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Full Length Research Paper

In-Vitro protein digestibility, amino acid profile, functional properties and utilization of white melon (*Cucumeropsis mannii*) protein isolates

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Abstract

Protein isolates was extracted from white melon (*Cucumeropsis mannii*) using alkali solubilisation and acid precipitation method. The amino acid composition, *in-vitro* protein digestibility and functional properties of the protein isolates were determined. The protein isolates were used to produce yogurt and ice cream, and the products analyzed for their physicochemical and sensory properties. Amino acid composition of the protein isolates revealed leucine as the most abundant essential amino acid, while limiting amino acids were methionine and cystine. I*n-vitro* protein digestibility of the seed flours ranged from 84.0 to 96.7%. Minimum solubility was observed at pH 4 and solubility increased with pH. The pH, titratable acidity and total solid of yogurt samples produced ranged from 4.23- 4.56, 0.68- 0.72% and 9.01- 9.87% respectively. Yogurt produced by substitution with 25% *Cucumeropsis mannii* protein isolates showed no significant difference in taste, colour and aroma from the control. The pH, specific gravity and over-run of the ice cream mixes ranged from 6.02- 6.20, 43.35- 45.80 and 0.83- 0.99 respectively. Ice cream produced by substituting with 50% *Cucumeropsis mannii* protein isolates showed no significant difference in all sensory qualities examined. White melon protein isolates may find use as functional ingredient in new food products formulation.

Keywords: Protein isolates, white melon, *in- vitro* protein digestibility, functional properties.

INTRODUCTION

Proteins are important in food processing and food product development because they possess desirable functional properties that influence consumer acceptability of food products. These properties include both physicochemical and nutritional properties. Protein solubility, water and oil absorption capacities, foaming capacity and stability, emulsion capacity and stability, viscosity and gelation potentials are some of the functional properties that influence protein quality. Amino acid composition and the ease with which digestive enzymes hydrolyze a food protein are major determinants of food quality (Sze-Tao and Sathe, 2000).

Legumes are noted to be cheap sources of protein and they contribute substantially to the protein intake of a significant proportion of the world population, particularly in the developing countries (Dairo *et al.,* 2007). Unfortunately, most developing countries are not producing enough of these legumes (Aletor and Aladetimi, 1989). Research attention is thus directed towards this area to identify and evaluate underexploited legumes and non-legume food sources such as oilseeds as alternative protein crop (Egbe and Akinyele, 1990). Such utilization would help to maximize available resources and alleviate the problem of malnutrition.

Cucumeropsis mannii is an oil rich seed plant belonging to the Cucurbitaceae. It is native to tropical Africa, where it is grown for food and as a source of edible oil. The seeds can be roasted and eaten as snacks and have found use as thickeners in soups, fermented melon as condiments are widely employed in domestic activities and have high nutritive value (Achu *et al*., 2005). Oilseeds of the cucurbitaceae are good sources of lipid and proteins and the defatted cake is capable of being used as protein supplement in human nutrition

(Chinyere *et al*., 2009). Many plant proteins usually in the form of protein extract are investigated and tested for new products such as low cost fabricated foods which are nutritious and acceptable like conventional foods from meat, fish and dairy products (Chavan *et al*., 2001).

The application of protein isolates in food trade as both protein supplement and functional ingredient are limited to those from whey and soya beans, whereas other protein sources are less used. Among these is protein rich oilseed white melon (*Cucumeropsis mannii*). The objective of this study was to evaluate the *in-vitro* protein digestibility, amino acid composition, functional properties and utilization of the potentials of white melon (*Cucumeropsis mannii*) seed protein isolates in food formulation.

MATERIALS AND METHODS

Collection and preparation of samples

White melon (*Cucumeropsis mannii*) seeds were bought from a farm in Omuo-Ekiti, Ekiti State, Nigeria. The seeds were manually shelled, washed and later dried in a Hot air oven at 50^0C. The seeds were pulverized using a Brabender blender, defatted continuously in a Soxhlet type apparatus for 8 hours using n-hexane solvent. Defatted meals were dried, pulverized and sieved to pass through a 500µm sieve.

Preparation of Protein Isolates

Defatted seed flours were added to distilled water at a meal to solvent ratio of 1:20 w/v. The mixture was stirred with a magnetic stirrer for 10min and the pH of the slurry was adjusted to 9.0 using 0.1M HCl or 0.1M NaOH drop wise. The extraction was allowed to continue for 2hours with constant stirring while the pH is kept constant. The slurry was centrifuged at 4000rpm for 20min. The residues were re-extracted with the same solvent under similar conditions. The supernatants were combined and proteins precipitated by adjusting the pH to 4.0 (pH of minimum solubility) with 0.1M HCl, followed by separation by centrifugation at 4000rpm for 20 min. The residue (proteins) was dispersed in distilled water, poured in dialysis tubing (Brooklyn, N.Y.11311, USA), and dialyzed against distilled water for 18 hours at room temperature. The dialyzing water was replaced at intervals of 3hours during the period of dialysis. The dialysate was freeze dehydrated using freeze dryer and later stored in an air-tight container in the deep freezer for future analysis.

Determination of *In-vitro* Multienzyme Protein Digestibility (IVPD)

The IVPD of the seed flours was determined following the procedure of Hsu *et al.* (1977) where Multienzyme solution was used. The enzymes used porcine pancreatic trypsin (ZF.93615.0025), bovine pancreatic chymotrypsin (ZF.27270) and porcine intestinal peptidases (Z.F.77163.0500) were purchased from Zefa lab service, GMBH Germany. The activity of the enzymes was initially determined before use by using them to digest casein. Full fat samples and protein isolates from White melon (*Cucumeropsis mannii*) were ground into fine powder and sieved. Each of the samples was dissolved in 50ml distilled water to give sample suspension of 6.25mg protein/ml. Each sample suspension was adjusted to pH 8.0 and incubated in water bath at 37^0C with constant stirring. Fresh Multienzyme solution was prepared to contain 1.6mg trypsin, 3.1mg chymotrypsin and 1.4mg peptidase dissolved in 1ml distilled water. The pH of enzyme solution was maintained at 8.0. Five millimeter (5ml) of the multienzyme solution was added to each sample suspension with constant stirring at 37^0C. The pH of each sample suspension was recorded at 10min and 15min respectively after adding the enzyme solution. The IVPD was calculated using the equation of Hsu *et al.* (1977).

Amino acid analysis

The amino acid profile of the protein isolates was determined using the Ion Exchange Chromatography (IEC). The sample was defatted, hydrolyzed and evaporated in a rotatory evaporator then injected into the Technicon sequential multisampling Amino Acid Analyzer (AAS). Tryptophan was determined as reported by Concon (1975) and modified by Ogunsua (1988). The amino acids obtained were used to evaluate the protein quality of the protein isolates. Predicted Biological value (BV) was calculated using regression equation of Morup and Olesen (1976). The predicted protein efficiency ratio (PER) was calculated using one of the equations developed by Alsmeyer *et al*. (1974).

Determination of Functional Properties

The water and oil absorption capacity (WAC, OAC) of the protein isolates were determined by using the procedure of Sathe *et al*. (1982) and the OAC was determined by replacing the distilled water with Executive Chef

Vegetable Oil (0.92g/ml) obtained from Jof Ideal family farm, Owo, Nigeria. The Bulk density (BD) of the seed flours was determined according to the procedure of Narayana and Narasinga Rao (1982), while the protein solubility (PS) of the seed flours was determined using the method described by Ige *et al*. (1982). The effect of pH on protein solubility of the seed flours was determined. The least gelation concentration (LGC) was determined using the method of Coffman and Garcia (1977) with slight modification.

Preparation and Analysis of Yogurt

Dano slim powdered milk (0% fat) Suspension was prepared in hot water at 90^0C. The milk was substituted with *Cucumeropsis mannii* protein isolates at the levels of 25, 50 and 75%. The suspension was cooled to 45^0C and old yogurt from Fan Milk Company (Fan Yogo) was added to introduce the starter culture. The mixture was incubated at 45^0C for about 12 hours and later cooled in the refrigerator for about 24 hours. The Total Solid of the yogurt samples was determined using the method of AOAC (2005), pH was measured using Bench top pH meter (model pH-016A) (Ruck, 1969) and titratable acidity was determined using the method of Ruck (1969). Sensory evaluation was carried out on the yogurt using panel of judges selected from their consistency in scoring and the samples were evaluated for taste, color, aroma and overall acceptability.

Preparation and Analysis of Ice cream

Ice milk base mixes were prepared to contain 16% fat, 16% sugar, with or without 0.5% gelatin and egg white, and 12% milk solid non fat (msnf). The milk solid non fat was substituted with White melon (*Cucumeropsis mannii*) protein isolates at the levels of 25, 50, and 75%. The mixes were homogenized, pasteurized at 90^0C and cooled rapidly to 5^0C, aged for about 12 hours and frozen in vertical batch freezer (Armfield model ATE-6073) and hardened for about 24 hours. The pH, specific gravity (at 20^0C) and overrun of the ice cream mixes were determined. The sensory evaluation of ice cream was carried out and sensory parameters examined were taste, colour, aroma and mouths feel.

Statistical analysis

All determinations were carried out in triplicates and errors were reported as standard deviation from the mean. Results were subjected to ANOVA and means separated by New Duncan Multiple Range Test using SPSS 15 computer programme.

RESULTS AND DISCUSSION

The *in-vitro* protein digestibility of white melon (*Cucumeropsis mannii*) seed flours ranged from 84.0 to 96.7% (Table 1). The protein isolates showed highest digestibility compare with the full fat and defatted flours. It has been reported that the presence of trypsin and chymotrypsin inhibitors and the globular structure of protein lower digestible enzymes activity. Thus, the removal of protease inhibitors during extraction increases the *in-vitro* protein digestibility observed in protein isolates (Richardson, 1991). In addition, seed proteins are denatured during isolation, rendering the protein isolates more accessible to digestive enzymes and improve the hydrolysis (Lynch *et al*., 1977). The *in-vitro* protein digestibility of *Cucumeropsis mannii* protein isolates (96.7%) compared favourably with the range of values (95.6- 96.1%) for chickpea (Sanchez –Vioque *et al*., 1999), but higher than the range of 86.3- 93.9% for lupin protein isolates (Lqari *et al*., 2002). *Cucumeropsis mannii* protein isolates have potential to be used in food system.

The amino acid composition of *Cucumeropsis mannii* protein isolates is shown in Table 2. The most concentrated amino acid in the protein isolates was glutamic acid (143.2mg/g protein) followed by aspartic acid (98.3mg/g protein). The most abundant essential amino acid was leucine (65.9mg/g protein). Glutamic acid and arginine were reported as the most concentrated amino acid and essential amino acid respectively in chickpea protein isolates (Sanchez –Vioque *et al*., 1999). The first and second limiting amino acid in *Cucumeropsis mannii* protein isolates is the sulphur amino acid (Cystine and Methionine) and valine respectively using egg reference amino acid FAO/WHO/UNU (1985). *Cucumeropsis mannii* protein isolates may find use in combination with cereal in weaning food formulation and as a protein supplement in the preparation of complementary diet.

The functional property of white melon (*Cucumeropsis mannii*) protein isolates is presented in Table 3. The water absorption capacity of white melon protein isolates (205.0%g/g) compared favourably with 220%g/g reported for cashew nut protein isolates (Ogunwolu *et al*., 2009) and 212%g/g reported for bitter lupin protein isolates (El-Adawy *et al*., 2001). The value is however lower 610%g/g for sesame protein isolates (Onsaard *et al*., 2010). Aletor *et al*. (2002) reported that water holding capacity of the range of values from 149 to 472.5g/g are considered critical in viscous food such as white melon protein isolates possess good water absorption capacity that can be used in food products requiring high water retention. The oil absorption capacity of white melon protein isolates (315%g/g) compared favourably with 294%g/g in sesame protein isolate (Tomotake *et al*., 2002), and 306.0%g/g in lupin protein isolate (Lqari *et al*., 2002) and

Table 1. *In-Vitro* Protein Digestibility (%) of *Cucumeropsis mannii* Seed Flours

Sample	*In vitro* Protein Digestibility (%)
Full fat	$84.00 \pm 0.30_b$
Defatted	$85.10 \pm 0.45_b$
Protein Isolates	$96.70 \pm 0.30_a$

Values with different subscripts on the same column are significant ($P \leq 0.05$)

Table 2. Amino Acid Composition of White Melon (*Cucumeropsis mannii*) Protein Isolates

Amino acid	Composition (mg/g protein)
Cystine*	11.90 ± 0.10
Methionine*	11.20 ± 0.20
Aspartic acid	98.30 ± 0.30
Threonine*	32.60 ± 0.20
Serine	39.80 ± 0.20
Glutamic acid	143.20 ± 0.20
Proline	32.6 ± 0.20
Glycine	44.10 ± 0.20
Alanine	44.10 ± 0.20
Valine*	36.40 ± 0.20
Isoleucine*	32.00 ± 0.20
Phenylalanine*	55.70 ± 0.30
Lysine*	34.80 ± 0.20
Arginine*	62.10 ± 0.30
Histidine*	27.40 ± 0.20
Leucine*	78.80 ± 0.30
Tyrosine*	33.20 ± 0.20
Tryptophan*	16.70 ± 0.10
Predicted Biological value (BV %)	27.62
Predicted protein efficiency ratio (PER)	3.2
1st limiting a. a	Cystine + Methionine
2nd limiting a. a	Valine

*Essential amino acid

Table 3. Functional Properties of *Cucumeropsis mannii* Protein Isolates

Functional Properties	Composition
Water Absorption Capacity (%g/g)	205.0 ± 5.00
Oil Absorption Capacity (%g/g)	315.0 ± 5.00
Foaming Capacity (%)	30.0 ± 1.00
Foaming Stability (%) (6 hours)	5.0 ± 1.00
Least Gelation Concentration (%g/ml)	18.0 ± 2.00
Emulsion Capacity (%ml/g)	58.0 ± 2.00
Emulsion Stability (5) (2 hours)	44.0 ± 2.00
Protein Solubility in water (%)	8.75 ± 0.30
Bulk Density (g/ml)	0.21 ± 0.01

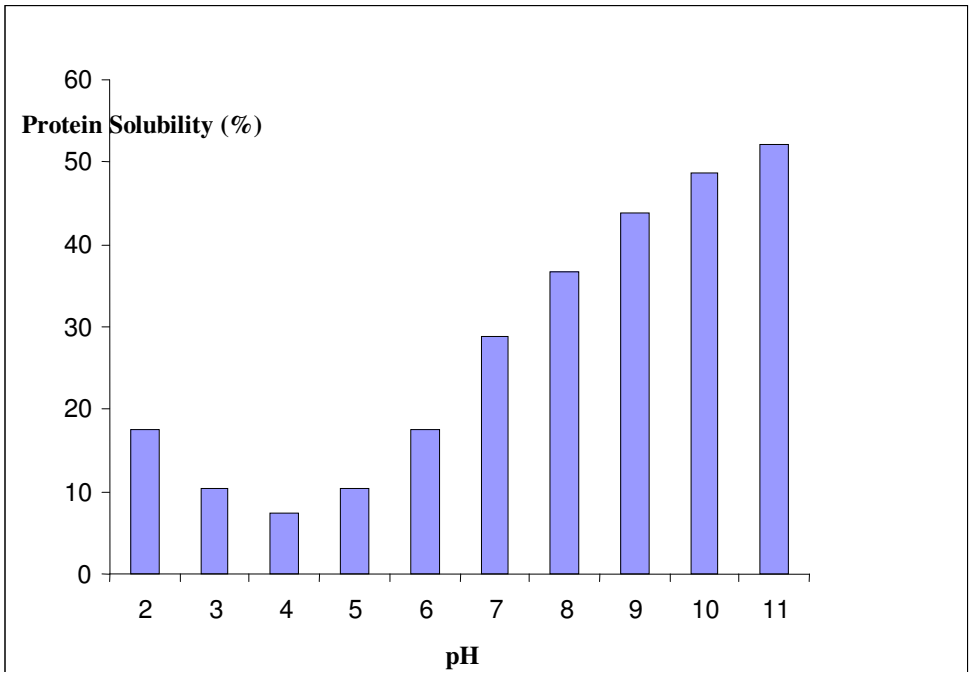

Figure 1. Effect of pH on the protein solubility of Cucumeropsis mannii protein isolates

Table 4. Physicochemical Properties of Yogurt Produced with *Cucumeropsis mannii* Protein Isolates

Yogurt Sample	pH	Titratable Acidity (%)	Total Solid (%)
Control (100% Milk)	$4.52\pm0.05_{ab}$	$0.71\pm0.01_{ab}$	$12..02\pm0.02_{a}$
25%	$4.56\pm0.10_{ab}$	$0.70\pm0.01_{a}$	$9.01\pm0.01_{d}$
50%	$4.30\pm0.05_{c}$	$0.72\pm0.01_{abc}$	$9.58\pm0.02_{c}$
75%	$4.23\pm0.05_{c}$	$0.68\pm0.01_{c}$	$9.87\pm0.03_{b}$

Values with different subscript on the same column are significant ($P \leq 0.05$)

higher than 102.29%g/g in bambara groundnut protein isolate (Eltayeb *et al*., 2011) but lower than 442%g/g reported for cashew nut protein isolates (Ogunwolu *et al*., 2009). Kinsella (1979) reported that the ability of protein to bind fat is very important for such applications as meat replacement and extenders principally because it enhances flavour retention and improve mouth feel. White melon protein isolates may be used as thickener and binder in food system.

Figure 1 shows the effect of pH on the protein solubility of white melon protein isolates. Minimum protein solubility was observed at pH 4, and the solubility increases with increase in pH of minimum solubility. Similar observation was reported for bambara groundnut protein isolate (Eltayeb *et al*., 2011).

The physicochemical properties of yogurt produced from white melon isolates is depicted in Table 4. The pH ranged from 4.23 to 4.56. The pH of yogurt sample substituted with 25% white melon protein isolates is not significantly ($P \leq 0.05$) different from the control (100% milk), however at higher level of substitution with the protein isolate the pH varied significantly. Decrease in pH of yogurt with increase level of protein isolates substitution may be due to increased protein content in the yogurt resulting into increased proteolysis by the proteolytic bacteria causing production of more lactic acid compared with the control. The range of titratable acidity of yogurt sample produced (0.68- 0.72) compared fairly with 0.68- 0.77% titratable acidity reported for low fat probiotic yogurt (Mazloomi *et al*., 2011). The total solid of yogurt produced decreased with increase level of substitution of milk solid non fat with white melon protein isolates. Harwalkar and kalab (1986) reported that non fat yogurt is normally low in total solid (10 to 12%) consequently suffers whey separation. The low solid content reported may be due to non fat dry milk solid (Dano slim with 0% fat) used for the preparation of yogurt samples.

Table 5. Sensory Quality of Yogurt produced with *Cucumeropsis mannii* protein isolates

Sensory Parameters	25%	50%	75%	Control
Taste	$4.20 \pm 2.47_b$	$3.70 \pm 2.91_b$	$4.60 \pm 2.41_b$	$7.00 \pm 1.94_a$
Colour	$6.10 \pm 1.85_{ab}$	$6.00 \pm 1.83_b$	$5.80 \pm 1.93_b$	$7.60 \pm 0.70_a$
Aroma	$5.50 \pm 2.12_{ab}$	$5.30 \pm 1.95_{ab}$	$4.90 \pm 2.42_b$	$7.00 \pm 1.33_a$
Overall acceptability	$5.80 \pm 1.14_b$	$4.49 \pm 2.13_b$	$4.60 \pm 2.55_b$	$7.80 \pm 0.92_a$

Values with different subscripts on the same row are significant ($P \leq 0.05$)

Table 6. Physicochemical Properties of Ice Cream Produced with *Cucumeropsis mannii* Protein Isolates

Ice cream sample	pH	Overrun (%)	Specific gravity
Control (100% milk)	$6.20 \pm 0.04_a$	$48.61 \pm 0.05_a$	$0.79 \pm 0.01_d$
25%	$6.10 \pm 0.03_b$	$45.80 \pm 0.03_b$	$0.83 \pm 0.01_c$
50%	$6.20 \pm 0.03_a$	$44.00 \pm 0.02_c$	$0.86 \pm 0.01_b$
75%	$6.02 \pm 0.02_c$	$43.35 \pm 0.0_d$	$0.99 \pm 0.01_a$

Values with different subscripts on the same column are significant ($P \leq 0.05$)

Table 7. Sensory Qualities of Ice Cream Produced with *Cucumeropsis mannii* Protein Isolates

Sensory Parameters	25%	50%	75%	Control
Taste	$6.90 \pm 2.28_{ab}$	$7.50 \pm 1.51_a$	$5.70 \pm 2.16_b$	$7.00 \pm 1.42_a$
Colour	$6.80 \pm 1.62_a$	$6.10 \pm 2.13_a$	$6.20 \pm 1.81_a$	$6.70 \pm 2.50_a$
Aroma	$6.60 \pm 2.12_a$	$6.20 \pm 2.70_a$	$6.70 \pm 1.89_a$	$7.20 \pm 1.23_a$
Mouth feel	$7.90 \pm 1.20_a$	$7.10 \pm 1.60_{ab}$	$5.00 \pm 2.62_b$	$5.80 \pm 2.97_{ab}$

Values with different subscripts on the same row are significant ($P \leq 0.05$)

White melon protein isolates substituted yogurt showed no significant difference from control in color and aroma at 25% levels of substitution, it however showed noticeable difference in taste and overall acceptability (Table 5). At higher levels of substitution, the products were inferior to the control in all the sensory parameters except 50% substitution that showed no significant difference in aroma from the control. White melon protein isolates may be substituted for milk solid up to 25% in the production of yogurt.

The physicochemical properties of yogurt produced by substituting milk solid non fat with white melon protein isolates are presented in Table 6. The range in pH of the ice cream (6.02- 6.20) compared favorably with the range of value from 5.8 to 6.1 reported for ice milk mixes produced with various levels of substitution with some oil seed protein concentrate (Salama *et al*., 2007). The overrun of 100 milk solid ice cream (control) is significantly higher than all the ice cream samples produced from various levels of substitution with white melon protein isolates. The specific gravity of the ice cream increases with addition of protein isolates. The decrease in overrun and increase in specific gravity of ice cream by increasing level of substitution with protein isolates may be attributed to increment of the mix's viscosity which affects the whipping rate of the mixes (Arbuckle, 1977).

Addition of white melon protein isolates up to 50% level of substitution of milk solid non fat showed no significant differences from the control in all sensory parameter evaluated (Table 7). White melon protein isolates may be used as stabilizer and protein enrichment in ice cream up to 50% substitution. White melon protein isolates may find use in ice cream as stabilizer and nutritional enhancer.

CONCLUSION

White melon protein isolates is mostly concentrated with the essential amino acid and thus may have applications in weaning food formulation. The study has also showed that extraction significantly enhanced the in vitro protein digestibility of white melon seed flours and that the isolates may find use in yogurt and ice cream as stabilizer and protein supplement.

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