26% and 55.74%, respectively, as the percentage of husks was higher than the percentage of cotyledons, and the percentage of seed weight was higher than what was found in the study of Mariod *et al.* (2015). Kalyanrao *et al.* (2016) mentioned that the weight of the husks and cotyledons is related to the period of seed maturity and harvest, and it is clear from Table 1 that the porosity of the seeds was 28.88%, on the other hand in Watermelon, the porosity percentage recorded by (Milani, and Razavi, 2006) was 39.143%. Koocheki *et al.* (2007) stated that the porosity of watermelon seeds ranges from 49.83 to 63.32%. The reason for the difference in the percentage of porosity and density of the seeds may be due to the roughness of the surface of the seeds or due to a decrease or increase in the percentage of moisture, which contributes to the change in the porosity and density of the seeds. The seeds wrinkle and are allowed to slide and roll over each other by forces of friction.

Table (1): Physical properties of seeds

Average weight of 100 seeds (g)	26.86 ±0.66
Seed volume (ml)	29.66 ±0.57
Cotyledon ratio (%)	44.26 ±2.10
Husk percentage (%)	55.74 ±2.09
Bulk density g/ml	0.263 ±0.01
True density g/cm ³	0.905 ±0.02
Porosity (%)	29.10 ±1.64

The values shown in the table represent the average of three replicates; ± standard deviation

The results of the study (Table 2) showed that the length of the whole seed was 17.17 ± 0.78 and its width was 9.7 ± 0.90 mm. As for the cotyledon, the length was 15.71 ± 0.44 and the width was 4.78 ± 0.77 mm. The results of this study were consistent with what was recorded by Buthelezi *et al.* (2019), where the average length of whole seeds was 17.6 mm, while the width of the seeds was 9.7 mm. These results were also close to what was mentioned by Pradhan (2014), where the length of whole seeds in the genus *L.siceraria* ranged from 11.20 to 17.40 mm, which is higher than what was stated in the study of the bitter melon by Ünal *et al.* (2013) in which it was stated that the length of the seed increased with increasing humidity from 12.05 to 15.77 mm, while its width increased from 6.34 to 9.21 mm, and in a study by Aydoğan-Coşkun *et al.* (2022) in a comparison between the length and width of the cotyledon in the genus *Citrullus lanatus* and *Lagenaria siceraria*, the length of the cotyledon was 8.87 and 9.05 mm respectively, while the width was 5.36 and 5.77 mm, respectively, and it was recorded (Sari *et al.*, 2021) the length of the cotyledon in *Lagenaria siceraria* ranged from 2.47 to 5.46 cm, while the width ranged from 1.5 cm to 3 cm.

Table (2): Length and width of whole seeds and cotyledon

Parameters	Whole seeds	Hulled seeds
Length (mm)	17.17 ^a ±0.78	15.71 ^b ±0.44
Width(mm)	09.70 ^a ±0.90	04.78 ^b ±0.77

Values shown in the table represent the mean of three replicates ± standard deviation; Values with the same letter in a row have no significant differences ($p \leq 0.05$)

3.2. Chemical composition

3.2.1. Moisture content

The results (Table 3) showed that the moisture percentage in the whole seeds was 3.4% ±0.20, which is the lowest moisture percentage ($p \leq 0.05$). As for the cotyledon, it was found that the moisture percentage was higher than the whole seeds, reaching 5.1 ±0.1 and 5.5 ±0.5% for non-defatted and defatted. These results were close to what was reported by Akusu *et al.* (2015) which stated that the moisture percentage in whole seeds was 3.81%, while the moisture percentage in the genus *Luffa cylindrica* was 5.84%. (Ogunyemi *et al.*, 2020). Dairo *et al.* (2007) reported that the moisture percentage in the cotyledon was 4.44%, and the difference in the moisture percentage may be attributed to the bottle gourd if it is in the maturity stage or Aging stage. Low moisture may also help preserve seed flour and cotyledon for a long time and is less susceptible to microbial contamination.

Table (3): Chemical composition of whole and hulled seeds

Component (%)	Whole seeds	Gotyledon	Defatted cotyledon
Total ash	4.21 ^b ±0.48	3.71 ^b ±0.12	7.55 ^a ±0.49
Crude fibre	15.33 ^a ±0.28	0.89 ^b ±0.07	1.00 ^b ±0.10
Protein	24.03 ^c ±0.19	26.43 ^b ±0.14	67.12 ^a ±0.44
Fat	38.11 ^b ±0.22	40.87 ^a ±0.49	0.83 ^c ±0.04
Carbohydrate	14.92 ^c ±0.17	23.09 ^a ±0.11	17.69 ^b ±0.22

Values shown in the table represent the mean of three replicates ± standard deviation;

Values with the same letter in a row have no significant differences ($p \leq 0.05$)

3.2.2. Total ash

As shown in Table 3, the percentage of total ash in the whole seeds was $4.21\% \pm 0.49$, and in the cotyledon it was $7.5\% \pm 0.50$, which is the highest ($p \leq 0.05$). The results of the whole seed ash converged with what was found by Ogundele *et al.* (2010) when he studied *L. Siceraria* seeds in which the percentage of ash was 4.07%, while the result of the cotyledon was close to what was mentioned by Anwar *et al.* (2011) in his samples of defatted seeds of the genus winter squash in which the percentage of total ash was 7.48%, and the percentage of ash in one of his samples reached 11.81%. Total ash is an indicator of mineral elements present in the sample.

3.2.3. Crude fibre

These results (Table 3) gave an indication and difference between the study samples of whole seeds and hulled seeds, where the percentage of crude fiber was higher ($p \leq 0.05$) in whole seeds $15.33\% \pm 0.28$, while its percentage in hulled seeds was $0.89\% \pm 0.07$ and $1\% \pm 0.1$ for defatted cotyledon. The percentage of fibers in the cotyledon was close to what was reported by Ogundele *et al.* (2010) in *L. Siceraria*, which was 1.10%. On the other hand, the results were different from what was shown by Akusu *et al.* (2015) in their study of *Citrullus vulgaris* seeds, in which the percentage of fiber was 3.83%. The percentage of fiber in whole seeds in this study was close to that of *Cucumeropsis manni* seeds in the study conducted by Ijarotimi *et al.* (2019), which was 13.07%. Seeds that contain a high percentage of crude fiber are useful when added to food products, as they work to reduce cholesterol in the blood, facilitate the exit of waste from the intestines, and reduce the incidence of many types of cancerous diseases. (Anhawange *et al.*, 2004).

3.2.4. Crude Protein

The results (Table 3) showed that crude protein constituted $24.03\% \pm 0.19$ of the whole seed and $26.43\% \pm 0.14$ of the defatted seed (cotyledon), while the results recorded $67.12\% \pm 0.44$ of the protein in the defatted cotyledon, which was highest percentage ($p \leq 0.05$), and in a study by Dairo *et al.* (2007), the protein percentage of Loofah Gourd seeds was close to this study, as it was 25.64%. Copeland (1976) mentioned that cotyledon contains a high percentage of protein and can be used as a nutritional supplement and can also be used when extracting protein and adding it as a fortifier to food products, adding a high

nutritional value to them, especially in poor countries whose food depends mainly on starches and also to supply the body with the required essential amino acids.

3.2.5. Fat

It is noted, as shown in Table 3, that the highest percentage of fat ($p \leq 0.05$) was in the cotyledon (the hulled seeds), where it reached $40.87\% \pm 0.49$, and $0.83\% \pm 0.04$ remained after removing the fat. On the other hand, the percentage of fat in whole seeds was $38.11\% \pm 0.22$. Ibeabuchi (2014) found that the percentage of fat in the cotyledon was 40.56% , which agrees with what was stated in this study. The seed oil can be utilized and used as a food ingredient or in other industrial products.

3.2.6. Carbohydrate

The result of carbohydrates was the difference in other chemical properties, where the lowest percentage of carbohydrates was in whole seeds, which was $14.92\% \pm 0.17$, while their percentage was highest ($p \leq 0.05$) in the cotyledon, where it reached $23.09\% \pm 0.11$, then in the defatted cotyledon, where it reached $17.69\% \pm 0.22$, and the result of the study about the whole seed sample was close to what was stated in the study of Ogunyemi *et al.* (2020), who stated that its percentage was 13.67% , and the percentage of carbohydrates in pumpkin seeds reached 25.19% (Gohari *et al.*, 2011), while in another study was 9.92% (Hassan *et al.*, 2008), and thus these seeds can be used as an energy source.

3.2.7. Mineral elements

The results (Table 4) showed that the highest percentage of mineral elements in seed flour was potassium, which reached $1397 \text{ mg}/100 \text{ g}$, followed by phosphorus, which reached $1,229 \text{ mg}/100 \text{ g}$, then sodium, $262.3 \text{ mg}/100 \text{ g}$, which was close to magnesium, which had a percentage of $260.1 \text{ mg.}/100 \text{ g}$ and the lowest percentages were for manganese and iron, which amounted to 12.1 and $6.9 \text{ mg}/100 \text{ g}$, respectively. The result was consistent with Ibeabuci (2014), where potassium was the most abundant element in *Lagenaria siceraria* seed flour, followed by phosphorus. The percentages differed in *Luffa cylindrica* which was studied by Ogunyemi *et al.* (2020), where the highest percentage in its seeds was for phosphorus, followed by magnesium. Mansour *et al.* (1993) indicated that the percentage of mineral elements is affected or varies due to some manufacturing treatments carried out on seed flour can reduce the percentage of these minerals.

Table (4): Mineral elements of seed flour based on dry weight

Mineral	mg/100g
Potassium	1397 \pm 4.11
Sodium	262.3 \pm 1.21
Calcium	113.4 \pm 1.03
Zinc	14.10 \pm 0.47
Phosphor	1229 \pm 1.66
Iron	6.9 \pm 0.11
Copper	14.6 \pm 0.42
Manganese	12.1 \pm 0.22
Magnesium	260.1 \pm 3.25

The values shown in the table represent the average of three replicates \pm standard deviation

3. Conclusions

Through this study, we conclude that peeled seeds contain high percentages of protein and fat and that the oil can be extracted from the seeds and then the protein is isolated. Accordingly, proteins can be used to fortify foods and increase their nutritional value, and whole seeds can be used to fortify foods with dietary fiber, such as biscuits and bread, as Fiber helps lower cholesterol and glucose in the blood. The percentage of carbohydrates is good, and therefore the study samples can be used as a means to increase the energy reserve of some foods.



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