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Research Article

Preparation and Physicochemical Properties of Cucurbit Protein Isolate from *Lagenaria siceraria* Defatted Seed Flour

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Abstract: Cucurbit seeds including *Lagenaria siceraria* seeds have been used from early times as a source of food. The protein constitutes about thirty-five percent of the weight of the decorticated seeds. Isolates are defined as the kind of protein that forms with a high concentration of protein that contains no dietary fiber. This study aims to investigate the preparation of protein isolated from *Lagenaria siceraria* defatted seed flour and to study their physicochemical properties. Protein was isolated from seed flours by using distilled water, salt solution, an alkaline solution, then precipitated with 0.1 N hydrochloric acid followed by centrifugation and freeze-drying. The findings reflected a high protein content (ranged from 80.67-91.04 g $100g^{-1}$) with the absence of fats. The functional properties of the protein isolate are said to be acceptable exhibiting a considerable range for all examined properties, water holding capacity 2.4-3.9 mL g^{-1} protein, fat binding ability 2.7-3.7 mL g^{-1} protein, emulsion capacity 51.0-57.8 mL of oil per g of protein, and bulk density 0.39-0.56 g mL^{-1}, for all samples. The results of foaming stability indicated that the seeds were possibly utilized in food applications such as beverages and cakes processing. The maximum protein solubility (95%) occurred at the alkaline range pH 10 and11. The SDS page test detected that the three major polypeptides are those with molecular weights 10, 23, and 38 KDa. This study is the first to take place on the protein isolation from eight Lagenaria siceraria defatted seeds grown in Sudan, opening the way for further studies on these seeds

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1. Introduction

Bottle gourds are available in two varieties, one is bitter bottle gourds and another is a sweet variety, a useful bottle gourd used as a vegetable or as a medicinal plant. The bitter variety is available as a wild plant and used for pharmacological applications (Shah et al., 2010). The bitterness in such bitter varieties is due to the presence of complex tetracyclic triterpenoid compounds known as cucurbitacin (Chen, et al. 2005). All the parts of *Lagenaria siceraria* involved fruits, leaves, stems, flowers, roots, and seeds have been traditionally used in the ointment for the ailment of various diseases throughout the world (Kumar, et al., 2012). The protein amount of *Lagenaria siceraria* seeds is like that of soybeans and pumpkin seeds and much more than groundnut (Fokou and Tchounguep, 2004). Due to the considerable space between the human populace and protein flexibly, it's necessary to look for and examine unconventional oilseeds as a future protein source (Fagbemi, 2007). Ogunbusola, et al. (2010) studied the composition of protein fractions and amino acids of *Lagenaria siceraria* defatted seed flours and found that the seed flours contained enough essential amino acids for young and adults, so, they suggested that the seeds have the importance as protein added value product in cereal diets or to be a protein source in animal proteins in well-known foods. Protein isolates were produced early in America in the late fifties (Jay and Michael, 2004). It is a high percentage of protein with the benefit of natural and useful qualities making it an ideal crude component to be utilized in refreshments, baby diets and kid's milk food, finished protein items, and specific kinds of functional foods (Olaofe, 1998). Protein isolates of the seeds of *Lagenaria siceraria* and other melons with regards to their functional characteristics have been investigated by many scientists (Ogundele, et al., 2015; Oyeleke, 2012). DeMan, (1999) defined the useful characteristics of proteins that influence the protein found in food frameworks during handling, storage, preparation, and utilization. That can refer to the texture, solubility, viscosity, water holding capacity, and fat binding ability, among different properties. According to their role in food systems, functional characteristics are classified into three groups including properties because of hydration, for example, dissolvability and wettability, properties identified with protein structure, for example, viscosity, and properties identified with protein surface, for example, emulsifying and foaming (Siong, et al., 2011). Ogunbusola, et al. (2013) determined the in-vitro protein digestibility of protein isolate prepared from white melon seed flours they reported the highest digestibility which is due to the removal of protease inhibitors during extraction. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) can determine the protein quantity laying on their molecule weights from 1 to 1,000 kDa. The proteins are typically dissolved in either a non-reducing or reducing buffer (Mohamed et al., 2009).

2. Material and Methods

2.1. Materials

2.1.1. Plant materials

Lagenaria siceraria dry fruits, (8 varieties) four of them are sweet gourds including African Kettle (AK), African Zulu (AZ), Snake Gourd (SG), Water Jug (WJ), and the other four are bitter gourds including Basket Ball (BB), Long Handle Dipper (LHD), Calabash Hybrid (CH) and Curved Dipper (CD) were collected from Omdurman market, Sudan.

2.1.2. Chemicals

All the chemicals utilized in this research are of a high analytical grade and quality. They were purchased either from (Sigma-Aldrich, UK) or (Thermo Fisher Scientific, USA) by the University of Malaya.

2.2. Seed preparation

The seeds were separated from the eight different types of *Lagenaria siceraria* dry fruits using a 4.8 mm knife, then, the obtained seeds were cleaned and washed off any adhering residue and sun-dried for 24 h. The dried seeds were then grounded to flour using a home blender. The grounded seeds were packed in plastic containers and kept in a deep freezer for further investigations.

2.2.1. Preparation of the seed extract

100 grams of the crushed materials of each seed flour were extracted sequentially into 500 ml of n-hexane, and the resulting extracts were evaporated and concentrated under reduced pressure using a Rotary evaporator at 50°C. The extracts were then kept in dark glass bottles for further use (Nyakudya et al., 2013).

2.2.2. Preparation of protein isolate

The protein isolates of eight *Lagenaria siceraria* varieties of defatted seed flour was processed following Adebowale et al. (2009) method. 500 mL of distilled water were added to 50 g of defatted seed flour, and the slurry 1:10 one part of the sample to 10 part of distilled water was stirred continuously with a magnetic stirrer for 2 hours at room temperature. The pH of the blend was controlled to pH 10 by the addition of 1% NaOH to make protein solubility to a maximum at pH 10. The slurry was centrifuged at 1800 rpm for half an hour.

The supernatant was collected into a plastic flask, and the protein in the residue was additionally extracted two times using distilled water. The supernatants were collected, and the pH changed with 1 M HCl to already determine the isoelectric points of the seeds.

The extract was additionally centrifuged at 6 000 rpm in a cold centrifuge at 4°C for a quarter an hour. The supernatant was decanted, and the protein isolate was dialyzed against distilled water for one day and afterward freeze-dried. The protein isolates were kept in airtight polythene bags.

2.2.3. Proximate analysis

Moisture, crude protein, fat, and ash contents were determined according to AOAC (2000). Total carbohydrate was determined by the difference for the total as follows:
total carbohydrate [g $100g^{-1}$] = 100 [g $100g^{-1}$] - moisture [g $100g^{-1}$] - protein [g $100g^{-1}$] - lipids [g $100g^{-1}$] - ash [g $100g^{-1}$]

2.3. Functional properties of protein isolates

2.3.1. Determination of water holding capacity

This was calculated utilizing the method reported by Rodriguez et al. (2005); 100 mg of each sample was blended with 1.0 mL of distilled water utilizing a magnetic stirrer. The protein suspension was then centrifuged at 1 800 rpm for 20 min at room temperature. The supernatant was collected, and the tube was drained at 45 angles for 10 min. Water holding capacity was determined by classifying the volume of water absorbed by the weight of the protein sample.

2.3.2. Fat binding ability (FBA)

Kaushik et al. (2016) method was followed to investigate FBA where a hundred milligrams of protein isolate were vortex-mixed with 1 mL of sunflower oil for 30 seconds. The emulsion was incubated at 25ºC for half an hour and afterward centrifuged at 13 600 rpm for 10 min. The supernatant was decanted and drained at a 45 angle for 20 min. The volume of oil absorbed was separated with the weight of the protein sample, to obtain the fat or oil binding ability of the sample.

2.3.3. Determination of foaming properties

The method of Vani and Zayas, (1995) was used to calculate Foaming Capacity (FC), where two grams of protein isolate were mixed with 50.00 cm^3 clean water in a mixer. The mixture was directly put into a 100.00 cm^3 measuring cylinder. The foam volume was measured directly after mixing. The Foaming Stabilities (FS) of the samples were calculated as a function of time for 0-2 hrs.

2.3.4. Determination of emulsifying properties

The method of Pedrocheet al. (2004) was used to measure the Emulsifying capacity (EC), where one gram of each sample was homogenized at a speed of 160 rpm for 1.0 min at room temperature in 25 cm^3 distilled water. The protein solution was mixed with 25 cm^3 of edible oil followed by homogenization at 160 rpm for 1.0 min and finally, the emulsion was centrifuged for 5 min. Emulsifying capacity was determined by measuring the height of the layer separated in the centrifuge tube, expressed as a percent of the total height of the liquid.

2.3.5. Protein solubility

The method of Klompong et al. (2007) was used, whereby 200 mg of the sample was dispersed in 20 mL of deionized water, and the pH was controlled to 2, 4, 6, 8, 10, and 12 with 1.0 or 0.1 N HCl and 1.0 or 0.1 N NaOH. The mixture was stirred at 20 °C for half an hour and then centrifuged at 7 500 g for 15 min. The protein percentage in the supernatant was calculated using the Bradford kit method (Bradford, 1976). Soluble protein was calculated as g $100g^{-1}$ DW sample.

2.3.6. Determination of in-vitro protein digestibility (IVPD)

The method of Hsu et al. (1977) was followed to determine the IVPD of the studied samples where Multienzyme solution was used. The IVPD was calculated using Hsu et al. (1977): IVPD = 65.66 + 18.1ΔpH 10min.

2.3.7. SDS–PAGE analysis SDS–polyacrylamide gel electrophoresis

Laemli (1970) method was followed to report SDS–PAGE by using a 4% stacking gel and a 15% separating gel. 60 µg of protein concentrate were added to the gel. Proteins were stained with 0.125% Coomassie brilliant blue R-250 and detained with 25% ethanol and 10% acetic acid. Electrophoresis was reported on plates of 100 mm 82 mm 1 mm at 25º C, for one hour.

2.4. Statistical analysis

All tests were run out in triplicate unless otherwise stated; results were reported as means ± SD. Statistical analysis was calculated utilizing a one-way ANOVA with a significance level of $P \leq 0.05$. SPSS for Windows statistical package (v.10.0.6; SPSS, Chicago, IL, USA) was used for analysis.

3. Results and Discussion

3.1. Proximate composition of gourd seed protein isolates

The proximate analysis of protein isolates prepared from eight Sudanese varieties of *Longenara siceraria* seeds is shown in (Table 1).

Table 1. Proximate composition (g $100g^{-1}$) of protein isolate of eight Sudanese *Lagenaria siceraria* gourd seeds (mean ±SD)

Sample	Moisture content	Protein content	Oil content	Ash content	Total CHO
AK	4.10 ± 0.13^{b}	90.32 ± 0.29^{a}	0.57 ± 0.28	2.88 ± 0.27^{c}	2.13 ± 0.08^{d}
AZ	4.96 ± 0.06^{b}	88.22 ± 0.16^{b}	1.41 ± 0.13^{a}	3.79 ± 0.02^{b}	1.62 ± 0.31^{e}
SG	4.00 ± 0.08^{b}	91.04 ± 0.07^{a}	0.68 ± 0.22^{b}	3.09 ± 0.18^{b}	1.19 ± 1.0^{e}
WJ	5.75 ± 0.11^{a}	85.93 ± 0.18^{c}	1.2 ± 0.16^{a}	4.33 ± 0.11^{a}	2.79 ± 0.47^{d}
BB	5.98 ± 0.16^{a}	79.50 ± 0.09^{e}	1.71 ± 0.14^{a}	3.21 ± 0.12^{b}	9.60 ± 0.39^{a}
LHD	5.03 ± 0.12^{a}	83.24 ± 0.19^{d}	1.14 ± 0.17^{a}	4.00 ± 0.17^{a}	6.59 ± 0.25^{b}
CH	5.27 ± 0.09^{a}	86.39 ± 0.20^{c}	1.66 ± 0.14^{a}	2.93 ± 0.15^{c}	4.29 ± 0.33^{c}
CD	5.44 ± 0.23^{a}	80.67 ± 0.15^{e}	0.86 ± 0.20^{b}	3.38 ± 0.17^{b}	9.65 ± 0.43^{a}

*Values are means ± SD of three (n = 3) measurements. Values with different superscript letters within a column indicate significant differences at $p \leq (0.05)$. AK=African Kettle, AZ=African Zulu, SG=Snake Gourd, WJ=Water Jug, BB=Basketball, LHD=Long Handle Dipper, CH=Calabash Hybrid, and CD=Curved Dipper.

The moisture content of the samples ranged between 4.10 g $100g^{-1}$ and 5.98 g $100g^{-1}$. The moisture content of the seed protein isolate depends on the drying conditions after protein isolation. The protein contents of the eight isolates (Table 1) were significantly different ($P \leq 0.05$) from each other, ranging from 80.67 for sample CD to 91.04 g $100g^{-1}$ for sample SG. This variation may be due to the variation in the protein contents of the starting raw gourd seeds (Mariod et al., 2015). On the other hand, the protein of the eight isolates (Table 1) was the predominant constituent, and in the range of protein contents of seed, protein isolates were reported in other works (Ahmed 1998; Wani et al., 2015). The low oil content of the eight protein isolates (0.57-1.66 g $100g^{-1}$) may be due to the defatting process of

gourd seeds prior to the preparation of the protein isolates. The ash content of the gourd seed protein isolates (Table 1) ranged from 2.88 to 4.33 g $100g^{-1}$, the ash content of the isolates reflects the presence of minerals in these products. The carbohydrate content of the eight isolates (Table 1) was significantly variable among the samples and may be inversely related to the protein content of these isolates as found by Ahmed (1998) for *Hibiscus sabdariffa* seed products.

3.2. Functional properties of protein isolate

3.2.1. Water holding capacity

The water holding capacity (WHC) of foods can be defined as the ability to hold its own and added water during the application of forces, pressing centrifugation, or heating (Zayas 1997). Table 2 shows the water holding capacities of eight protein isolates prepared from Sudanese gourd seeds, they ranged from 2.4 to 3.9 mL g^{-1} protein. The lower water holding capacity can be due to less availability of polar amino acids. The quinoa protein showed an absorption capacity of 3.94 mL g^{-1} protein (Elsohaimy et al., 2015), they commented that the good ability of quinoa protein isolates to absorb water encourages its use in bakery products to enhance their functional properties. Referring to the results in Table 2, the water absorption capacity of the isolates may be affected by conformation and environmental factors. Kinsella and Melachouris (1976) related that conformational changes in the protein molecules may expose previously enclosed amino acid side chains, thereby making them available to interact with water. The Sudanese gourd seeds protein isolates in this study are good at absorbing water, they can be used in bakery products to improve their functions.

3.2.2. Fat binding ability

Fat binding ability (FBA) is a very important functional characteristic that contributes to improving the mouth feel while preserving the flavor of the food (Iwe et al., 2016). The fat binding ability of protein isolates of gourd seeds of eight Sudanese varieties are shown in Table 2, they range from 2.7 to 3.7 mL oil g^{-1} protein. These results are higher than that reported by Ashraf et al. (2012) for the fat binding ability of quinoa protein, wheat protein, and soy protein which were 1.88, 1.58, and 2.1 mL g^{-1}, respectively. The FBA is always related to the presence of hydrophilic and polar amino acids on the surface of the protein molecules (Garba, & Kaur, 2014). The variation in FBA of seed proteins because of protein percentage, surface area, hydrophobicity, the solubility of the oil, and the method of analysis utilized, as far as to lay on protein capacity to capture the oil (Wani et al., 2015).

3.2.3. Emulsion capacity

Emulsion capacity (EC) of foods is related to the quantity of oil, non-polar amino acid residues on the surface of the protein, water, and other food ingredients (Godswill, et al. 2019). Emulsion capacity is a crucial factor in deciding which protein to utilize in industrial food processing (Elsohaimy, et al. 2015). The emulsion capacity of the protein isolates in this study (Table 2) ranged from 51.0 for sample CD to 59.0% for sample SG. These results are comparable to those of other works (Tang, 2007; Yust, et al., 2010; Malomo and Aluko, 2015).

3.2.4. Bulk density

Bulk densities of seed protein isolates of eight Sudanese gourd varieties are reported in Table 2. They ranged from 0.39 to 0.56 g mL^{-1}. Freeze dried lentil protein isolate has a bulk density of 0.28 g mL^{-1} (Joshi et al., 2011). A high bulk density value of 0.94 g mL^{-1} was reported by Rajesh and Prakash (2008) for the albumin fraction of lentil. Bulk density depends on particle size. It is also depending on the method of measurement, and surface properties (Iwe et al., 2016).

Table 2. Functional properties of seeds protein isolates of eight Sudanese *Lagenaria siceraria* gourds

Sample	Water holding capacity (g $100g^{-1}$)	Fat binding ability (g $100g^{-1}$)	Emulsion Capacity (mL of oil per g of protein)	Bulk Density (g mL^{-1})
AK	3.7±0.43[a]	3.5±0.72[a]	57.8±0.74[b]	0.51±0.26[b]
AZ	3.5±0.87[a]	3.5±0.61[a]	55.3±0.36[c]	0.47±0.88[c]
SG	3.9±0.90[a]	3.7±0.52[a]	59.0±0.62[a]	0.56±.0.94[a]
WJ	3.2±0.46[b]	3.0±0.66[b]	55.0±0.17[c]	0.45±.92[d]
BB	2.4±0.81[c]	2.8±0.36[b]	51.5±0.82[e]	0.40±0.16[e]
LHD	2.8±0.74[b]	3.0±0.78[b]	51.7±1.0[e]	0.43±0.28[e]
CH	3.0±0.43[b]	3.2±0.33[b]	53.6±076[d]	0.49±0.76[d]
CD	2.4±0.29[c]	2.7±0.66[c]	51.0±0.33[e]	0.39±0.75[e]

*Values are means ± SD of three (n = 3) measurements. Values with different superscript letters within a column indicate significant differences at $p \leq (0.05)$. AK=African Kettle, AZ=African Zulu, SG=Snake Gourd, WJ=Water Jug, BB=Basketball, LHD=Long Handle Dipper, CH=Calabash Hybrid and CD=Curved Dipper.

3.2.5. Foaming stability

Table 3 shows values of foaming stability as a function of time for protein isolates provided from gourd seeds. Foam stability results are expressed in terms of foam height measured in milliliter (mL), for every sample foam stability corresponding to a time interval (from 0.00 to 120.00 minutes) was calculated. Foaming stability for the AK sample was ranged from 67.0±0.74 in zero time to 58.5±0.28, 57.5±0.33, 56.0±0.80, and 54.5±0.26 after 30, 60, 90, 120 min, respectively (Table 3). The difference between 0.00 to 120.00 min with respect to foam height (mL) for AK, AZ, SG, WJ, BB, LHD, CH, and CD samples was as follows: 12.5, 12.0, 12.0, 9.0, 6.0, 7.5, 10.5 and 6.0 mL, respectively, these results may indicate good foam stability for the samples. Foaming stability was ranged from more than 65% at zero time to more than 55% after 60 min for AK, AZ, and SG. These results were found less than foaming stability for quinoa protein that was ranged from 83.55 as zero time to 54.54% after 60 min. The result of the high ability of quinoa protein to form foam with high stability qualifies it for use in food processing (Elsohaimy et al., 2015). Further, Wani et al. (2015) commented that the formation of foam is much needed in food processing techniques such as syrups, beverages, cakes, and whipped toppings.

Egg albumin is an excellent foaming agent which is considered the standard reference, its foaming capacity, and foaming stability were reported in the literature (Lomakina and Mikova, 2006). So, we can say that the protein isolates from gourd seeds had the ability to make foam less than egg albumin but showed foam stability like it.

3.2.6. Protein solubility

Good protein solubility is important to use a product in manufacturing foods. The improvement of other functional properties is essential, underpinning the appropriate initial solubility of proteins (Yust et al., 2010). Figure 1. shows the protein solubility profiles of eight protein isolates prepared from gourd seeds. SG, AK, AZ, and WJ samples have very low solubility between pH 4 and 5 (the approximate isoelectric point for the proteins). Samples CD and CH have 25-30% solubility at pH 4 and 5. An increase in protein solubility is remarkable at pH 2 (Acidic range) for all the samples, but the maximum solubility (≈95%) occurred at pH 10 and 11 (alkaline range). The same solubility examples were shown in other seed protein isolates (Tang, 2007; Yust et al., 2010; Malomo and Aluko, 2015; and Wani et al., 2015). Protein solubility at different pH values shows the importance of the protein isolates to be applied to different food systems.

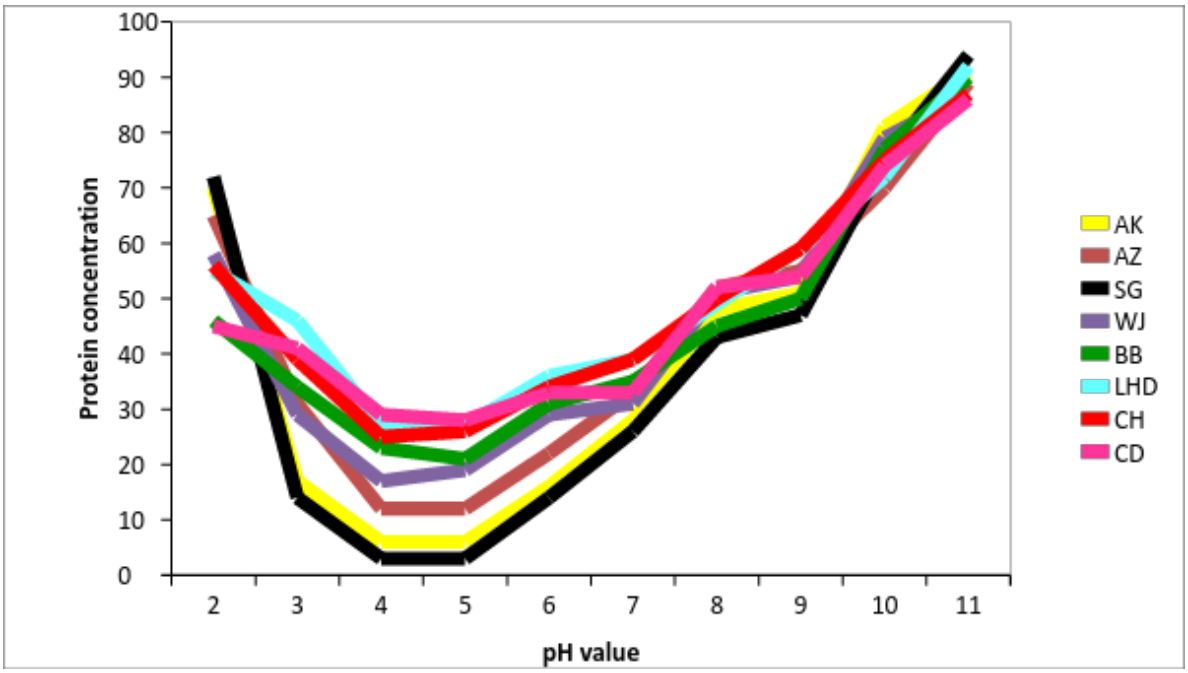

Fig. 1. Protein solubility of eight Lagenaria siceraria seeds flour protein isolate. AK=African Kettle, AZ=African Zulu, SG=Snake Gourd, WJ=Water Jug, BB=Basket Ball, LHD=Long Handle Dipper, CH=Calabash Hybrid, and CD=Curved Dipper.

Table 3. Foaming stability as a function of time for *Lagenaria siceraria* seeds flour protein isolate

Time (min)	Foam height (mL) of the seed flours Protein isolates							
	AK	AZ	SG	WJ	BB	LHD	CH	CD
0.00	67.0±0.74^{b}	65.0±0.36^{a}	68.0±0.62^{a}	60.5±0.17^{a}	56.5±0.82^{a}	59.0±1.0^{a}	62.5±076^{a}	57±0.33^{a}
0.25	65.0±0.46^{a}	63.0±0.66^{b}	66.5±0.74^{b}	58.0±0.90^{b}	53.5±0.59^{b}	57.0±0.8^{b}	60.0±0.6^{b}	54.5±0.1^{b}
0.50	63.5±0.58^{c}	62.0±0.59^{c}	64.5±0.33^{c}	55.5±0.44^{c}	52.5±0.62^{c}	56.0±0.5^{c}	58.5±0.1^{c}	53.5±0.6^{c}
1.00	62.5±0.36^{c}	61.0±1.50^{d}	63.5±0.49^{d}	55.0±0.66^{c}	51.5±083^{d}	55.5±0.6^{d}	57.5±0.7^{d}	52.5±0.4^{d}
1.50	62.5±0.77^{c}	61.0±0.55^{d}	63.5±0.71^{d}	55.0±1.10^{c}	51.5±0.47^{d}	55.5±0.9^{d}	57.5±0.2^{d}	52.5±0.4^{d}
2.00	62.5±0.66^{c}	61.0±0.66^{d}	63.5±0.47^{d}	55.0±0.69^{c}	51.5±0.4^{d}	55.5±0.1^{d}	57.5±0.6^{d}	52.5±0.6^{d}
15.00	60.0±0.64^{d}	59.5±0.15^{d}	60.5±0.76^{e}	53.0±0.85^{d}	51.0±0.66^{d}	53.0±0.6^{e}	55.0±0.7^{e}	52.5±0.9^{d}
30.00	58.5±0.28^{e}	59.5±2.00^{d}	59.0±0.29^{e}	52.0±0.77^{d}	50.5±0.57^{e}	52.0±0.4^{f}	54.5±0.6^{f}	52.0±0.4^{d}
60.00	57.5±0.33^{e}	56.5±0.95^{e}	58.5±0.18^{e}	51.5±0.33^{e}	50.5±0.8^{e}	51.0±0.3^{g}	53.0±0.5^{f}	52.0±0.8^{d}
90.00	56.0±0.80^{e}	54.5±0.37^{f}	57.0±1.30^{f}	51.5±0.63^{e}	50.5±0.77^{e}	51.5±0.7^{g}	52.0±03^{g}	51.0±0.6^{e}
120.00	54.5±0.26	53.0±0.88	56±.0.94	51.5±.92^{e}	50.5±0.16^{e}	51.5±0.2^{g}	52.0±0.7^{g}	51.0±0.7^{e}

*Values are means ± SD of three (n = 3) measurements. Values with different superscript letters within a row indicate significant differences at $p \leq (0.05)$. AK=African Kettle, AZ=African Zulu, SG=Snake Gourd, WJ=Water Jug, BB=Basketball, LHD=Long Handle Dipper, CH=Calabash Hybrid and CD=Curved Dipper.

3.2.7. In vitro protein digestibility

In vitro protein digestibility is rapid and inexpensive compared to animal assays, in vitro assays are suitable for routine monitoring of the nutritional quality of protein foods (Ahmed, 1998). The in vitro protein digestibility of eight gourd seed protein isolates is AK 94.78, AZ 93.66, SG 95.35, WJ 92.96, BB 92.03, LHD 92.24, CH 93.11, CD 91.86, these values ranged from 91.86 for sample CD to 95.35% for the sample SG. The in vitro protein digestibility of the eight samples is high compared to quinoa seed protein isolate which was 78.37% (Elshohaimy et al., 2015), and compared to in vitro protein digestibility of *Hibiscus sabdariffa* seed protein isolate (87.09%) as reported by Ahmed (1998). High in vitro digestibility indicates high susceptibility of the protein to the proteolytic enzymes used and a high percentage of readily digested protein. The presence of trypsin and chymotrypsin amino acids, which act as inhibitors, in addition to the nature of the structure of *Lagenaria siceraria* seed proteins, undoubtedly, all of them limit the work of digestive enzymes, which affects protein digestion (Clemente, et al. 2019).

3.2.8. SDS-PAGE profiles

The SDS-PAGE profiles of protein constituents of the eight protein isolates are shown in Fig 3. The electrophoretic pattern of the eight samples was almost identical, with some minor variation among the samples. Four major polypeptides with molecular weights 10, 23, 38, and 47 KDa were identified in samples AK, AZ, WJ, LHD, and CD. On the other hand, three major polypeptides with molecular weights of 10, 23, and 38 KDa were identified in samples SG, BB, and CH. The protein band with MW 23 KDa in samples AZ and WJ has higher intensity, indicating the high concentration of the polypeptide in the mentioned samples. The disappearance of protein components of high MW (>70 KDa) was observed in the eight samples (Fig. 2). Chango et al. (1995) stated that the increase in heating temperature, used for coagulation of soluble lupin proteins, resulted in the removal of high molecular weight proteins.

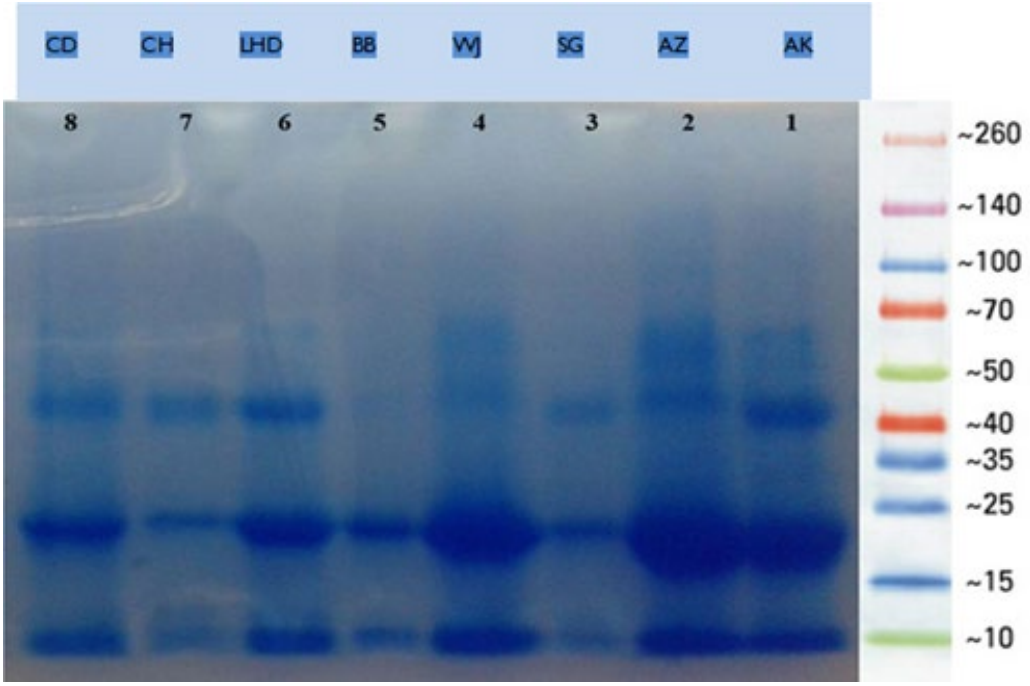

Figure 2. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of eight *Lagenaria siceraria* Seeds flour protein isolate. AK=African Kettle, AZ=African Zulu, SG=Snake Gourd, WJ=Water Jug, BB=Basket Ball, LHD=Long Handle Dipper, CH=Calabash Hybrid, and CD=Curved Dipper.

4. Conclusion

The protein isolates prepared from bottle gourd seeds are of good functional properties, such as water holding capacity, fat binding ability, foaming stability, protein solubility, and emulsion capacity. These findings gave basic information on the nutritional and potentiality of these isolates and achieving the hypothesis of the study and ensuring that they can be utilized as supplemented materials in food products to enhance their nutritional values especially for improving protein content and quality.

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