SUNFLOWER LEAVES PROTEIN CONCENTRATE AND RESIDUE WITH THEIR NUTRITIONAL AND ANTI-NUTRITIONAL CONTENTS

[1]BASHIR S.Y., [2] O.A. SOGBESAN, [3]M.M. KUTTE, AND [4]U. MUHAMMAD
[1]Department of Fisheries, Adamawa State Polytechnic, P.M.B. 2146, Adamawa State, Nigeria;
[2]Department of Fisheries, Modibbo Adama University, P.M.B. 2076, Yola, Adamawa State, Nigeria;
[3]Department of Fisheries, Adamawa State College of Agriculture, P.M.B. 2088, Adamawa State, Nigeria;
[4]Department of Fisheries, Adamawa State Polytechnic, P.M.B. 2146, Adamawa State, Nigeria.
Corresponding Author: bashirsumayyayahya@gmail.com, 08103805198, 08142640895

ABSTRACT
Sunflower is an annual dicotyledonous plant that belongs to the family Asteraceae. It roots, stems, leaves and seeds contained phenols, flavonoids and alkaloids, the florets contain fibre and phenolic acid while the petals contains triterpene glycosides which had anti-inflammatory activities. The sunflower leaves were collected, washed, and blended to produce the protein concentrates and residue, which were analyzed in the laboratory for nutritional and anti-nutritional profiles. The result of the analysis revealed that 38.76% CP was the highest value in SLPC and 26.72% was the lowest in SLR. SLPC was higher in terms of TEAA, TNEAA and TEAA/CP with the values 46.08mg/100g, 73.09mg/100g and 119.00mg/100g respectively. The most abundant mineral in SLPC was calcium (437.73mg/100g) and potassium (385.44mg/100g) in SLR, and copper (0.43mg/100g and 0.41mg/100g) was the least abundant in both the SLPC and SLR. Oleic was the highest fatty acid in both the SLPC and SLR with the values 61.07% and 59.21% and the lowest was arachidic (1.15 and 0.91%) respectively with significant difference ($p<0.05$). The highest anti-nutritional factor was saponins with values (11.03 and 14.23) while the lowest was flavonoids with the values (6.12 and 6.98) in both the SLPC and SLR respectively with significant difference at $p<0.05$. Sunflower leaves enough nutritional components that can be in the diets of humans and animals.
Keywords: proximate, amino acid, minerals, fatty acid, phytochemicals.

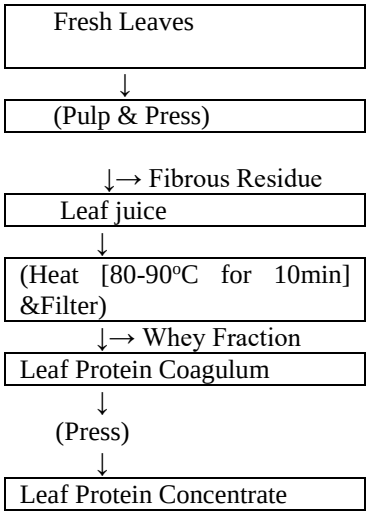
INTRODUCTION

Sunflower leaf meal is rich in protein and used in the feed of livestock, but has some limitations due to the presence of antinutritional factors such phytin and tannins with some traces of alkaloids, saponins, oxalates and flavonoids (Fasuyi *et al.*, 2010). Sunflower proteins are characterized with high nutritive value such as lysine, and contained well-balanced amino acids. The proteins are rich in sulphur-containing amino acids, which is deficient in most protein with plant origin (Ivanova *et al*., 2013). Sunflower is an annual dicotyledonous plant that belongs to the family Asteraceae. It roots, stems, leaves and seeds contained phenols, flavonoids and alkaloids, the florets contain fibre and phenolic acid while the petals contains triterpene glycosides which had anti-inflammatory activities (Liu *et al.*, 2020). Sunflower is a resilient plant that is drought-resistant and can grow in many types of soils but prefers deep well-drained soil that is not waterlogged and slightly acidic with a pH range of 6.0 and 7.5. Sunflower leaves are rough, egg-shaped with a hairy surface and measure about 30cm long. The upper surface of the leaf is dull green and sandpaper-like texture (Blog, 2019). Sunflower is currently cultivated for its seeds and is the fourth largest oil seed crop in the world. Sunflower meal is a potential source of protein for human consumption due to its high nutritional value and lack of anti-nutritional factors (Liu *et al.*, 2020). *Helianthus annus* has a nutritional quality like that of other oilseed proteins including soybean and other convectional legumes (Adesina, 2018). Plant protein are more availability and economic benefits, and has less amount of phosphate and nitrogen than animal proteins (Rajesh, 2019). Leaves are good sources of protein although, they content high amount of fibre and anti-nutritional factor. By separating the fibre and removing anti-nutritional factors, fibre-free protein (protein concentrate) could be yield and can be fed to humans, ruminants and non-ruminants which enhances food production and consumption (Agbede, 2005). With superior performing plant materials and suitable extraction techniques, leaf protein yield per hectare can exceed four times that of seed-derived protein (Xie, 2017). Plant leaves have been reported to contain higher crude protein and extractable protein than plant stems (Solati *et al.*, 2018). Depending on the plant species and extraction methods, the total protein content in leaf protein concentrates varies from 40 to 70%. While a significant quantity of minerals and vitamins, including β-carotene (pro-vitamin A) and vitamin E, have been recovered in leaf protein concentrates (Badar *et al.*, 2011). Hence, the leaf protein concentrates can therefore be used as protein, vitamin, mineral supplements in animal or human diets, and attend by scientific finding by Ngugi (2017). This study had analyzed the nutritional and anti-nutritional contents of sunflower leaves protein concentrate and residue.

MATERIALS AND METHODS

Production of Leaf Protein Concentrate and Residue:

The leaves were obtained from one farm at Dukku Local Government Area of Gombe State. 20kg of the leaves were washed to removed dirt, blended with distilled water and then separate the leaf juice from the residue using sieve. The separated juice of sunflower leaves was heated to 80-90^{o}C for about 20minutes, and centrifuged at 15000 rpm for 10min to coagulate the leaf protein. The coagulum was separated from the whey fraction through cloth filter, the leaf protein concentrate obtained was then partially oven dry and then completely air dried (Fagbenro *et al.,* 2017). 3.62% of the sunflower leaves protein concentrate and 21.05% of residue were obtained.



Leaf protein concentrate schemata (Fagbenro *et al.,* 2017)

Nutrient Analysis

Analysis for crude protein, moisture, fibre, ash, ether extract and nitrogen free extracts contents according to Association of Analytical Chemistry method (A.O.A.C., 2010) sunflower leaves protein concentrates and residue, were carried out at the laboratory.

Moisture

12g of the sample was weighted and pour in porcelain evapourating dish. The dish was covered with aluminium foil paper and weighed to nearest mg. The sample is dry uncover at 105^{0} C for 16h. It will be remove from the oven, cover tightly, cool and weigh. The percentage of moisture content was calculated as:

$$\%\text{moisture} = 1 - \left(\frac{\text{weight of dried sample}}{weight\ of\ wet\ sample}\right) \times 100$$

Lipid

Determination of lipid content was performed following Soxtec method, using a SoxtecTM 2050 automated analyzer (FOSS Analytical, Hillerød,Denmark). Petroleum ether will be used for the extraction, whereas percentage of lipid will be obtained following equation below:

$$\%\ \text{Lipid} = \frac{weight(extraction\ cup + residue) - weight\ \ (extraction\ cup)}{weight\ of\ sample} \times 100$$

Protein

The total nitrogen amount in the sample was determined using KjeltecTM 2200 Auto Distillation Unit (FOSS Tecator, Höganäs, Sweden). A nitrogen-to-protein conversion factor of 4.4 was used for the determination of protein present in the samples.

Ash

A dry ashing method was used to determine the ash content. The samples will be incinerated in a furnace at 550°C until become completely ash. The remaining inorganic material was cooled, weighed and then calculated with the formular;

$$\%\text{Ash} = \frac{\text{weight of ash}}{weight\ of\ sample} \times 100$$

Carbohydrate

The total carbohydrate content (%) in the samples was calculated by difference method (subtracting (%) crude protein, (%) moisture, (%) fat, (%) fibre and (%) ash from 100).

% carbohydrate = 100- (% crude protein + % lipid +% moisture + % fibre + % ash)

Amino Acid Analysis

The essential amino acid of the sample was analyzed using the method of AOAC (2010).

Defatting of sample: 0.5g of each dried sample was transferred into an extraction thimble and the fat was extracted with chloroform and methanol mixture using soxhlet extraction apparatus for eight hours.

50mg of the defatted sample was weighed into a glass ampoule for hydrolysis. 7ml of $6NH_3Cl$ was added to the sample and nitrogen was passed into the ampoule for 20 minutes to expel oxygen (in order to prevent possible oxidation of some amino acid during hydrolysis). The glass ampoule was then sealed with Bunsen burner flame and put in an oven at $105^0C \pm 5^0C$ for 22hours. The ampoule was allowed to cool before breaking at the tip and the content filtrated.

The filtrates were allowed to evaporate to dryness at 40^0C under vacuum in a rotary evaporator. The residues were dissolved in 5ml acetate buffer (pH 2.0) and transferred into plastic specimen bottles and stored in the deep freezer.

10 microliters of hydrolysate were dispensed into the cartridge of the TSM analyzer for analysis for 75 minutes. TSM is an automated instrument design to separate, detect, and quantify amino acids (Eyo, 2001). The system can separate and analyze free acidic, neutral and basic amino acids from a protein hydrolysate in $1\frac{1}{4}$ hours.

The net heights each peak representing an amino acid produced by the chart record of the TSM was measured. The half-height of the peak on the chart was determined, and the width of the peak at the half height was measured and recorded. The appropriate areas each peak was obtained by multiplying the net height with the width of the half height.

The Norleucine Equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$\frac{Area\ of\ Norleucine\ peak}{Area\ of\ each\ amino\ acid}$$

From the Norleucine Equivalent above, a constant S was calculated for each amino acid in the standard mixture as follows:

$S_{std} = NE_{std}$ x Mol. Weight x µ MAA_{std}

S_{std} = constant for the standard mixture

Mol. Weight = Molar Weight of each sample

NE_{std} = Norleucine equivalent of the standard

MAA_{std} = Mole of the amino acid of the standard

Finally, the amounts of each amino acid present in the sample was calculated in g/16g N using the following formula:

$$\text{Concentration in g/16g N} = \frac{\text{NH x Sstd x C}}{2}$$

$$\text{where C} = \frac{\text{Dilution x 16}}{\text{Sample Wt.(g) x N2\% x vol.Loaded}}$$

NH=Net height of the park on the chart for each amino acid

Essential amino acid indices: The essential amino acids indices were calculated using whole hen egg crude protein and essential amino acids composition documented in cud Derfold (1983) and Sogbesan (2014) as references. The essential amino acids indices were determined as:

a) $\text{Chemical score (\%)} = \frac{\text{Essential amino acid of the sample}}{\text{Essential amino acid of whole hen egg}} \times 100$

b) Total essential amino acid to crude protein content ratio (EAA:CP)% =

$$\frac{\text{Total essential amino acid}}{\text{Crude protein of the animal meal/100g of diet}} \times 100$$

Mineral Analysis

The minerals elements include calcium, iron, magnesium, phosphorus, sodium, manganese, zinc, copper and potassium will be determined following the method in AOAC (2010).

Determination of Mineral Elements

The remaining inorganic material for ashing was further used for the determination of mineral contents. An ash solution was prepared by dissolving the ash in 100 mL of 1 MHCl. Sample was digested by concentrated nitric acid and sulfuric acid (3:1, v/v). An atomic absorption spectrometer was used for absorbance measurements at different wavelengths. Phosphorus (p) was measured by spectrophotometer then, converting phosphates into phosphorus molybdenum blue pigment and measured at 410nm.

The results for mineral contents were expressed as mg/100 g DW.

Fatty Acids Analysis

The lipid was extracted from samples by soxhlet extractor using hexane as a solvent. Fatty acids were transformed into methyl ester according to the ISO procedure (ISO, Method 5509, 1978). The fatty acid methyl esters (FAMEs) was extracted with petroleum ether and analyzed by high pressure liquid chromatography (HPLC) (Buck scientific BLC 10/11 USA) equipped with flame ionization detector and integrator. The mobile phase is (59:41) Acetonitrile: 2-propanol and the column (prevail C-18, 5u, 150 x 4.6mm) flow rate was 1 ml/min. the oven temperature was maintained at 210^0C for 45 min. The fatty acids were identified by comparing their retention times with those of standards.

Phytochemical Analysis

1. Qualitative analysis

Test for flavonoids

0.5 mL of sample was shaken with pet ether to remove the fatty materials. The defatted residue was dissolved in 20 mL of 80% ethanol and filtered. Then 3 mL of the filtrate was mixed with 4 mL of 1% KOH. A dark yellow colour indicated the presence of flavonoids (Sofowara, 1993).

Test for saponins

0.5 mL of sample was dissolved in 2 mL of boiling water in a test tube, it then allowed to cool and shaken to mix thoroughly. Appearance of Foam indicate the presence of saponins (Sofowara, 1993).

Test for alkaloids

0.5 mL of sample was mixed with 8 mL of 1% HCl, warmed on steam bath for 5 minutes and filtered. Then 2 mL of the filtrate was treated separately with Mayer's reagent. Turbidity indicated the presence of alkaloids (Trease and Evans, 1989).

Test for tannins

2.0 mL of sample was mixed with 2.0 mL of distilled water then few drop of $FeCl_3$ solution was added. The formation of a green precipitation, indicates the presence of tannins (Trease and Evans, 1989).

2. Quantitative analysis

Determination of Tannins

0.20 g of the aqueous sample, 20 mL of 50% methanol was added, shaken thoroughly and placed in a water bath at 80^0C for 1 hour for uniform mixing. The extract was filtered into 100 ml volumetric flask, followed by addition of 20 mL of water, 2.5 mL of Folin-Denis reagent and 10 mL of 17% Na_2CO_3 and mixed. The mixture will be made up to 100 mL with distilled water, mixed and allowed to stand for 20 minutes. The bluish-green colour developed at the end of the reaction mixture of different concentration was range from 0 to 10 ppm. The absorbance of the tannic acid solution and sample was measured at 760 nm. The concentration of tannins will be estimated using a standard curve of tannic acid.

Determination of Total Alkaloids

The method described by Harbone (1973) was used. The aqueous Pterocarpus erinaceus stem bark sample weighing 5.0 g was added into a 250 mL beaker, 200 mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. The filtrate was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide will be added drop wise to the extract until a precipitate is formed. The whole solution was allowed to stand, the precipitate was collected and washed using 0.1% ammonium hydroxide. The residue was dried and weighted.

$$\%\ Total\ Alkaloids = \frac{Final\ weight\ of\ sample}{initial\ weight\ of\ extract}\ X\ 100$$

Determination of flavonoids

The aqueous sample weighing 10.0 g was extracted using 100 mL of 80% aqueous methanol at room temperature. The solution was filtered through Whatman's filter paper no 42. The filtrate was transferred into crucible and dried in a water bath and weighed to a constant weight (Bohm and Koapai-Abyazan, 1994).

Determination of Total Saponins

The method described by Obadoni and Ochuko, (2001) was used. The powdered sample (20 g) was added to 100 mL of 20% aqueous ethanol and kept in a shaker for 30 minutes. The sample was heated on a water bath for 4 hours at 55^0C. The mixture was filtered and the residue re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL on the water bath at 90^0C. The concentrate was transferred into 250 mL separating funnel and extracted twice with 20 mL diethyl ether.

The ether layer was discarded while the aqueous layer was retained and 60 mL of n-butanol was added. The n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. The sample was dried in hot air oven at 40^0C to a constant weight. The saponins content was calculated using the formular:

$$\% \, Saponins = \frac{Final\ weight\ of\ sample}{initial\ weight\ of\ extract} \ X\ 100$$

Statistical Analysis

Data was analyzed using one-way analysis of variance (ANOVA) at 95% level of significance while differences between the means will be compared using Duncan Multiple Range Test.

RESULTS

Table 1 shows that the crude protein (38.76%), lipid (4.17%) and ash (6.38%) were higher in SLPC and lower (26.72, 3.81 and 4.10% respectively) in SLR. While fibre (8.30%), DM (8.20%) and NFE (58.87%) were higher in SLR and lower (4.95, 4.16 and 41.18% respectively) in SLPC.

Table 1: Proximate Compositions of the Sunflower Leaves Protein Concentrate and Residue

Sample	CP(%)	Lipid(%)	Fibre(%)	Ash(%)	DM(%)	NFE(%)
SLPC	38.76±.02[a]	4.17±.01[a]	4.95±.00 [b]	6.38±.02[a]	4.16±.01[b]	41.18±.01[b]
SLR	26.72±.02[b]	3.81±.00[a]	8.30±.01 [a]	4.10±.02[a]	8.20±.01[a]	58.87±.01[a]

Mean with different superscripts on the same column differ significantly at p<0.05

SLPC- sunflower leaves protein concentrate, SLR-sunflower leaves residue, CP- crude protein, DM-dry matter, NFE- nitrogen free extract.

Table 2 presents the essential amino acids of sunflower leaves protein concentrate and residue. The highest essential amino acid was leusine in both SLPC and SLR (11.13 and 7.34 respectively) and the lowest were histidine (0.84) and methionine (1.13) respectively. TEAA and TEAA/CP ration were higher in SLPC (46.80 and 119.00) than in SLR (29.68 and 111.00) respectively

Table 2: Essential Amino Acids Indices of Sunflower Leaves Protein Concentrate and Residue

Amino Acids(mg/100 gprotein)	Lys	Met	Thre	Isol	Leu	Phen	Val	Tryp	His	**TEAA**	**TEAA /CP**
SLPC	3.56 ±.01 [a]	3.64 ±.01 [a]	7.04± .01[a]	4.14± .01[a]	11.13 ±.01[a]	4.11± .01[a]	6.50± .01[a]	5.1±. 012[a]	0.84 ±.01	**46.08**	**119.00**
SLR	2.62 ±.01 [a]	1.13 ±.01 [b]	2.25± .01[b]	2.84± .02[a]	7.34±. 01[b]	3.84± .01[a]	4.01± .01[b]	3.78± .01[b]	1.87 ±.02	**29.68**	**111.00**

Mean with different superscripts on the same column differ significantly at p<0.05

SLPC-sunflower leaves protein concentrate, SLR-sunflower leaves residue, Lys-lysine, Met-methionine, Thre-threonine, Isol-isoluesine, Phen-phenylalanine, Val-valine, Tryp-tryptophan and His-histidine, TEAA/CP- total essential amino acid, TEAA/CP- total essential amino acid to crude protein ratio

Table 3 presents the essential amino acids, essential amino acids indices, and chemical score of sunflower leaves protein concentrate sunflower leaves residue. The essential amino acid index and chemical score of SLPC (136.69 and 1.78) was higher than SLR (72.37 and 0.94) respectively.

Table 3: Essential Amino Acids (EAA), Essential Amino Acids Indices (EAAI), and Chemical Score (CS) of Sunflower Leaves Protein Concentrate (SLPC) and Sunflower Leaves Residue (SLR), and Essential Amino Acids Requirement Level for Nile Tilapia

Essential Amino Acid	SLPC	SLR	Tilapia
Lysine	3.56	2.62	5.12
Methionine	3.64	1.13	3.75
Threonine	7.04	2.25	3.75
Isoleusine	4.14	2.84	3.11
Leusine	11.13	7.34	3.39
Phenylalanine	4.11	3.84	2.68
Valine	6.50	4.01	2.80
Tryptophan	5.12	3.78	1.00

Histidine	0.84	1.87	1.72
Total	**56.06**	**29.68**	**27.32**
EAAI	1.78	0.94	-
CS	136.69	72.37	-

Table 4 presents the non-essential amino acids of sunflower leaves protein concentrate and residue. Glycine was the highest non-essential amino acid with the value 17.72mg/100g while cysteine was the lowest (0.38mg/100g) in SLPC. In the residue, aspartic acid was the highest (6.52mg/100g) and cysteine was the lowest (0.70mg/100g). TNEAA was higher in SLPC (73.09) than in the SLR (30.29).

Table 4: Non-essential Amino Acids Indices of Sunflower Leaves Protein Concentrate and Residue

Amino Acids(mg/100gprotein)	SLPC	SLR
Arginine	5.77±.02 [a]	4.23±.01 [a]
Serine	16.44±.01[a]	3.13±.01 [b]
Cysteine	0.38±.01[a]	0.70±.01[a]
Tyrosine	5.15±.01[a]	4.62±.02[b]
Alanine	6.02±.01[a]	2.71±.01[b]
Aspartic acid	8.31±.01[a]	6.52±.01[b]
Glutamic acid	9.13±.01[a]	3.14±.01[b]
Glycine	17.72±.02[a]	3.41 ±.01[b]
Proline	4.17±.02[a]	1.83±.02[b]
TNEAA	**73.09**	**30.29**

Mean with different superscripts on the same row differ significantly at p<0.05

SLPC-sunflower leaves protein concentrate, SLR-sunflower leaves residue, TNEAA/CP- total non-essential amino acid, TNEAA/CP- total non-essential amino acid to crude protein ratio.

Table 5 shows that the calcium and potassium were the highest minerals in both the SLPC (437.73 and 424.08 mg/100g respectively) and SLR (385.44 and 317.34mg/100g respectively), while cupper was the lowest (0.43 and 0.41mg/100g) mineral with significant difference (p<0.05).

Table 5: Mineral Content of Sunflower Leaves Protein Concentrate and Residue

Mineral(mg/100g)	Mg	K	Ca	Na	Fe	P	Mn	Zn	Cu
SLPC	54.16.±.01 [a]	424.08±.01 [a]	437.73±.01 [a]	37.93±.01 [a]	14.85±.01 [a]	157.37±.01 [a]	54.02±.02 [a]	5.18±.03 [a]	0.43±.02 [a]
SLR	38.25±.01 [b]	385.44±.01 [b]	317.34±.02 [b]	25.55±.01 [b]	8.25±.01 [b]	118.05±.01 [b]	51.22±.02 [a]	3.44±.02 [a]	0.41±.01 [a]

Mean with different superscripts on the same column differ significantly at p<0.05

SLPC-sunflower leaves protein concentrate, SLR-sunflower leaves residue, Mg-magnessium, K-potassium, Ca-calcium, Na-sodium, Fe-iron, P-phosphorus, Mn-manganese, Zn-zinc and Cu-cupper.

Table 6 presents the fatty acid profile of SLPC and SLR. Oleic was the highest fatty acid in both the SLPC and SLR with the values 61.07% and 59.21% and the lowest was arachidic (1.15 and 0.91%) respectively with significant difference (p<0.05).

Table 6: Fatty Acids Profile of Sunflower Leaves Protein Concentrates and Residue

Fatty acids(%)	Capric	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Arachidic
SLPC	2.05±.01 [a]	3.10±.01 [a]	1.87±.03 [a]	18.46±.01 [a]	24.15±.01 [a]	61.07±.02 [a]	29.92±.01 [a]	1.98±.01 [a]	1.15±.01 [a]
SLR	1.58±.01 [a]	2.23±.03 [a]	1.46±.01 [a]	15.35±.01 [b]	19.53±.02 [b]	59.21±.01 [a]	27.86±.01 [a]	1.36±.01 [a]	0.91±.01 [a]

Mean with different superscripts on the same column differ significantly at p<0.05

SLPC-sunflower leaves protein concentrate, SLR-sunflower leaves residue.

Table 7 shows the anti-nutritional contents of SLPC and SLR. The highest anti-nutritional factor was saponins with values (11.03 and 14.23) while the lowest was flavonoids with the values (6.12 and 6.98) in both the SLPC and SLR respectively. There is a significant difference at $p<0.05$ between them.

Table 7: Quantitative Phytochemicals Screening of Sunflower Leaves Protein Concentrates and Residue

Phytochemical	Flavonoids	Tannins	Alkaloids	Saponins
SLPC	6.12±.01 [a]	8.74±.01 [a]	7.95±.01 [a]	11.03±.02 [a]
SLR	6.98±.02 [a]	9.15±.01 [a]	8.15±.01 [a]	14.23±.01 [b]

Mean with different superscripts on the same column differ significantly at $p<0.05$
SLPC-sunflower leaves protein concentrate, SLR-sunflower leaves residue.

DISCUSSION

The proximate composition of SLPC in this study was in support with Omolola (2020). But not supported by Abifarin *et al.* (2021) and Adeyeye and Omolayo (2011) in which the crude protein and crude fibre were higher than 35.4% and 1.6% while lipid and ash were lower than 10.7% and 12.3% of *Telfairia occidentalis* and *Amaranthus hybridus*. In SLR, the crude protein (26.72), crude fibre (8.30), lipid (1.81) and ash (4.10) were lower than that of Akaeze and Paul-Osagie (2023) and Nwokoro *et al.* (2022) except crude protein which was higher.

Threonine was abundant while histidine and cysteine were limited amino acids in SLPC. This was not supported by Adeyeye and Omolayo (2011) were aspartic acid was abundant and cysteine was limited in *A. hybridus* while in *T. occidentalis* glutamic acid was abundant and aspartic acid was limited, and Bunda *et al.* (2015) were tryptophan was the most limiting and leusine was the most abundant in Rhizoclonium meal.

The EAAI of this study (1.78 and 0.94) were similar to 1.02 and 0.97 of Rhizoclonium meal obtained by Bunda *et al.* (2015), and (1.14 and 0.80) for sunflower meal and canola meal respectively as reported by Kirimi *et al.* (2020). The CS of SLPC (136.69) was higher while SLR (72.37) was similar to (72.37) of Rhizoclonium for the study of Bunda *et al.* (2015).

Calcium (437.73mg/100g) was the most abundant while copper (0.43mg/100g) was the least abundant mineral in SLPC with significant difference ($p<0.05$). And this was similar to Adeyeye and Omolayo (2011) and Abifarin *et al.* (2021) but lower than Omolola (2020). In SLR, potassium (385.45mg/100g) was the most abundant mineral while zinc and copper were limited and it was supported by Nwokoro *et al.* (2022).

The fatty acid composition of SLPC and SLR indicates that oleic was the major component, this was not supported by Nazir and Shah (1990) and Kaszas *et al.* (2020), in which palmitic acid (>15%) and linoleic acid (>40%) respectively were the components in both the studies.

The phytochemicals values of the SLPC and SLR in this studies were similar to Omolola (2020) for *Tithonia diversifolia* but higher than the one obtained by Harris *et al.* (2017). Saponins was the highest phytochemical (11.03 and 14.23) recorded while steroids were the lowest (6.12 and 6.933) respectively in both SLPC and SLR with significant difference at $p<0.05$. Saponins binds with proteins to form digestible complexes that inhibit protein digestion, while tannins interfere with digestion and reduces vitamin B_{12} absorption.

CONCLUSION

Sunflower leaves protein concentrate and residue can be safely utilized by humans and animal due their nutritional contents and low level of anti-nutritional factors and heavy metals (zinc and copper). The sunflower leaves residue can be use in ruminant and pseudo-ruminant feeds because of its high fibre content.

RECOMMENDATIONS

Further studies should be carry out on the best extraction method that will enhances the nutritional contents and reduces the anti-nutritional factors.

Further studies should be carry out on the best inclusion levels that can use in feeds

REFERENCES

Abifarin, T.O., Otunola, G.A. and Afolayan, A.J. (2021). Nutritional Composition and Antinutritrient contents of *Heteromorpha arborescens* (Spreng) Cham.Schtdl. Leaves: An Underutilized Wild Vegetable. *Food Science and Nutrition*, 9(1):172-179.

Adesina, S.A. (2018). Effect of Processing on the Proximate Composition of Sunflower (*helianthus annus*) Seeds. *Journal of Tropical Agriculture, Food, Environment and Extension*. 17(3): 27-33.

Adeyeye, E.I. and Omolayo, F.O. (2011). Chemical Compositions and Functional Properties of Leaf Protein Concentrates of *Amaranthus hybridus* and *Telfairia occidentalis. Agriculture and Biology Journal of North America*, 2(3): 499-511.

Agbede, J.O. (2005). Nutritional significance of leaf meal, protein concentrates and residue from some tropical leguminous plants. *Journal of food, Agriculture and Environment*, 3(1): 25-30.

Akaeze, N.C. and Paul-Osagie, M.A. (2023). Physicochemical Composition of Leaf

Protein Concentrates and Bagasse Obtained from Cassava (*Manihot esculenta)* Leaves Using Three Different Processing Methods. *Journal of Applied Science and Environmental Management*, 27(8): 1733-1738.

Akaeze, N. C., Nwokoro, S. O., and Imasuen, J. A. (2014). "Performance of growing rabbits offered rubber leaf protein replacement for soya bean meal". *Nigerian Journal of Agriculture, Food and Environment*, 10(4), pp. 55-59.

AOAC (2010). Association of Official Analytical Chemists International. Official methods of analysis, 17th edition AOAC International Gaithersburg, MD, USA 2000, 25-28pp.

Badar, K. V., and Kulkarni, A. U. (2011). Leaf Protein Concentrate is Novel Source of Protein for Human Health and Nutrition: A review. *Current Botany*, 2(1).

Blog (2019). 50 Important Uses of Sunflower Leaves. www.allusesof.com. Book of Abstracts, Volume 2. World Aquaculture 2003. Salvador, Brazil, World Aquaculture Society, Baton Rouge, Louisiana, USA. pp. 592.

Bunda, M.G.B., Tumbokon, B.L.M. and Serrano Jr. A.E. (2015). Composition, Chemical Score (SC) and Essential Animo Acids Index (EAAI) of the Crinkle grass Rhizoclonium specie as Ingredient for Aquafeeds. Jiue=rnal of Bioflux Society, 8(3): 738-743.

Eyo, A.A. (2001). Chemical composition and amino acid content of the commonly available feedstuff used in fish feeds in Nigeria. *Fish* Nutrition and Fish feed technology in Nigeria. In: *Eyo, A.A. (eds) symposium on fish Nutrition and fish technology NIOMR Lagos*, 5: 58-71.

Fagbenro, O., Oresegun, A., Nwanna, L., Ilona, P., OLuroyimi, O. and Adebayo, A. (2017). Performance of Diets for *Oreochromis niloticus and Tilapia zilli* by Inclusion of Cassava Leaf Protein Concentrate as Partial Replacement for Solvent-extracted Soybean Meal. *Applied Tropical Agriculture*. 22(2): 16-23.

Fagbero, A.O., Oresegun, A. and Olurole, O.A. (2018). Equi-protein Dietary Replacement of Solvent- extracted Soybean Meal with Cassava Leaves Protein Concentrate for Nile tilapia (*Oreochromis niloticus* Linnaeus 1758) and African Catfish (*Clarias gariepenus* Burchell 1822). *Nigerain Journal of Fisheries and Aquaculture*, 6(1): 68-77.

Fasuyi O.A., *et al* (2010). Ensiling Wild Sunflower (*tithonia diversifolia*) Leaves with Sugar Cane Molasses. Livestock Research for Rural Development.

Ivanova, P., Chalova, V., Koleva, L. and Pishtiyski, I. (2013). Amino Acids Composition and Solubility of Proteins Isolated From Sunflower Meal Produced in Bulgaria. *International Food Research Journal.* 20(6): 2995-3000.

Harris, K.K., Devshree, V. and Sahu, M. (2017). Phytochemical Analysis of *Helianthus annus Lin.* (Angiosperm: Asteraceae). *World Journal of Phermacy and Pharmaceutical Sciences*, 6(3):825-846.

Kaszas, L., Alshaal, T., El-Ramady H.R. and Kovacs, Z. (2020). Identification of Bioactive Phytochemicals in Leaves Protein Concentrate of Jerusalem Artichoke (*Helianthus tuberosus L.*). *Plants*, 9(7): 889.

Kirimi, j.G., Musalia, L., Magana, A.E. and Mugnguti, J. (2020). Protein Quality of Rations for Nile Tilapia (*Oreochromis niloticus)* Containing Oilseed Meal. Journal of Agricultural Science, 12(2): 82.

Liu Xin-sheng, Bo Gao Xin-Lu, Li Wan-Nan Li, ZI-An Qiao and Lu Han (2020). Chemical Composition and Anti-microbial and Anti-oxidant Activities of Essential Oil of Sunflower (*Halianthus annus L*) Receptacle. PubMed Central, 25(22): 5244.

Nazir, M. and Shah, F.H. (1990). Stability of Fatty Acids and Leaves Protein Concentrate of Persian clover (*Trifolium resupinatum). Plants Food for Human Nutrition*, 40:283-288.

Ngugi, C. C., Oyoo-Okoth, E., Manyala, J. O., Fitzsimmons, K., & Kimotho, A. 2017. Characterization of the Nutritional Quality of Amaranth Leaf Protein Concentrates and Suitability of Fish Meal Replacement in Nile tilapia Feeds. *Aquaculture Reports*, 5: 62-69.

Nwokoro, S.O., Agbonghae, O.W., Akaeze, N.C., and Onojeta, E.E. (2022). Chemical Compositions of Leaves Protein Concentrate and Bagasse of Pride of Barbados (*Caesalpinia pulcherrima)* Leaves obtained from three Different Locations in Benin City, Nigeria. *Journal of Applied Science and Environmental Management*, 26(5): 845-849.

Omolola, T.O. (2020). Phytochemical, Proximate and Elemental Composition of *Tithonia diversifolia* (Hemsley). A Grey Leaves. *International Annals of Science*, 8(1): 56-61.

Rajesh Singh, (2019). Use of Plant Leaves AS Fish Meal. Pashudhan Praharee. rajeshsinghvet@gmail.com.

Sofowara, A. (1993). Phytochemical Screening of Medicinal Plants and Traditional Medicine in

Africa Edition. Spectrum Books Ltd., Nigeria, 150-156.

Sogbesan, O.A. (2014). Non- conventional Animal Protein Feedstuffs for the Culture Catfish. Ibadan University-Press, Publishing House University of Ibadan, Ibadan-Nigeria. Pp. 99.

Solati Z., Jorgensen, U., Eriksen, K. and Soegaard K. (2018). Estimation of Extractable Protein in Botanical Fractions of Legume and Grass Species. *Grass Forage Sci*. 73: 572-581.

Trease, G.E. and Evans W.C. (1989). Trease and Evans Pharmacognosy. 11th edition, Bailliere Tindall, London, 45-50.

Xie Runshi (2017). Evaluation of Leaf Protein Content, Extraction, and Purification in Napiergrass (*Pennisetum purpureum* SCHUMACH.). A Thesis Submitted to the Office of Graduate and Professional Studies of Texas A&M University in partial fulfillment of the requirements for the degree of master of science.