

Evaluating the Extracts of Water Melon Rind, *Alayyaho*, *Yakuwa* and *Karikashi* as remedy for Protein Energy Malnutrition

Onuoha Ogbonnaya Gideon^{1,*}, Oyeniyi Patience Fumilayo¹, Adamu Hauwau Zakari², Muhammad Sumayya Thani², Maidawa Chongda Zakmi¹, Adama Isaiah Ufedo¹, Lamido Abubakar Umar¹

¹Department of Food Science and Technology, the Federal Polytechnic, Bauchi, Nigeria

²Department of Nutrition and Dietetics, the Federal Polytechnic, Bauchi, Nigeria

Email address:

reachgideon@yahoo.co.uk (O. O. Gideon)

*Corresponding author

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Abstract: 50% of world hungry people live in the third world countries with the worrisome implication among children under age five as a result of malnutrition. This situation persists because cheaper available sources of quality and quantity protein and energy low-cost foods (leaf vegetables and agricultural waste) and simple technologies are not exploited. Result of yield and mass loss showed loss in mass in the study with Method 3 (with *Tsamiya* as coagulant) having the least value. The results of chemical composition showed energy content; WRE had the highest value (363.19 Kcal) and least value in KKB (309.63 Kcal). Protein content; YIE had the highest value (33g) and least value in AXT (21g) while carbohydrate content WRE and KKB had the highest value (51.6g) and least value in AXT (44.91g/100g). Result of amino acid profile showed eighteen amino acids (which were higher in quantity than their respective RDA values) of which ten were essential amino acids. The first limiting and second limiting amino acids were methionine and isoleucine respectively. The results of the blood analysis showed albumin value ranged from 3.6 - 3.8 (g/dL), Hematocrit parameters (ASA, ALA and creatinine) and all showed no significant difference ($p>0.05$) between the diets and the control. Cholesterol values were within the normal range (<150 mg/dL). Result of food intake showed that the rats fed the control had the highest value (327 g) and least value in YIE (276g). PER of all the diet were higher than 2.7. Weight gained were between 38-44 g; EFU (34% - 48%), NPR (3.2 - 4.5) and TD (82% - 95%) and showed no significant difference with the control diet. The result of the functional properties showed BD was highest in sample AXT (12.5) and least in sample YIE (6.61), WAC was highest in sample AXT (12.5) and least in sample YIE (6.61), OAC was highest in sample AXT (12.5) and least in sample YIE (6.61), MD was highest in sample WRE (89.65) and least in sample KKB (85.07), EC was highest in sample KKB (9.115) and least in sample WRE (3.25), FS was highest in sample AXT (28.27) and least in sample WRE (20.75) and PS was highest in sample KKB (23.96) and least in sample WRE (20.64). This study showed that the concentrates can support growth and development especially in infants.

Keywords: Protein Concentrate, Leafy Vegetables, Leaf Concentrate Technology, *Tsamiya* (Tamarind), Agricultural Waste

1. Introduction

It has been reported that 50% of the world hungry people live in most third world countries [37; 44]. The level of

hunger is most exacerbated by the number of deaths recorded annually as a result of problems emanating from protein energy deficiency related diseases especially among infants and children. Malnutrition according to the UN Children's Fund (UNICEF) constitutes a global "silent emergency,"

killing millions every year. In *The State of the World's Children 1998*, UNICEF urges intensive efforts by governments to counter the scourge, which it also regards as a violation of children's rights [21]. The consequences of this scourge include; varying degrees of brain damage, stunted growth, skin wastages and iron deficiency anemia which causes 20 per cent of post-birth maternal deaths in Africa and Asia [25, 21]. While this condition is caused by poor diet and frequent infections; delayed motor development, impaired cognitive function and poor school performance, which are effects of stunting, are largely irreversible. Many researchers have traced and related the problem of protein energy malnutrition to the economic level or standard of living of people in these third world countries [25; 21]. This regrettable situation persists because some other cheaper remedies have not been exploited. Remedies that make available good sources of quality and quantity protein, vitamins and minerals from alternative cheaper and abundantly available low cost foods and simple technologies.

Leaf concentrate is an extremely nutritious food made by mechanically separating indigestible fiber and soluble anti-nutrients from much of the protein, vitamins and minerals in certain fresh green plant of leaves. It is a good source of beta-carotene, iron and high quality protein. Leaf concentrate is very effective in combating malnutrition especially anemia and vitamin A deficiency which are prevalent among children and pregnant women in most developing countries [41]. The aim of this research is to produce leaf concentrates from four differently abundant leaf crops in Northern parts of Nigeria, assay it for their chemical and identify the functional characteristics (bulk density, water absorption capacity, measure of dispersibility) of the concentrates. Evaluate the protein quality of the concentrates using the Protein Efficiency Ratio (PER) of Wister rats and assess the blood parameters of Wister rats fed the concentrates

2. Materials and Method

2.1. Experimental Design

The Completely randomized design was used. The primary variable considered was the effect of protein concentration. The model for response is

$$Y_{ij} = \mu + T_i + \text{random error}$$

Where;

Y_{ij} = observations for which $X_1 = i$

μ = general location parameter

T_i = effect of having treatment level i

And statistical tests for levels for X_1 was used for a one-way ANOVA

2.2. Materials Required

Three (3) leaf vegetable namely Alayyaho (*Amaranthus hybridus*), Yakuwa (*Hibiscus sabdariffa* Linn), Karikashi (*Sesamum radiatum* Schum) and water melon (*Citrullus lanatus*) rind were used. Three (3) leaf vegetable produced

and consumed in abundance in the Northern part of Nigeria namely *Karikashi*, *Alayyaho*, *Yakuwa*, and one (1) fruit remain water melon rind was used. The leaves were bought from the local market (Muda Lawal Market in Bauchi metropolis). Leaf concentrate were analyzed for proximate, amino acid profile, mineral content, vitamin content and sensory evaluation. The flow diagram (Figure 1) below describes the methodology for production that will be used.

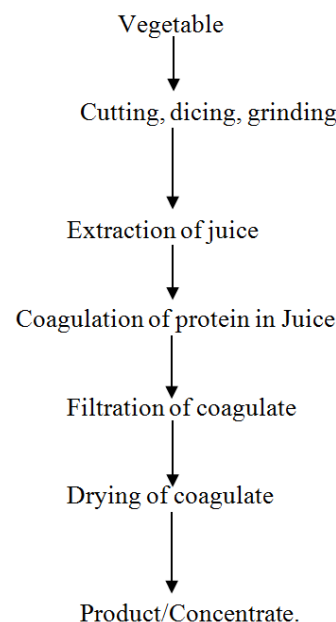


Figure 1. Flow Chart for Leaf Concentrate Production.

2.3. Obtaining of the Protein Concentrate

To obtain the protein concentrate from the leaves, we tested three methods of leaf extraction adopting the methods described by Plummer, (1978) with slight modifications [43]. In the evaluation of the methods, in steps of obtaining the leaf juice 100g of leaves in 1000ml of distilled water resulting in the ratio 1:10 (w/v).

2.3.1. Method 1- Extraction by Isoelectric Precipitation

The method of Cereda and Vilpoux, (2003) was adopted [10]. To 100g of each leaf was added 1000ml of distilled water and then subjected to adjust the pH value to 8.0 with 0.1 N NaOH. It was then milled and allowed to stand. After filtration, the pH of the juice was again corrected to 4.0 with 0.1N HCl. It was then centrifuge with the fabricated 16liter cyclo-centrifuge for 10 minutes at 3200 rpm. The precipitate is sieved out and dried in an oven with air circulating at 60°C to a constant weight.

2.3.2. Method 2- Fermentation of Filter Leaf Juice

The method of Chaves, (1987) was adopted [11]. 100g of each leaf was triturated with distilled water for 5 minutes and then subjected to adjust the pH value to 8.0 with 0.5 N NaOH. The extract was sieved using a muslin cloth. The filtrate was left to ferment naturally in a stainless vessel for 48 hours at room temperature. With the fermentation the pH dropped naturally promoting the separation of the fractions.

Then the solution was centrifuged with the fabricated 16liter cyclo-centrifuge for 10 minutes at 3200 rpm. The precipitate is sieved out and dried in an oven with air circulating at 60°C to a constant weight.

2.3.3. Method 3- The Traditional Method of Using *Tsamiya* (*Tamarindus indica*)

100g of each leaf was added to 1000ml of distilled water. It was then milled and allowed to stand. After filtration, the juice was then cooked and *Tsamiya* was added until coagulants are formed. It was cooled and then centrifuge with the fabricated 16liter cyclo-centrifuge for 10 minutes at 3200 rpm. The precipitate was sieved out and dried in an oven with air circulating at 60°C to a constant weight.

2.4. Determination of % Yield of Extracts

The permanent regime for the calculation of the mass balance

2.4.1. The Extraction Yield (EY) of Protein

The extraction yield (EY) of protein was calculated by the method described by Priscila *et al.*, (2013) was adopted [44].

$$EY (\%) = \frac{CPPC}{CPBE} \times 100$$

Where CPPC = Crude Protein of the protein concentrate mass (g)
and CPBE = Crude Protein present in the beginning of the Extraction mass (g)

2.4.2. The Mass Yield of the Protein Concentrate (MYPC)

The Mass Yield of the protein concentrate (MYPC) was calculated by the method described by Plummer, (1978) was adopted [43].

$$MYPC (\%) = \frac{PCM}{CLM} \times 100$$

Where PCM = Protein Concentrate mass (g) in dry basis
and CLM = Leaf Vegetables mass present in the start of the extraction process (g) on dry weight basis

2.5. Determination of the Chemical Composition of the Extracts

The method of AOAC, (1990) were adopted in the determination of the moisture and fat contents of the meal samples while the method described in AOAC, (1998) were adopted in the determination of crude fiber and protein contents of the meal samples [5, 4]. The method of Ebuehi, *et al.*, (2006) was adopted in the fatty acid analysis and the method described by Miller and Bender, (1955) was adopted in amino acid analysis respectively [15, 33].

2.6. Determination of Chemical Score of Amino Acid

The chemical score of a protein was determined as amino acid profile rated to some standard or reference protein, each amino acid is rated on a scale indicating how much of that amino acid is present compared to the reference protein.

2.7. Determination of the Functional Properties the Weaning Food Formulae

2.7.1. Bulk Density

The method described by Onweluzo and Nwabugwu, (2009) was adopted [39]. A 10 ml capacity graduated measuring cylinder was pre-weighed. The cylinder was then filled gently with the sample. The cylinder was tapped gently several times on the laboratory bench severally until no further diminution of the sample level after filling to the 10 ml mark. It was then weighed. It was then calculated as:

$$\text{Bulk density (g/ml)} = \frac{\text{weight of sample (g)}}{\text{Volume of Sample}}$$

2.7.2. Swelling Power

This was determined by the method described by Leach *et al.*, (1959) with modification for small samples [28]. 1g of the flour sample was mixed with 10 ml distilled water in a centrifuge tube and heated at 80°C for 30 min. This was continually shaken during the heating period. After heating, the suspension was centrifuged at 1000 × g for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as:

$$\text{Swelling power} = \frac{\text{weight of the paste}}{\text{weight of dry flour.}}$$

2.7.3. Determination of Wettability of Sample

The method described by Onweluzo and Nwabugwu, (2009) was adopted [39]. 1g of sample was added into a 25ml graduated cylinder with a diameter of 1cm. And placing a finger over the open end of the cylinder, it was inverted and clamp at a height of 10cm from the surface of a 600ml beaker containing 500ml of distilled water. The finger was removed and the time required for the sample to become completely wet recorded as its wettability.

2.7.4. Determination of Water Absorption Capacity

Water absorption capacity was determined using the method described by Onweluzo and Nwabugwu, (2009) was adopted [39]. 1g of the sample was weighed in a conical graduated centrifuge tube. It was then mix thoroughly with 10ml-distilled water using a warring whirl mixer for 30 seconds. The sample was allowed to stand for 30 minutes at room temperature and then centrifuge at 5000 x g for 30 minutes. The volume of free water (supernatant) was then read directly from the graduated centrifuge tube.

2.7.5. Measurement of Dispersibility

The method described by Balami *et al.*, (2004) was adopted [6]. 50cm³ of distilled water was added to 3g of the sample and stirred to mixture for a minute at room temperature. The mixtures were filtered through dried cheesecloth of known weight then rinse in a beaker with 50cm³ of distilled water and pour through the cheesecloth. The sieve and residue was dried in a hot air oven at 100°C for 10 minutes. The dispersibility was expressed as the percentage of the solids dissolved.

Table 1. Composition (g/100g) of experimental diets.

Ingredients	Diet composition				
	N-free	Egg White	Diet 1	Diet 2	Diet 3
Corn starch	66.5	55.7	56.5	56.5	56.5
Egg White	-	10.8	-	-	-
WRE (31.4%)	-	-	10	-	-
AXT (21.1%)	-	-	-	10	-
YIE (33.23%)	-	-	-	-	10
Glucose	10	10	10	10	10
Sucrose	5	5	5	5	5
Olive oil	10	10	10	10	5
Wheat fiber	5	5	5	5	5
Bone meal	2	2	2	2	2
Vit/premix	1	1	1	1	1
NaCl	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Source: [37]

Key: N-free = Nitrogen free (basal diet); Casein = control; Diet 1 = PSE (15%); Diet 2 = WRE (15%); *100g contains Vitamin A (500,000 IU), Vitamin B1(100mg), vitamin B2 (250mg), Vitamin B6 (100mg), Vitamin E (150mg), Vitamin C (200mg), Vitamin K3 (25mg), Pantothenic acid (200mg), Carnithine HCL (150mg), Folic acid (5mg), Nicotinic acid (300mg), Methionine (750mg), Anhydrous Glucose Q.S (100g).

2.8. Biological Assay

Biological evaluation was done using methods of Onuoha, (2014) with modification [38]. It was done by measuring the protein quality of the extracted formulae into a composite flour and a control namely egg white (protein diet) fed to Wister rats (Table 1). Weaning male Wister rats at 21 days of age was used. The rats were put on stock diet for 7 days prior to the experiment. They were arbitrarily divided into experimental units of 6 rats each and each weighed. The rats were fed the allotted diet *ad libitum* for a period of 21 days. During this period fresh, clean water was made available at all times and room temperature was maintained at 24–27°C. The weight of each replicate was recorded weekly. The faecal matter from each cage was collected daily, dried to a constant weight and stored in glass bottles for nitrogen determination. At the end of 21 days trial, the rats were weighed, then bleed for biochemical analysis and then weighed again before been sacrificed with an overdose of chloroform and their cranial and abdominal cavities will be opened. The nitrogen content of the diet and faeces of each group were determined by Kjeldahl method [5]. Data obtained will be used to determine the protein efficiency ratio (PER) and feed efficiency ratio (FER) [32].

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein consumed}}$$

$$\text{Feed Efficiency Ratio (FER)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

2.9. Biochemical Analysis

2.9.1. Collection of Blood Samples

The Wister rats were anaesthetized with chloroform and bled by cardiac puncture procedure. Portions of whole blood were expressed from each rat into sample bottles containing

EDTA (1mg/ml) for parameters that required the use of whole blood. The remaining blood samples were allowed to clot for 20 minutes, before centrifuging at 3000rpm for 15 minutes in a refrigerated centrifuge, (to obtain serum for parameters determined in sera). Serum was carefully transferred with Pasteur pipettes into clean, dry labeled light-shielded sample bottles and stored frozen until required.

2.9.2. Haematological Parameters

Packed Corpuscular Volume (PCV) and Haemoglobin (Hb) concentration were determined from a portion of the whole blood collected.

2.9.3. The Packed Cell Volume

The method described by Baker *et al.*, (2001) was adopted in the determination of the Packed Cell Volume [7]. The Packed Cell Volume (PCV) was used as a measure of the relative mass of erythrocytes present in the blood of rat samples as an index of protein utilization. 2 cm³ of blood was extracted from the rats and inserted into a centrifuge tube. The tube was placed in a centrifuge and centrifuge at approximately 12000g for 10mins. The PCV was subsequently determined by measuring the height of the erythrocyte column and expressed as a fraction of the height of the total blood column.

$$\text{PCV} = \frac{\text{height of packed cell column}}{\text{height of whole blood column}}$$

2.9.4. Determination of Albumin Content

The method of Doumas, *et al.*, (1971) was used to determine Albumin level in the blood obtained from the experimental rat [13]. 2 ml each of blood samples, a calculated standard sample and blank sample was brought to room temperature. The specimen was pipette into labeled tubes. It was then mixed and allow to stand for 1 min at room temperature. The absorbance of the samples and the standards were read at 630nm against the reagent blank in a colorimeter. Amount of albumin was calculated as:

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times C_{\text{standard}} = \text{g/dL albumin}$$

2.9.5. Serum Alanine Aminotransferase (ALT)

The assay is based on the method of Proll *et al.*, (1998) with slight modification [45]. In labeled test tubes, was added 0.5ml each of ALT buffered substrate solution and incubated at 37°C for 3 minutes. 0.1ml of the different serum samples was added to the tubes labeled 'test,' 0.1ml working pyruvate standard into tube labeled 'standard' and 0.1ml distilled water into tube labeled 'standard blank.' Tube contents were mixed and incubated at 37°C for 60 minutes, after which 0.5ml of 2, 4-DNPH was added to each tube and mixed. All mixtures were allowed to stand at room temperature for exactly 5 minutes. Absorbance of the resultant coloured complex of each mixture was read at 510nm.

The pyruvate formed (mMoles) per minute per litre of serum is the unit of enzyme activity at 37°C given by:

$$\frac{T - TB \times 0.4 \times 1/60 \times 1000}{S - SB \times 0.1}$$

Where: T is the absorbance of test serum

TB is the absorbance of the test blank

S is the absorbance of the standard pyruvate

SB is the absorbance of standard blank

0.4 is the concentration of standard.

60 is the time of incubation in minutes

0.1 is the volume of serum used

1000 is to allow for the expression of enzyme activity per litre of serum.

2.9.6. Serum Aspartate Aminotransferase (AST)

The method of Proll *et al.*, (1998) was used in this assay [45]. The procedure and assay conditions were the same as for ALT assay except that aspartate was used as substrate and the period of incubation was 30 minutes instead of 60 minutes. The oxaloacetate formed (mMoles) per minute per litre of serum is the unit of enzyme activity calculated as follows:

$$\frac{T - TB \times 0.4 \times 1/30 \times 1000}{S - SB \times 0.1}$$

Where all terms are as explained earlier, except that the incubation time in this assay was 30 minutes.

2.9.7. Determination of Creatinine in Serum of Rats Fed the Diets

This assay was achieved by the method described by Thenmozhi and Subramanian (2011) and involved two stages [47]. In the first stage 2ml of each serum was pipetted into labeled test tubes followed by 2 ml distilled water. These were then left to stand for 5 minutes, 3ml 5% sodium tungstate was added followed by 2ml 2/3N H₂SO₄. The mixtures were centrifuged for 15 minutes at 3000rpm.

In the second stage, 3.0ml of the supernatants were pipetted into another set of clean dry test tubes one of which contained 3.0ml standard creatinine (1mg/100ml) and 3.0ml distilled water as blank. To all the mixtures, 1.0ml 0.04M picric acid solution and 1.0ml 0.75M NaOH solution were added and mixed after each addition. These were left at room temperature for 15 minutes and absorbance read at 540nm against the blank.

Calculation

Creatinine in serum of rats fed the experimental diets were calculated as follows:

$$\frac{\text{Absorbance of serum} \times \text{conc. of standard} \times 100}{\text{Absorbance of standard} \times 2}$$

2.9.8. Determination of Total Cholesterol

Cholesterol concentration in serum of rats fed the experimental diets was determined using the CHOD-PAP method of Allain *et al.*, (1974) with slight modification [2]. The determination was achieved using a reagent kit (code 80106) purchased from Biolab Diagnostic, France. The

reagent vials contained after reconstitution, phosphate buffer and chlorophenol (vial R 1), cholesterol oxidase, cholesterol esterase, peroxidase, 4-amino-antipyrine (vial R 2), and cholesterol standard (200mg/dl) (vial R 3). Just before use, the reagents were reconstituted by transferring the entire contents of Vial R2 (enzymes) into Vial R1 (buffer) mixed gently and allowed to stand for 5 minutes. This served as the working reagent. Into four sets of 3 test tubes labeled 'samples,' 'standard', and 'blank', 10ml of sample (serum) standard (Vial R3) and distilled water were separately pipetted respectively. 1ml each of the reconstituted reagent was added to all test tubes, mixed, and incubated for 5 minutes at 37°C.

Absorbencies of the sample and standard were measured against reagent blank at 500nm. Triplicate determinations were carried out on each serum sample.

Calculation

The cholesterol concentration in the serum of rats fed the different diets was calculated as follows:

$$\text{Cholesterol conc} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{conc. of standard}$$

2.10. Statistical Analysis

The data obtained for protein efficiency ratio (PER) will be used for analysis of variance while the mean separation will be done using the Duncan's Multiple Range test in the SPSS (15) statistical package [26].

3. Results and Discussion

3.1. Analysis of the% Yield of the Extracts

The result of the mass loss in each extraction method was calculated by the reduction of the initial mass of leaves in each phase of separation and the result is shown in Table 2. There was a decrease in mass losses in the studied methods. Method 3 however had higher values. This is probably as a result of the addition of Tsamiya, the plant food used as coagulant that was used in extraction process. And since it was not removed from the medium and thus constitute the final material used in the analysis could have contributed significantly to mass and material (chemical composition) of this fraction. Tsamiya is rich in protein (13–20%) and oil (4.5–16.2%) and fibre as well [40]. However, the purpose of applying more than one extraction phase is to minimize the losses in the process and thus increase yield. There was remarkable difference in the fractions in the traditional methods (using Tsamiya as a coagulant) than in the conventional method. However, mass losses in the processes of protein extract of fresh leaves were greater in percentage terms than using dried leaves. This was similar to results that suggested that probably the larger part of mass loss could be in the filtration phase [43].

Table 2. Percentage Yield and mass loss of the Extracts.

Samples	Wt of initial sample(g)			Wt of initial sample (d/b) (g)			Wt of extract (d/b)(g)			EY(%)		MYPC(%)			
	MTD1	MTD2	MTD3	MTD1	MTD2	MTD3	MTD1	MTD2	MTD3	MTD1	MTD2	MTD3	MTD1	MTD2	MTD3
WRE	1000	1000	1000	134.6	138.2	188.2	36	48.67	64	280.93	281.93	286.3	23.31	24.71	26.2
AXT	1000	1000	1000	193.8	194.2	212.4	34	42.3	62	159.32	157.23	161.4	10.89	11.2	12.4
YIE	1000	1000	1000	153.4	157.2	391.4	24.9	26.4	42.34	871.08	869.08	878.2	17.88	18.2	19.1
KKB	1000	1000	1000	234.6	232.8	282.4	42	46	76	928.23	928.23	933.3	13.45	14.2	14.9

Key:

WRE: Watermelon rind extract

AXT: Alleho extract

YIE: Yakuwa extract

KKB: Kirkashi extract

MTD 1: Extraction by Isoelectric Precipitation

MTD 2: Fermentation of Filter Leaf Juice

MTD 3: The traditional method of using *Tsamiiyaa* (*Tamarindus indica*)

EY (%): Extraction yield (EY) of protein

MYPC (%): The Mass Yield of the protein concentrate (MYPC)

3.2. Chemical Composition of the Leaf Concentrates

The results of the chemical composition of the extracts are shown on Table 3. The energy content of the extracts is shown in Table 3. Sample WRE had the highest energy value of 363.19 Kcal while the least energy value of 309.63 was obtained in sample KKB. However, statistical analyses show that there was no significant difference between all the

samples. This implies that they are lightly to provide high amount of energy. Moreover, all the values obtained are higher than values required for daily energy consumption by weaning infants. Infants need energy from food for activity, growth and normal development stipulated that weaning foods should have a composite energy density range from 1.07 to 1.46 kcal/g [49].

Table 3. Chemical composition of the extracts (dry weight basis).

SAMPLE	Energy (Kcal)	Crude protein (g/100g)	Crude Fat (g/100g)	Crude fiber (g/100g)	Ash (g/100g)	Moisture (g/100g)	Carbohydrate (g/100g)
WRE	363.19	31.38±0.36	3.47±0.05	5.46±0.11	6.97±0.15	1.13±0.03	51.60±0.14
AXT	314.21	21.11±0.24	5.57±0.05	14.63±0.03	12.52±0.03	1.26±0.03	44.91±0.11
YIE	354.97	33.23±0.33	3.13±0.03	8.07±0.14	6.61±0.24	0.50±0.02	48.17±0.11
KKB	309.63	31.56±0.36	2.75±0.03	7.81±0.14	17.08±0.26	0.42±0.03	51.60±0.14

Key:

WRE: Watermelon rind extract

AXT: Alleho extract

YIE: Yakuwa extract

KKB: Kirkashi extract

Protein content result is shown in Table 3. The sample YIE had the highest value (33g/100g) while sample AXT had the least value (21g/100g). Notwithstanding all the values obtained were higher in comparison to the value of protein in most vegetables and cereals. The results indicated a concentrate that provides above the recommended dietary allowance (RDA) and thus can be used as used to enrich poor protein stable food such as pap (Ogi) and supplementation for infant weaning foods or complementary foods in our quest to defeat malnutrition. The optimum recommended dietary allowance (RDA) with respect to protein for infants is 10 to 12% by the [49]. However, infants require high quality protein from breast milk. Crude Fat content is shown in Table 3. The sample AXT had the highest value (5.57g/100g) while sample KKB had the least value (2.75g/100g). Fat is important in the diets of infants and young children because it provides essential fatty acids, facilitates absorption of fat-soluble vitamins, and enhances dietary energy density and sensory qualities.

Dietary fiber results are shown in Table 3. The sample AXT had the highest value (14.62g/100g) while sample WRE

had the least value (5.46g/100g). Infants generally do not consume fiber in the first 6 months of life. However, as complementary foods are introduced to their diet, fiber intake increases. Some researchers recommended that from 6 to 12 months whole grain cereals, green vegetables and legumes be gradually introduced to provide five grammes of fiber per day by one year [1].

Percent of ash content was highest in sample AXT (12.5) and least in sample YIE (6.61) in Table 3. This is indicative of the potential of the extracts to provide quantity and quality minerals essential for proper growth and development of infants. Moisture content observed in all the samples are relatively low. This suggest potential for the extracts to keep for long with minimal recourse to preservation process and overt preservation procedures.

The Carbohydrate content is shown in Table 3. The sample WRE and KKB had the highest value (51.6g/100g) while sample AXT had the least value (44.91g/100g). In infants the amount of energy ingested is usually more than they expend since they use the rest of the energy to build up their bodies. It is crucial that the level of carbohydrates (starch) should be high.

3.3. Amino Acid Profile and Chemical Scores of the Six Weaning Formulae

The amino acid profile and the chemical scores of the Essential Amino Acids (EAA) in the weaning formulae and control are shown in Tables 4 and Table 5. Eighteen amino acids were detected, out of which ten were essential amino acids, namely, Isoleucine, leucine, lysine, methionine, threonine, valine, tyrosine, phenylalanine, histidine and tryptophan. On the whole, the values observed in the extracts were higher than the recommended dietary allowance (RDA). This implies that the extracts provide good quality and quantity proteins. Amino acids are building blocks of protein and by inference life. These amino acids perform important

functions in the synthesis of protein, nitric oxide (a vasodilator) and key regulatory hormones and as a neurotransmitter [16]. Thus, the abundance of these amino acids in these extract flour may be of nutritional and physiological importance, warranting further studies.

The calculated chemical scores of the essential amino acids indicated that the extract had higher value for the RDA values (Table 5). This suggests that the extracts can provide good quality and quantity protein to support growth and development. However, the first limiting and second limiting amino acids are methionine and isoleucine respectively. This doesn't however imply that this amino acid is not in sufficient amount and would require supplementation to meet this deficit.

Table 4. Amino Acid Analysis of the extracts (g/100g).

Amino acid	WRE	AXT	YIE	KKB	RDA
Leucine	9.51	8.58	7.30	7.82	4.20
Lysine	4.93	5.41	5.04	4.61	4.20
Isoleucine	3.73	4.58	3.93	3.99	4.20
Phenylalanine	4.97	4.26	4.08	4.61	2.80
Tryptophan	1.10	1.21	0.94	1.05	
Valine	4.21	5.20	3.98	3.92	4.20
Methionine	1.55	1.33	1.23	1.28	2.20
Proline	4.06	3.86	3.25	3.05	3.30
Arginine	6.36	5.33	4.64	5.33	4.10
Tyrosine	3.10	3.44	3.44	2.75	
Histidine	2.30	2.36	2.24	2.04	3.30
Cystine	1.21	1.09	0.97	1.09	2.00
Alanine	4.47	4.32	4.17	3.94	1.20
Glutamic acid	12.26	13.48	11.66	11.96	8.50
Glycine	4.90	5.32	3.90	4.37	1.40
Threonine	4.00	3.39	3.83	4.22	
Serine	3.56	4.00	3.40	3.29	3.20
Aspartic acid	10.61	10.98	9.68	9.99	7.50

Key:

WRE: Watermelon rind extract

AXT: Alleho extract

YIE: Yakuwa extract

KKB: Kirkashi extract

Table 5. Chemical Scores of Amino Acids (%).

Essential amino acid	WRE	AXT	YIE	KKB	RDA
Leucine	135	121.9	137.0	111.1	7.04
Lysine	117.4	128.8	120.0	109.8	4.20
Isoleucine	88.8 ^b	109.1	93.6 ^b	95.0	4.20
Phenylalanine+Tyrosine	132.7	126.6	123.7	121.1	6.08
Tryptophan	114.6	126.04	97.9	100.8	0.96
Valine	100.2	123.8	94.8	93.3 ^b	4.20
Methionine+Cystine	58.5 ^a	51.3 ^a	46.6 ^a	50.2 ^a	4.72
Threonine	100.0	84.8 ^b	95.8	105.5	4.0

Key:

[17].

*% Chemical score = Value of amino acid in diet (g/100 protein) x 100

FAO Ref. Value

a= first limiting amino acid

b=second limiting amino acid

WRE: Watermelon rind extract

AXT: Alleho extract

YIE: Yakuwa extract

KKB: Kirkashi extract

Table 6. Functional Properties of the Weaning Food Formulae.

Samples	Bulk Density	Water Absorption Capacity(g/g)	Oil Absorption Capacity (g/g)	Measurement of Dispersibility (%)	Emulsifying Capacity (ml/g)	Foaming stability ml/min	% Solubility
WRE	5.889 ^a ±0.098	7.3 ^b ±0.712	1.65 ^b ±0.047	89.65 ^a ±3.37	3.25 ^b ±1.04	20.75 ^b ±0.5	20.64 ^b ±1.225
AXT	6.32 ^b ±0.126	2.8 ^b ±0.13	1.43 ^b ±0.10	89.29 ^a ±2.15	8.83 ^a ±1.04	28.27 ^b ±0.58	21.5 ^b ±0.215
YIE	6.696 ^b ±0.359	2.00 ^b ±0.047	5.7 ^a ±0.3	87.35 ^a ±2.6	7.45 ^a ±5.075	25.51 ^b ±0.045	21.36 ^b ±1.235
KKB	6.476 ^b ±0.103	2.62 ^b ±0.283	6.02 ^a ±0.45	85.07 ^a ±0.89	9.11 ^a ±2.06	43.8 ^a ±0.02	23.96 ^{ab} ±0.18
Control	5.56 ^a ±0.03	2.31 ^a ±0.24		40 ^b ±0.0103	3.63 ^b ±0.048	27.21 ^b ±0.015	40 ^b ±0.0103

Key:

*Figures bearing the same superscripts along columns are not significantly different from each other ($p \geq 0.05$).

** Means (\pm SD) of triplicate analyses

***Cerelac used as control

WRE: Watermelon rind extract

AXT: Alleho extract

YIE: Yakuwa extract

KKB: Kirkashi extract

3.4. Effect of Consumption of Concentrates on Serum Enzymes on Rats

The results of the biochemical analysis of the blood samples of rats fed the leaf extract diets is shown in Figure 2. The albumin value in the formulae ranged from 3.6 - 3.8 (g/dL). Normal albumin levels generally range from 3.4 to 5.4 grams per deciliter of blood [31]. Dieticians use albumin as an indicator of nutritional status of an individual. Inadequate dietary protein intake may cause decreased levels of blood albumin. Low albumin results from not consuming sufficient protein and calories. Low serum albumin may lower the resistance of infants and makes them more susceptible to infections, according to the National Kidney Foundation.

The results of haematocrit and creatinine showed no significant difference ($p > 0.05$) between the different concentrates diets and the control. Therefore, the consumption of concentrates wouldn't have any negative effect on blood and reticula endothelial system. Hepato protective effect of extracts maybe mainly attributed to its antioxidant capacity to scavenge free radicals and reduced inflammation in liver [46]. Similarly, the ASA values showed significantly difference at ($p < 0.05$) for rats fed with the modified diets with the extracts and the control diet. Serum enzymes (ALAT, ASAT and creatinine) are often used in clinical diagnosis to assess the integrity of certain body organs. Furthermore, the relative weights of liver and kidney compares favorably with those of the control. Total cholesterol of rats fed with diets of the extracts showed no significant difference ($p > 0.05$) results obtained with feeding the rats with a control diet (Figure 2). The values obtained were within the normal range (< 150 mg/dL for TC). A researcher collaborated a relationship between fiber-rich foods such as grains and legumes and effects on serum cholesterol [31].

3.5. Effect of Extracts on Food intake, Weight Gain and Efficiency Ratios

The food intake of the rats fed with the weaning formulae and the control is shown in Figure 2. The rats fed the control highest value (327 g) while the extract diet YIE had the least value (276g). The food intake consumed by the rats fed on the control diet however compared favorably with the

concentrate diet WRE which probably suggests that the weaning formulae were acceptable to the rats. The growth rate of rats is shown in Figure 3 which shows that rats fed with the concentrate diet WRE had comparable growth rate to the control group. Again, the values in the results from the concentrate diets compared favorably with that of the control. This agrees with observations hat the PER of food should be higher than 2.7 [9]. Again, this suggests that the concentrate diets have the ability to support growth in rats.

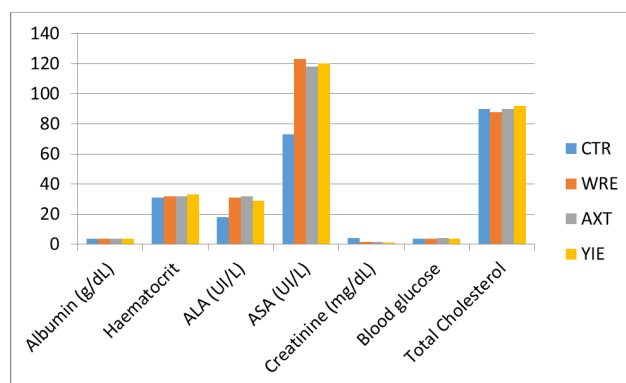


Figure 2. Effect of experimental diet on few parameters (Haematocrit, ASAT, ALAT).

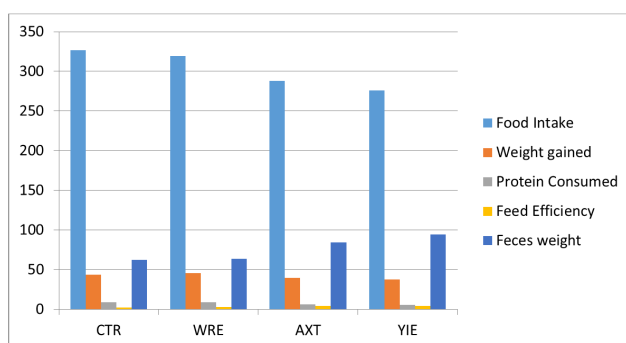


Figure 3. Performance of Rat fed with experimental diet.

3.6. Protein Quality and Digestibility of Differently

The processing technology involve in the leaf protein concentration required boiling of the filtrate leading to

coagulation of protein. This probably is responsible for the high degree in improvement of the protein quality and digestibility of the extracts. The food intake, weight gained, EFU, PER, NPR and TD values were higher for all in the extracts and showed no significant difference with the control diets (Figure 4). A researcher who evaluated the effect of different processing methods also found similar results

showing that protein processing methods affect quality of proteins in plant foods [14]. The feed efficiency (FE) which is the feed consumed per unit weight gain differ among all the treatment groups. Since FE is a measure of the efficiency of rats to convert feed consumed into flesh, the lower the value, the better the FE. Thus, the results of this study indicate that the FE for the concentrates were efficient.

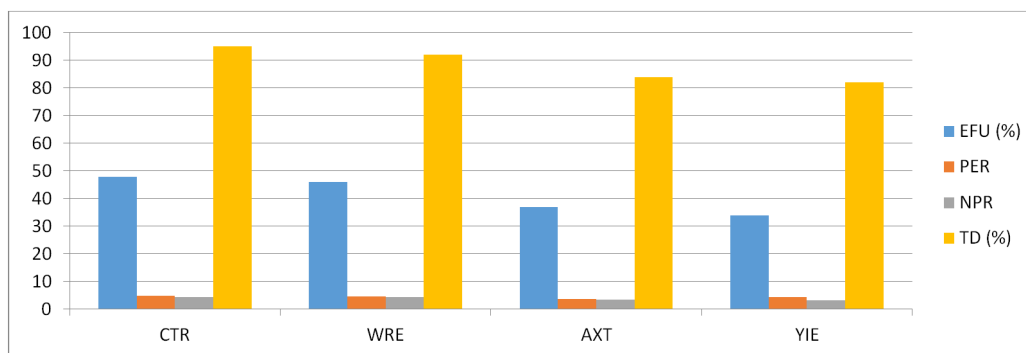


Figure 4. Efficiency of Food Utilization (EFU), Protein Efficiency Ratio (PER), Net Protein Ratio (NPR) and True Digestibility (TD).

The result of the functional properties of the extracts is shown in Table 6. The functional properties of the food materials are very important for the appropriateness of the diet, particularly, for the growing children [36]. The consistency of energy density (energy per unit volume) of the food and the frequency of feeding are also important in determining the extent to which an individual will meet his or her energy and nutrient requirements [36]. The extract sample had the highest value of 7.953 while sample had the least value of 5.889 for bulk density. There were significance differences statistically between the extracts and the control at $p \leq 0.05$. Bulk is an index of nature of the starch and its water-holding capacity and invariably the releasing the water trapped in the when involved in a gelation process or the gel structure and producing a more liquid gruel. The advantage of a low bulk density for instance in complementary diets is that the gruel or porridge made from this diet will have a lower dietary bulk. This is important in complementary foods because high bulk limits the caloric and nutrient intake per feed per child and infants are sometimes unable to consume enough to satisfy their energy and nutrient requirements. A diet with low dietary bulk implies that a thick gel will not be formed in the complementary diet. This is important especially as it can affect the gastric system of the child since they have limited gastric capacity to metabolize thick or viscous foods. The importance of this lower bulk density to the complementary diet is however that the diet will have reduced viscosity, plasticity and elasticity hence the diet is highly favourable as a good complementary diet [12].

The result of functional properties on WAC, OAC, EC and FS are presented in Table 6. The functional properties in percentage reveals that water absorption capacity, oil absorption capacity, foaming capacity, foaming stability and emulsion stability were higher in the extracts in comparison to control value. It is observed that the water absorption capacity and the oil absorption capacity values suggested a

less hydrophobic and more viscous flours could have a better flavor retention and mouth feel potentials [34]. Furthermore, interactions of water and oil with proteins are very important in the food systems because of their effects on the flavor and texture of foods. Generally, emulsion stability is important for stabilization of additives in production of foods like soup and cakes. The high foaming capacity value of the extracts suggests its importance as an aerating agent in food system [35]. The phenomena can be explained based on the capacity of hydrophilic peptides binding to water molecules and high hydrogen bonding.

The leaf proteins were found to be good emulsifying agents. Their ability to emulsify water-oil dispersion had good industrial applications. Moreover, the water absorption capacity (WAC) and oil absorption capacity (OAC) values can be attributed to the higher crude fiber of the extracts. The increase in WAC could also be attributed to the depolymerization of starch into short chain dextrin with a higher affinity for water [48]. A researcher reported that high WAC of flours is advantageous in the preparation of pastries such as bread and sausages, to maintain freshness and for easy handling [8]. Oil absorption capacity is a measure aimed inferring on effect of denaturation of the protein of extracts which destroyed the hydrophobic domain of the extract and reduced fat binding. The mechanism of oil absorption involves the physical entrapment of oil by food component and the affinity of non-side chain for lipids [27]. Emulsifying capacity and food emulsions are thermodynamically unstable mixtures of immiscible liquids (water and oil). The formation and stability of emulsion is very important in food systems such as salad dressings. Proteins constitute an important group of emulsifiers because they reduce interfacial tension, form rigid interfacial films and possess charged groups. The emulsification capacity is an important property if the extracts can be used in products such as salad dressings. Protein, carbohydrates and fat play an important role in

WAC, FAC and EC. Fat absorption plays an important role in food preparations, because fat improve the flavor and mouth feel of products. High WAC and FC can also be useful applications in the preparation of bakery products such as cakes and biscuits. Foam stability depends on protein content with hydrophilic nature of peptides and these characteristics are desirable in preparation of beverage.

4. Conclusion

In conclusion, This study has shown that the use of simple technology like the leaf protein concentration technology has remarkably improve on all the parameters (Proximate, functional and biochemical properties) assessed on the concentrates of water melon rind (WRE), Alleyyaho leaf (AXT), Kakuwa leaf (YIE) and Kirkashi leaf (KKB). The results showed that the extracts can support growth and development especially in infants. These concentrates will greatly impact on the economy of low income earners and rural people as the technology (The Leaf concentrate technology) is easily assessable. Furthermore, the application a local spice, 'Tsamiya' (Tamarind) provides a veritable tool as a cheaper source of coagulation or coagulant (used in Method 3 in this research) and requires further study.

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