Section A-Research paper
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Amino Acid Profile and Biological Value of Aqueous Seed Extract of *Cucumis melo Linn Cucurbitaceae*.

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Abstract*—Malnutrition is one of the most pressing challenges for developing countries. Cucurbitaceae are known for their nutritive and medicinal values. The objective ofthis study is to evaluate the amino acids profile which will prompt the need to develop varieties of protein-rich plant products for use as nutritional supplements. Cucumis melo seeds were bought washed, dried, and milled into flour. Defatted seed flour and protein precipitate were prepared using standard methods by AOAC, and analysed using the Applied Biosystems PTH Amino Acid Analyzer to determine the amino acids contents. The amino acid profile of C. melo seed flour is comparable to that of egg, fish, and beef but have higher contents of arginine, glutamic acid, aspartic acid, methionine, and histidine. The protein efficiency ratio calculated ranged from 2.32 through 4.13 indicating high-quality protein. The total essential amino acids ranged from 41.39-52.7 % and amino acid scores were from 102% to 575% values compared to whole egg. The defatted sample (POP) had lower amino acid contents compared to the whole flour (HW) and the protein recipitate (PP) however, POP significantly ($p<0.05$) higher glutamic acid. There is potential of developing nutraceuticals and meat analogs with the whole-seed flour of Cucumis melo.

Index Terms— ***Cucumis melo, amino acids, supplements, protein biological value.***

I. INTRODUCTION

The importance of protein in the human diet cannot be underestimated. Proteins play important roles in bodybuilding and are responsible for the formation of enzymes, transcription factors, antibodies, neurotransmitters, hormones, and other factors that help to maintain health status [1]. Moreover, functional amino acids, regulate key metabolic pathways that are necessary for maintenance, growth, reproduction, and immunity. These include arginine, cysteine, glutamine, leucine, proline, and tryptophan [2]. The function of proteins goes beyond their nutritional value and role in biological metabolism. There are important in stabilizing food systems, giving structure to emulsions, foams, and gels as well as binding water in foods [3]. Protein diet malnutrition ultimately results in death from illnesses such as kwashiorkor and marasmus. A large proportion of deaths from protein malnutrition is very comman to developing countries [1]. With the average population of the world estimated to exceed 9 billion by 2050, global food insecurity looms. This may present a condition of emergency for Africa which is currently unable to feed its population. It has been expressed and is obvious that conventional protein production from poultry, cattle, and fish may not be sufficient to match the current rate of population increase [1], [3].

Several studies have sought alternative protein sources that are sustainable and have the potential of replacing the conventional sources of proteins. Soy, whey, insect, algae, mycoprotein, and many others have been explored [1], [3]. Furthermore, recent investigations have revealed a shift in consumer perception toward sources of proteins. Consumers have become more aware of the effect of their activities on their environment [4]. Plant-based protein substitutes are now preferred to animal sources (vertebrate) for moral and ethical reasons [5] while consumer acceptability of microbial proteins is still in the developing stage.

Cucumis melo Linn Cucurbitaceae is a perennial crop belonging to *the Cucurbitaceae* family. It is grown in North Africa, India, and the Mediterranean basin. According to Boulanouar *et al* [6], studies have suggested the utilization of the seeds for medicinal purposes especialyin the treatment of diabetes mellitus [7]. The bioactive compound known as cucurbitacin glucoside has been shown to exhibit anti-inflammatory, antipyretic, and antitumor activities as well as cytotoxic and insecticidal effects [7]. However, the crop remains underutilized in food applications. Melons used as vegetables are rich in flavonoids, alkaloids, and bitter principles, which are known to promote healthy living [8], [9] Vitamin E, omega-3 fatty acids, carotenoids, and polyphenols found in C. melo seeds have excellent antioxidant activity. [10]. Cucurbitacins are triterpenoids with an oxygenated tetracyclic triterpene found in the cucurbitaceae family and the genus *cucurmi*s with various structures.

Adam *et al*. [11] previously highlighted the nutritional composition of *Cucumis melo Linn Cucurbitaceae*. The study revealed high contents of carbohydrates, proteins, and fat of 11.89, 27.67, and 52.48%. [12] studied the proximate composition of selected species of the *Cucurbitaceae* family and reported similar observations. The goal of this study

was to emphasize the potential of *Cucumis melo Linn Cucurbitaceae* seed flour protein extracts as a sustainable protein source in food product development by determining their amino acid profile and assessing their biological value and antioxidant characteristics.

II. MATERIALS AND METHODS

A. Freeze-dried whole-seeds

Cucumis melo Linn Cucurbitaceae seeds were purchased at a community market in Biu,and Garkida North Eastern , Nigeria. Other materials include; alcohol (Sigma Aldrich, Germany). The seeds were washed and freeze-dried at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. First, the seeds were dried for 24h at −15 °C and p=12 Pa. Followed by the removal of adsorbed water. To achieve this, the temperature was increased for an additional 24 h to 20 °C and the pressure was lowered to p=1 Pa to produce the freezedried whole seeds. The dried pulverized seed flour (500g) were macerated in 90% Hexane material at room temperature for 72 h. After maceration, the mixture was filtered using amuslin cloth, concentrated in a Rotary evaporator (Bibby, Germany) and further concentrated over a water bath (Karl Kolb, Germany) at 40°C. The resulting product; Hexane extract, The dried mark was then extracted with distilled filtered and freeze dried kept in the refrigerator at 4°C until further use. The freeze-dried whole seeds were treated with solvents resulting in the fractions; defatted flour, protein oil, and protein precipitate according to the process shown in Figure 1.

B. Defatted flour

The seeds (500g) were grounded and defatted by maceration in Hexane for 78 hours. The resultant solution was filtered through Whatman filter paper (No. 1). The filtrate was recovered under pressure in a rotary evaporator to produce protein oil. The residue (defatted portion) was dried in a water bath.

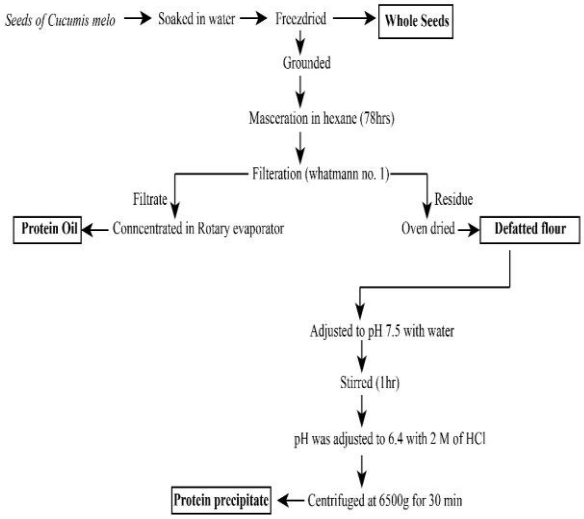

Figure 1 Processing of protein rich fractions of Cucumis melo Linn Cucurbitaceae

C. Protein Precipitate

The protein precipitate was prepared according to the methods previously described with modifications. Briefly, a total of 50g of defatted *Cucumis melo Linn Cucurbitaceae* seeds was mixed with a 15-fold volume of distilled water to get the mixtures and the pH of the mixtures was adjusted to 7.5 [13]. The aqueous protein solution was acidified to a range of pH levels (6.8 to 4.0) [14] with 2M HCl. The solution was stirred for one hour and centrifuged at 2 at 6500g for 30 min. The resultant solution was concentrated in a water bath to produce the protein precipitate.

D. Amino acid profile

As described by [15] with modifications, the samples (HW: whole seed flour, POP: Defatted flour, POL: protein oil, and PP: protein precipitate) were dried at 70°c to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator, and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

E. Protein efficiency ratio (PER)

The protein efficiency ratio (PER) of the amino acid composition was predicted based on three equations described by [16]:

$$PER_1 = -0.684 + 0.456 \times Leu - 0.047 \times Pro \quad (1)$$

$$PER_2 = -0.468 + 0.454 \times Leu - 0.105 \times Tyr \quad (2)$$

$$PER_3 = -1.816 + 0435 \times Met + 0.78 \times Leu + 0.211 \times His - 0.944 \times Tyr \quad (3)$$

F. Amino acid scores

The Amino Acid Scores (AAS) were calculated for adults based on whole hen's egg as recommended by the FAO [17]. In this method described by [18], methionine + cysteine, and phenylalanine + tyrosine as regarded as two distinct units.

$$AAS = \frac{\text{mg amino acid/g of test protein}}{\text{mg amino acid/g of reference protein}} \times 100\%.$$

G. Free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging test

Using a method previously described by [19], the total antioxidant capacity of the three fractions was assessed using the DPPH radical scavenging assay. The assay method is based on 1,1-diphenyl-2-picrylhydrazyl's (DPPH) propensity to discolor when present with antioxidants with scavenging activity. Then, after being incubated for 2, 5, 10, 15, 20, and 30 minutes at room temperature, 100 L of the test extracts at concentrations ranging from 10 to 150 g/mL were combined with 0.1 mL of the DPPH solution (0.2 mg/mL in ethanol). Positive controls included ascorbic acid (Vit. C), a well-known standard with potent antioxidant properties.

H. Free radical (2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic Acid) scavenging test

The method of [20] was modified to determine the fractions' ABTS radical-scavenging capacities. In MQ water, 7.4 mM ABTS and 2.6 mM potassium persulfate were dissolved to create a stock solution. The phosphate-buffered saline (PBS), pH 7.4, was used to dilute the concentrated ABTS stock solution after 16 hours, resulting in an absorbance measurement at 734 nm. Then, 10 L of the examined samples were combined with 990 L of the ABTS radical

solution, with concentrations ranging from 10 to 150 g/mL, and the absorbance was calculated.

I. Ferric Reducing Power Assay

In an acidic condition, antioxidants reduce the ferric ion (Fe^{3+})-ligand complex to the deeply blue ferrous (Fe^{2+}) complex, which is the basis for FRAP (Ferric Reducing Ability of Plasma) [21]. Similar to this, samples with concentrations between 10 and 150 g/mL were employed in a 1:30 test sample to reagent ratio. The following equation was used to calculate the scavenging activity:

J. Statistical analysis

All results were subjected to analysis of variance (ANOVA) using IBM Statistical package for social sciences (SPSS) version 21 to determine statistically significant differences at $p<0.05$. Duncan Multiple Range Test was used to separate the mean values.

Table 1 Essential amino acid profile of lyophilized and protein-rich fractions of *Cucumis melo Linn Cucurbitaceae*.

Amino Acid	Concentration g/100g			
	HW	**PP**	**POL**	**POP**
Leucine	9.64^{a}±0.15	7.63^{b}±0.02	1.25^{d}±0.01	7.01^{c}±0.01
Lysine	6.74^{a}±0.13	4.14^{c}±0.01	0.73^{d}±0.01	4.59^{b}±0.01
Isoleucine	7.09^{a}±0.08	3.88^{b}±0.01	0.65^{d}±0.01	3.58^{c}±0.01
Phenylalanine	10.21^{a}±0.15	4.80^{b}±0.01	1.06^{d}±0.00	4.18^{c}±0.01
Tryptophan	3.45^{a}±0.12	1.04^{b}±0.01	0.26^{c}±0.01	0.94^{b}±0.02
Valine	7.93^{a}±0.47	4.23^{b}±0.03	0.67^{c}±0.00	4.56^{b}±0.01
Methionine	3.33^{a}±0.14	1.74^{b}±0.01	0.38^{d}±0.01	1.67^{b}±0.01
Histidine	5.78^{a}±0.06	4.04^{b}±0.01	0.68^{d}±0.01	3.04^{c}±0.01
Arginine	11.27^{a}±0.51	8.88^{b}±0.02	1.21^{d}±0.01	8.00^{c}±0.01
Tyrosine	7.44^{a}±0.56	3.63^{b}±0.02	0.87^{c}±0.01	3.10^{b}±0.01

Values are mean ± standard deviation of duplicate determinations.
Mean values with different superscripts are statistically significantly different ($p<0.05$).
Keys;
HW: Whole seed; POL: Protein Oil; POP: Defatted flour; PP: Protein precipitate.

Table 2 Nonessential amino acid profile of lyophilized and protein rich fractions of *Cucumis melo Linn Cucurbitaceae*.

Amino Acid	Concentration g/100g			
	HW	**PP**	**POL**	**POP**
Alanine	4.65^{a}±0.25	3.88^{b}±0.01	1.03^{d}±0.01	3.16^{c}±0.01
Glutamic acid	18.17^{b}±0.68	19.18^{a}±0.02	3.19^{d}±0.01	15.29^{c}±0.01
Glycine	4.68^{a}±0.18	4.38^{b}±0.02	1.28^{d}±0.02	3.70^{c}±0.02
Threonine	6.46^{a}±0.15	3.27^{b}±0.04	0.89^{d}±0.01	2.84^{c}±0.01
Serine	5.15^{a}±0.11	4.02^{b}±0.03	1.36^{d}±0.01	3.66^{c}±0.02
Proline	6.71^{a}±0.30	3.97^{b}±0.01	0.62^{d}±0.01	3.05^{c}±0.01
Aspartic acid	13.23^{a}±0.58	8.02^{b}±0.01	2.62^{d}±0.01	7.36^{c}±0.02

Values are mean ± standard deviation of duplicate determinations.
Mean values with different superscripts are statistically significantly different ($p<0.05$).
Keys;
HW: Whole seed; POL: Protein Oil; **POP: Defatted flour**; **PP: Protein precipitate.**

$$Scavanging\ activity\ \% = \frac{A_c - A_s}{A_c} \times 100\%$$

where A_s is the absorbance of the samples at different concentrations, A_c is the absorbance of control. At 50% scavenging (IC_{50}) was the concentration required to scavenge free radicals, which was calculated by plotting the percentage of scavenging activity against the different concentrations of the samples (10–150 µg/ml).

III. RESULTS AND DISCUSSIONS

A. Amino acid composition

The essential and dispensable amino acid contents of *Cucumis melo Linn Cucurbitaceae* seeds are shown in Tables 1 and 2 respectively.

The defatted flour (POP) had significantly ($p<0.05$) lower amino acid content compared to the whole seeds (HW) and

the protein precipitate (PP) indicating the loss of some proteins to the hexane (fat) layer. The protein precipitate, however, had significantly ($p<0.05$) higher glutamic acid content.

The amino acid profile revealed that arginine (essential in infants) was high in all samples. The least amino acid content in g/100g was methionine in the whole seed flour and tryptophan in the protein precipitate, protein oil, and the defatted flour. While the total amino acid profile indicates good quality protein and is comparable to meat and egg, the relatively high content of arginine in the whole seed, protein precipitate, and defatted flour samples suggest suitability in formulating infant food formula.

The amino acid profile of *Cucumis melo Linn Cucurbitaceae* is comparable to that of fish [22], egg [23], and meat [24] proteins. It was observed that *Cucumis melo Linn Cucurbitaceae* whole seed flour has higher contents of arginine, glutamic acid, aspartic acid, methionine, and histidine than egg proteins [23] and in most species of fish reported by [22].

The amino acid profile, biological value and antioxidant properties of whole C. melo, it's defatted flour, protein precipitate and protein oil was determined. It was found that the amino acid profile is rich both essential and nonessential amino acids. The protein quality was found to be high and comparable with meat, whole hen's egg and fish.

Table 3 Amino Acid Scores (AAS) of freezedried and protein rich fractions of *Cucumis melo* Linn *Cucurbitaceae*.

Amino Acid	RAA (mg/g)	Amino Acid Scores (%)			
		POL	POP	PP	HW
Leucine	59.00	21.19	118.81	129.32	163.39
Isoleucine	20.00	32.50	179.00	194.00	354.50
Histidine	15.00	45.33	202.67	269.33	385.33
Lysine	45.00	16.22	102.00	92.00	149.78
Met. + Cys.	22.00	31.36	123.18	131.82	238.18
Threonine	23.00	38.70	123.48	142.17	280.87
Phe.+Tyr.	38.00	50.79	191.58	221.84	464.47
Tryptophan	6.00	43.33	156.67	173.33	575.00
Valine	39.00	17.18	116.92	108.46	203.33
Total	267.00	29.03	136.89	148.16	262.10

RAA – Reference Amino Acid Composition (WHO/FAO, 2007).

Keys;

HW: Whole seed flour; POP: Defatted flour; PP: Protein precipitate; POL: Protein oil

B. Biological Value of *Cucumis melo Linn Cucurbitaceae* Protein-rich Fractions

The Amino Acid Scores (Table 3) revealed that the whole seed, protein precipitate, and defatted flour samples have more than 100% of the egg standard value for all the essential amino acids. In whole-seed flour, tryptophan has the highest score of 575% amino acid scores, while Lysine has the least score of 163%. It was also observed that defatting reduced

50% equivalent of whole egg standard of Phenylalanine and tyrosine while there was a total of 29% equivalent of whole egg protein in terms of essential amino acids found in the hexane layer. This indicates that 29% equivalent of whole egg protein was reduced as a result of defatting the whole flour. The biological value of protein can be estimated by calculating the Protein Efficiency Ratio (PER). The calculation is based on the concentration of either leucine and proline (PER_1); leucine and tyrosine (PER_2); or methionine, leucine, histidine, and tyrosine (PER_3). The result (shown in Table 4) revealed that the (PER) for *Cucumis melo Linn Cucurbitaceae* whole seed flour, defatted flour and protein precipitate as described by [16] ranged from 2.32 through 4.13. Protein efficiency ratios of greater than 2.00 suggest a high-quality value of protein.

Table 4 Protein efficiency ratio of protein rich extracts of *Cucumis melo Linn Cucurbitaceae*

	POP	PP	HW
PER1	2.32	2.65	3.40
PER2	2.39	2.61	3.13
PER3	3.90	4.13	3.16

Keys;

HW: Whole seed; POP: Defatted flour; PP: Protein precipitate.

C. In-vitro Antioxidant Activity Assay

The result of antioxidant activity is shown in table 5. The IC_{50} values represent the concentration of the test sample that can scavenge 50% of the free radical. This implies that, samples with low IC_{50} values will require lower concentrations to scavenge free radicals and vice versa.

All the samples were found to have activities for DPPH with the exception of the protein oil (POL). The defatted has the least IC_{50} value while the whole seed has the highest IC 50 value for DPPH. The protein oil has the least IC_{50} value (699.68 µg/ml) for the Ferric reducing power assay while the whole seeds have the highest IC_{50} value for FRAP. The protein precipitate (PP) has the least IC_{50} value for ABTS while the protein oil has the highest.

Samples have higher IC_{50} values for DPPH and FRAP and low concentrations for ABTS. The observed antioxidant activity shows the activity of functional amino acids in the sample. The observed scavenging ability of the protein oil of *Cucumis melo Linn Cucurbitaceae* suggests the presence of functional free fatty acids.

The extracts showed antioxidant activities with DPPH, FRAP and ABTS with the exception of the protein oil in

DPPH. The antioxidant activity exhibited by the protein oil suggests the presence of functional fatty acids while the whole extract and protein precipitate has high content of functional amino acids viz This is similar to the reports of [25], [26] and includes tryptophan, methionine, histidine, lysine, cysteine, arginine, and tyrosine, which are referred to as antioxidative amino acids. Tryptophan and tyrosine are two examples of amino acids with aromatic side chains rich in electrons that are easily oxidized. Because the pyrrole ring in the tryptophan molecule has five ring atoms (four of which are readily oxidized), The pyrrole ring, which has five carbon atoms and one nitrogen atom sharing six electrons, is what makes the tryptophan molecule sensitive to oxidizing agents [26]. Methionine and cysteine are two examples of sulfur-containing amino acids that are readily oxidized. This is due to the fact that the sulfur atom has two solitary electron pairs and is also considerably oxidized. [26]. The high content of arginine in the extracts of C.melo indicates the suitability in formulating infant foods since it is an essential amino acid for infants. The rich amino acid profile of the extracts *C.melo* also shows its suitability in formulating meat analogs and utilization as a sustainable protein ingredient.

Table 3 Antioxidant capacity assay of lyophilized and protein rich fractions of *Cucumis melo Linn Cucurbitaceae*.

Antioxidant	IC50 (µg/ml)			
Assay	HW	PP	POL	POP
DPPH	1792.24[a]	1659.61[b]	NA	1485.26[c]
FRAP	4070.51[a]	1763.75[b]	699.68[c]	1749.63[b]
ABTS	10.15[b]	3.80[d]	23.016[a]	4.63[c]

Keys;
HW: Whole seed; POP: Defatted flour; PP: Protein precipitate; POL: Protein oil

Conclusion

The amino acid profile, biological value and antioxidant properties of whole *Cucumis melo Linn Cucurbitaceae*, it's defatted flour, protein precipitate and protein oil was determined. It was found that the amino acid profile is rich both essential and nonessential amino acids. The protein quality was found to be
high and comparable with meat, whole hen's egg and fish. The extracts showed antioxidant activities with DPPH, FRAP and ABTS with the exception of the protein oil in DPPH. The antioxidant activity exhibited by the protein oil suggests the presence of functional fatty acids. The high content of arginine in the extracts of *Cucumis melo Linn Cucurbitaceae* indicates the suitability in formulating infant foods since it is an essential amino acid for infants. The rich amino acid profile of the extracts *Cucumis melo Linn Cucurbitaceae* also shows its suitability in formulating meat analogs and utilization as a sustainable protein ingredient.

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