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Effects of Extraction Media on Protein Isolates of Some Gourd Melon (Egusi) Seeds

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Abstract: Protein isolates of *Citrullus colocynthis*, *Citrullus vulgaris*, *Lageneria siceraria I* (African Wine Kettle gourd), *Lagenaria siceraria II* (Basket Ball gourd) and *Lageneria siceraria III* (Bushel Giant Gourd) melon seeds were prepared using sodium sulphite extraction followed by precipitation at the isoelectric points of the seeds. Proximate composition of the isolates were determined using standard methods. Protein contents (%) of the protein isolates are relatively high, with values: 89.04, 89.44, 93.96, 90.42 and 90.63 (%) for *Citrullus colocynthis*, *Citrullus vulgaris*, *Lageneria siceraria I*, *Lagenaria siceraria II* and *Lageneria siceraria III* melon seeds protein isolates, respectively. The fat, fibre and carbohydrate contents of the protein isolates are however, relatively low.

Key words: Sodium sulphite, extraction media, protein content, gourd melon (egusi) seeds

INTRODUCTION

Plant proteins are an abundant and relatively inexpensive source of protein that is widely recognized due to their nutritional values and excellent physicochemical properties (Issoufou *et al*., 2010). The most commonly used method for preparing plant protein concentrates or isolates is alkali extraction followed by precipitation of the extracted protein either by decreasing the pH to the iso-electric point or by heating (Aremu *et al*., 2007). Alkalis such as Sodium hydroxide and sodium sulphite are of interest in protein isolate extraction. Sodium sulfite (Na_2SO_3) are allowed as food ingredients in the U.S and may be used as a food preservative. These substances are considered as GRAS (Generally Recognized as Safe) (Timbo *et al*., 2004). There is a growing interest in the utilization of plant proteins for the formulation of new food products. The varieties of products that can be obtained from soy-proteins, oil seeds, *Adenopus breviflorus benth* seed flour, lima bean flour and African yam bean are now good indications of the possible uses of foods of plant source to overcome the acute food shortage of animal protein (Ige *et al*., 1984; Oshodi, 1992; Oshodi and Adeladun, 1983; Oshodi, 1997). Gourd seeds (Cucurbitaceae) are versatile and include hundreds of species of vine bearing coiled climbing tendrils and some of the most unusual fruits in the world (Wayne, 1996). The gourd seeds for this work: *Citrullus colocynthis*, *Citrullus vulgaris*, *Lageneria sicerararia I*, *Lagenaria siceraria II* and *Lageneria siceraria III* are melons and are consumed by rural dwellers in the Southern part of Nigeria among the Yorubas as soup thickeners (Ogundele and Oshodi, 2010). These gourd seeds are underutilized. However, their flours and protein isolates have been reported to contain relatively high protein contents (Ogundele and Oshodi, 2010; Ogundele *et al*., 2013a). Ogunbusola *et al*. (2010) reported on the amino acid and protein fraction of the bottle gourd variety of *Lageneria siceraria*. This research work is therefore on the impact of sodium sulphite as an extraction medium on the protein isolate of these seeds.

MATERIALS AND METHODS

Samples: The seeds used in this work are gourd melon seeds. The names of the seeds are listed in Table 1 stating their local names, English names and scientific names (Omotoye, 1984). Seeds of Samples 1 to 3 were bought from markets in Akure, Ondo state while samples 3 to 5 were brought from Ilora town, Oyo State, Nigeria. The seeds were identified at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. Part of the seeds were planted and the fruits harvested after three months (Fig. 1-5). The harvested seeds were dehulled, dried, milled into fine flour, kept in polythene containers and labeled appropriately.

Preparation of protein isolate 1 with sodium hydroxide (PI_1): The protein isolates of the seeds were prepared according to Adebowale *et al*. (2009) with little modification. Portion of the flour samples were defatted in a soxhelet's apparatus with n-hexane for 9 h. A slurry, 1:10 of the defatted flour to distilled water was stirred

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continuously with a magnetic stirrer (Gulfex Medical and Scientific, England) for 2 h. The pH of the slurry was adjusted with 1% sodium hydroxide to around pH10 for maximum solubulization. The slurry was centrifuged at 1800 rpm for 30 min. Decanting the supernatant into a clean plastic container, the residue protein in the sample cake was further extracted twice with half the initial volume of distilled water. The three supernatant portions were pulled together and the pH adjusted with 1.0 M HCl to the predetermined iso-electric point of the gourd melon seeds to precipitate the soluble protein in the solution. The extract was further centrifuged at 6,000 rpm in a refrigerated centrifuge (Centurion Scientific Limited) at 4°C for 15 min. The supernatant was decanted and the protein isolate dialyzed against distilled water for 24 h and then freeze dried in a lyophilizer. The Protein isolates of the seeds were kept in air-tight polythene bags and labeled. *Citrullus colocynthis* Protein isolates 1 ($CcPI_1$), *Citrullus vulgaris* Protein isolates 1 ($CvPI_1$), *Lageneria siceraria I* Protein isolates 1 ($LsIPI_1$), *Lageneria siceraria II* Protein isolates 1 ($LsIIPI_1$) and *Lageneria siceraria III* Protein isolates 1 ($LsIIIPI_1$) (Ogundele *et al*., 2013b).

Preparation of protein isolate 2 with sodium sulphite (PI_2): Protein isolate 2 (PI_2) was prepared in the same way as PI_1 using 0.25% sodium sulphite solution instead of distilled water.

Determination of proximate composition: Standard procedures recommended by Association of Official Analytical Chemists were used for sample treatment and analysis (AOAC, 1990). The fat content (FC) was determined using solvent extraction method with n-hexane (b.p. 40-60°C) in a soxhlet extractor. The moisture content (MC) was determined using air oven as weight difference after oven-drying for 4-5 h at 105°C. Crude Protein (CP) was determined by Kjeldahal's method as N x 6.25. Total ash Content (TAC) was determined by weight difference after incinerating a known weight to ash in a muffle furnace. The Crude Fibre (CF) was determined according to Pearson (1981). Carbohydrate was determined by difference (100-OFC, MC, CP, TA and CF). The Proximate analysis was carried out in replicates and the results are in percent dry weight of flour (Ogundele and Oshodi, 2010).

Statistical analysis: One way analysis of Variance (ANOVA) and least significance difference (LSD) were carried out on the data generated using SPSS 15 and 18 packages. The results are expressed as mean±standard deviation. Duncan was also used to determine values that are significantly different with $p \leq 0.05$ (Ogundele and Oshodi, 2010).

RESULTS AND DISCUSSION

Table 2 and 3 show the proximate composition of protein isolates extracted with sodium hydroxide and sodium sulphite, respectively. In comparison, the protein contents of Protein isolates 1 (extracted with only sodium hydroxide) ranged from 88.19 (*C. colocynthis*) to 90.91% for *L. siceraria I* [AWG]) (Table 2) (Ogundele *et al*., 2013a) while the protein contents of protein isolates 2, (extracted with sodium sulphite) of the gourd seeds in Table 3, ranges from 89.04 (*Citrullus colocynthis*) to 93.96% (*Lageneria siceraria I* [AWG]) protein isolates, respectively. Extracting the protein with sodium sulphite gives protein isolates with slightly higher protein contents than those extracted with only sodium hydroxide. These results are similar to the report made for protein isolates from chickpea (*Cicer arietium* L.) by Sanchez-Vioque *et al*. (1998). The values of the crude protein (%) of the raw flours for *Citrullus colocynthis, C. vulgaris, Lageneria siceraria I* (AWK), *L. siceraria siceraria II* (BBG) and *L. siceraria III* (BGG) seed flours are 24.37±2.13, 32.96±2.53, 34.64±0.08, 27.71±0.41 and 32.70±0.03, respectively (Ogundele *et al*., 2012; Ogundele and Oshodi, 2010). There is therefore very

Fig. 1: *Citrullus colocynthis* fruit

Fig. 2: *Citrullus vulgaris* fruits with leaves

Table 1: Names of varieties of gourd melon seed samples

S/N	Local name	English name	Scientific name	Abbreviation
1	Egusi Bara	Melon	*Citrullus colocynthis*	*C.c*
2	Egusi Sofin	Melon	*Citrullus vulgaris*	*C.v*
3	Egusi Akeregbe	African wine kettle melon (AWK)	*Lageneria siceraria I*	*L.s.I*
4	Egusi Ademu	Basketball gourd melon (BBG)	*Lageneria siceraria II*	*L.s.II*
5	Egusi igbaje	Bushel gourd melon (BGG)	*Lageneria siceraria III*	*L.s.III*

Table 2: Proximate composition (%) of protein isolates 1 (extracted with only sodium hydroxide)

	Sample				
Parameter	*C. colocynthis*	*C. vulgaris*	*L. siceraria I*	*L. siceraria II*	*L. siceraria III*
Protein	88.19±1.20^{a}	89.34±1.91^{a}	90.91±3.0^{a}	88.62±1.95^{a}	89.14±5.58^{a}
Fat	1.14±1.41^{a}	1.67±2.89^{a}	0.65±1.13^{a}	1.03±0.79^{a}	0.84±1.45^{a}
Moisture	4.81±0.46^{a}	3.73±2.23^{a}	5.13±0.22^{a}	5.13±0.14^{a}	5.02±1.13^{a}
Ash	4.70±0.92^{a}	4.84±1.17^{a}	3.29±1.16^{a}	4.24±1.73^{a}	4.54±2.25^{a}
Carbohydrate	1.14±0.47^{a}	0.41±0.54^{a}	0.02±0.57^{a}	0.98±0.48^{a}	0.46±1.46^{a}

Values with different superscripts on the same row are significant at ($p \leq 0.05$). Ogundele *et al*. (2013a)

Table 3: Proximate composition (%) of protein isolates 2 (extracted with sodium sulphite)

	Sample				
Parameter	*C. colocynthis*	*C. vulgaris*	*L. siceraria I*	*L. siceraria II*	*L. siceraria III*
Protein	89.04±2.66^{a}	89.44±3.37^{a}	93.96±4.46^{a}	90.42±2.92^{a}	90.63±3.23^{a}
Fat	0.71±0.25^{a}	0.83±1.01^{a}	0.76±1.32^{a}	0.67±0.34^{a}	1.06±1.17^{a}
Moisture	6.21±1.15^{b}	5.43±0.37^{b}	2.45±1.77^{a}	4.46±1.21^{b}	4.70±0.10^{b}
Ash	3.90±0.25^{ab}	4.03±1.48^{ab}	2.30±1.23^{a}	4.11±0.74^{b}	3.15±0.60^{ab}
Carbohydrate	0.14±1.62^{a}	0.26±1.09^{a}	0.92±0.78^{a}	0.34±0.86^{a}	0.75±1.55^{a}

Values with different superscripts on the same row are significant at ($p \leq 0.05$)

Fig. 3: *Lageneria siceraria I* (Africa Wine Kettle Gourd)

Fig. 4: *Lageneria siceraria II* (Basket Ball Gourd)

high increase in the protein contents of the gourd seeds due to protein isolation with sodium hydroxide and sodium sulphite with percentage increase of 162.44 to 261.88% and 171.25 to 265.37%, respectively.

Deshpande (1992) reported that grass pea protein isolate contained 83.3-92.1% protein depending on the solvent used in their preparation. The protein values of the protein isolates of these gourd seeds are comparable with the protein values of protein isolates of varieties of mucuna beans ranging from 92.19 to 95.53%, African yam bean (92.50±0.45%) and higher than the protein contents of protein isolates of chick pea and (78.0±2.10 to 88.10±2.70)% (Adebowale, 2006; Adebowale *et al*., 2009; Sanchez-Vioque, 1998). The high protein contents of *C. colocynthis*, *C. vulgaris*, L. *siceraria I* (AWK), *L. siceraria II* (BBG) and *L. siceraria III* (BGG) protein isolates will go a long way in meeting the protein need of the growing world population, serve as a substitute for animal protein and meet the need as protein supplement in human and animal nutrition.

The fat content in Table 2 of the protein isolates 1 (extracted with only sodium hydroxide), protein isolates 2 (extracted with sodium sulphate) in Table 3 are relatively low, with values ranging from 0.65 to 1.67% and 0.67 to 1.06%, respectively. This is an indication of the effectiveness of the oil extraction (defatting) process.

Fig. 5: *Legeneria siceraria III* (Gaint Bushel Gourd)

There is no appreciable variation in the fat contents of the protein isolates. The fibre content in the protein isolates are relatively lower as presented in Tables 2 and 3. This is possibly due to the fact that reasonable quantities of the crude fibre and carbohydrate in the defatted sample have been hydrolyzed in the process of extracting the protein isolates. This is consistent with the report of Adebowale (2006) on the fibre content of velvet bean protein isolates.

The carbohydrate contents of the protein isolates in Tables 2 and 3 represent the carbohydrates by difference of protein isolates 1 and protein isolates 2, on dry matter weight (Ogundele *et al*., 2010). These are very low with values ranging from 0.02 to 1.14% and 0.14 to 0.92%, respectively. Similar results have been observed for the carbohydrate contents of chickpea protein isolates 1.1% with sodium hydroxide extraction and 3.5% with sodium sulphide extraction (Sanchez-Vioque *et al*., 1998). Hence the degree of elimination of fibre and carbohydrate from the final protein isolates is quite appreciable with 90% removal of carbohydrate on the average, during protein isolation.

Conclusion: These gourd melon seeds, though underutilized, are rich in protein (Ogundele and Oshodi, 2010; Ogundele *et al*., 2012) and produces protein isolates of higher protein contents. Production of protein isolates with sodium sulphite increased the protein contents of the isolates more than when ordinarily extracted with sodium hydroxide. The production of these protein isolates as very good sources of protein and will go a long way in meeting the protein need of the World's increasing population, reducing malnutrition in man and useful in the formulation of animal feeds.

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