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QUALITY EVALUATION OF NON-TRADITIONAL HALAWA TAHINIA

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ABSTRACT

In an attempt to develop the processing of the traditional sesame halawa tahinia, to increase the nutrient diversity, consumer preferences and to reduce the production costs, tahina and halawa tahinia were produced from the roasted seeds of sesame, soybean, peanut, and sunflower. The quality characteristics of the produced halawa tahinia were assigned by applying the sensory, physico-chemical, and microbiological analyses. Soybean halawa tahinia had the lowest overall acceptability (6.8^{b}), brilliant- yellowish color, the hardiest, the gummiest, and the chewiest texture**,** more resistant to the rancidity, oil separation, and microbial instability over the storage period of 6 months/25º C. Peanut halawa tahinia had overall acceptability of 7.6^{ab}, brilliant- yellowish color, the most significant adhesive texture (extremely sticky), and close similarity in chemical composition to sunflower and sesame halawa tahinia. Sunflower halawa tahinia showed no significant differences in overall acceptability (8.0^{a}) and texture with sesame halawa tahinia, and it had the darkest color, the highest significant peroxide values (0.046-26.5meq/kg oil), acid values (0.04-24.2mg/g oil), and microbial instability over the storage period of 6 months/25º C. All samples were free of pathogenic microorganisms. The soybean, peanut, and sunflower reduced the total purchasing cost of the raw materials by 83.6, 13.64, and 83.5 %, respectively. Results could be useful in developing halawa tahinia processing, however, the emulsifiers, ingredients, processing, and storage conditions should be justified, to prevent oil separation, rancidity, and microbial instability.

KEYWORDS:
Confectionary halawa, Sensory acceptability, physico-chemical properties, Storage stability

INTRODUCTION

Halawa tahinia (also called halva, halaweh, havah) is one of the oldest traditional confectioneries, which is very popular in the Middle East, Indian, Balkan, North African and Central East European countries [1]. It is a traditional dessert, consumed generally at breakfast, and at dinner and sometimes introduced to the students at school feeding programs, because it has a high dense nutrient (32 % oil, 47 % carbohydrates, 13 % proteins) and a pleasant nutty taste, besides it is available all over the year at a reasonable price [2-4].The basic ingredient in halawa tahinia is tahina (tehena, tehineh, tabena, tehineh), a paste made of the milled, husked and roasted white sesame seeds. Tahina is mainly composed of 57-65 % oil (rich in $C_{18:1}$ and $C_{18:2}$), 23-27 % protein (rich in sulfur amino acids, tryptophan), and some minerals, such as calcium, phosphorous, potassium, and magnesium [5-6].

Generally, in the manufacturing of halawa tahinia, tahina is mixed with natural nutritive sugars /sweeteners (glucose, sucrose, and fructose), and with soapwort viscous extract and citric acid or tartaric acid to obtain solid texture consistency. A wide variety of ingredients (cacao powder, chocolate, pistachio, peanut, almond, milk powder, dehydrated fruits) and flavorings (vanilla, rose water, and bergamot) could be added to this mixture to enhance its nutritional value and satisfy consumer preferences [6]. Infrequently, manufacturers add titanium dioxide (E171) and palm oil as whitening and emulsifying agents [7].

Halawa tahinia is an oil-based confection with a low-moisture content that subjected to economic and credibility losses caused by oil separation and microbial instability. In these concerns, the emulsifiers, packaging materials, storage, and transportation conditions should be recognized [5, 8].

As tahina is a paste made of roasted sesame seeds, it is typically has a homogenous smooth texture, similar to nut butter, so sesame seeds may be replaced by other nut seeds in tahina production. For example, in some Eastern European countries, sunflower halawa is made of sunflower tahina instead of sesame tahina [9].

The utilization of sunflower seeds in halawa tahinia manufacturing may be of great interest because it characterized by a balanced nutritional composition. Sunflower seeds are rich in lipids (46 – 55%), proteins (23 – 24%), carbohydrates (8 – 12%), and ash (3 – 4%). However, proper sunflower hybrids, roasting conditions, tahina granules, and storage techniques must be assessed to prevent oil

separation and rancidity of halawa produced from sunflower seeds [10].

The utilization of soybean products in the food industry has increased steadily over the past decades. Soybean offers many health benefits with nutritional significances as well as technological and functional properties [11]. There were investigations on fortification of spreads and cereals with soy flour with some beneficial nutrients and evaluated consumer preferences. Zahedi and Mazaheri-Tehrani [12] developed spreadable halawa fortified with soy flour. Mazaheri-Tehrani et al. [13] and Abd-Elsattar and Abdel-Haleem [11] carried out successful studies on substitution of peanut butter by soy flour/ soy nut up to 20 and 100%, respectively.

Peanuts are a highly concentrated source of protein (24 to 36%), oil (47 to 50%), and are abundantly used in the roasted form as a snack food by all age groups, but more so by the preschool and school-going children [13]. Peanuts have been developed into a variety of products like roasted peanuts, peanut butter, peanut oil, peanut paste, peanut sauce, peanut flour, peanut milk, peanut beverage, peanut snacks (salted and sweet bars) and peanut cheese analog [14]. E1-Shirbiny et al. [15] studied the process for the production of peanut butter and its possible uses in halawa tahinia making. They found that the texture of the peanut butter was too dry and unsuitable for mixing directly with the syrup used for halawa manufacture and the addition of vegetable oil was necessary to increase the oil content by about 58%. Also, Sumainah et al. [16] developed a new product from peanut (as a nutraceutical additive) via adding sesame paste with high antioxidative capacity.

Nowadays, the market of halawa tahinia is expanding broadly because of the ever-increasing consumption in the countries of origins, and in the importing countries (including the USA and some European countries), due to its high nutritive value and its health properties [4]. On the other hand, due to the importance of the cost of raw materials in the food industry, there is a need for the effective use of alternative raw materials that offer both the possibility of significantly lowering production costs and improving nutritive value [10]. Nevertheless, storage stability, as well as the sensory acceptability, must be fulfilled if halawa tahinia produced from alternative raw materials to accomplish its promise of becoming a cost-effective alternative to sesame seeds. Accordingly, this paper was carried out in an attempt to develop traditional sesame halawa tahinia via utilizing roasted seeds of soybean, peanut, and sunflower in the production of both tahina and halawa tahinia, then assessing the quality characteristics of the produced halawa tahinia by applying the sensory, physicochemical, and microbiological analyses.

MATERIALS AND METHODS

Materials. Sesame and peanut seeds, soapwort root, vanilla, vinegar, fine granulated cane sugar, and salt were obtained from the local market, Giza- Egypt. Soybean seeds were obtained from Soybean Factory, Food Technology Research Institute, Agricultural Research Center, Al-Giza- Egypt. Peeled sunflower seeds were obtained from El Ashraf Company, factory # 8, block 9, 4$^{th.}$ industrial zone, New Borg El Arab Alexandria, Egypt. The standard plate count (SPC) Agar medium was obtained from Conda Pronadisa, Spain. MacConkey Agar medium was obtained from Biolife, Milano, Italy. Yeast and mold agar medium was obtained from the Difco™ Co USA. Agar, Salmonella Shigella Agar and Mannitol Salt agar (MSA) were obtained from Hi Media, Mumbai, India. Glucose syrup with 83-84 % Brix and candy test of 145 C° was obtained from Al Monairy for Corn products Factory, 10$^{th.}$ of Ramadan City, 6A Industrial zone. Lot#110, Egypt. Soy lecithin with a melting point of 37ºC produced by AAK California Oils Corporation, Richmond, USA. All other chemicals used were of analytical reagent grade.

Processing of halwa tahinia. The flow process for the manufacturing of halwa tahinia samples is illustrated in Figure 1. Sesame, soybean, and peanut seeds were sorted and cleaned of impurities. Soybean seeds were soaked in acidified water (20 ml vinegar/ 100 ml water) for 12 h at 25°C. Then, the excess water was drained, and seeds were further rinsed with distilled water. All seeds were roasted separately, in a convection oven (Memamert, Cambridge, UK) at 130° C for 1h. For tahina preparation, the roasted seeds were milled separately in a laboratory scale colloidal mill, then transferred to glass jars. The soapwort liquid extract was obtained by filtration, after cooking of grounded soapwort root with water (1: 10) in a closed pot at 115 °C until the viscous liquid was formed. The caramel mass was formed by boiling granulated cane sugar, glucose syrup, and water in a proportion of (3: 1: 2). The temperature was observed during cooking using a digital Pen thermometer (WT-1, Elitech® International, USA). The citric acid (0.2 g/ 100g), the soy lecithin (0.2 g/ 100g), and the soapwort extract (3 g/ 100g) were added at 100º C, 117º C, and 120º C, respectively. The vanilla (1 g/ 100g) was added after the caramel mass was formed at 220º C, and 67 % Brix (RHBO-80 Gain Express Refractometer, USA). halwa tahinia samples were manufactured by kneading 50 g/ 100g caramel mass with 50 g/ 100g tahina in an upside-down movement using a pastry scraper, then molded in plastic containers (4cmx4cmx4cm) with straight bottoms, to avoid oil separation during storage, then samples were let to cool down, and finally stored at 4º C for

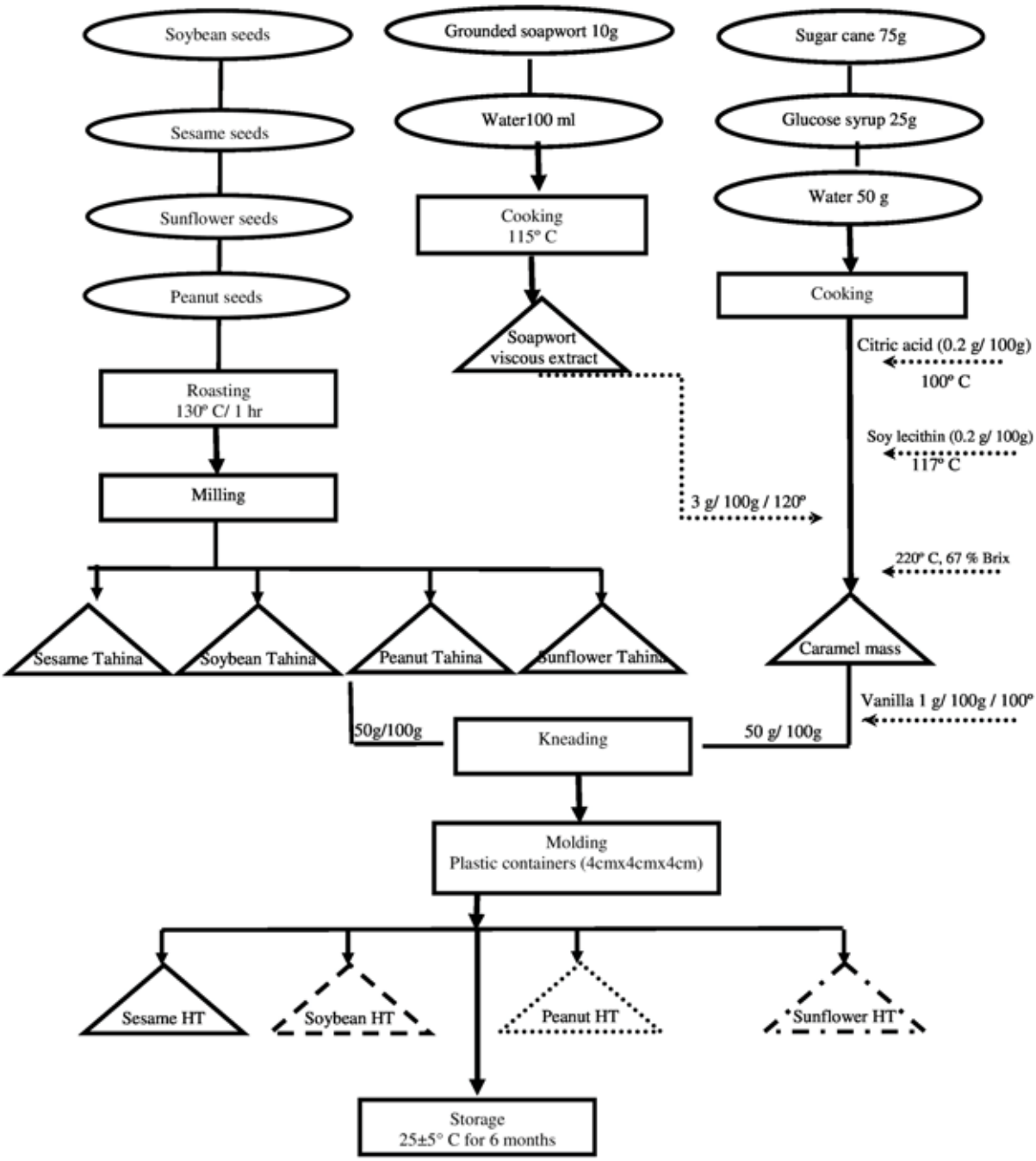

FIGURE 1
Flow process of halawa tahinia manufacturing

HT- halawa tahinia.

24 hrs to assure complete shaping and then at 25±5° C for 6 months.

Sensory evaluation of halawa tahinia. The sensory acceptability scores were performed on halawa tahinia samples using a 9-point hedonic scale according to Sanja et al. [1]. A panel of fifteen members from the Food Technology Research Institute, Agricultural Research Center, Al-Giza-Egypt. The panel was asked to judge the samples for color, odor, taste and melting, texture consistency, a puncture in cross-section, oiliness and the overall acceptability.

Proximate analysis. Moisture, protein, oil, crude fiber and ash contents of the tahina, and the produced halawa tahini were determined according to the methods of AOAC [17]. The nitrogen content was estimated by the micro Kjeldahl method, using a conversion factor of 6.25. The total carbohydrate content was calculated by subtracting the contents of crude protein, fat, ash, and moisture from 100 g of samples. The proximate compositions were averaged from three replicates. The results were expressed on a dry basis.

The energy of halawa tahinia. The energy of halawa tahinia samples was calculated according to the following equation:

$$Energy\ (Kcal) = [Fat\ x\ 9\ +\ Protein\ x\ 4\ +\ Total\ carbohydrate\ x\ 4] \quad (1)$$

Color measurement of halawa tahinia. The color of halawa tahinia samples was measured in triplicate using a colorimeter (CR-400, Konica Minolta Sensing Inc., Japan). The color values were recorded as L^* =lightness (0 = black, 100 = white), a^* ($-a^*$ = greenness, $+a^*$ = redness) and b^* ($-b^*$ = blueness, $+b^*$ = yellowness). The visual colors were assigned using computer software Hue Angle Program.

Textural profile analysis (TPA) of halawa tahinia. The textural profile of halawa tahinia samples was analyzed according to Bourne [18] sing Universal Testing Machine, Cometech, B type, Taiwan. Two cycles were applied at a constant velocity of 1 mm / s, 25 % depth, and 35mm compression. Firmness (N), cohesiveness, gumminess (N), chewiness (N), adhesiveness (Ns), and adhesiveness force (N) were calculated using the TPA forced- time curves. The texture measurement of each sample was averaged from five replicates.

Storage stability analyses. Peroxide value of halawa tahinia. The peroxide value of halawa tahinia was determined at intervals from zero time until 6 months, each of two weeks using technique Ja 8-87 of the official methods of the American Oil Chemists' Society AOCS [19]. One-gram oil extracted from halawa tahinia was dissolved in 10 ml of glacial acetic acid/chloroform and 0.2 ml of potassium iodide solution. Samples were stand in the dark for a minute and then titrated with 0.1N sodium thiosulphate solution using 20 ml of the starch indicator. The peroxide value was expressed as meq/ kg of halawa tahinia.

The acid value of halawa tahinia. The acid value of the halawa tahinia was determined at intervals from zero time until 6 months, each of two weeks using technique Ac 5-41of the official methods of the American Oil Chemists' Society AOCS [19]. One-gram oil extracted from the halawa tahinia was dissolved in 50 ml ethyl alcohol, and then the mixture was heated in a water bath at 80º C for 15min. The free fatty acids present in the oil of the halawa tahinia were neutralized with 0.1 N potassium hydroxide using one ml of phenolphthalein indicator solution. The acid value was expressed as mg/g oil of the halawa tahinia.

Oil separation of halawa tahinia. Fifty grams of the halawa tahinia were placed into a funnel that was covered with a glass watch. The oil separated was allowed to drop down from the funnel into a glass tube. The amount of oil separated (gm/100g oil) was periodically (monthly) weighed during the storage period of 6 months at room temperature of 25±5° C, and then calculated against the total oil present in the halawa tahinia.

Microbiological evaluation. The growth of the total bacteria, fecal Coliforms, yeast and mold, *Staphylococcus aureus* and Salmonella *spp.* was performed in halawa tahinia at intervals, from zero time and 1- 6 months according to the American Public Health Association [20] guidelines. A series of dilutions were performed from twenty- five grams of halawa tahinia for obtaining dilution factors 10^{-1}- 10^{-5} (each dilution plated in triplicate). Ten ml of tempered (45°C) Standard plate count Agar, MacConeky Agar, yeast and mold Agar, Mannitol Salt agar (MSA) and Salmonella Shigella Agar were added to Petri dishes containing the test dilutions. The plates were incubated at 37 °C/ 48 h for bacteria and at 25°C/ 5 days for yeast and mold. The colonies were counted by multiplying the average number of colonies per plate by the reciprocal of the dilution used as cfu/ml/g.

Statistical analysis. All data were analyzed using computer software CoStat 6.303, CoHort, USA, 1998–2004 for Windows; an analysis of variance (ANOVA) followed by Duncan's multiple range tests at $P \leq 0.05$ to compare between means.

RESULTS AND DISCUSSION

Sensory quality attributes of halawa tahinia. Table 1 displays the sensory acceptability scores of halawa tahinia (HT) samples. There were no significant differences ($P \leq 0.05$) in most sensory properties among HT samples, except for color, and oiliness properties. Sunflower seeds significantly ($P \leq 0.05$) decreased the color acceptability score, where the panelists tend to accept (*like very much*) lighter color HT than darker (*like slightly*) color HT [1]. Also, soybean and peanut seeds influenced ($P \leq 0.05$) the color acceptability of HT, where the panelists favored lighter than moderately color.

The soybean HT received the lowest significant ($P \leq 0.05$) scores (tended to *like moderately*) in odor, taste and melting, and OAA, compared with the sesame HT, and the other two HT samples. These results may be ascribed to the fact that the appearance properties of food products play an important role in consumer perception, and they may not change their habits easily, especially when traditional food is concerned [8].

TABLE 1
Sensory acceptability of halawa tahinia samples

Samples	Color (9)	Odor (9)	Taste & melting (9)	Texture consistency (9)	Puncture in cross section (9)	Oiliness (9)	OAA* (9)
Sesame HT	8.5±0.65^{a}	8.32±0.99^{a}	8.0± 0.7^{a}	8.0±0.49^{a}	8.3±0.95^{a}	6.6±0.6^{c}	8.2±0.63^{a}
Soybean HT	7.4±0.94^{b}	7.3±1.3^{b}	6.8± 1.3^{b}	7.96±0.84^{a}	8.1±0.95^{a}	4.3±0.8^{d}	6.8±1.2^{b}
Peanut HT	7.8± 0.94^{b}	8.0±0.75^{ab}	7.7±1.0^{a}	7.9±0.97^{a}	7.8±1.4^{a}	8.6±1.4^{a}	7.6±1.2^{ab}
Sunflower HT	6.0±1.2^{c}	8.0±0.94^{ab}	8.0±0.85^{a}	8.2±0.64^{a}	8.3 ±1.0^{a}	7.3±0.86^{b}	8.0±0.94^{a}

HT: halawa tahinia, *OAA: overall acceptability.
Data are presented as means ± SDM (n = 15, a 9-point hedonic scale: 1 (9= like extremely, 8= like very much, 7= like moderately, 6= like slightly, 5= neither like nor dislike, 4= dislike slightly, 3= dislike moderately, 2= dislike very much, 1= dislike extremely) & Means within a column with different letters are significantly different at $P \leq 0.05$.

TABLE 2
Chemical composition (g/100g) and energy (Kcal/100 g) of tahina and halawa tahinia samples

Samples	Moisture (g/100g)	Protein (g/100g)	Oil (g/100g)	Ash (g/100g)	Crude fiber (g/100g)	T.C* (g/100g)	Energy (Kcal/100 g)
Sesame tahina	1.4±0.1^{c}	17.23±0.74^{d}	55.89±1.6^{a}	3.84±0.51^{a}	5.99±0.45^{ab}	27.63±0.05^{a}	NC
Soybean tahina	2.96±0.1^{a}	43.44± 0.73^{a}	26.86±0.22^{d}	4.10±0.09^{a}	6.68±0.16^{a}	29.33±1.1^{a}	NC
Peanut tahina	2.24±0.4^{b}	22.82±1.6^{c}	51.73±0.4158^{b}	2.69±0.04^{b}	3.95±1.0^{c}	24.47±0.75^{b}	NC
Sunflower tahina	1.42±0.28^{c}	28.15±0.28^{b}	48.0±0.7^{c}	4.14±0.25^{a}	5.0 ±0.21^{b}	23.35±1.5^{b}	NC
Sesame HT	0.9±0.12^{c}	12.2±1.37^{c}	26.6± 0.93^{a}	1.59±0.09^{b}	3.2±0.36^{a}	61.9±1.34^{a}	536±6.8^{a}
Soybean HT	1.99±0.19^{b}	22.4± 0.49^{a}	12.1±0.24^{c}	1.9±0.07^{a}	2.9±0.22^{a}	64.5±0.67^{a}	456±1.9^{c}
Peanut HT	1.81±0.82^{b}	13.4±0.57^{c}	23.9± 1.3^{b}	1.4±0.08^{c}	1.4±0.21^{b}	60.89±1.6^{b}	512±4.3^{b}
Sunflower HT	2.23±0.13^{a}	15.3±1.3^{b}	22.9±0.70^{b}	1.9±0.07^{a}	1.7±0.17^{b}	59.37±1.09^{b}	505±3.5^{b}

HT: halawa tahinia, *TC: Total carbohydrates was calculated by difference, NC- not calculated.
Data are presented as means ± SDM (n=3) & Means within a column with different letters are significantly different at $P \leq 0.05$.

The peanut HT received the highest significant ($P \leq 0.05$) score in oiliness property followed by sunflower HT; implies that they had a noticeable amount of the separated oil, where oil separation is one of the most common problems in HT manufacturing, which may lead to a decrease in consumer acceptability [12].

Chemical composition of tahina, and halawa tahinia samples. Table 2 exhibits the chemical composition of tahina produced from different roasted seeds. Moisture, protein, oil, ash, crude fiber, and total carbohydrate contents were ranged from 1.4 to 2.96; 17.23 to 43.44; 26.86 to 55.89; 2.69 to 4.14; 3.95 to 6.68; and 23.35 to 29.33 g/100g, respectively. These results are comparable with those obtained by El-Adawy and Mansour [21] for sesame tahina, and with Boriy [22] for sunflower tahina. Significant differences were observed in protein and oil contents, being the highest ($P \leq 0.05$) in soybean tahina and sesame tahina, respectively. However, peanut tahina has the lowest significant ($P \leq 0.05$) amounts of protein, ash, and crude fiber.

Table 2 shows the chemical composition (g/100g) and the energy (Kcal/100 g) of halawa tahinia samples. The sesame HT contained 0.9 g/100g moisture, 12.2 g/100g protein, 26.6 g/100g oil, 1.59 g/100g ash 61.9 g/100g total carbohydrate, and 536 Kcal/100 g energy. These values are within the satisfactory quality limits of sesame halawa sets by The Egyptian Standard (ES: No. 384, 992, 1332/2005) [23] and the Regional Standard of Codex Alimentarius Commission (CODEX STAN CXS 309 R-2011) [24], where the percentage of fat (sesame oil) shall not be inferior to 25% of the weight and ash shall not exceed 2.5% of dry weight, moisture at most 2.5%, sugar at least 45 %, protein at least 8.5%.

Soybean HT has the lowest significant ($P \leq 0.05$) contents of oil and energy. This result is due to lower potential contents of oil in its tahina. On the other hand, soybean HT has the highest significant ($P \leq 0.05$) protein content, and significant ash, crude fiber, and total carbohydrate contents.

There were no significant differences ($P \leq 0.05$) between peanut HT and sunflower HT in oil, crude fiber, total carbohydrate, and energy contents, and they showed close similarity to that of sesame HT, rather than that of soybean HT.

Color of halawa tahinia. The color is an important factor in the quality evaluation of halawa tahinia, where it refers to the freshness or the staleness of the product. It depends on various factors, including the cultivars of kernel used, concentrations of tahina and caramel mass, and other additives [7]. Table 3 represents the color values of halawa tahinia samples. The results showed that soybean HT, peanut HT, and sunflower HT were significantly different ($P \leq 0.05$) in color values compared to those of sesame HT. The lightness (L) values of the halawa tahinia samples ranged from 67.37 to 49.33, while redness (a) and yellowness (b) values ranged between 0.84–5.531 and 19.6–30.54, respectively. These values are close to those obtained by Öğütcü et al. [8] who found L, a, and b

TABLE 3
Color and texture of halawa tahinia samples

Samples	*L*	*a*	*b*	visual color	Firmness (N)
Sesame HT	67.37±0.06^{a}	0.84±0.02^{c}	19.6±1.297^{c}	Light Yellow	17.11±0.08^{c}
Soybean HT	56.77±0.53^{b}	5.3±0.26^{b}	30.54±0.56^{a}	Brilliant-Yellow	50.6±0.04^{a}
Peanut HT	58.67±0.47^{b}	5.28±0.07^{b}	30.38±0.3^{a}	Brilliant-Yellow	33.59±0.02^{b}
Sunflower HT	49.33±0.46^{c}	5.52±0.0^{a}	23.38±0.5^{b}	Vivid-Orange Yellow	12.8±0.05^{cd}

Samples	Gumminess (N)	Chewiness (N)	Adhesiveness (NS^{-1})	Adhesiveness force (N)
Sesame HT	8.82±0.03^{c}	5.7±0.05^{c}	11.17±0.02^{b}	4.9±0.01^{b}
Soybean HT	25.87±0.14^{a}	19.5±0.37^{a}	6.2±0.30^{c}	5.79±0.05^{b}
Peanut HT	18.62±0.10^{b}	13.72±0.04^{b}	28.52±0.09^{a}	9.32±0.12^{a}
Sunflower HT	8.1±0.20^{c}	5.52±0.56^{c}	12.68±0.01^{b}	5.05±0.07^{b}

HT: halawa tahinia.
*L** = lightness (zero = black, 100 = white), *a** (- *a** = greenness, + *a** = redness) and *b** (- *b** = blueness, + *b** = yellowness).
Data are presented as means ± SDM (n=5) & Means within a column with different letters are significantly different at $P \leq 0.05$.

values of sesame halawa tahinia were 75.23, 0.95 and 19.67, respectively.

Our findings indicated that using sesame in the manufacturing of HT produces the lighter-yellowish product while sunflower gives the darker or vivid- orange yellowish product [1, 25]. This is attributed to the chlorogenic acid present in sunflower seeds produce a greenish-grey color [2]. On the other hand, the soybean and the peanut give brilliant- yellowish products. The results of color measurements are coherent with those obtained from the sensory color scores in Table 1.

Texture profile of halawa tahinia. The texture of halawa tahinia highlights quality improvement opportunities by studying the mechanical and the physical properties of the final products as affected by alternative ingredients [26]. Table 3 presents the texture profile properties of different HT samples. There were statistical differences ($P \leq 0.05$) between soybean HT, peanut HT, and the sesame HT. However, sunflower HT underwent the same patterns of the sesame HT in texture profile properties.

The soybean HT was significantly ($P \leq 0.05$) the hardiest, the gummiest, and the chewiest one, followed by peanut HT. In a study conducted by Abd-Elsattar and Abdel-Haleem [11], the hardening effect of soy nut butter was more than commercial peanut butter. The effect of hardness is attributed to the lowest oil content of soybean HT (Table 1), where oil in foods provide smooth and homogeneous structure [27], and to the hardening effect of soy proteins on dimensional structure stability of HT [12]. The gumminess and the chewiness values of the HT samples followed a similar trend that of hardness, where both of them are parameters dependant on hardness calculation.

Peanut HT was the most significant ($P \leq 0.05$) adhesive HT, meaning that the amount of force needed to pull the compressing plunger away from the peanut HT was significantly ($P \leq 0.05$) higher than those needed for sesame HT and the other HT samples.

Perfect halawa tahinia should have a smooth and homogeneous surface, consistent texture slightly sticky and easy to cut without crumbling [25]. Our results indicated that soybean seeds produced more hardened, gummy, and chewy HT (may be its much better to add extra soybean oil to the soybean tahina to prevent the hardening effect of the soy proteins and to provide smooth and homogenous texture to soybean). Peanut seeds produced extremely sticky HT. Both hardness and stickiness are prevalent problems in food processing operations that cause reduced product recovery and operational problems [28]. For these reasons, formulations, additives, and instrumental optimizations are needed to improve the texture quality of the soybean HT, and the peanut HT.

Chemical and microbiological storage stabilities of halawa tahinia. Peroxide value (PV) and acid value (AV) of halawa tahinia samples. The peroxide value (PV) is the most widely measure that used as an indication of the early stages of oil oxidation and to assess the efficiency of the storage conditions [6]. On the contrary, there is a synergistic relationship between the PV and the acid value (AV). The AV shows the information on the freshness of oil-based food, and it increases due to the improper storage conditions [27]. Figure 2 (a and b) depicts the trends in the peroxide value (PV, meq/ kg oil) and the acid value (AV, mg/g oil) of the halawa tahinia samples during the storage period of six months at 25±5° C. The results indicated that the sesame HT and the soybean HT had the lowest significant ($P \leq 0.05$) PV and AV, which were more resistant to the rancidity. This is due to the antioxidant effect of sesamol, sesaminol, and tocopherols compounds of sesame oil [6] and due to the selective and the synergistic antioxidant effect of the added soy lecithin with only the γ- and δ-forms of tocopherols in soybean oil [29]. However, the sunflower HT had the highest significant PV

and AV than those of the other halawa tahinia samples. The PV and AV of sunflower HT increased on prolonged storage, reaching the maximum values after 14 and 16 weeks, respectively. Similar observations were obtained by Damir [30] and Damir and Abdel-Nabey [2], who compared and evaluated the storage stability of the sesame HT versus the sunflower HT. The authors found that the PV was higher in sunflower HT than that in sesame HT during a storage period of 90 days at 25º C. This might be due to that sunflower oil is rich in unsaturated fatty acids (UFA) that make it more susceptible to oxidation. It is well-known that the greater number of the double bonds contained in UFA, the more it is susceptible to oxidative deterioration [31], besides, its higher water content (Table 1); where the exits of moisture progress the oil oxidation [5].

The PV and the AV of peanut HT followed the same trend that of the sunflower HT, but with lesser extent. The fatty acid composition of peanut oil is majorly palmitic acid and oleic acid that might be the reason for the lower sensitivity of peanut HT to oxidation than sunflower HT [32].

At the end of the storage period, the PV and the AV of peanut HT and sunflower HT were (1.9, 1.6) and (2.39, 1.8) folds, respectively, than that of sesame HT. Besides, the PV of sesame HT reached 11.05 meq/ kg after 16 weeks of the storage period at 25±5° C, whilst that of the sunflower HT reached 14.25 meq/ kg and underwent the onset of rancidity just after 14 weeks of storage at 25±5° C. The CODEX STAN CXS 309 R-2011 [24] stipulated that the peroxide value and the acidity for extracted oils from the halwa tahinia shall not exceed 10 meq oxide /kg and 2%, respectively.

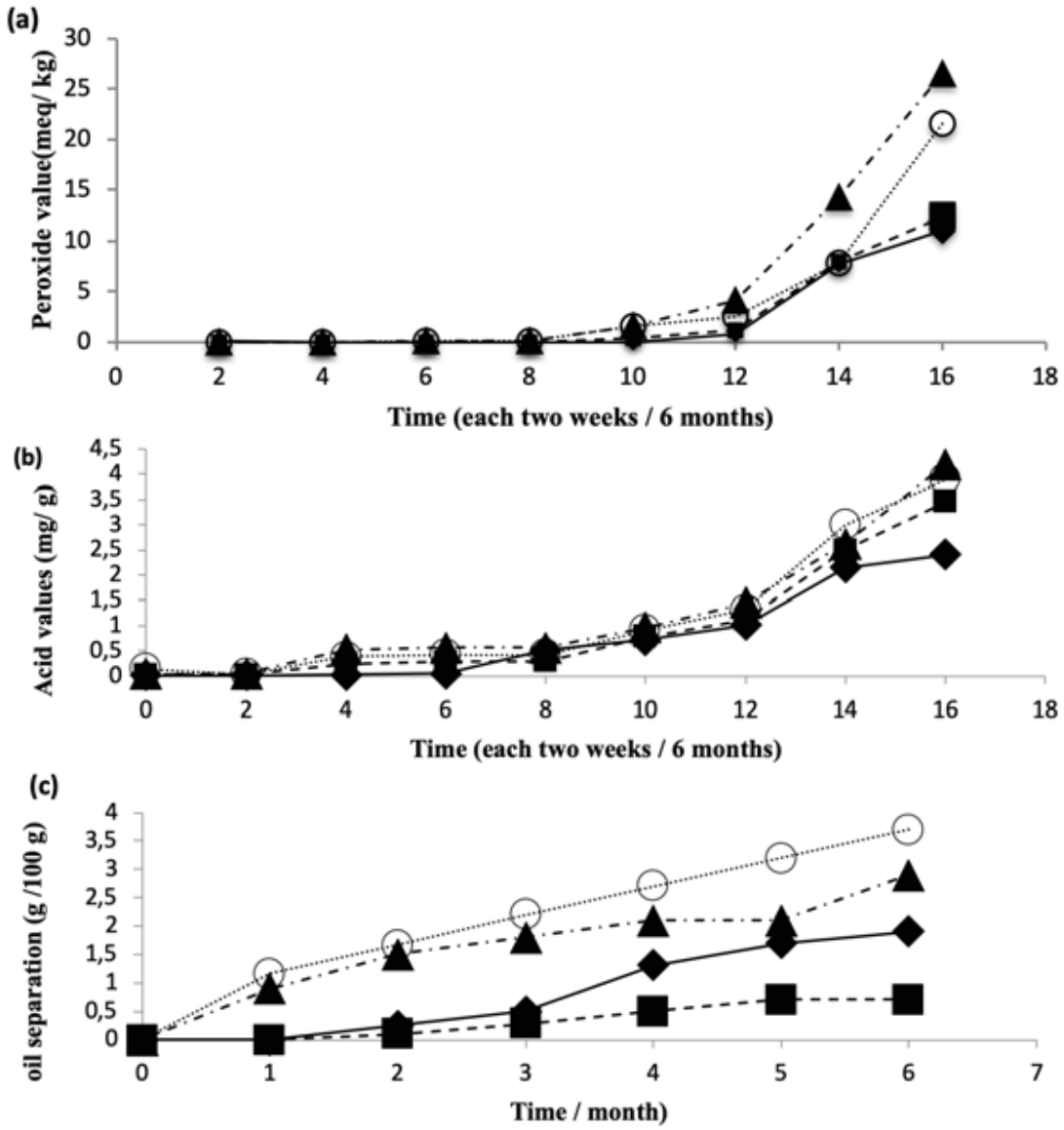

FIGURE 2

Chemical storage stability of halawa tahinia samples stored for six months

Sesame HT- (—♦—), Soybean HT- (---■---), Peanut HT- (···○···), sunflower HT- (-·-▲-·-). (a) Peroxide value (meq/ kg), (b) Acid values (mg/ g), (c) Oil separation (g/ 100 g), HT-halawa tahinia

Oil separation of halawa tahinia samples. Figure 2 (c) depicts the amount of separated oil (g/ 100 gm) from the halawa tahinia samples during the storage period of six months at 25±5° C. The results indicated that the soybean HT had the lowest significant ($P \leq 0.05$) amount of separated oil over the storage period of six months at 25±5° C. This result is due to its lower oil content (Table 2). However, peanut halawa tahinia showed the highest significant ($P \leq 0.05$) amount of separated oil, followed by sunflower halawa tahinia, where oil separation reached 3.7 g/ 100g for peanut HT and 2.9 g/ 100g for sunflower HT at the end of the storage period at 25±5° C.

The oil separation problem mainly occurs in halawa tahinia because it has melted sugar particles surrounded by a protein layer, and the sesame oil physically fills the spaces between the sugar and the protein in a non-emulsified form, which is easy to exudates from the inner surface to the outer surface of the product. In consequences, changes in color, texture, and sensory properties takes place and the packaging container contaminates with the exuded oil and the marketability is reduced [25]. Oil separation cannot be effectively terminated, but it can be delayed with proper emulsifiers, additives, and fibers [33].

In the present study soy lecithin of 0.2 % was added to the caramel mass for delaying this phenomenon, nevertheless, it seems only efficient with sesame HT and soybean HT, and not effective with peanut HT and sunflower HT. This effect may be due to the synergistic of the soy lecithin with only the γ- and δ- forms of tocopherols in soybean oil [29], besides lecithin is a mixture of phospholipids composed mainly of phosphatidylcholine with hydrophilic character [3] that failed to effectively restrict the oil separation. On the other hand, the fatty acid composition of peanut oil is majorly palmitic acid and oleic acid [32] that unhydrogenated palm oil, sorbitan monopalmitate, sorbitan monooleate, and sorbitan tristearate might be more effective than soy lecithin [25]. For the sunflower HT, Damir [30] and Damir and Abdel-Naby [2] found that glycerol monostearate (1%) had a pronounced stabilizing action in comparison with the untreated halawa. However, Boriy [22] found that there was no oil separation at 4º C, and the amount of oil separated reduced from 6 to 5% at 35 º C when lecithin of 0.1% was added. Furthermore, Mureşan et al. [33] established that 3% of palm oil was more favorable than 1, 2, 4 and 5% in sunflower halawa tahinia stability and texture.

Microbiological evaluations of halawa tahinia. Figure 3 (a) depicts the aerobic plate count (APC, log CFU/ g) of halawa tahinia samples stored at 25±5º C for 6 months. The Sunflower HT recorded the highest estimated APC (3.6×10^2 to 5.2×10^2). This is maybe due to its higher water content (Table 2) which might correlate to water activity (a_w) that helps the microorganisms to grow and affects the shelf life [5]. In addition to its higher oil separation (Fig. 3), that causes the growth of microorganisms [6]. Soybean HT and peanut HT underwent the same APC patterns for sunflower HT, but with very little extent, ranged from 0.13×10^2 to1.7×10^2, and from 1×10^2 to 2.9×10^2 CFU/ g, respectively. On the other hand, the APC of sesame HT was increased during the first two months and then decreased gradually during the storage period of the 6 months at 25±5° C. This result is comparable with those obtained by Eissa and Zohair [5] who found that the APC of sesame halawa tahinia stored at 25º C did not increase significantly during the storage period of 6 months, and is considerably lower than those APC reported by Kahraman et al. [6], which averaged 4.3×10^4 CFU/g.

The ES: No. 384, 992, 1332 [23], and ODEX STAN CXS 309 R-2011 [24], sets a satisfactory limit of the APC not to exceed than 1×10^5 CFU/g, indicating that all HT samples were within the acceptable limits.

Figure 3 (b) displays the growth rate for yeasts/molds (log CFU/ g) of halawa tahinia samples stored at 25± 5º C for 6 months. Data showed that the growth rate for yeasts/ molds followed the same pattern that of APC, and ranged from 0.15×10^2 to 2.75×10^2 CFU/g, being the highest in sunflower HT, with an average growth of 2×10^2 CFU/g. Our findings showed similarities to the results of Sengun et al. [34], however, in another study conducted by Eissa and Zohair [5], higher yeasts/ molds growth ranged from 30×10^3 to 1.3×10^3 CFU/g of were detected. Based on the ES No. 384, 992, 1332 [23] and the CODEX STAN CXS 309 R-2011 standard [24], it is seen that the yeasts/ molds growth levels were lower than the permitted limit (1×10^4 CFU/g).

The yeasts that grew in oily and sugar-dense confectioners are called the osmotolerant yeast. Consideration should be given to control these osmotolerant yeasts during the processing of halawa tahinia by limiting the water activity (a_w) to 0.60 [35].

In this study, the results showed that all halawa tahinia samples were free of *Salmonella spp*. and *Staph. aureus*, as well as, *Coliform* group, indicates the good hygienic conditions during preparing, manufacturing, and storage of halawa tahinia samples. This is also due to the higher temperature of the roasting process, and the caramel mass formation. Similarly, no pathogenic bacteria (*Salmonella spp*. and *Staph. aureus*) detection was reported by Eissa and Zohair [5]. In contrast, Kahraman et al. [6] detected 3×10^1, 28 and < 3 CFU/g of *Staph. aureus*, *Coliform, and E. coli*, respectively in halawa tahinia samples collected

from different retail markets and producers in Turkey.

A lot of notifications have been received at Food and Feed Safety Alerts Portal- RASFF [36] due to the presence of *Salmonella* in halawa tahinia; from
Syria (28/01/2020), the Czech Republic (20/12/2018), Lebanon (02/04/2013), Saudi Arabia (13/05/2009), Turkey (30/03/2007), and Egypt (23/12/2003). In addition to the outbreaks associated with the consumption of halawa tahinia contaminated with *Salmonella spp., Escherichia coli O157: H7 and Listeria monocytogenes,* particularly, Salmonellosis (DT104) with 27 cases in Sweden and 14 cases in Australia [4, 34] Therefore, it is of utmost importance to implement the good manufacturing practice (GMP) and the hazards analysis (HACCP) based on the risks assessment program to assure the safety of the halawa tahinia processing chain [37].

Cost benefits. The volume of halwa tahinia production in Egypt is around 22,800 tons in 2017, which accounted for 34% of total sugar confectionery sales [38]. From the economic point of view, sesame is in short supply; to satisfy the increasing demands, Egypt imported 26362 tons of sesame seeds, costing about 46.133 million US$. The price of soybean, peanut, and sunflower seeds were 361, 1900, and 363.75 US$/ ton, respectively compared with 2200 US$/ ton for sesame seeds according to FAOSTAT [39], which may offer a reduction of 83.6, 13.64, and 83.5 % of total purchasing cost of raw materials, respectively.

CONCLUSION

In an attempt to develop the processing of the traditional sesame halawa tahinia, to increase the nutrient diversity, consumer preferences and to reduce the production costs, tahina and halawa tahinia were produced from the roasted kernels of sesame, soybean, peanut, and sunflower. Soybean HT had moderate satisfactory acceptability, shiny yellowish color and extended chemical and microbiological stability. However, extra soybean oil may be added to the soybean tahina to prevent the hardening effect of the soy proteins and to provide smooth and homogenous texture to soybean HT. Peanut HT and sunflower HT they might, however, be highly liked by the panelists, had proximate composition comparable with that of sesame HT and reduced the purchasing costs, but whitening agents, emulsifiers, acidity regulators and storage conditions should be perfected to enhance the color of the sunflower HT and the texture of the peanut HT, to prevent oil separation, and to extend their chemical and microbiological stability.

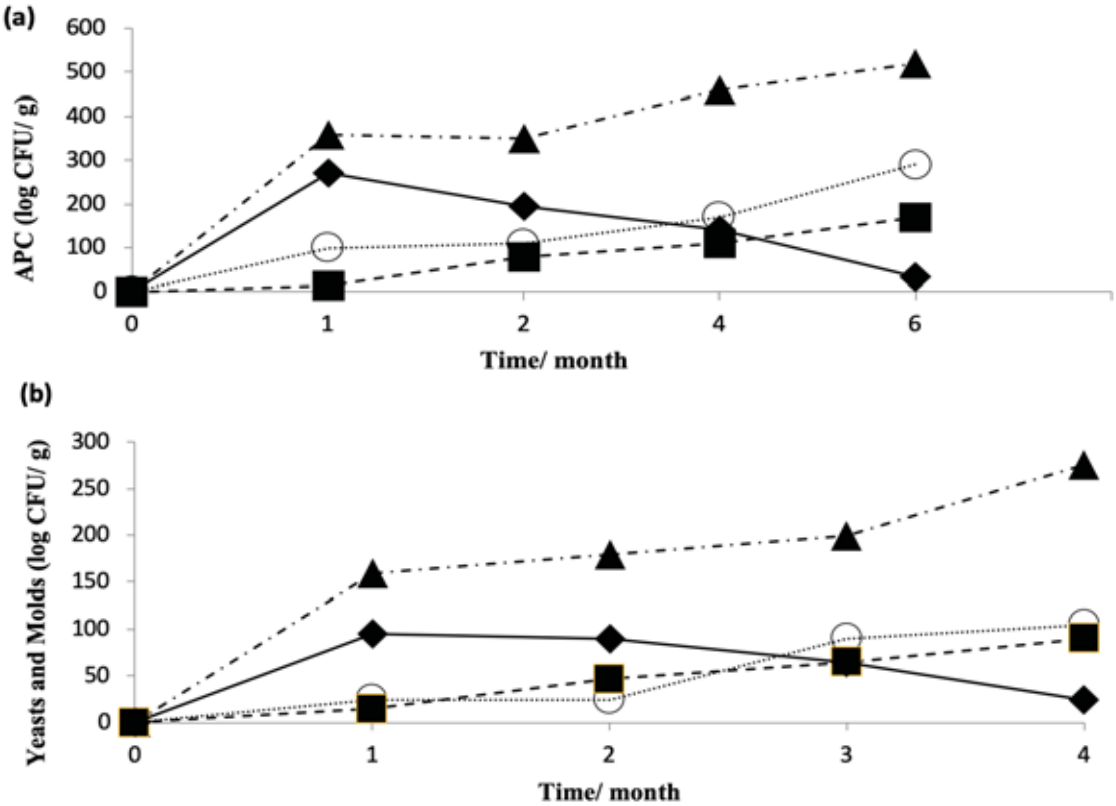

FIGURE 3

Microbiological stability of halawa tahinia samples stored for six months

Sesame HT- (—♦—), Soybean HT- (---■---), Peanut HT- (···○···), sunflower HT- (-·-▲-·-). HT-halawa tahinia
(a) APC- Aerobic Plate Count (log CFU/g), **(b)** Yeasts and Molds (log CFU/ g)

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BIOPHYSICAL PROPERTIES OF PERSIAN LIME (*CITRUS LATIFOLIA*) SEEDS AS A NEW OIL AND PROTEIN SOURCE AT DIFFERENT MOISTURE CONTENT

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ABSTRACT

In order to oil and protein production, biophysical properties of Persian lime seeds were studied at different moisture levels for designing and development of equipment for further processing. Seeds contained 44.18% oil, 20.56% protein, 32.15% carbohydrate and 2.15% ash (all dry basis). By increasing in moisture content, Bulk density, true density, angle of repose and thousand seed mass were increased but porosity decreased (p<0.05). The type of slipping surface significantly (p<0.05) affected static coefficients of friction. Static coefficients of friction increased with an increase in moisture content, and at all moisture content, the highest and the lowest value belonged to rubber surface and the glass sheet, respectively. Moisture content had not any significant effect on the sphericity and aspect ratio of seeds (p>0.05). Persian lime seed could not be regarded as sphere and would not roll on flat surfaces. Moisture dependence of the tested properties is described using regression equations, as well.

KEYWORDS:
Biophysical Property, Persian lime Seed, Density, Coefficient of Friction, moisture content

INTRODUCTION

Persian lime (*Citrus latifolia,* also known as Tahiti lime or Bearss lime), is a medium-sized, nearly thornless tree in the *Rutaceae* family that produces the most commonly sold lime fruit. The Persian lime is of hybrid origin (*C. aurantiifolia* × *C. limon).*

During the processing of lime fruits for juice or other purposes, valuable by-products such as peels, seeds, and pulp are produced. These materials are approximately 50% of the fresh fruit weight, and therefore, could be valorized. Citrus seeds have been recognized as an important source of vegetable oils and proteins. It contains 34 to 43% oil (dry basis), which can be of great importance especially for countries with insufficient domestic edible oil production such as Iran [1, 2, 3]. Accordingly, some researchers have investigated various citrus seeds oil properties [1, 2]. Juhaimi et al. [4] analyzed seed oils of some citrus genus from Turkey and Saudi Arabia and reported that palmitic (19.6- 26.2%), oleic (21.3-31.4%) and linoleic (32.3- 43.7%) acids were the main fatty acids. The protein fraction of the citrus seeds, on the other hand, is also of great importance. Yilmaz and Güneşer [1] repoted 19.41% (dry basis) protein for *Citrus limon* L. (Kütdiken variety). Citron, orange and mandarin seeds have protein content in the range of 15.9-19.9% (dry basis). Citrus seeds protein showed interesting water and fat absorption capacities, gelation, foaming and emulsification properties, as well. Generally, amino acids profile of citrus seed is excellent, particularly from the point of view of sulfur containing and total essential amino acids [3].

Considering the potential of citrus seed for oil and protein/hydrolyzate production, elementary information about biophysical characteristics of the seed are needed for further processing. Food scientists and engineers, processors, plant breeders and other scientists can use such basic information to find new uses [5]. Designing of various separating, handling, storing and drying equipment requires data about main biophysical characteristics such as shape, size, volume, surface area, thousand seed mass, density, porosity, angle of repose, static coefficient of friction.

Approximately 98% of edible oil consumed in Iran is imported in the form of crude oil and oil-bearing seeds. Therefore, agricultural by-products, including citrus seed, are suitable cases for providing little part of this deficiency and need. Iran is one of the propounded countries in the production of citrus and it is usually found among the top 10 countries of the main citrus producers. According to the FAO [6] the annual lemon and lime production in 2014 was 13172.3 thousand tons in the world. In the same year, Iran produced 1024.0 thousand tons of lemon and lime and was the fifth biggest produc-

er in the world. At present, there is no report about the biophysical properties of any of the citrus seed. The objective of this research was to determine the biophysical properties of Persian lime (*Citrus Latifolia*) seeds in the moisture range of 7.5 – 45% (wet basis) in order to designing and development of cleaning, grading, drying, separating, storage, handling and oil and protein extraction equipment.

MATERIALS AND METHODS

Sample preparation. Persian lime seeds were procured from a lime juice extraction plant (Asiashoor Food Industry and Trade Inc., Tabriz, Iran) as a waste composed of a mixture of peels, seeds, and pulp. The waste which contained about 8-10% seed was first poured in water and agitated. The seeds deposited because of higher density and then separated. After separation of seeds, they were rinsed and dried by direct solar drying for 48 hours. The seeds contained 41.65 ± 0.26 and 4.66 ± 0.17% (wet basis) moisture before and after drying, respectively. The seeds were then packed in black nylon bags and stored in 4 ºC.

To achieve the desired moisture content (7.5, 12.5, 17.5, 22.5 and 45% (wet basis), distilled water was added to the seeds. The required distilled water for preparation of samples with the desired moisture content was calculated using the following equation [7]:

$$Q = \frac{W_i(M_f - M_i)}{100 - M_f} \quad (1)$$

Where, W_i = initial mass of sample in Kg, M_i = initial moisture content (% dry basis) of sample, M_f = final moisture content (% dry basis) of sample. After addition of the required water content, seeds were conditioned by packing in polyethylene bags and storing at 4 °C in a refrigerator for 48 h.

Proximate composition. Moisture, protein, ash, fat and total carbohydrate content of seeds were analysed according to methods of Ca 2d-25, Ba 4d-90, Ba 5a-49, Am 2-93 [8] and Herbert et al. [9], respectively.

For kernel and shell determination, 50 gr of seeds weighted and deshelled manually. Kernel and shell percentage calculated as follow:

$$h = (w_h / w_s) \times 100 \quad (2)$$

$$k = (w_k / w_s) \times 100 \quad (3)$$

where h = shell percent, K = kernel percent, W_h = mass of shell (gr), W_k = mass of kernel (gr) and W_s = initial mass of seed (gr).

Dimensions, surface, volume and 1000 seed mass. The geometrical dimensions namely length (L), width (W) and thickness (T) of *Citrus limon* seeds were measured with a Verrnier caliper (Mitutoyo, Japan) with an accuracy of ± 0.01 mm. The arithmetic mean diameter (Da), geometric mean diameter (Dg) and sphericity (Φ) of seeds were calculated from the geometrical dimensions as described by Mohsenin [5]:

$$D_g = (L \times W \times T)^{\frac{1}{3}} \quad (4)$$

$$D_a = \frac{L+W+T}{3} \quad (5)$$

$$\Phi = \frac{(L \times W \times T)^{\frac{1}{3}}}{L} \quad (6)$$

The aspect ratio (which relates the width to length of the seed, indicative of tendency toward oblong shape) was calculated as Altuntaş et al. [10]:

$$R_a = \frac{W}{L} \times 100 \quad (7)$$

Surface (S) and volume (V) was calculated using the following equation [11]:

$$S = \frac{\pi \times B \times L^2}{2L - B} \quad (8)$$

$$\text{where } B = (W \times T)^{\frac{1}{2}} \quad (9)$$

$$V = 0.25[(\frac{\pi}{6})L(W + T)^2] \quad (10)$$

The 1000 seed mass was determined by random selection of samples containing 100 seeds and weighing using an electronic balance with a sensitivity of 0.001 g. The weight was then converted into 1000 seed mass (M_{1000}).

Bulk density, true density and porosity. Bulk density (ρ_b) of seeds was determined by filling a cylindrical container of 500 ml volume with the seeds at a constant rate and the height of 15 cm and then weighing the contents without any manual compaction of seeds [7]. The mass of the seeds and the volume of the container were used for calculation of the bulk density as follow:

$$\rho_b = \frac{m}{V} \quad (11)$$

where, m = mass (g) of seeds and V = cylindrical container volume (mm^3).

True density (ρ_s) was determined using Toluene displacement method [12]. 500 milliliters of toluene (C_7H_8) were placed in a 1000 ml graduated measuring cylinder. Then 50 g seeds were immersed in toluene. The amount of displacement in toluene was recorded quickly. Since the toluene is absorbed to a lesser extent by the seeds and also it fills even shallow dips in the seeds because of its low dissolution power and surface tension, it was used instead of water [5].

Porosity (ε) was calculated using the following relationship [5].

$$\varepsilon = (1 - \frac{\rho_b}{\rho_s}) \times 100 \quad (12)$$

Coefficient of static friction and repose angle of emptying and filling. Coefficient of static friction (μ_s) of seeds was determined against surfaces of steel, galvanized iron, plywood, glass and rubber at different moisture contents. A wooden topless and bottomless box of 100 × 100 × 40 was

TABLE 1
Proximate composition of dried Persian lime (*Citrus Latifolia*) seeds (% dry basis).

Moisture	Protein	Oil	Carbohydrate	Ash
4.66 ± 0.17	20.56 ± 1.07	44.18 ± 1.64	32.15 ± 0.14	2.15 ± 0.05

Note: Values given are the means of three replicates ± standard deviation.

filled with the seeds and placed on a changeable inclined plate, faced with the test surface. To prevent contact of the box with sloping surface it was lifted slightly (5–10 mm). The inclination angle of the test surface was increased gradually with a screw device until the box just started to slide down and the angle of slope (α) was read from a graduated scale. For each replication, the sample in the box was evacuated and refilled with a new sample of seeds [13]. The coefficient of static friction was calculated using the following relationship:

$$\mu_s = \tan\alpha \quad (13)$$

In order to obtain the repose angle of emptying, the seeds were filled in a 15×15×15 cm handmade wooden box with a sliding side door. The angle of repose was then calculated by measurement of the depths of the free surfaces (h_1 and h_2) of the seeds at two known horizontal distances (x_1 and x_2) from one end of the box. The angle of repose of emptying (θ_e) was obtained using the following equation [14].

$$\theta_e = \text{Arctan}\frac{(h_2 - h_1)}{(x_2 - x_1)} \quad (14)$$

To obtain the angle of repose of filling (θ_f), seeds were poured from 15 cm height on a wooden horizontal surface. The height of seeds stack above the floor (H) and the diameter of the heap (D) were measured and used to determine the angle of repose for filling with the following relationship [15]:

$$\theta_f = \text{Arc tan}\frac{(2h)}{D} \quad (15)$$

Data analysis. Analysis of variance (ANOVA) method and Duncan's means comparing test at 5% significance level was used to data analysis using IBM SPSS Statistics software (Ver. 24, Armonk, New York, USA). The relationship between seeds properties and moisture content was determined by linear regression analysis using Excel (Microsoft Office 2016, Redmond, Washington, USA).

RESULTS AND DISCUSSION

Chemical composition. Proximate composition of Persian lime (*Citrus Latifolia*) seeds is shown in Table 1. Persian lime seed was composed of 72.39% ± 0.01 kernel and 27.61% ± 0.01 shell. Oil was the main constitute (almost close to half) of Persian lime seed and it constituted 53.28 % ± 0.22 (dry basis) of deshelled kernel. Oil content of Persian lime seed was almost similar to that of castor seed and canola (35 – 42%) and corn germ (about 40%); higher than soybean (18 – 21%), cotton seed (15 – 25%), whole sunflower (22 – 36%) and rice bran (16 – 20%); and lesser than sesame (45 – 55%) and groundnut (46 – 51%). Fiber and protein were the other major constitute of lime seeds. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) content of Persian lime seed was 21.04 and 25.45% (wet basis), respectively. Ajewole [16] reported 41.1 % (dry basis) oil from Nigerian *Citrus aurantifolia* seeds, while Malacrida et al. [2] reported 34.92% (dry basis) oil from lemon (*Citrus limon Osbeck*) seeds. In another study [17], oil content in the Kütdiken and Interdonato variety of lemon (*Citrus limon*) seeds from Turkey was found to be 45.1 and 45.75% (dry basis), respectively. Total ash content of Egyptian lime seeds was reported as 2.19% (dry basis) [18], which was almost similar to our finding (2.15%) for Persian lime. Total ash contents of other citrus seeds were reported as follows: orange seed 2.95%, grapefruit seeds 2.60%, mandarin seeds 3.53% (dry basis) [18]; orange seed 3.17%, citron seed 3.39 and mandarin 3.14% ash (dry basis) [3]. Habib et al. [18] reported that Egyptian lime contained 42.65% lipid, 13.75% protein, 40.56% carbohydrate and 2.19% ash (dry basis). Yilmaz and Güneşer [1] reported that *Citrus limon L.* (Kütdiken variety) contained 34.55% oil, 19.41% protein and 1.41% ash (dry basis). Different harvest year, variety, methods of analysis and oil extraction could be the reason for these differences.

Dimension (length, width, thickness, geometric and arithmetic mean diameter). Mean values and standard deviation of dimensions and geometric and arithmetic mean diameter of Persian lime seeds at vaious moisture contents are presented in Table 2. The average length, width, thickness, geometric mean diameter (D_g) and arithmetic diameter (D_a) of Persian lime seeds were 8.39-8.50 mm, 3.31-3.39 mm, 5.13-5.46 mm, 5.22-5.40 mm, and 5.61-5.78, respectively. The effect of moisture content on seeds length and width was not significant (p>0.05). It seems gradual drying (sun drying for 48 h) and rigid shell structure prevented seeds shrinkage. Accordingly, no significant change was observed in seed length and width during rehydration. However, increase of seed moisture content increased thickness, geometric mean diameter and arithmetic mean diameter of Persian lime seeds (p<0.05). These data could be of importance in theoretical determination of seeds volume at various moisture contents. The effect of moisture content of seeds on geometric mean diameter and

arithmetic mean diameter was insignificant (p>0.05), so both of these parameters can be used in determination of the average diameter of Persian lime seeds. The insignificant effect of moisture content on dimensional characteristics of other seeds such as bitter gourd [19], Anardana [15] and hemp seeds [7] was reported previously. Daraei Garmakhany et al. [20] showed that moisture content have insignificant (p>0.05) effect on length of pomegranate seeds but width and height of pomegranate seeds increased by increaseing of moisture content (p<0.05). The comparison of the data with the works done on the other seeds can be adequate in making symmetrical projections towards process equipment adaptation. Axial dimensions are important in determining aperture size in designing of handling and sieving machinery. Arithmetic mean diameter and effective mean diameter are useful in determination of sieve holes diameter [21]. The major axis (the longer axis of seed) can indicate the natural rest position of the material. This dimension will be beneficial in the application of compressive force to induce mechanical fracture and applying shearing force during slicing [22]. Size and shape are important characteristics for electrostatic separation of seeds from undesirable materials, development of sizing and grading machinery and prediction of seeds drying behavior [5].

Sphericity, aspect ratio, surface, volume and thousand seed mass. Sphericity, aspect ratio, surface, volume and thousand seed mass data are presented in Table 3. Sphericity of lime seeds was in the range of 62.24 - 63.55%. The sphericity of a particle is the ratio of the surface area of a sphere (with the same volume as the given particle) to the surface area of the particle. Likewise, it is regarded as the degree of closeness of seed to a sphere. Furthermore, it also describes the rolling ability of seed during processing. Moisture content had not any significant effect on the sphericity and aspect ratio of seeds (p>0.05), that could be due to proportional increase of length, width and thickness by moisture increase. The sphericity of kidney bean seeds and faba bean grains has been reported as 61.03-61.31% and 63.47-65.78%, by Altuntas and Demirtola [23] and Altuntas and Yıldız [13], respectively, which were close to that of the Persian lime seeds. Sacilik et al. [7] and Dursun and Dursun [24] considered the grain as spherical when the sphericity value was higher than 80, 73% and 70%, respectively. Therefore, in this study, Persian lime seed could not be regarded as sphere. Furthermore, it may be deduced that Persian lime seeds would not roll on flat surfaces. The tendency to either roll or slide is very important in design of hoppers and deshelling equipment for the seed because most flat seeds slide easier than spherical seeds. Sphericity of Persian lime seeds was greater than those reported for lima bean [25] and bitter gourd [19], but lower than that of kenaf seeds [12], lathyrus grain [26], and close to that of faba bean grain [13] and kidney bean [23].

Surface area of the seeds increased from 71.92 to 77.02 mm^2 (p>0.05) when the moisture content increased from 7.5 to 45% (wet basis) (Table 3). A similar trend has been reported for hemp seed [7] and caper seed [24]. Altuntas and Demirtola [23] reported that the effect of moisture content on projected area of kidney bean, pea, and black-eyed seeds was not statistically significant. Data of grain surface is important for modeling of heat and mass transfer during grain drying, aeration, heating and cooling. The surface area is an applicable tool in determining the shape of the seeds. This will actually be an indication of the way the seeds will behave on oscillating surfaces during processing [21].

Volume of Persian lime seeds are shown in Tabbe 3. Seed volume increased from 78.24 to 87.37 mm^3 with the increase in the moisture content; however, the changes were insignificant (p>0.05). Increasing of seed volume, by increasing of seed moisture content, were reported for kenaf [12], hemp seed [7] and legume seeds [23].

Characteristic length (The ratio between surface area and volume) is especially important in the case of irregularly shaped objects. Determination of projected area of particles moving in turbulent air stream, which can be useful in designing grain cleaners, separators, and pneumatic conveyors is one of its applications. By increasing the ratio of surface area to volume, the rate of heat and mass transfer from kernel increases, which affects some

TABLE 2
Effect of moisture content on dimensions of Persian lime *(Citrus Latifolia)* seed.

Moisture (% wet basis)	L (mm)	W (mm)	T (mm)	D_g (mm)	D_a (mm)
7.5	8.39 ± 0.19 ^{a}	3.31 ± 0.26 ^{a}	5.13 ± 0.13 ^{a}	5.22 ± 0.06 ^{a}	5.61 ± 0.03 ^{a}
12.5	8.42 ± 0.15 ^{a}	3.32 ± 0.16 ^{a}	5.28 ± 0.16 ^{ab}	5.28 ± 0.03 ^{ab}	5.67 ± 0.05 ^{ab}
17.5	8.44 ± 0.05 ^{a}	3.34 ± 0.19 ^{a}	5.36 ± 0.04 ^{ab}	5.32 ± 0.12 ^{ab}	5.71 ± 0.08 ^{ab}
22.5	8.46 ± 0.08 ^{a}	3.36 ± 0.07 ^{a}	5.41 ± 0.15 ^{ab}	5.36 ± 0.10 ^{ab}	5.74 ± 0.09 ^{b}
45	8.50 ± 0.06 ^{a}	3.39 ± 0.04 ^{a}	5.46 ± 0.09 ^{b}	5.40 ± 0.05 ^{b}	5.78 ± 0.04 ^{b}

L: length; W: width; T: thickness; D_g: geometric mean diameter and D_a: arithmetic mean diameter.
Note: Values given are the means of three replicates ± standard deviation. Means with different letters within a column are significantly different at p<0.05 (using Duncan's Multiple Range Test).

TABLE 3

Effect of moisture content on sphericity, aspect ratio, surface, volume and 1000 seed mass of Persian lime (*Citrus Latifolia*) seed.

Moisture (% wet basis)	Φ (%)	Aspect ratio (%)	S (mm^2)	V (mm^3)	M_{1000} (g)
7.5	62.24 ± 2.17 [a]	39.53 ± 4.14 [a]	71.92 ± 1.73 [a]	78.24 ± 1.62 [a]	41.13 ± 1.99 [a]
12.5	62.72 ± 1.23 [a]	39.42 ± 2.56 [a]	73.69 ± 1.06 [ab]	81.63 ± 2.22 [ab]	43.48 ± 1.99 [a]
17.5	63.12 ± 1.22 [a]	39.61 ±2.19 [a]	74.95 ± 3.49 [ab]	83.84 ± 4.8 [ab]	48.24 ± 2.64 [b]
22.5	63.36 ± 0.61 [a]	39.76 ± 0.49 [a]	75.90 ± 2.85 [ab]	85.48 ± 4.89 [b]	54.75 ± 3.51 [c]
45	63.55 ± 0.81 [a]	39.92 ± 0.66 [a]	77.02 ± 1.45 [b]	87.37 ± 2.6 [b]	63.90 ± 1.10 [d]

Φ: sphericity; S: surface; V; volume and M_{1000}: thousand seed mass.
Note: Values given are the means of three replicates ± standard deviation. Means with different letters within a column are significantly different at $p<0.05$ (using Duncan's Multiple Range Test).

TABLE 4
Effect of moisture content on poosity, true and bulk density of Persian lime (*Citrus Latifolia*) seed.

Moisture (% wet basis)	ε (%)	ρ_s (g/cm^3)	ρ_b (g/cm^3)
7.5	42.14 ± 0.61 [a]	0.745 ± 0.026 [a]	0.431 ± 0.011 [a]
12.5	40.09 ± 0.81 [b]	0.786 ± 0.008 [b]	0.471 ± 0.002 [b]
17.5	39.68 ± 0.70 [bc]	0.885 ± 0.007 [c]	0.533 ± 0.007 [c]
22.5	38.71 ± 0.63 [c]	0.978 ± 0.003 [d]	0.599 ± 0.007 [d]
45	35.43 ± 0.40 [d]	1.210 ± 0.007 [e]	0.781 ± 0.001 [e]

ρ_s: true density; ρ_b: bulk density; ε: porosity.
Note: Values given are the means of three replicates ± standard deviation. Means with different letters within a column are significantly different at $p<0.05$ using Duncan's Multiple Range Test.

unit operations such as drying, cooling, and heating [21]. In this study, increasing of moisture content from 7.5 to 45% (wet basis) increased characteristic length from 1.08 to 1.13 mm ($p>0.05$).

Thousand seed mass of Persian lime increased from 41.13 g to 63.90 g by increase of moisture content from 7.5% to 45% (wet basis) ($p<0.05$) (Table 3). Similar results were reported for pigeon pea grain [27], mahogany [28] and kidney bean, pea and black-eyed pea [23]. The relationship between moisture content (m_c) and thousand seed mass (M_{1000}) could be described using the following regression equations:

$$M_{1000} = 0.618m_c + 37.327 \quad (R^2 = 0.95) \quad (16)$$

Bulk density, true density and porosity. The true density of a particulate solid or powder, is the density of the particles of the powder. Bulk density measures the average density of a large volume of the powder in a particular medium (usually air). Bulk and true densities of Persian lime seeds are shown in Table 4. Bulk density of seeds increased from 0.431 to 0.781 (g/cm^3) with the increase of moisture content from 7.5% to 45% (wet basis) ($p<0.05$). This was due to the higher rate of increase of seed mass (35.64%) as compared to the volume (10.44%). Kingsly et al. [15] and Daraei Garmakhany et al. [20] found similar results for Anardana and pomegranate seeds respectively. The bulk density was lower than true density because the air spaces in seeds bulk increase the volume while the weight remains unchanged. The bulk density of seeds was found to have the following regression relationship with moisture content:

$$\rho_b = 0.0094m_c + 0.3659 \quad (R^2 = 0.99) \quad (17)$$

Bulk density is important because it determines the capacity of storage and transport systems. Since the bulk density affects the resistance to airflow of a stored bulk, it is also used in design of drying and aeration systems. True density increased from 0.745 to 1.210 (g/cm^3) as the moisture content increased from 7.5 to 45% (wet basis) ($p<0.05$). The increase of true density with increase of moisture content might be attributed to the relatively low volume increase as compared to the corresponding mass increase. The increase of true density with increase of moisture content have been reported by Aydin and Ozcan [29] for myrtle fruit kernel, Pradhan et al. [30] for karanja kernel, Ünal et al. [19] for bitter gourd seed, Kingsly et al. [15] for Anardana and Daraei Garmakhany et al. [20] for pomegranate Seeds. The moisture dependence of the true density was described by a linear equation as follows:

$$\rho_s = 0.0126m_c + 0.6565 \quad (R^2 = 0.98) \quad (18)$$

Bulk density, true density, and porosity affect the rate of heat and moisture transfer during aeration and drying processes and are useful in sizing grain hoppers and storage facilities. The density values of seeds are used in design of storage bins and silos, separation of impurities from desirable materials, cleaning and grading and quality evaluation of the products [20].

True density of Persian lime seeds was found to be less than that of kenaf seeds [12], faba bean grains [13] and Karanja (Pongamia pinnata) kernel [30] and greater than that of Caper seeds [24].

As can be seen from Table 4, at moisture contents of 7.5, 12.5 and 17.5%, true density of the Persian lime seeds became less than the density of water (1 g/m^3). However, at moisture content of

45%, true density of seeds was higher than density of water. According to the Eq 17, at a moisture content of 27.26%, the true density of Persian lime seed equals to 1 g/m^3. This means that at moisture contents greater than 27.26% (e.g. in fresh form), the seeds sediment in water while at moisture content lower than 27.26 % (e.g. in dried form) the float in water. This characteristic can be used for separation of lime seeds from other impurities by sedimentation or floatation.

Porosity depends on the bulk density as well as on true density. The porosity of Persian lime seeds was found to decrease from 42.14 to 35.43% (p<0.05) as the moisture content increased from 7.5 to 45% (wet basis). The porosity value is often needed in air and heat flow studies. The relationship between porosity (ε) value and moisture content (m_c) of the Persian lime seed could be described as:

$$\varepsilon = -0.1658m_c + 42.695 \quad (R^2 = 0.96) \quad (19)$$

Kingsly et al. [15] and Balasubramanian and Viswanathan [31] observed a negative linear relationship between porosity and moisture content for Anardana, and minor millet, respectively. In contrast, a positive correlation between moisture content and porosity was reported for karanja kernel [30] and bitter gourd seed [19]. It must be noted that the resistance of the mass of seeds to airflow during aeration and drying process is determined by porosity.

Angle of repose and static coefficients of friction. The angle of repose is an indicator of the product's ability to flow. The high value of the angle of repose may be due to the large size of the grains and their relatively rough surface, which prevent easy sliding of the grains on one another. The experimental results of the angle of repose with respect to moisture content are shown in Table 5. The values of angle of repose of filling (θ_f) and emptying (θ_e) were found to increase from 20.47 to 32.00 and 15.54 to 27.84, respectively (p<0.05), with the increase of moisture from 7.5 to 45% (wet basis). θ_e was lower than θ_f at each moisture level (p<0.05). All biological materials exhibit an increase in angle of repose with moisture increase [5]. At higher moisture content seeds might tend to stick together resulting in better stability and less flow ability, so angle of repose increases by increase of moisture content.

The values of the angle of repose of Persian lime seeds showed the following linear relationship with moisture content (m_c):

$$\theta_f = 0.3099\ m_c + 18.991 \quad (R^2 = 0.90) \quad (20)$$

$$\theta_e = 0.3212x + 14.737 \quad (R^2 = 0.84) \quad (21)$$

Increase of θ_f and θ_e of Persian lime seed with increase of moisture content were similar to those reported for Lathyrus, Anardana and minor millets [26, 15, 31].

The static coefficients of friction (μ_s) of Persian lime seeds on five various surfaces (steel, galvanized iron, plywood, glass and rubber) at different moisture contents are presented in Table 5. It was observed that the static coefficient of friction increased with increase of moisture content on all contact surfaces (p<0.05). In fact, Due to the presence of water, a sticky and cohesive force is created on the surface of contact resulting in better stability and less flow ability. Increase of μ_s by increase of moisture content for different contact surfaces have been reported for pigeon pea grains [27], some grain legume seeds [23] and Karanja kernel [30].

The effect of type of contact surface material on static coefficient of friction was greater compared to moisture content (p<0.05). This was previously reported by Shafaei et al. [32]. At all moisture content, the maximum friction was offered by rubber, followed by plywood, galvanized iron, steel and glass surface. This difference could be due to the different roughness of the various surfaces. In fact, smoother surfaces of glass and steel show lower resistance against the motion of the seeds. Rubber offered the maximum friction for pea, kidney bean and blacked-eyed [23], faba bean grain [13], pomegranate seed [20] and bitter gourd seed [19]. Glass offered the maximum friction for lima bean [25] and mahogany seed [28]. Knowledge about static coefficient of friction and angle of repose on various contact surfaces is useful in sizing motor requirements for material displacement, achievement of consistent flow of materials, designing of storage bins, hoppers, pneumatic conveying system, screw conveyors, forage harvesters and threshers.

The relationships between static coefficient of friction (μ_s) and moisture content of seeds (m_c) on steel (s), galvanized iron (gi), plywood (pw), glass (g) and rubber (r) can be represented by the following equations:

$$\mu_s = = 0.0033m_c + 0.2942 \quad (R^2 = 0.98) \quad (22)$$

$$\mu_{gi} = 0.0049m_c + 0.3215 \quad (R^2 = 0.98) \quad (23)$$

$$\mu_{pw} = 0.0048m_c + 0.3477 \quad (R^2 = 0.97) \quad (24)$$

$$\mu_g = 0.0044m_c + 0.2278 \quad (R^2 = 0.92) \quad (25)$$

$$\mu_r = 0.0042m_c + 0.4413 \quad (R^2 = 0.94) \quad (26)$$

Friction coefficient directly affects the friction force. Thus, knowing the friction coefficients is essential to determine friction force value. From industrial standpoint, it indirectly helps in optimization of equipment designing to prevent mechanical damages to the product during mechanical processes. During post-harvest process of lime seed, it is desirable to prevent seed from slipping and escaping from the defined location of equipment. Thus, according to level of moisture content of samples and type of structural surface, estimation of holding force seems to be of great importance [32].

TABLE 5
Angle of repose and coefficient of static friction of Persian lime (*Citrus Latifolia*) in different moisture and surface material.

Moisture (%)	Angle of repose		Coefficient of static friction (μ_s) at different Surface material				
	Filling (θ_f)	Emptying (θ_e)	Glass	Steel	Galvanized iron	Plywood	Rubber
7.5	20.47 ± 0.54 ^{a}	15.54 ± 0.34 ^{a}	0.247 ± 0.041 ^{aE}	0.314 ± 0.017 ^{aD}	0.349 ± 0.032 ^{aC}	0.367 ± 0.031 ^{aB}	0.457 ± 0.019 ^{aA}
12.5	21.79 ± 0.42 ^{b}	17.57 ± 0.68 ^{b}	0.267 ± 0.039 ^{bE}	0.331 ± 0.030 ^{bD}	0.378 ± 0.023 ^{bC}	0.409 ± 0.030 ^{bB}	0.485 ± 0.028 ^{bA}
17.5	24.88 ± 0.58 ^{c}	21.46 ± 0.33 ^{c}	0.318 ± 0.037 ^{cE}	0.353 ± 0.017 ^{cD}	0.409 ± 0.026 ^{cC}	0.438 ± 0.028 ^{cB}	0.528 ± 0.016 ^{cA}
22.5	28.33 ± 0.47 ^{d}	24.97 ± 0.21 ^{d}	0.353 ± 0.017 ^{dE}	0.380 ± 0.027 ^{dD}	0.448 ± 0.027 ^{dC}	0.470 ± 0.043 ^{dB}	0.554 ± 0.028 ^{dA}
45	32.00 ± 0.59 ^{e}	27.84 ± 0.58 ^{e}	0.413 ± 0.017 ^{eE}	0.438 ± 0.019 ^{eD}	0.533 ± 0.027 ^{eC}	0.555 ± 0.037 ^{eB}	0.618 ± 0.025 ^{eA}

Note: Values given are the means of three replicates ± standard deviation. Means with different capital letters within a row and small letters within a column are significantly different at $p<0.05$ using Duncan's

CONCLUSION

Characteristics of Persian lime seed as a valuable new source of oil and protein were studied. Compared to other oil-bearing seeds, the Persian lime seed has relatively high oil content. Most biophysical properties increased with increase in moisture content but Porosity of seeds decreased. Persian lime seed could not be regarded as sphere and would not roll on flat surfaces. At all moisture content, the highest value of static friction coefficient was found on the rubber surface and the lowest on the glass sheet. Immersion in water and aeration can be used to separate seeds from lighter and heavier foreign materials. Rubber surface is suitable for moving and transportation of seeds.

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HEALTH RISK ASSESSMENT OF LEVEL OF ACIDITY AND SWEETENERS THROUGH BEVERAGES MARKETED IN ALGERIA

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ABSTRACT

The aim of our study is to determine a physico-chemical quality of beverages (pH, titratable acidity, Sugar content (Brix) and level of sweeteners) marketed in Algeria. 66 analyzed samples are divided into five categories: 33 soft drinks, 23 fruit drinks, 4 energy drinks, 3 light beverages, 3 juices and nectar. pH analysis is performed by pH-meter, titratable acidity by increment of 0.1N NaOH were titrated until neutrality reached. The 1st part is to use an Abbe refractometer to measure the sugar content (Brix). The 2nd part, using high performance liquid to analyze aspartame saccharin and acesulfame k. The physicochemical results of all the analyzed samples show mean value of pH was between 2.42 to 3.94. With mean titratable acidity of 0.38±0.233g/100mL. The brix rate is showed an average value of 10.76±3.112%. On average, beverages contained of 22.11 ± 46.062mg/L of Aspartame, 16.15 ± 21.424mg/L of saccharin and 47.47 ± 53.32mg/L of acesulfame k. All results of chemical quality present a significant difference between mean (*p-value* <0.005).

KEYWORDS:
Algeria, beverages, HPLC, physico-chemical, quality, sweeteners

INTRODUCTION

The consumption of sugar drinks become increasingly a major issue in Health risk because of its ability to excess weight gain and risk of chronic diseases [1, 2]. The researchers have shown that the children are the major consumers of sugary drinks, which cause them real public health issues such as obesity and the early appearance of other pathologies.

Large-scale epidemiological studies have focused in a positive association between consumption of sugary drinks and risk of death from cardiovascular [3]. Consequently, the scientists develop artificial sweeteners to reduce the amount of calories that people frequently consume. However, many reports have proven the negative side effects if the artificial sweeteners are consumed excessively.

On concern, the annual consumption of sugary drinks per person in Algeria is about 57.4 liters, including 22.2 liters of soft drinks [4]. The daily and successive consumption of theses drinks is susceptible to increase the health risk.

Due to the incessant search for health, the notion of changing eating habits and lifestyle become the most needed thing. With increased consumer interest in reducing sugar intake, food products made with sweeteners rather than sugar have become more popular. The discovery of artificial sweeteners has triggered the development of sugar-free products, particularly in diabetes, energy-controlled diet, special diets and obesity [5].

The purpose of this study is to assess the pH, acidity and sugar level. Then, the presence and the levels of the sweeteners in the drinks were determined, in order to verify compliance with Algerian legislation concerning the maximum authorized levels.

MATERIALS AND METHODS

Sampling. A total of 66 commercial drinks from different markets in Bechar city (Algeria). The products are randomly selected according to the market availability. The study examined 33 soft drinks, 23 fruit drinks, 4 energy drinks, 3 light beverages, 3 juices and nectar. The labelling and packaging contained only qualitative information without mentioning the kind of sweeteners and their concentrations. They were evaluated by a randomized experiment with 3 measurements for each sample, recording the mean of three values. All soft drinks were decarbonated.

Determination of pH, titratable acidity and sugar content. The pH of the sample was measured using a pH meter, after being calibrated. Titratable Acidity (TA) was measured according to the method

of French standard NF V 05-101 [6]. Sugar content (Brix) was determined by refractometry using an Abbe refractometer [7].

Determination of sweeteners. HPLC conditions. Analytical separation of the sweeteners was measured according to the method of French standard NF V 03-074 [8] with some modifications. beverages were analyzed by using HPLC system (Agilent Technology 1200 series) equipped with a quaternary pump, an inline degasser, a column oven, and a UV detector. A Teknokroma C18 column analytical column (250 × 4.6 mm, 5 µm) was used for the separation.

The mobile phase consisted of a phosphoric acid in 0.02M Potassium dihydrogen phosphate buffer (solvent A, pH 3.98) and acetonitrile (solvent B) 90: 10 v/v. The detector was set at 196nm, 227nm and 235nm for aspartame, acesulfame k and saccharin respectively. External standard method was used for quantification. Integration was performed with an integrator OpenLab.

Preparation samples and standards. Deionized water, acetonitrile and Acesulfame were purchased from Sigma. Saccharin and aspartame was obtained from Thermo Fisher. Aspatrtame, Saccharin and Acesulfame were prepared at 1mg/mL with deionized water. Standards and samples were filtered through a 0.45µm.

Statistical analysis. The mean values and standard deviation were analyzed statistically using the IBM SPSS statistics software. One-way ANOVA was carried out to test for any significant difference. Difference between means at *P-value* < 0.05 level were considered significant.

RESULTS AND DISCUSSION

For all the samples analyzed N = 66, the results obtained were presented under form of an average framed by the calculated standard deviation. Table 1 shows mean results of pH, titratable acidity and sugar content of beverages. The mean value of pH is between 2.42 to 3.94. With mean titratable acidity of 0.38 ± 0.233 g/100mL. This difference in pH and acidity may be due to the amounts of ingredients for each beverages. Mean pH of beverages are significantly different (*P-value* <0.05). The brix rate is showed an average value of 10.265±3.48 (P< 0.0001). It varies between 1% and 15%.

It should be mentioned that the pH of saliva lies within the range of 5.5-6.5. In their study, Shellis et al. [9] proved the threshold level for the development of dental caries is pH of 5.5. In addition, Von Fraunhofer and Rogers [10] affirmed the dental caries are developed under the threshold pH of 5.5. In the analyzed samples showed the pH range of most beverages is 2.42 - 3.94. Therefore, it influences the erosive potential of the beverage while it is being consumed [11]. Most beverages contain one or more food acidulants phosphoric and citric acid are common but malic, tartaric and the organic acids may be present [12]. The dietary acids present in low pH beverages are presumably the major erosion ingredients [13]. Many studies in vitro indicate that low pH beverages and particularity fruit-based drinks cause erosion to enamel and dentine [14, 15]. Beverages have a relatively low pH, which makes soft drinks and fruit juices not suitable for people with stomach ulcers. Low pH has effects on the enamel, which can affect teeth development in infants [16].

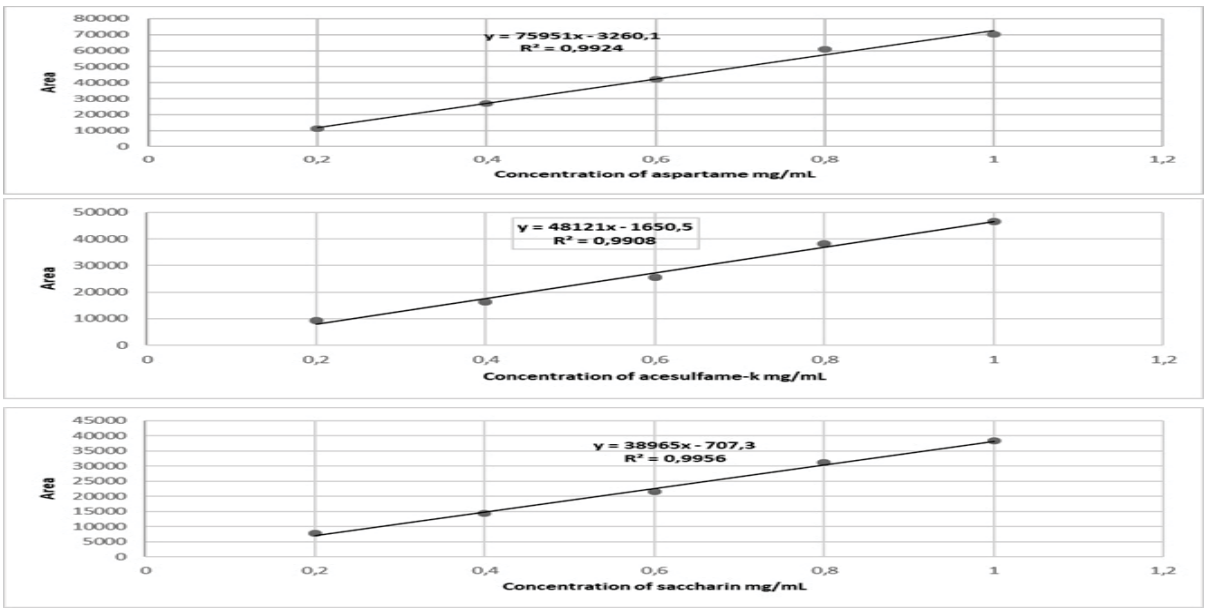

FIGURE 1
Calibration curve obtained using sweeteners standards

TABLE 1
Mean of pH, titratable acidity and content sugar of beverages

Samples	pH	Acidity g/100mL	Brix %
Soft drinks	2,96 ± 0,336	0,288 ± 0,125	10,69 ± 3,092
fruit drinks	3,21 ± 0,302	0,434 ± 0,29	11,85 ± 1,28
Juices and nectar	3,72 ± 0,23	0,451 ± 0.00	11,8 ± 1,361
Light drinks	3,26 ± 0,17	0,35 ± 0,09	2 ± 0,87
Energy drinks	3,14 ± 0,259	0,735 ± 0.132	10,75 ± 2,26

Values are means ± SD (triplicate)

TABLE 2
Mean of Sweeteners in Beverages

Sample	Aspartame mg/L	Saccharin mg/L	Acésulfame-k mg/L
Soft drinks	6,82 ± 38,77	11,50 ± 10,058	45,86 ± 54,816
fruit drinks	23,84 ± 55,97	21,37 ± 33,124	31,69 ± 14,825
Juices and nectar	96,39 ± 144,59	20,49 ± 1,82	39,86 ± 3,32
Light drinks	132,21 ± 107,81	20,43 ± 1,58	160,60 ± 52,80
Energy drinks	0.00	18,05 ± 11,53	72,4 ± 89,24

Values are means ± SD (triplicate)

According to ICMSF [17], Sugar levels in soft drinks and fruit juices range from 5 to 15% Brix. Although sugar is a source of energy in human diet, it is also deleterious to health. It is a long- term chemical poison. Sugar is by far the leading cause of dental deterioration-cavities in the teeth, bleeding gums, failure of bone structure, and loss of teeth. It is the main cause of diabetes, hyperglycemia and hypoglycemia. It is either a significant or a contributory cause of heart disease, arteriosclerosis, mental illness, depression, hypertension and cancer [18]. Importantly, Beverages can play an essential role in energy intake due to the evidence that high intake of liquid carbohydrates may lead to weight gain because of a lack of dietary compensation compared to that observed in similar amounts of solid carbohydrates [19, 20]. Findings from studies of Ariza et al., [21] and Andersen et al. [22] suggest a positive trend in the relationship between sugar-sweetened beverages intake and obesity. The deleterious effect of sugary drinks is attributed in particular to their hyperglycemic power. Indeed, several studies indicate that a high glycemic load diet increases the risk of diabetes mellitus. In addition, research shows that children who consume many sweetened drinks are more likely to be overweight or obese [23].

The Table 2 shows the obtained results of sweeteners. The mean value of sweeteners is 22.11 ± 64.062 mg/L, 16.15±21.42 mg/L and 47.47±53.32 mg/L of aspartame, saccharine and acesulfame-k respectively (*p-value* <0.05). Out of 66 samples, 3 analysed contained aspartame, saccharin and acesulfame-k. 3 analysed do not contain sweeteners in beverages destined to children. The others were contained one or two sweeteners.

According to the Algerian legislation relating to the conditions of use of sweeteners in foodstuffs [24], the acesulfame-k content must not exceed 350 mg/L, aspartame 600 mg/L, saccharin 80 mg/L for flavored drinks and 100 mg/L for soft drinks. Levels of aspartame and acesulfame were not exceeded in all samples analyzed. One sample was exceeded the maximum permitted level for saccharin in fruit drinks with concentration of 172,65mg/L.

The average Acesulfame content of our samples has appeared to be higher than that found by Orawan and Jaroon [25] which is been estimated at less than 3.6mg/L in beverages. Some beverages contained aspartame and acesulfame-k. Mukhopadhyay et al. [26] showed the absence of a genotoxic effect of Acesulfame-K in combination with aspartame.

The concentration ranged between 0 - 225.8 mg/L for aspartame and 0 - 28, 16 mg/L for saccharine in soft drinks. Our results are close to those found by Croitoru et al., who determined the concentrations ranged between 9.94–296.82 mg/L in case of aspartame and 17.96–50.94 mg/L for saccharine in soft drinks [27].

Bergamo et al. [28] found an average content of saccharin in soft drinks of the order of 32mg/l. This value is higher than the average grade of our samples; it estimated at 10.11 mg/L.

Consumption of beverages loaded with sweetener imbalances the metabolism that ultimately causes obesity [29]. Ballard et al. found the use of the two sweeteners aspartame and saccharin rapidly increases weight [30].

CONCLUSION

The high concentration of sugar content founded in almost beverages associated to low pH have can increase health risks. They have a great impact on the human body in case the children consume them in excess. In addition, sweeteners, which have replaced sugars to eliminate health risks, are not effective in maintaining good health if used in an unacceptable or suspicious manner. Therefore, consumers' awareness of the health risks of mishandling soft drinks is becoming insufficient to reduce risks like diabetes and obesity, which ultimately lead to cancer.

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AMINO ACIDS, PHYTOCHEMICALS, ANTIOXIDANT, ANGIOTENSIN CONVERTING, α-AMYLASE AND α-GLUCOSIDASE ENZYME INHIBITORY POTENTIALS OF AFRICAN LOCUST BEANS (*PARKIA BIGLOBOSA* JACQ BENTH) BIOACTIVE PROTEINS

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ABSTRACT

The use of plant food bioactive compounds to prevent and manage chronic diseases has gained prominence; due to antioxidant activities of these compounds. Hence, this study aimed to determine antioxidant activities, alpha-amylase-glucosidase and angiotensin-1-converting enzyme inhibitory potentials of bioactive compounds in *Parkia biglobosa* seed flours. *Parkia biglobosa* seeds were processed as undefatted (ULB), defatted (DLB), protein isolate (LPI) and protein hydrolysate (LPH) flours. Chemical compositions, antioxidant activities, alpha-amylase-glucosidase and angiotensin-1-converting enzyme inhibitory potentials of the flours were determined. Essential amino acid compositions (mg/100g protein) of flours ranged between 27.82 and 56.40, essential amino acid index (52.33 - 82.63%) and biological values (45.34 - 78.36%). The arginine/lysine ratios and branched chain amino acids ranged from 0.02 - 8.53 and 13.89 - 16.54, respectively. Phytochemicals in *Parkia biglobosa* flours were lower than critical levels. The *Parkia biglobosa* protein hydrolysate exhibited higher antioxidant activities in DPPH, FRAP, Fe+2 chelation; whereas, ULB had higher antioxidant activities in ABTS and OH free radicals than other samples. The LPH exhibited lowest IC50 (inhibition concentration-50%) and highest inhibitory activities on alpha-amylase-glucosidase and angiotensin-1-converting enzymes than other flour samples, respectively. This study provided information on chemical composition, antioxidant activities, inhibitory activities on alpha-amylase-glucosidase and angiotensin converting enzymes *P. biglobosa* seed flours. The protein hydrolysate of *P. biglobosa* (LPH) exhibited higher antioxidant activity, inhibition on apha-amylase-glucosidase and angiotensin-1-converting enzymes than other samples. Therefore, LPH sample may be suitable as antioxidant, antidiabetic and antihypertensive agent in form of nutraceutical supplement or functional food application.

KEYWORDS:
Parkia biglobosa, bioactive proteins, Amino Acid profile, Antioxidant activities, Enzymes inhibitory potentials

INTRODUCTION

African locust bean (*Parkia biglobosa*), an underutilized seed, belonging to the family of *Leguminosae* and subfamily of *Mimosoideae,* is commonly found in many parts of West African countries with different names, that is, "dawadawa" in Ghana; "soumbala" in Burkina Faso; and "iru" in Benin and Nigeria [1]. African locust bean is usually consumed among low-income families who cannot afford animal protein inform of spice [2, 3], due to its quality protein content and other essential nutrients [4]. In recent times, scientific studies reported on the medicinal properties of different parts of *P. biglobosa* tree in traditional medicine to prevent or manage metabolic diseases [3, 5]. The pharmacological and nutritional benefits of *P. biglobosa* has been associated with its bioactive compounds like peptides and phytochemicals, which acted independently or in synergy to manage diet related diseases [3, 5].

The bioactive compound is present in small quantities in functional foods, and usually provides health benefits besides its basic nutritional value [6]. Epidemiological studies indicate that high consumption of foods rich in bioactive compounds enhance human health by reducing risks of age-related diseases [7]. The health benefits of bioactive compounds like peptides and phytochemicals depend on their bioavailability, antioxidant effect, and ability to inhibit enzymes [8].

In recent decades, scientific studies established that bioactive proteins can serve as nutrients, and also perform physiological functions [9] by the peptides that are encrypted in the native protein, which were released from the sequences either by digestion, during food processing, or by microbial fermentation [9, 10]. It is evident that peptides can regulate important physiological functions through

their various activities including antioxidant, etc. [11, 12].

In the last decades, the use of bioactive peptides in form of nutraceuticals supplements [13] or in functional food productions [14] has gained much interest. For instance, several efforts have been recently geared towards production of bioactive peptides from various food materials like cereals, legumes and oil seeds in the production of functional foods [14]. For the *P. biglobosa,* there is scanty information on its utilization in diabetic and hypertensive management. In view of this, the study present study was carried out to evaluate nutritional composition, antioxidant activities, angiotensin converting and alpha amylase-glucosidase enzyme inhibitory potentials of undefatted, defatted, protein isolate and hydrolysate of *P. biglobosa* seed.

MATERIALS AND METHODS

Plant material and authentication. The raw *P. biglobosa* seeds were purchased from a local market in Akure, Ondo State, Nigeria. The raw seeds were identified and authenticated by the Chief Technologist in the Department of Crops, Pests and Soil, Federal University of Technology, Akure, Nigeria.

Processing of *P. biglobosa* flour samples. Undefatted African locust bean flour. Raw African locust bean seeds were processed into flour using Bolajoko [14] method. The seeds were separated from foreign materials, and were manually dehulled, washed with distilled water and allowed to drain. Thereafter, the dehulled seeds were hot-air oven dried at 60 ºC for 20 h (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK), milled with a laboratory blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK) and sieved (60 mm mesh, British Standard) to obtain undefatted African locust bean seed flour, and stored in an airtight plastic container at room temperature (~27ºC).

Defatted African locust bean flour. The oil in African locust bean flour was extracted using hexane solvent [14]. The seed flour (100 g) was placed in a soxhlet apparatus and extracted severally with hexane for 24 h. After extraction, the defatted African locust bean flour was separated from hexane by air-drying under a fume hood chamber, and the defatted flour was further dried in hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) until hexane odour was completely removed. The defatted seed sample was stored in an airtight container at room temperature (~27ºC).

African locust bean Protein Isolate. The African locust bean protein isolate was prepared as described by Wolf [15]. Defatted African locust bean flour was dispersed in distilled water (water: flour ratio, 1:25 (w/v)), the mixture was maintained at alkaline medium of pH 11.0 using 1 M NaOH to solubilize the proteins and the temperature of the mixture with continuous stirring was maintained at 37 ºC for 2 h. After, the slurry was centrifuged at 15,000 x g for 20 min at 4 °C. The residue was discarded, while the supernatant was adjusted to pH 4.0 (isoelectric point) with 1 M HCl to precipitate the proteins. The precipitated protein was washed twice times with distilled, centrifuged at 15,000 xg for 20 min at 4 °C, re-dissolved in water, neutralized to pH 7 with 0.1 N NaOH, and then freeze-dried (Fig. 1). The African locust bean protein isolate was stored for further analysis.

African locust bean Protein Hydrolysate. The African locust bean Protein hydrolysate was produced using the method described by Wang et al. [16] with slight modification. The white melon protein hydrolysate flour was produced using *Lactobacillus fermentus* BGT10 obtained from the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria. The mixture of protein isolate and lactic acid bacteria (7log cfu/g in flour after inoculation) were dispersed in distilled water, and were thoroughly mixed in a food processor mixer. After mixing, the mixtures were placed in incubator at 37 °C for 72 h; and thereafter, the lactic acid bacteria were inactivated at 98 °C for 10 min. After inactivation, the mixture was centrifuged at 6000 x g for 30 min and filtered through a 0.45 µm membrane filter. The filtrates were then freeze-dried and stored at 4 °C before further analysis.

Amino acid analysis. Amino acid composition was determined using method described by Jeong and Shim [18]. The sample (0.1 g) was placed in test tubes, 5 mL of 6 N HCl was added, and vortex (TSS2, IKA, Korea) for 30 s. The tube was flushed with N gas for 1 min, sealed and incubated in a hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at 110 °C for 24 h. After the reaction was completed, the supernatant was passes through glass filter, and the filtrate was evaporated in a vacuum rotary evaporator (HS-2005S, Jisico Co, Ltd, Korea) to remove HCl. The concentrate was then dissolved in 3 mL of sodium citrate buffer (pH 2.2), filtered with 0.45 lm PTFE filters (Sigma-Aldrich, Seoul, South Korea) and analyzed using an auto amino acid analyzer (Technicon Instruments Corporation, New York) at 570 nm. The tryptophan content was determined in a separate analysis as described by Hariharan et al. [19].

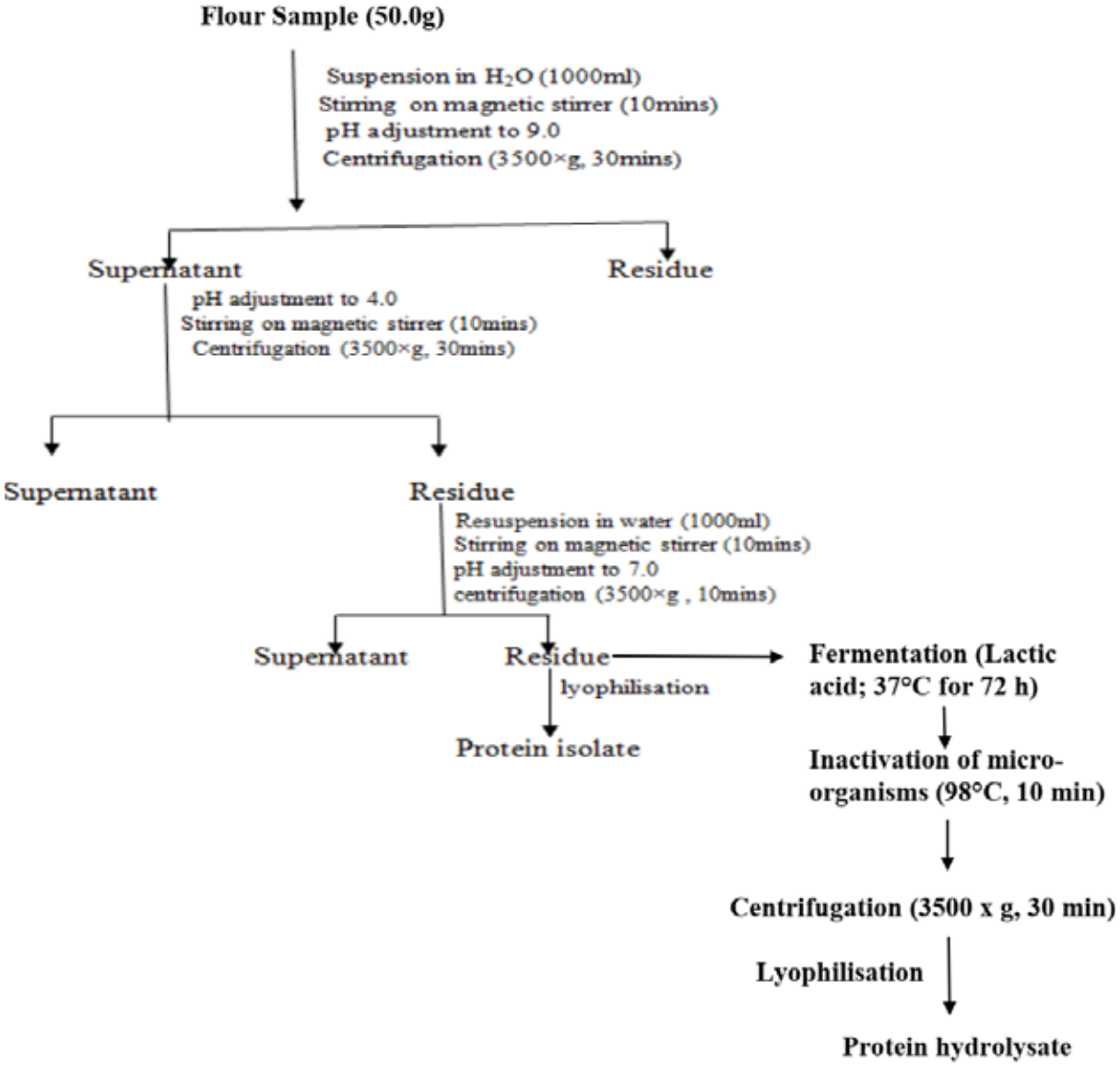

FIGURE 1
Flowchart showing production of protein isolate and hydrolysate flour from African locust bean seeds [16, 17].

Protein Quality Indices. Protein quality indices were calculated with reference to amino acid profiles, that is, essential Amino Acid Index (EAAI) according to the equation below Labuda et al. [20]:

$$EAAI = \sqrt{\frac{[\text{lys x try x isoleu x val x threo x leu x phenylal x hist x meth}]a}{[\text{ [lys x tryp x isoleu x val x threo x leu x phenyla x hist x meth] b}} * 100$$

where:

[lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine and methionine] an in test sample and [lysine, tryptophan, isoleucine, valine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cystine] b content of the same amino acids in standard protein (%) (egg or casein), respectively.

The nutritional index of the food samples was calculated :

$$\text{Nutritional index (\%)} = \left[\frac{EAAI\ x\ \%protein}{100}\right]$$

The biological value [21, 22]:

BV = 1.09 x Essential amino acid index [EAAI] – 11.7

Protein Efficiency Ratio (PER) [22, 23]:

PER = -0.468 + 0.454(LEU) – 0.105(TYR)

Phytochemicals composition of African Locust Beans Flour samples. The phytochemical compositions of African locust bean flour samples were determined using standard methods, that is, phytic acid [24], Tannin content [25, 26]. Oxalate [27], Total flavonoid content [28], total saponin content [29], total phenolic content [30].

Antioxidant activity of African Locust Beans Flour samples. Antioxidant potentials of the African locust bean flour samples were evaluated using DPPH radical scavenging, ABTS, OH free radicals, Iron chelation and FRAP assays.

Ferric reducing antioxidant power (FRAP) assay. The ferric reducing antioxidant power (FRAP) assay was performed as described by Benzie and Strain [31] with some modifications. Freshly prepared FRAP reagent (30 mL) was heat to 37 °C, a reagent blank reading was measured at 593 nm. Each of the samples (150 µL) was added and the volume made up to 1 mL with distilled water. The absorbance (A) readings were measured (Jenway Vis Spectrophotometer 6305) after 0 s and 4 min. Thereafter, the change in absorbance (A_{593} nm) between the final reading selected and the blank reading was calculated for each sample and related to A_{593} nm of a Fe^2 standard solution tested in parallel. The readings were selected for calculation of FRAP values.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical *scavenging activity assay*. The DPPH was determined as described by Pownall et al. [32] with slight modification. The DPPH stock solution (0.6 mM) was prepared by adding 10 mL to 45 mL of methanol. To each of the extracts, 1 mL methanol DPPH was added to extract at different concentrations. The absorbance reading was measured after 30 min at 517 nm using a spectrophotometer (Jenway 6305). The inhibition activity (%) on DPPH radical was calculated using the equation below:

$$\text{Scavenging effect (\%)} = \left[\frac{(\text{A517c} - \text{A517s})}{\text{A517c}}\right] * 100$$

where c and s represent control and sample, respectively.

Ferrous ion-chelating activity. The chelating activity of the samples on Fe^{2+} was determined according to the method of El and Karakaya [33], with some modifications. To the sample aliquots (200 µL), 740µL of deionized water and 20 µL of 2 mM FeCl2 solution were added, and the mixture was incubated for 30 min at room temperature. After incubation, 5 mM ferrozine (200 µL) was added and the mixture was re-incubated for 10 min in the same conditions. The absorbance was measured at 562 nm. And distilled water was used as control.

$$\text{Fe chelating capacity (\%)} = \left[\frac{(\text{A562c} - \text{A562s})}{\text{A562c}}\right] * 100$$

where c and s represent control and sample, respectively.

2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS). The ABTS was determined as described by Re et al. [34]. The ABTS solution was prepared (7 mM ABTS and 2.5 mM potassium persulfate) 12 h before used. The solution was diluted with 200 mM phosphate buffer pH 7.4, and 5 mL of diluted solution was mixed with 50 mL of extract solution to obtain concentration of 0.1 mg/mL. Absorbance at 734 nm was read after 10 min using water instead of sample as a control. ABTS scavenging activity was calculated according to formula:

$$\text{ABTS Scavenging (\%)} = \left[\frac{(\text{A734c} - \text{A734s})}{\text{A734c}}\right] * 100$$

where c and s represent control and sample, respectively.

Hydroxyl (OH) Free Radicals scavenging activity. The hydroxyl radical scavenging activity of the samples was determined as described by Klein et al. [35]. The reaction mixture (1.0 mL) was measured from the mixture of different concentration of samples (2-10 mg/ml), 0.5 mL of 0.018% EDTA, 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulphate 0.26% EDTA), 1.0 mL of DMSO (0.85 % in 0.1 M phosphate buffer pH 7.4) and 0.5 ml of 0.22% ascorbic acid into tubes. The tubes were tightly capped, heated (80-90ºC for 15 min.) in a water bath; and the reaction was terminated by adding 1.0 mL of ice-cold TCA (17.5 %). To the above reaction mixture, 3.0 ml of Nash reagent (75.0 g of ammonium acetate, 3.0 ml of glacial acetic acid and 2.0 ml of acetyl acetone were mixed and distilled water was added to a total volume of 1.0 L); and incubated at room temperature for 15 minutes for color development. The intensity of the yellow color formed was measured at 412 nm against a reagent blank. Ascorbic acid and gallic acid were used as standards.

$$\text{OH radical inhibition (\%)} = \left[\frac{(\text{A412c} - \text{A412s})}{\text{A412c}}\right] * 100$$

Where c and s represent control and sample, respectively.

Determination of Angiotensin Converting Enzyme (ACE) inhibition activity. The ACE-inhibitory activity was determined *in vitro* using spectrophotometric method described by Zhang et al. [36] with slight modifications [37]. This method was based on the liberation of hippuric acid from hippuryl-L-histidyl-L-leucine (Hip-His-Leu) catalyzed by ACE. For the assay, 42.5 µL of the sample solution (2 mg/mL) was pre-incubated at 37 ◦C for 5 min with 10 µL ACE (0.6 mU/mL) enzyme. The mixture, 20 µL of the substrate (5 mM HHL in 10 µM zinc chloride containing 100 mM sodium trizma base and 300 mM NaCl at pH 8.3) was added and incubated (37 ◦C for 60 min). After incubation,

12.5 µL of 5 M HCl. was added to terminate the reaction. The ACE-inhibition (%) was determined by HPLC system with a 486 tunable UV detector. The average value from three determinations at each concentration was used to calculate the ACE-inhibition (%) rate as follows:

$$\text{ACE} - \text{inhibition}\ (\%) = \left[\frac{(\text{A228a} - \text{A228b})}{\text{A228a} - \text{A228c}}\right] * 100$$

where A is the absorbance (Abs) of HA generated in the presence of ACE-inhibitor, B the Abs of HA generated without ACE-inhibitor and C the Abs of HA generated without ACE (corresponding to HHL autolysis in the course of enzymatic assay). The IC50 value (mg/mL) was defined as the concentration of inhibitor required to reduce the HA peak by 50% (corresponding to 50% inhibition of ACE activity). The captopril was used as a positive control for ACE-inhibition.

Alpha-Amylase Inhibition Assay. The activity of α-amylase was determined with reference to starch-iodine colour changes described by Mao and Kinsella [38]. The starch solution was prepared by adding 1.0 g of soluble potato starch to 10 mL distilled water; the mixture was boiled for 2 minutes and cooled. To the cooled mixture, water was added to obtain final volume of 100 mL. α-amylase solution (0.1 mL of 15 μg/mL in 0.1 M acetate buffer at pH 7.2 containing 0.0032 M sodium chloride) was added to a mixture of 3 mL of 1% soluble starch solution and 2 mL of acetate buffer (0.1 M, pH 7.2) pre-equilibrated at 30 ∘C in a water bath. Substrate and α- amylase blank were allowed to incubate at zero time (t=0 min) and at the end of the incubation period (t = 60), 0.1 mL of reaction mixture was withdrawn from each tube after mixing and transferred into 10 mL of an iodine solution (0.254 g iodine and 4.0 g potassium iodide in 1 L). After mixing, the absorbance of the starch-iodine mixture was measured immediately at room temperature at 565 nm using a spectrophotometer. Percentage inhibition was calculated using the equation below.

The samples were diluted as appropriate to establish dose-dependent effects and for calculation IC_{50} values, that is, the concentration of α-glucosidase inhibitor to inhibit 50% of its activity :

$$\text{Inhibition}\ (\%) = \left[(Ao - At)control - \frac{(\text{Ao} - \text{At})sample}{(\text{A0} - \text{At})\text{control} -}\right] * 100$$

Alpha-Glucosidase Inhibition Assay. The α-glucosidase inhibitory activity was determined using modified method of Pistia-Brueggeman and Hollingsworth [39]. In test tubes, sample of varying concentrations (200 μL) was mixed with 500 μL of phosphate buffer (50 mM, pH 6.9), and 100 μL of α-glucosidase (1 U/mL) was pre-incubated at 37 ^{0}C for 5 min. After incubation, 200 μL of 1 mM PNPG (4-nitrophenyl-alpha-D-glucopyranoside) substrate was added to the mixture. The mixture was re-incubated at 37 ^{0}C for 30 min, and 500 μL of Na_2Co_3 (0.1 M) was added to terminate the reaction of the enzyme. The yellow colour produced (due to pnitrophenol formation) by the mixture and blank were read at absorbance of 405 nm. The percentage inhibition was calculated using the formula below:

$$\text{Inhibition}\ (\%) = \left[\frac{(\text{Acontrol} - \text{Asample})}{(\text{Acontrol})}\right] * 100$$

Statistical analysis. Data were analysed and values were expressed as mean of triplicate determinations ±standard error of mean (SEM). The data were subjected to a one-way analysis of variance (ANOVA) and the significant difference between mean values was determined by Duncan's multiple range test (p<0.05) using SPSS (Statistical Package for the Social Sciences) version 13.0. (SPSS Inc., Illinois, USA).

RESULTS AND DISCUSSION

The amino acid compositions of undefatted, defatted, protein isolate and fermented hydrolysate of African locust beans flour samples were analyzed and are presented in Table 1. The results indicated a variation in amino acid compositions in different African locust bean flour samples. The non-essential and essential amino acid compositions (mg/100g protein) ranged between 24.79 in African locust bean hydrolysate (LPH) and 61.62 in undefatted African locust bean (UBL) and 27.82 in UBL and 56.40 in LPH, respectively. The non-essential amino acid composition had glutamic acid (2.08 – 18.78 mg/100g) as the highest, while essential amino acid had leucine the most abundant for UBL, DLB and LPI and histidine for LPH. Valine was the first limiting amino acid for UBL, DLB and LPI, while arginine was limiting in LPH. The present study agreed with the reports that glutamic acid is the most abundant amino acid in plant based foods [40]; and the finding also agreed with the report that African locust bean is rich in protein and essential amino acids necessary for growth and development in children and with protein-energy malnutrition (PEM) ameliorating potentials [41].

The protein quality indices of African locust bean flour samples are presents in Table 2. The protein quality results indicated that protein efficiency ratio (PER), essential amino acid index (EAAI) and biological values (BV) of undefatted, defatted, protein isolate and hydrolysates sample of African locust beans ranged as 2.26 - 3.44, 52.33 - 82.63% and 45.34 - 78.36%, respectively. While that of arginine/lysine ratios and branched chain amino acids (BCAAs) ranged from 0.02 - 8.53 and 13.89 - 16.54, respectively. This study established

TABLE 1
Amino acid profiles (mg/100g protein) of African locust bean flour samples

Parameters	UBL	DLB	LPI	LPH
Non-Essential Amino Acids				
Glycine	2.35^{d}	4.53^{a}	2.46^{c}	3.04^{b}
Alanine	4.60^{b}	3.65^{c}	4.80^{a}	3.66^{c}
Serine	2.53^{c}	4.22^{a}	2.66^{b}	2.26^{d}
Proline	3.14^{d}	4.18^{b}	3.24^{c}	4.57^{a}
Aspartic	15.81^{a}	9.44^{c}	12.08^{b}	4.65^{d}
Cysteine	1.98^{b}	1.23^{c}	2.18^{a}	0.34^{d}
Glutamic	18.78^{a}	16.53^{b}	15.66^{c}	2.08^{d}
Tyrosine	2.11^{d}	2.82^{b}	2.24^{c}	3.75^{a}
Arginine	10.32^{a}	6.41^{c}	8.64^{b}	0.44^{d}
ΣNEAAs	61.62^{a}	53.01^{b}	53.96^{b}	24.79^{c}
Essential amino acids (EAAs)				
Phenylalanine	3.65^{d}	5.25^{b}	3.79^{c}	8.61^{a}
Histidine	2.45^{b}	2.14^{c}	2.53^{b}	7.45^{a}
Methionine	1.11^{b}	1.05^{b}	1.15^{b}	1.54^{a}
Tryptophan	2.02^{a}	1.23^{b}	2.17^{a}	0.39^{d}
Threonine	3.49^{b}	3.64^{b}	4.61^{a}	1.73^{d}
Valine	2.71^{c}	4.60^{b}	2.79^{c}	5.04^{a}
Isoleucine	4.35^{a}	4.06^{b}	4.43^{a}	3.07^{c}
Leucine	6.83^{b}	6.89^{b}	6.91^{b}	8.43^{a}
Lysine	1.21^{c}	5.75^{b}	1.44^{c}	20.14^{a}
ΣEAAs+Histidine	27.82^{d}	34.61^{b}	29.82^{c}	56.40^{a}
1st Limiting Amino acid	Valine	Valine	Valine	Arginine
Abundant Amino acids	Arginine	Tryptophan	Arginine	Histidine

Means with different alphabetical superscripts in the same row are significantly different at P<0.05.
Key: ULB: Undefatted Locust Bean; DLB: Defatted Locust Bean; LPI: Locust Protein Isolate; LPH: Locust Protein Hydrolysate. (ΣNEAAS) Non-essential amino acid, Essential amino acids (ΣEAAs) [57]

that the PER, EAAI and BV of African locust bean protein hydrolysate (LPH) were significantly higher than other flour samples, that is, UBL, DLB and LPI. This finding could be due to the breaking down of large protein molecules in hydrolysate into smaller molecular size peptides, which enhances its bioavailability. This observation agreed with the findings that peptides are more active and biologically available than when trapped in naïve protein [16]. Similarly, total branched chain amino acids, that is, valine, leucine and isoleucine in LPH were higher than that of UBL, LPI and DLB, respectively. Studies have reported that branched chain amino acids are essential in management of type - 2 diabetes [42, 43], hence, African locust bean protein hydrolysate sample (LPH) which high in these amino acids may be suitable as antidiabetic agent. The arginine/lysine ratio was greater than one (>1), and was higher in UBL, LPI and DLB than that of LPH. This indicates that arginine concentration is high in locust bean flour samples; and this may be beneficial in the management of high blood pressure. Tejero et al. [44] established that arginine plays important roles in the production of nitric oxide in the body, which helps in the relaxation of the arteries to enhance easy flow of blood, and thereby reduces the risk of high blood pressure.

The results of analysed phytochemicals in African locust bean samples ranged as follow: 4.50 - 6.30 for oxalate, 6.47 - 13.06 phytate, 150.48 - 400.48 saponin and 0.35 - 0.74 flavonoid, while range values of phenol and tannin were 7.32 - 15.04 and 0.44 - 0.98, respectively (Table 2). The present study showed that saponin had the highest concentration of all analysed phytochemicals in African locust bean flour samples; and that, the phytochemicals in African locust bean flour were similar in composition to soybean [45]. In recent decades, study had reported that phytochemicals, particularly flavonoids, saponin and polyphenol, have strong antioxidant activities and good therapeutic potentials [46].

The antioxidant activities of African locust bean flour are presented in Table 4. The results show that the free radicals scavenging abilities of African locust flour samples in DPPH ranged between 34.27% in ULB and 67.09% in LPH; and for the ABTS, FRAP and Fe^{+2} chelation the values ranged were 3.40 - 6.790 mg/g, 11.91- 28.21 mg/g and 5.81 - 16.15%, respectively. While hydroxyl (OH) free radical ion scavenging potential of the flour samples ranged 47.41 – 85.19%. The presents study established that African locust protein hydrolysates exhibited higher antioxidant and free radical scavenging abilities in DPPH, FRAP, Fe^{+2}

chelation than undefatted, defatted and protein isolate African locust flour samples, whereas, undefatted sample (ULB) had higher antioxidant activities in ABTS and OH free radicals. This finding could be attributed to the lower molecular weight of the peptides in the hydrolysates, which are more active than when they are in native protein, that is, without being hydrolysed. This observation agreed with other findings, who reported that enzymatic hydrolysis of protein enhanced antioxidant capacity, which was related to the release of bioactive peptides [47]. According to García et al. [48], antioxidant activity of proteins depends on the structure, bioavailability and composition of the amino acids. For instance, amino acids with antioxidant potential may be buried within the protein core and thereby inaccessible to pro-oxidants. And except such protein are hydrolysed by enzymes or during food processing to expose the peptides before it can be more active as antioxidant agent. And for the higher antioxidant activity observed in undefatted flour sample (ULB) in ABTS and OH free radicals, it could be as a result of synergistic effects of bioactive compounds in undefatted African locust bean flour. It is well established that antioxidants, that is, chemical substances that inhibit oxidation process by preventing the formation of free radicals play major roles in preventing chronic degenerative diseases like diabetes, hypertension and cancers [49, 50].

TABLE 2
Protein Quality Indices of African Locust Beans Flour Samples

Parameters	UBL	DLB	LPI	LPH
TEAA+His+Arg/TAA%	45.38^{c}	49.26^{b}	48.93^{b}	79.08^{a}
TEAA/TAA%	31.11^{d}	39.50^{b}	35.59^{c}	69.32^{a}
TNEAA/TAA%	68.90^{a}	60.50^{c}	64.41^{b}	30.68^{d}
TSAA(Meth+Cys) (mg/100g protein)	3.09^{b}	2.28^{c}	3.33^{a}	1.88^{d}
ArEAA (Phe+Tyr) (mg/100g protein)	5.76^{d}	8.07^{b}	6.03^{c}	12.36^{a}
TEAA/TNEAA	0.45^{c}	0.65^{a}	0.55^{b}	2.26^{d}
PER	2.26^{c}	2.44^{b}	2.28^{c}	3.44^{a}
EAAI (%)	52.33^{d}	62.31^{b}	56.06^{c}	82.63^{a}
BV (%)	45.34^{d}	56.22^{b}	49.40^{c}	78.36^{a}
Arg/Lysine	8.53^{a}	1.12^{c}	6.00^{b}	0.02^{d}
BCAAs (mg/100g protein)	13.89^{d}	15.55^{b}	14.13^{c}	16.54^{a}

Means with different alphabetical superscripts in the same row are significantly different at P<0.05.
Key: ULB: Undefatted Locust Bean; DLB: Defatted Locust Bean; LPI: Locust Protein Isolate; LPH: Locust Protein Hydrolysate. Total essential amino acids (ΣEAA), Total essential amino acid, Total non- essential amino acids (ΣNEAA), Total sulphur amino acid (ΣSAA), Total Aromatic essential amino acids (ΣArEAA), Essential amino acid index (LAAI), Biological value.

TABLE 3
Phytochemical concentrations (mg/g) of African locust bean flour samples

Samples	ULB	DLB	LPI	LPH
Oxalate	4.50±0.00^{c}	6.30±0.01^{a}	4.52±0.00^{c}	5.42±0.02^{b}
Phytate	6.47±0.03^{c}	13.06±0.02^{a}	7.61±0.01^{b}	6.61±0.00^{b}
Saponin	400.48±0.77^{a}	224.12±0.11^{c}	150.48±0.29^{d}	299.74±0.60^{b}
Flavonoid	0.74±0.01^{a}	0.55±0.05^{b}	0.25±0.08^{c}	0.35±0.02^{c}
Phenol	7.32±0.45^{b}	7.40±0.67^{b}	7.67±0.26^{b}	15.04±0.16^{a}
Tannin	0.44±0.02^{d}	0.98±0.03^{a}	0.62±0.01^{b}	0.54±0.02^{c}

Means with different alphabetical superscripts in the same row are significantly different at P<0.05.
Key: ULB: Undefatted Locust Bean; DLB: Defatted Locust Bean; LPI: Locust Protein Isolate; LPH: Locust Protein Hydrolysate.

TABLE 4
Antioxidant activities of African locust bean flour samples

Samples	ULB	DLB	LPI	LPH
DPPH (%)	34.27±0.16^{d}	42.84±0.08^{c}	41.16±0.15^{b}	67.09±0.10^{a}
ABTS (mg/g)	6.90±0.35^{a}	3.40±0.11^{b}	1.18±0.13^{c}	0.58±0.01^{c}
FRAP (mg/g)	18.18±1.80^{b}	13.82±0.40^{c}	11.91±0.40^{c}	28.21±0.11^{a}
Iron Chelation (%)	16.15±0.11^{b}	4.70±0.51^{d}	5.81±0.13^{c}	34.43±0.05^{a}
OH Radical (%)	85.19±0.23^{a}	47.41±23.72^{a}	76.93±0.70^{a}	47.85±1.40^{a}

Means with different alphabetical superscripts in the same row are significantly different at P<0.05.
Key: ULB: Undefatted Locust Bean; DLB: Defatted Locust Bean; LPI: Locust Protein Isolate; LPH: Locust Protein Hydrolysate.

TABLE 5
α-amylase and α-glucosidase enzyme inhibitory activities of African locust bean flour samples

Sample	Concentration (mg/g)						
	0.04	0.08	0.12	0.16	0.20	IC_{50}	R^2
α – Amylase inhibitory activities (%)							
ULB	36.48^{d}±0.29	40.00^{c}±0.32	41.01^{b}±0.84	42.36^{c}±0.37	43.26^{c}±0.29	0.1641^{a}	0.8858
DLB	39.00^{c}±0.73	41.14^{b}±0.30	43.47^{a}±0.47	46.27^{ab}±0.22	46.37^{b}±0.26	0.1082^{b}	0.9832
LPI	41.29^{b}±0.37	42.38^{b}±0.34	44.32^{a}±0.35	45.29^{b}±0.35	46.28^{b}±0.26	0.1026^{c}	0.9735
LPH	44.63^{a}±0.91	45.26^{a}±0.30	44.90^{a}±0.53	45.59^{a}±0.31	47.26^{a}±0.33	0.0841^{d}	0.9635
α - Glucosidase inhibitory activities (%)							
ULB	62.80^{a}±0.30	64.51^{a}±0.35	65.50^{a}±0.30	69.91^{a}±0.29	70.65^{a}±0.33	0.1458^{a}	0.9347
DLB	62.51^{a}±0.30	62.63^{b}±0.32	63.46^{b}±0.32	63.50^{d}±0.30	64.08^{b}±0.36	0.1270^{b}	0.9417
LPI	61.94^{a}±030	62.50^{b}±0.25	65.03^{a}±0.06	66.76^{c}±0.31	71.37^{a}±0.70	0.1201^{c}	0.8680
LPH	52.65^{b}±0.40	55.16^{c}±0.30	65.30^{a}±0.26	67.70^{b}±0.77	70.47^{a}±0.46	0.1062^{d}	0.9914

Means with different alphabetical superscripts in the same row are significantly different at P<0.05.
Key: ULB: Undefatted Locust Bean; DLB: Defatted Locust Bean; LPI: Locust Protein Isolate; LPH: Locust Protein Hydrolysate.

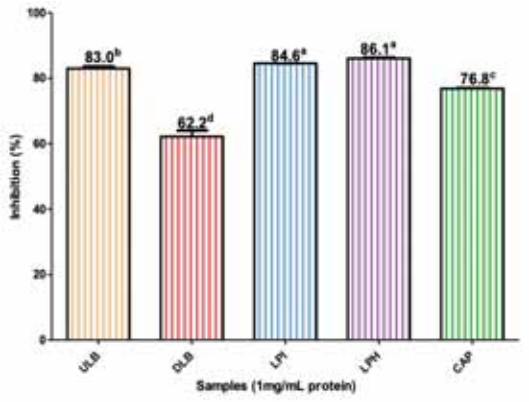

FIGURE 2
Angiotensin Converting Enzyme inhibitory potential of African locust beans (undefatted, defatted, protein isolate and hydrolysate) samples

Means with different alphabetical superscripts in the same row are significantly different at P<0.05.
Key: ULB: Undefatted Locust Bean; DLB: Defatted Locust Bean; LPI: Locust Protein Isolate; LPH: Locust Protein Hydrolysate; CAP: Captopril.

The α-amylase and α-glucosidase enzyme inhibitory activities of African locust bean flour samples are presented in Table 5. The percentage α-amylase enzyme inhibitory activities of African locust bean flour samples were concentration dependent, that is, as the concentration of the flour samples increases the percentage inhibition also increased. The LPH had the highest percentage α-amylase and α-glucosidase enzyme inhibition properties (44.63 - 47.26%), while ULB had the lowest enzyme inhibition properties (36.48 - 43.26%). The inhibitory concentration-50% (IC_{50}) that is, the concentration at which the bioactive agents reduce physiological reactions by 50%, showed that LPH (protein hydrolysate) had the least value, this indicates that the sample had more power to inhibit α-amylase enzyme activity when compared with undefatted, defatted and protein isolate of African locust bean flour samples. Similarly, the α-glucosidase enzyme inhibitory activities of African locust bean flour samples were concentration dependent. For instance, at 0.04 mg/g concentration the percentage enzyme inhibition ranged from 52.65% in LPH to 62.80% ULB, while at 0.20 mg/g concentration the values ranged between 64.08% in DLB and 71.37% in LPI. The IC_{50} of LPH had the least value, this also indicates that the sample had more power to inhibit α-glucosidase enzyme activity when compared with undefatted, defatted and protein isolate of African locust bean flour samples. A higher α-amylase and α-glucosidase enzyme inhibition rate or lower IC_{50} value indicates stronger α-amylase and α-glucosidase enzyme inhibitory activities of the hydrolysate. This present study established that African locust bean flour samples, particularly hydrolysates, had properties to inhibit α-amylase and α-glucosidase enzyme activities, and hence it may be suitable as antidiabetic agent. Recent reports have established that plant bioactive compounds like phytochemicals and proteins have the potentials to inhibit α-amylase and α-glucosidase activities [51]. Inhibition of α-amylase and α-glucosidase contributes to improve symptoms of type-2 diabetes by preventing breakdown of starch, disaccharide and oligosaccharide substrates into absorbable monosaccharaides, and thereby reduce rate of glucose absorption in the gastrointestinal tracts (GIT) [52, 53].

The angiotensin converting enzyme (ACE) inhibitory potential of African locust beans (undefatted, defatted, protein isolate and hydrolysate) samples is shown in Fig. 2. The result showed that African locust bean samples, except DLB, had higher percentage of inhibition on angiotensin converting enzyme than CAP (Captopril, a synthetic antihypertensive agent). However, LPH, a hydrolysate sample, (86.1%) had higher angiotensin converting enzyme inhibitory activities when compared to ULB (83.0%), DLB (62.2%) and LPI (84.1%) samples. This observation may be attributed to hydrolysed peptides smaller molecular weight in

the hydrolysates samples; and this finding agreed with the reports of Ahn et al. [54], Bhat et al. [55] and Aluko [56] who reported that hydrolysate or bioactive peptides have strong inhibitory properties against the conversion of angiotensin I into angiotensin II. This action facilitates vasodilation of the arteries, and thereby lowering blood pressure.

CONCLUSION

This study provided information on amino acid profiles, phytochemical composition, antioxidant activities, inhibitory activities on α-amylase, α-glucosidase and angiotensin converting enzymes of undefatted, defatted, protein isolate and hydrolysate from *P. biglobosa* seed flours. The finding established that *P. biglobosa* protein hydrolysate exhibited high antioxidant activity, percentage inhibitory activities on α-amylase, α-glucosidase and angiotensin-1enzyme than protein isolate and other flour samples. Therefore, *P. bilogbosa* seed protein hydrolysate may be suitable for the production of nutraceutical supplements and in functional foods for the treatment of oxidative stress, diabetes and hypertension.

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