



Research Article

Proximate Composition, Amino Acids and Fatty Acids Profile of *Citrullus Vulgaris* (Guna) Seeds

Ndahi Tarhyel Ezekiel¹, Hyelvidzira Haruna Mshelia², Mshelia Ladi Peter¹, Milala Muhammad Adamu², Peter Danjuma¹, Badau Mamudu Halidu¹, Kassum Afodia Litini¹, Kyari Bawagana¹ and Lawan Hadiza Kubra¹

¹Department of Food Science and Technology, Faculty of Engineering, University of Maiduguri, Borno State, Nigeria

²Department of Biochemistry, Faculty of Life Sciences, University of Maiduguri, Borno State, Nigeria;

*Corresponding author's Email: ndahitarhyel10@yahoo.com, doi.org/10.55639/607.333231

ARTICLE INFO:

Keywords:

C. vulgaris seeds,
Essential amino acids,
Fatty acids,
Plant-based nutrients

ABSTRACT

Citrullus vulgaris (guna) seeds have significant potential to meet the growing nutritional demands in Sub-Saharan Africa due to their rich nutrient composition. This study aimed to determine the proximate composition, amino acid, and fatty acid profiles of these seeds. Using AOAC methods, the analysis revealed that *C. vulgaris* seeds contain 4.75% moisture, 3.12% ash, 23.74% protein, 28.44% fats, 19.46% fiber, 20.99% carbohydrates, and 434.88 kcal/100g energy. The amino acid profile showed high levels of glutamic acid (10.12g/100g protein), aspartic acid (8.28g/100g protein), and leucine (6.80g/100g protein), with cysteine (0.50g/100g protein) being the lowest. Essential amino acids like leucine (6.80g/100g protein), lysine (3.89g/100g protein), valine (3.85g/100g protein), phenylalanine (3.46g/100g protein), and isoleucine (3.31g/100g protein) were abundant, while tryptophan (1.17g/100g protein) and methionine (1.31g/100g protein) were limiting. The seeds contained 70.25g/100g protein of total amino acids, with 40.53% essential and 59.47% non-essential amino acids. The fatty acid profile indicated high oleic acid (42.04%), linoleic acid (20.84%), palmitic acid (13.02%), and stearic acid (10.95%). The total unsaturated fatty acids (63.30%) exceeded saturated fatty acids (36.70%), and polyunsaturated fatty acids (PUFA) (20.84%) were lower than monounsaturated fatty acids (MUFA) (42.46%). The study concludes that the high protein, fat, and essential amino acid content of *C. vulgaris* seeds and oil can improve dietary protein quality and energy density, thus helping to alleviate protein-energy malnutrition and promoting healthy diets.

Corresponding author: Ndahi Tarhyel Ezekiel, **Email:** ndahitarhyel10@yahoo.com

Department of Food Science and Technology, Faculty of Engineering, University of Maiduguri, Nigeria,

INTRODUCTION

Malnutrition due to inadequate intake of balanced diet is a global challenge. The shift towards plant-based nutrient sources in meeting the growing nutritional demand sustainably amidst limited natural resources for the future promotes local sourcing and consumption of local indigenous edible seeds especially oilseeds and legumes, which have been found to be rich in nutrients (Sarwar *et al.*, 2013). There are available opportunities to diversify plant production to widen the range of ingredients in diets promoting nutrition, health and the environment (FReSH, 2020).

Globally, the projected demand for oilseeds and oilseed products has translated into an increase in oilseeds production. In general, soybean is highly cultivated compared to other oilseeds, notably with the former having an estimated increase of 411 Mt of production and later (rapeseeds, sunflower seeds and ground nut combined) at 179Mt of production respectively (OECD/FAO, 2021). In Sub-Saharan Africa, other oilseeds production is more cultivated compared to solely soybeans (OECD/FAO, 2021).

The significance of oilseeds as source of plant protein in meeting the protein-energy nutrients demand for healthy diets cannot be overemphasized. According to Millward (2011), one-third of the consumed proteins in developed countries comes from plant-protein sources while plant-protein sources constitute almost 80% of consumed proteins in the developing countries. Oilseeds contain high protein content (25.33 - 39.82%) which can be used as protein sources (Akinyele and Oloruntoba, 2013; Egbegi, 2014; Kowalska *et al.*, 2020). Studies have shown that oilseeds are good sources of protein-energy and amino acids nutrients for human and animal nutrition (Adebisi *et al.*, 2017, Jannathulla *et al.*, 2017; Ndahi *et al.*, 2019). Correspondingly, oilseeds products have been found to contain high fat and protein contents (Adua *et al.*, 2019 and Adeyeye *et al.*, 2020).

Wu (2013) remarked that the source of essential amino acids for humans is dietary protein. Oilseeds are considerable sources of essential amino acids. According to Kotecka-Majchrzak *et al.* (2020), oilseeds are high in leucine and valine contents in essential amino acids profile while the limiting essential amino acids are tryptophan, methionine (and cysteine).

Plant seeds such as oilseeds are potential sources of fatty acids for human diets and their prospect in production scalability (e Silva *et al.*, 2018). Oilseeds are known to contain a considerable amount of oil. Vegetable oil from plant sources is of interest due to the increased awareness of healthy diets, global wealth and biofuel feedstock supply (US FDA, 2013; Kijima *et al.*, 2016). Vegetable oils are important in human nutrition. Fatty acids are the major constituents of these oils playing critical roles in physiological functions and serve as absorbent for fat-soluble nutrients (Jafari *et al.* 2022). The Utilization of vegetable oils depends on fatty acid constituents which reflect its stability and application value (nutritional and industrial) (Ramadan, 2015; Aremu *et al.*, 2015). Vegetable oil are known to supply the essential fatty acids that are of significant benefits in nutrition.

In recent years, oilseeds such as soybeans, rapeseeds, sunflower seeds, ground nuts, cotton seeds, melon seeds among others have been increasingly studied as nutrient potential sources for food application and industrial purposes (McKevith, 2005; Aremu *et al.*, 2015). They are utilized in various processed forms such as whole seeds, meals, vegetable oil, oleochemicals and biofuels (Kojima *et al.*, 2016; Igwenyi *et al.*, 2017). Currently, soybean, chia seeds, flaxseed, rapeseeds among other oilseeds are used in food application such as bakery products, cakes, energy bars, dairy and egg substitutes, processed meat products, salad dressings, sauces and flavourings (FAO, 1992; Guo *et al.*, 2010; Kajla *et al.*, 2015; Kotecka-Majchrzak *et al.*, 2020).

Citrullus vulgaris (*guna*) plant also referred to as melon belongs to the family *Cucurbit*. Studies have shown that this family are rich in nutrients for nutritional uses (Abiodun and Adeleke 2010; Adua *et al.*, 2019; Akansha *et al.*, 2018; Adeyeye *et al.*, 2020). *C. vulgaris* is called *guna* in Hausa, *egusiito* in Yoruba, *ugbogoro* in Igbo and called *hela* in the Babur/Burah language in southern Borno state. Fresh *guna* seeds were documented to contain moisture (11.44%), ash (2.64%), protein (21.82%), fat (26.58%), fibre (18.21%), carbohydrate (19.31%) and energy value (403.74kcal/100g) contents (Ndahi *et al.*, 2023). In addition, *C. vulgaris* seeds as an oilseed have been noted to be a good source of protein, fat, minerals and phytochemicals (Penuel *et al.*, 2013; Ndahi *et al.*, 2023). It is processed into valuable edible products as oil, snacks, *guna* flour, *guna* paste, *guna* cake etc. It has been studied that expectant and breastfeeding mothers in some part of Borno state, Nigeria, consume *guna* porridge, which was meant to improve their nutrition during breastfeeding as reported by Wakshama (2016).

Various oilseeds have the potentials in meeting the growing nutritional demands especially in Sub-Saharan Africa, hence the need for nutrient characterization of the local available oilseeds for wide utilization. The objective of this study was to determine the proximate composition, amino acids and fatty acids profile of *C. vulgaris* (*guna*) seeds.

$$\% \text{ Moisture} = \frac{(w_1 - w_2) \times 100}{w_0} \quad \text{-----} \quad 1$$

Where; w_0 = weight of sample used
 w_1 = weight of petri dish with the sample in grams before oven drying
 w_2 = weight of petri dish with the sample in grams after oven drying

Ash Content Determination

Two grammes of *guna* seed sample flour was poured into separate crucible that were initially clean dried, cooled (in a desiccators) and weighed (w_1) and weighed (w_2), respectively. It was

MATERIALS AND METHODS

Sample Collection

Five (5) kilogrammes of *C. vulgaris* (*guna*) seeds were bought from Biu LGA Central Market, Borno state, Nigeria and identified at the Department of Biological Sciences, University of Maiduguri, Nigeria.

Sample Preparation

The *guna* seeds sample was sorted, cleaned and ground using an electric blender (Sonik, model SB-566 (made in china)). The ground sample flour was packaged in an airtight plastic container and stored in a cool and dry place prior to the analyse.

Proximate Composition Determination

The proximate composition of the *guna* seeds sample was carried out according to the methods described by AOAC (2005). The sample was analyzed for moisture, crude protein (%N x 6.25 conversion factor), crude fats, ash and fibre contents. While the carbohydrate content was obtained by difference.

Moisture Content Determination

A clean flat petri dish was dried in an oven and cooled in a desiccator. Five (5) grammes of each seed sample flours (w_0) was introduced and spread into the petri dish, weighed accurately (w_1) and transferred into the air oven at 80°C to dry for about 3 hours. A pair of tongs was used to transfer the dish into a desiccator, allowed to cool and weighed to a constant weight (w_2). The moisture content of the sample was calculated as follows:

transferred using a pair of tongs into a Muffle furnace at 650°C until fully ashed and weighed (w_3) respectively. The ash content was calculated as follows:

$$\% \text{ Ash} = \frac{(w_3 - w_1) \times 100}{(w_2 - w_1)} \quad \text{-----} \quad 2$$

Where

w_1 = weight of empty crucible

w_2 = weight of crucible + sample before ashing

w_3 = weight of crucible + sample after ashing

Protein Content Determination

Protein content determination was carried out using the kjeldahl method. One (1) gram of the sample was put into a digestion flask and 20ml of concentrated sulphuric acid (H_2SO_4) and one digestion tablet which act as a catalyst were added and put on a digester. The temperature was set at about $35^{\circ}C$ and the samples allowed to digest for 5 hours during which the sample became colourless. The digester was allowed to cool and made up to 100ml with distilled water. The diluted sample (5ml) was introduced into a

distillation unit and 20mls of 40% sodium hydroxide (NaOH) added and heated until it began to boil. It then condensed in a condenser and collected into 4% baric acid in which 4 drops of mixed indicators (bromocresol green and methyl red) have been incorporated. The distillation process was completed when the volume of the content of the conical flask reached 70mls and collected. The collected distillate was titrated against 0.01N HCl until a faint pink colouration was achieved.

The Crude protein was calculated using the formular:

$$\% \text{ Protein} = \frac{(A - B) \times N \times F \times 6.25 \times 100}{\text{mg of sample}} \quad \text{-----} \quad 3$$

where

A = Acid used for titrating the sample (ml)

B = Acid used for titrating blank sample (ml)

N = Normality of acid used for titration

F = 14.007

6.25 = Protein conversion factor

Fat Content Determination (Soxhlet method)

One (1) gram of *guna* seed sample flour was weighed (w_0) and put into a thimble separately and covered with a loosed mass of cotton wool to check spillage. Using Soxhlet extraction apparatus, petroleum ether was added and allowed to reflux in a cleaned weighed flask (w_1) for about 6hours.

The petroleum ether in the extracted solution was distilled off and the sample in the flask, dried in the oven for one hour at $10^{\circ}C$ and then cooled in desiccators and weighed (w_2). Increase in weight of the flask was taken as the weight of the extracted fat

$$\% \text{ Fat} = \frac{(w_2 - w_1) \times 100}{w_0} \quad \text{-----} \quad 4$$

Where

w_0 = weight of sample used

w_1 = weight of flask

w_2 = weight of flask + fat

Crude Fibre Content Determination

Two (2g) (w_0) of *guna* sample and 50mls of tri-chloroacetic acid reagent (TCA) were mixed in a flask and refluxed for 40 minutes. It was filtered with a gilter paper washed with water and then rinsed with petroleum ether. The filter paper (with the filter cake) were dried at 30°C – 60°C for 24 hours, cooled and weighed (w_1). The resultant was then ashed at 650°C, cooled in a desiccator and reweighed (w_2).

Formula:

$$\% \text{ Fibre} = \frac{(w_1 - w_2) \times 100}{w_0} \text{ ----- } 5$$

Where

W0= weight of sample used

W1= weight of sample plus filter paper after drying

W2= weight of sample plus filter paper after ashing

Carbohydrate Content Determination

The carbohydrate content was determined by taking the difference when all other components of proximate were added and was subtracted from hundred. i.e.;

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Fat} + \% \text{ Protein} + \% \text{ Ash} + \% \text{ Fibre}) \text{ ----- } 6$$

Energy Value Determination

The energy values (Kcal) of the sample were calculated by applying AtWater factors 4, 9, and 4 for each gram of protein, lipid, and carbohydrate, respectively (FAO, 2003) as shown below:

$$\text{Energy value (Kcal/100g)} = (4 \times \% \text{ Carbohydrate}) + (4 \times \% \text{ Protein}) + (9 \times \% \text{ Fat}) \text{ } 7$$

Determination of Amino Acid Profile

The Amino acid profile was determined as described by AOAC (2005) with modifications. The sample was dried at 70°C to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer. The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 4g of the sample was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus. Nitrogen determination by micro Kjeldahl Method. Hydrolysis was carried out using 7ml of 6N HCL addition and oxygen expelled by passing nitrogen into the ampoule (this was to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystine) (note: Tryptophan was destroyed during acid hydrolysis, hence, the

tryptophan in the known sample was hydrolyzed with 4.2 M Sodium hydroxide (Maria *et al*, 2004) and subsequent steps (evaporation and loading into the analyzer) were taken separately for its determination). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°C± 5°C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer. The amount loaded was 60 microlitre. This was dispensed into the cartridge of the analyser (the analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate). An integrator attached to the Analyzer calculated the peak area proportional to the concentration of each of the amino acids.

Oil Extraction

Solvent extraction method was used to extract the oil from *C. vulgaris* seeds as described by Anwar *et al.* (2008) with modifications. Four hundred and fifty grammes (450 g) of ground *guna* seed

was fed into the Soxhlet extractor fitted with a 1-L round bottomed flask and a condenser. The extraction was done using a 1000ml of n-hexane on a water bath (40-60°C) for 6h. The oil was obtained after the solvent was distilled off under vacuum.

Fatty Acid Profile Determination

Half (0.5) ml of the *C. vulgaris* seeds oil sample was dissolved in 10mL hexane and 1mL of alcoholic potassium hydroxide was added. Fatty acid methylation was prepared according to IUPAC (1987) and analyzed using gas chromatography/mass spectrometry (GC/MS). One (1µm) of the sample was analyzed using Agilent GC (7890B), equipped with a 30 m x 250 µm x 0.25 µm Column; coupled with Agilent MSD (5977A MSD). The carrier gas helium was at a flow rate of 1 ml/min. The GC oven was initially set at 70 °C and then ramped at the rate of 20 °C/min to 110 °C and held for 1 min. The temperature was then ramped at the rate of 5 °C/min to 230 °C and then held for another 5 min. Finally, the oven temperature was ramped to 280°C at the rate of 20°C/min and held for further 5 minutes. Equilibration time, MSD Transfer Line, MS Source and MS Quad were set at 0.5 minutes, 250 °C, 230 °C and 150 °C respectively.

The identification and characterization of chemical compounds in the sample was based on GC relative retention times with those of authentic standards of FAMES (Sigma Chemical Co., St Louis, MO, USA). The FAMES were further identified and confirmed using their mass spectroscopy spectra were computer-matched with those of standards available in NIST mass spectrum libraries. The percentage composition of the sample constituents was expressed as a percentage by peak area (Anwar *et al.*, 2008).

Statistical Analysis

Data (in triplicates) generated from the analyses for proximate composition and amino acid profile were expressed as mean (±) standard deviation while the fatty acid profile result was expressed in percentage using MS Excel 2019 Software.

RESULTS

The proximate composition of the *C. vulgaris(guna)* seeds sample was presented in Table 1. *C. vulgaris* seeds contained moisture (4.75%), ash (3.12%), protein (23.74%), fat (28.44%), fibre (19.46%), carbohydrate (20.99%) and energy value (434.88kcal/100g). The sample had high fat content followed by protein and carbohydrate contents. Together, the protein and fat contents of the *C.vulgaris* seeds was more than 50 percent of the total proximate contents

Table 1: The Proximate Composition of *C. vulgaris (guna)* seeds

Parameters	<i>C. vulgaris</i> seeds
Moisture (%)	4.75 ± 0.05
Ash (%)	3.12 ± 0.03
Protein (%)	23.74 ± 0.09
Fat (%)	28.44 ± 0.05
Fibre (%)	19.46 ± 0.02
Carbohydrate (%)	20.99 ± 0.09
Energy value (kcal/100g)_	434.88 ± 0.11

The amino acid profile of *C. vulgaris* seeds was presented in Table 2. It was observed that glutamic acid (10.12g/100g protein) recorded the highest content followed by aspartic (8.28g/100g protein), leucine (6.80g/100g protein) and arginine (6.33g/100g protein) contents while cystine (0.50g/100g protein) recorded the least amino acid in *C. vulgaris* seeds. Among the essential amino acids, leucine (6.80g/100g protein) was the highest followed by lysine (3.89g/100g protein), valine (3.85g/100g protein),

phenylalanine (3.46g/100g protein), isoleucine (3.31g/100g protein), threonine (2.43g/100g protein) and histidine (2.25g/100g protein) while tryptophan (1.17g/100g protein) was the first limiting essential amino acid followed by methionine (1.31g/100g protein) in *C. vulgaris* seeds. The total amino acids (TAA), percent total essential amino acids (TEAA%) and percent total non-essential amino acids (TNEAA%) of *C. vulgaris* seeds were observed to be 70.25g/100g protein, 40.53% and 59.47% respectively.

Table 2: The Amino Acids Profile of *C. vulgaris* (*guna*) seeds

Amino Acids Composition	<i>C. vulgaris</i> (<i>guna</i>) seeds (g/100g protein)	Reference Protein (g/100g protein) ¹
Essential Amino Acids (EAA)		
Leucine (Leu)	6.80 ± 0.02	5.90
Lysine (Lys)	3.89 ± 0.02	4.50
Isoleucine (Ileu)	3.31 ± 0.01	3.00
Phenylalanine (Phe)	3.46 ± 0.01	-
Tryptophan (Tryp)	1.17 ± 0.02	0.60
Valine (Val)	3.85 ± 0.05	3.90
Methionine (Met)	1.31 ± 0.04	2.20 (Met + Cyst)
Threonine (Threo)	2.43 ± 0.06	2.30
Histidine (His)	2.25 ± 0.02	1.50
Total	28.47	
Non-Essential Amino Acids (NEAA)		
Arginine (Arg)	6.33 ± 0.06	
Alanine (Ala)	3.34 ± 0.05	
Cystine (Cyst)	0.51 ± 0.02	
Proline (Pro)	3.05 ± 0.01	
Tyrosine (Tyro)	3.30 ± 0.01	
Glycine (Gly)	3.16 ± 0.03	
Serine (Ser)	4.39 ± 0.02	
Aspartic acid (Asp)	8.28 ± 0.04	
Glutamic acid (Glu)	10.12 ± 0.01	
Total	41.78	
Summary of Amino Acids		
TAA	70.25	
TEAA (%)	40.53	
TNEAA (%)	59.47	
First Limiting EAA	Tryptophan	
Second Limiting EAA	Methionine	

TAA: Total Amino Acids, TEAA: Total Essential Amino Acids, TNEAA: Total Non-essential Amino Acids

¹: Adopted from WHO/FAO/UNU, 2007

The fatty acid composition of *C. vulgaris* seeds oil was shown in Table 3. Among the saturated fatty acids, palmitic acids (13.02%) had the highest content followed by stearic acid (10.95%), myristic acid (4.45%), capric acid (3.02%), lauric acid (2.58%), caprylic acid (2.12%) and trace amount of tridecanoic acid (0.56%) content. *C. vulgaris* seeds oil contained oleic acid (41.34%) as the highest unsaturated fatty acid followed by linoleic acid (20.84%) and

trace amount of palmitoleic acid (0.70%) and malvalic acid (0.42%). It was observed that oleic acid had the highest fatty acid content followed by linoleic acid, palmitic acid and stearic acid contents in *C. vulgaris* seeds oil. The study showed that *C. vulgaris* seeds oil contains total saturated fatty acids (TSFA) (36.70%), total unsaturated fatty acids (63.30%), monounsaturated fatty acids (42.46%) and polyunsaturated fatty acids (20.84%).

Table 3: The Fatty Acids Profile of *C. vulgaris* (*guna*) Seeds Oil

Fatty Acids Composition	Chemical Formula	<i>C. vulgaris</i> Seeds Oil (%)
Saturated Fatty Acids		
Caprylic acid	C8:0	2.12
Capric acid	C10:0	3.02
Lauric acid	C12:0	2.58
Tridecanoic acid	C13:0	0.56
Myristic acid	C14:0	4.45
Palmitic acid	C16:0	13.02
Stearic acid	C18:0	10.95
Unsaturated Fatty Acids		
Palmitoleic acid	C16:1	0.70
Oleic acid	C18:1	41.34
Malvalic acid		0.42
Linoleic acid	C18:2	20.84
Summary of Fatty Acids		
TSFA		36.70
TUSFA		63.30
MUFA		42.46
PUFA		20.84

TSFA: Total saturated fatty acids, TUSFA: Total unsaturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

DISCUSSION

Moisture content is a significant parameter in predicting the storage stability of food products. The low moisture content noted of *C. vulgaris* seeds (4.75%) implies that they will be shelf-stable under good storage conditions. The moisture content of *C. vulgaris* seeds falls within the range reported for groundnut cultivars (4.14 -

5.46%) in Nigeria (Olayinka *et al.*, 2022) and was close to that reported for *C. vulgaris* (4.45%) by Akinyele and Oloruntoba (2013) and flaxseeds (4.71%) by Khan and Saini (2016) and However, higher moisture content of fresh *C. vulgaris* seeds (11.44%) was recently reported (Ndahi *et al.*, 2023). Again, Egbebi, (2014) who studied three different species of melon seeds also reported a

lower moisture content (1.44 – 1.55%). This variation in moisture content could be due to the varietal, method of drying process and analytical processes (Kiin-Kabari and Akusu 2014; Sa *et al.*, 2021).

The amount of ash content signifies the amount of mineral in a sample. In this study, the ash content of *C. vulgaris* seeds was 3.21%. This is similar to previous study where the ash content obtained for *C. vulgaris* was 3.15% (Egbebi, 2014) and also similar to another study although in groundnut where the ash content was observed to be 3.20% (Oguntoyinbo *et al.*, 2023). Conversely, higher ash content was noted for soybean (4.61%) by Datti *et al.* (2019). Hence, *C. vulgaris* seeds could be a good source of minerals owing to their ash content.

Proteins are macromolecules of significant nutritive value and play a range of structural and function role in the body including hormone, blood coagulation, enzymes, tissue building, etc (Loveday, 2019; Jose, 2022). The protein content in a food source is important in alleviating protein-energy malnutrition through diets. The protein content of *C. vulgaris* seed (23.74%) in this study falls within the range of selected soybean genotypes (16.06 - 41.92%) in Nigeria (Nwosu *et al.*, 2019). However, in a previous study by Penuel *et al.* (2013), a higher protein content was reported for *C. vulgaris* (36.58%) and groundnut cultivars ranging from 25.27 - 30.22% (Olayinka *et al.*, 2022). The high protein content noted in this study agreed that *C. vulgaris* could be used as a protein source as suggested by Adua *et al.* (2019). In addition, It has been studied that expectant and breastfeeding mothers in some part of Borno state, Nigeria, consume *C. vulgaris* (*guna*) seeds porridge, which was meant to improve their nutrition during breastfeeding as reported by Wakshama (2016).

Fats in food is significant in nutritional aspects and organoleptic attributes. They are high caloric macromolecules, serve as nutrient absorbents (fat-soluble nutrients) and play important roles in

physiological processes. The fat content of *C. vulgaris* seeds (28.44%) falls within the range of 21.12 -30.45% reported for soybeans (Nwosu *et al.*, 2019). It is noteworthy to state that *C. vulgaris* seeds as an oilseed is a good source of oil. However, the different observations reported for *C. vulgaris* as 26.85% (Akinyele and Oluruntoba 2013) and 49.00% (Penuel *et al.*, 2013) could be due to ecological and processing methods. The high fat content of *C. vulgaris* seeds suggest that it is a good source of oil and an energy dense seed for consumption.

The fibre content of *C. vulgaris* seed was noted as 19.46%. This result was higher compared to that of flaxseed (9.34 - 9.63%) (Khan and Saini 2016), *C. lanatus* (14.92 - 15.81%) (Jackson *et al.*, 2013) and soybean (6.84%) (Datti *et al.*, 2019). The higher fibre content observed was due to the utilization of the undehulled whole seeds. The carbohydrate content of *C. vulgaris* seeds (20.19%) obtained in this study was higher compared to that of soybean (16.31%) (Etiosa *et al.*, 2018) and groundnut cultivars (9.89 -15.76%) (Olayinka *et al.*, 2022). Conversely, the carbohydrate content of flaxseed (26.72 - 29.06%) (Khan and Saini 2016).

In this study, the energy value of *C. vulgaris* seeds was 434.88 kcal/100g (Table 1). This value is slightly below the caloric content of soybean ranging from 459.97 - 496.37 kcal/100g (Nwosu *et al.*, 2019) and above that of *C. vulgaris* (411.94 kcal/100g) (Adebisi *et al.*, 2017). The high energy content was due to the high protein, fat and carbohydrate contents of the seeds. *C. vulgaris* seeds supplementation in animal diets has been shown to positively increase the protein and energy density of the diets as well as improve the milk yield and composition of lactating ewes (Ndahi *et al.*, 2019). Hence, as the search for nutrient sources increases, it is important to point out that the observed high-caloric nutrients in *C. vulgaris* seeds could be significant in improving the nutrient density of diets where protein-energy malnutrition is a menace.

The quality of a protein source is viewed in its amino acid profile. Hertzler *et al.* (2020) argued that consuming protein from plant sources can help to increase the intake of essential amino acids which are significant in healthy diets. The high content of glutamic acid, aspartic acid, leucine and arginine in *C. vulgaris* seeds of this study agreed with the reported studies of the amino acids profile of *C. vulgaris* seeds, pumpkin seed meal, chia seeds meal and sesame seed meal where the highest amino acid contents were glutamic acid content was the highest amino acid followed by aspartic acid, although, arginine was noted to be higher than leucine (Penuel *et al.*, 2014; Sa *et al.*, 2022). Among the essential amino acids (EAA) in this study, leucine, lysine and valine contents were found higher compared to the others. Similarly, the contents of leucine, lysine and isoleucine were the highest among the EAA profile as reported for flaxseeds and chia seeds (Nitrayova *et al.*, 2014) and *Hura crepitans* seed and *Vigna unguiculata* (Adebisi *et al.*, 2020). However, different results were observed for sesame seeds and soybeans (Adebisi *et al.*, 2017). According to Kotecka-Majehrzak *et al.* (2020), sulphur-containing amino acids (methionine and cystine) and hydrophobic tryptophan are the least amounts in oilseeds which is in line with this study where the first two limiting EAA are tryptophan and methionine.

The data (Table 2) showed that the leucine (6.80g/100g protein) content was higher compared to the leucine content for *C. vulgaris* (5.66g/100g protein) as reported by Penuel *et al.*, (2014). In addition, the tryptophan (1.17g/100g protein) content found in this study was comparable to the tryptophan content for *C. vulgaris* (1.10g/100g protein) whereas the other essential amino acids in this study were found to be lower than their respective reported values by Penuel *et al.*, (2014). Notably, the leucine, lysine, isoleucine and valine contents of *C. vulgaris* seeds of this study fall within the range observed for groundnut cultivars recording 6.69 - 7.53,

3.49 - 4.52, 3.11 - 4.29 and 2.62 - 3.93 g/100g protein respectively (Olayinka *et al.*, 2022). Moreover, the methionine and tryptophan contents of *C. vulgaris* seeds of this study recorded higher values compared to groundnut cultivars (0.10 - 0.97 and 0.11 - 0.95g/100g protein respectively) while the phenylalanine, threonine, and histidine contents in this study were lower than the observed range 4.16 - 5.88, 2.53 - 2.75 and 2.47 - 2.89g/100g protein respectively (Olayinka *et al.*, 2022).

The TEAA% (40.53%) of *C. vulgaris* seeds obtained in this study was close to that reported although for groundnut cultivars (39.07%) (Olayinka *et al.*, 2022) and higher compared to chia seeds (37.88%) and flaxseeds (33.76%) (Nitrayova *et al.*, 2014).

From Table 2, the study observed that leucine, isoleucine, threonine, tryptophan, and histidine of *C. vulgaris* seeds recording 6.80, 3.31, 2.43, 1.17, 2.25 g/100g protein were above the reference protein as 5.9, 3.0, 2.3, 0.6 and 1.5 g/100g protein respectively for maintenance of amino acid pattern, while valine (3.85g/100g protein) in this study was close to the reference protein content for val (3.9g/100g protein) (WHO/FAO/UNU, 2007). However, the lysine and methionine + cystine contents as 3.89 and 1.81g/100g protein were lower compared to the reference protein as 4.5 and 2.2 g/100g protein respectively. Although the sulphur-containing amino acids and tryptophan are the limiting amino acids in *C. vulgaris* seeds in this study, their contents together with lysine are not too low compared to the reference protein. Thus, this result implies that *C. vulgaris* seeds could be used to complement other food sources as it has a higher value of limiting amino acids.

As building constituents of lipids, fatty acids function as a source of energy, nutrient absorbent (fat-soluble vitamins), in the biosynthesis of other compounds, among others, which are fundamental for nutrition, health and

reproduction (Kaur *et al.*, 2014; Jafari *et al.*, 2022).

Plant oil has been known to contain more unsaturated fatty acids and saturated fatty acids (Ivanova *et al.*, 2016) which is in agreement to the findings of this study. *C. vulgaris* seeds oil had oleic acid (41.34%) as the highest fatty acid content followed by linoleic acid (20.84%), palmitic acid (13.02%) and stearic acid (10.95%) contents. Similar observations were reported for rapeseed oil and peanut oil samples recording oleic acids (59.0% and 51.0%) as the highest fatty acid followed by linoleic acids (21.0% and 27.0%) and palmitic acids (4.7% and 10.1%) respectively, whereas that of soybean oil and corn oil samples noted linoleic acids (55.3% and 55.7%) as the highest fatty acid followed by oleic acids (21.0% and 30.5%) and palmitic acids (10.0% and 10.7%) respectively was different (Jafari *et al.*, 2022).

The TUSFA (63.30%) content of *C. vulgaris* seeds oil was higher than the TSFA (36.7%) content (Table 3). This result is in line with the fatty acids composition of groundnuts, flaxseeds, chia seeds, walnut, and canola oils samples (Nitrayova *et al.*, 2014; Ivanova *et al.*, 2016; Olayinka *et al.*, 2022; Katunzi-Kilewela *et al.*, 2022). Saturated fatty acids elevates cholesterol level where as unsaturated fatty acids functions include; cholesterol level reduction, blood viscosity reduction, brain cell activity and improving memory, cell membrane fluidity, etc (Freitas and Campos, 2019; Mannard and Dulloo, 2021). The result of this study implies that the consumption of *C. vulgaris* seed oil could be of health benefit due to its high contents of TUSFA. PUFA are essential fatty acids that cannot be synthesized by the body hence, vegetable oils are important source of dietary fatty acids. The consumption of these fatty acids are significant in the mental development and growth of children, increasing beneficial cholesterol, liver fat reduction, anti-inflammatory, dementia prevention, bone health, ect (Nguyen *et al.*, 2018;

Chen and Liu 2020; Jafari *et al.*, 2022). The main component of the PUFA noted in *C. vulgaris* seeds oil is linoleic acid. Similarly, Ivanova *et al.* (2016) also reported that the major component of PUFA in walnut, pumpkin, salad mix, and sunflower oil is linoleic acid. In this study, the PUFA (20.84%) content of *C. vulgaris* seed oil is lower compared to the MUFA (42.46%) content. This result was lower compared to the findings of PUFA contents reported for nuts' oil (49 - 55%) (Jafari *et al.*, 2022), linseeds oil (57 - 76%) (Juric *et al.*, 2022) and some oil samples ranging from 54.3 - 79.1% (Orsarova *et al.*, 2015) and was higher than coconut oil (1.6%) (Jafari *et al.*, 2022). *C. vulgaris* seed oil could be used as a dietary source of essential fatty acids for health and nutrition due to its noted quantity of PUFA.

CONCLUSION

C. vulgaris seeds as an oilseed contained high caloric nutrients and ash content. In the EAA composition, histidine (2.25 g/100g protein), leucine (6.80 g/100g protein), isoleucine (3.31 g/100g protein), threonine (2.43 g/100g protein), valine (3.85 g/100g protein) and tryptophan (1.17 g/100g protein) contents were close and/or above the reference protein for maintenance of amino acids pattern in nutrition. Although, lysine (3.89 g/100g protein) and methionine+cystine (1.82 g/100g protein) were lower compared to the reference protein, the noted values were higher compared to some reported plant protein sources such as some groundnut cultivars in Nigeria, sandbox seeds, black eyed pea and red kidney bean. Notably, *C. vulgaris* seeds oil has higher unsaturated fatty acids (63.30%) than saturated fatty acids (36.70%) where oleic acid (41.34%) and linoleic acid (20.84%) were the major components of unsaturated fatty acid and polyunsaturated fatty acid respectively. Thus, the high protein and fat contents and considerable essential amino acid and fatty acids compositions of *C. vulgaris* seeds and its oil revealed that it has the potential to improve the protein quantity and quality and energy density of diets which is

paramount in alleviating protein-energy malnutrition and for therapeutic purposes towards promoting healthy diets.

REFERENCES

- Abiodun, O.A. and Adeleke, R.O. (2010). Comparative Studies on Nutritional Composition of Four Melon Seeds Varieties. *Pakistan Journal of Nutrition*. 9 (9): 905-908.
- Adebisi, A. K., Stephen, E. C., Chinedu, I. and Emmanuel, U. (2017). Quantification of Protein and Amino Acid Composition of Some Oilseeds. *World Journal of Biochemistry and Molecular Biology*. 2(2): 8 – 11.
- Adebisi, A.K., Abiodun, P.C., Ejeh, Y.O., Chinekwa, U.K. and Falila, A. (2020). Proximate composition and amino acids contents in selected legumes and *Hura crepitans* seeds in Wukari, Taraba State. *Covenant Journal of Physical and Life Sciences*. 8(1): 14 – 22.
- Adeyeye, S. A. O., Bolaji, O. T., Adegunde, T. A., Adebayo-Oyetaro, A. O., Tiamiyu, H.K. and Idowu-Adebayo, F. (2020). Evaluation of nutritional composition, physicochemical and sensory properties of ‘Robo’ (a Nigerian traditional snack) produced from watermelon (*Citrullus lanatus* (Thumb.)) seeds. *Food Research* 4 (1) : 216 – 223.
- Adua, E. A., Adzitey, F. and Abu, A. (2019). Nutrient Composition of Whole Grain and Processed “Niri” (*Citrullus vulgaris*) seeds. *International Journal of Food Science and Nutrition*. 4(2): 76 -79.
- Akansha, Kriti, S., Aditi, S. and Ekta, S. C. (2018). Nutritional and Phytochemical Evaluation of Egusi Melon Seeds: *Citrullus colocynthis* L. *International Journal of Creative Research Thoughts*. 6(2): 1 – 6.
- Akinyele, B.J. and Oloruntoba, O.S. (2013). Comparative studies on *Citrullus vulgaris*, *Citrullus colocynthis* and *Cucumeropsis mannii* for Ogiri production. *British Microbiology Research Journal*. 3:1-18
- Anwar, F., Naseer, B., Bhangar, M.I., Ashraf, S., Talpur, F.N. and Aladedunye, F.A. (2008). Physico-chemical characteristics of citrus seeds and seed oils from Pakistan. *Journal of American Oil Chemists Society* 85: 321 – 330.
- Aremu, M.O., Ibrahim, H. and Bamidele, T.O. (2015). Physicochemical characteristics of the oils extracted from some Nigerian plant foods – A Review. *Chemical and Process Engineering Research*. 32: 36 – 52.
- Association of Official Analytical Chemists AOAC (2005). Official method of analysis of the AOAC W. Horwitz Editor, 18th Edition, Washington: D.C.
- Chen J, and Liu H. 2020. Nutritional indices for assessing fatty acids: a mini-review. *International Journal of Molecular Science*. 1(16):5695.
- Datti, Y., Bayero, A.S., Abdullhadi, M., Yahya, A.T., Salihu, I., Lado, U.A, Nura, T. and Imrana, B. (2019). Proximate composition and mineral composition of Soya bean (*Glycine max*) available in Kano State, Nigeria. *ChemSearch Journal*. 10(2): 62 – 65.
- e Silva A.P., da Costa W.A., Salazar, M.D., do Nascimento Bezerra, P., Pires, F.C., Ferreira, M.C., Menezes, E.G., Urbina, G.R., Barbosa, J.R., and de Carvalho, R.N. (2018). Commercial and therapeutic potential of plant-based fatty acids. In: Biochemistry and health benefits of fatty acids. DOI:<http://dx.doi.org/10.5772/intechopen.81122>
- Egbebi, A. O. (2014). Comparative Studies on the three different Species of Melon seeds: (*Citrullus vulgaris*, *Cucumeropsis mannii* and *Leganaria siceraria*). *Sky Journal of Food Science*. 4 (1): 1 – 4.
- Etiosa, O.R., Chika, N.B. and Benedicta, A. (2017). Mineral and proximate composition of soya bean. *Asian Journal*

CONFLICT OF INTERESTS

The authors declared no conflict of interests.

- of Physical and Chemical Sciences*. 4(3): 1 – 6.
- FAO, (1992). Technology of Production of Edible Flours and Protein Products from Soybean. Ed. by Zek, B. FAO Agricultural Service Bulletin, No. 97.
- FAO, (2003). Food Energy – Methods of analysis and conversion factors. FAO Food Nutrition. Pp 7 – 17.
- Freitas, R. D. and Campos, M.M. (2019). Protective effects of omega-3 fatty acids in cancer-related complications. *Nutrients*. 11(5): 945.
- Food Reform for Sustainability and Health (FReSH) (2020). Plant Protein: A Key Lever to Accelerate Food System Transformation. WBCSD, 1211.Geneva, 1, Switzerland.
- Guo, X., Tian, S., and Small, D. M. (2010). Generation of meat-like flavourings from enzymatic hydrolysates of proteins from *Brassica sp.* *Food Chemistry*, 119, 167–172.
- Hertzler, S. R., Lieblein-Boff, J. C., Weiler, M. and Allgeier, C. (2020). Plant Proteins: Assessing Their Nutrition Quality and Effects on Health and Physical Function. *Nutrients*, 12, 3704 pp 1 – 27.
- Igwenyi, I.O., Enyi, N.A., Igwenyi, I.P., Famurewa, A.C. and Obasi, N.A. (2017). Amino acids composition, fatty acids profile and characterization of vegetable oil from *Hura crepitans* seeds. *International Journal of Biology, Pharmacy and Allied Sciences*. 6(9): 1781 – 1791.
- International Union of Pure and Applied Chemistry (IUPAC). (1987). In: Paquot C, Hautfenne A (eds) Standard methods for the analysis of oils, fats and derivatives, 7th revised and enlarged edn. Blackwell Scientific, London.
- Ivanova S, Marinova G, and Batchvarov V. (2016). Comparison of fatty acid composition of various types of edible oils. *Bulgarian Journal for Agricultural Sciences*. 22(5):849–56.
- Jackson, B. A., Adamade, C. A., Azogu, I. I. and Oni, K. C. (2013). Melon pod fermentation and its effects on physiochemical characteristics of melon seeds. *Scientific Research and Essays*, 8(17): 664 – 669.
- Jafari, S., Ebrahim, M. Assatarakul, K. and Jafari, S.M. (2022). Plant oils rich in essential fatty acids. Handbook of Food Bioactives Ingredients. Jafari S. M. et al. (eds.). Springer Nature, Switzerland.
- Jannathulla, R., Dayal, J. S., Vasanthakumar, D., Ambasankar, K. and Muralidhar, M. (2017). Effect of Fermentation Methods on Amino Acids, Fibre Fractions and Anti-nutritional Factors in Different Plant Protein Sources and Essential Amino Acids Index for *Panaeus vannamei* Borne, 1931. *Indian Journal Fish*. 64 (2): 40 – 47.
- Jose, S. (2022). Protein importance and their function in the human body. *Journal of Biomedical Research and Therapeutics*. 11(1):194.
- Jurić S, Jurić M, Siddique MA and Fathi M. (2022). Vegetable oils rich in polyunsaturated fatty acids: Nanoencapsulation methods and stability enhancement. *Food Review International*. 38(1):32–69.
- Kajla, P., Sharma, A., and Sood, D. R. (2015). Flaxseed - a potential functional food source. *Journal of Food Science & Technology*, 52(4), 1857–1871.
- Katunzi-Kilewela, A., Kaale, L.D., Kibazohi, O., Fortunatus, M. and Rweyemamu, L.M.P. (2022). Fatty acids and amino acids profiling of Chia seeds and physicochemical characterization of Chia seeds oil. *African Journal of Food Science*. 16(11): 269 – 278.
- Kaur N, Chugh V, and Gupta AK. (2014). Essential fatty acids as functional components of foods – a review. *Journal of Food Science and Technology*. 51(10):2289–303.
- Khan, A. and Saini, C. S. (2016). Effect of Roasting on Physico-chemical and Functional Properties of Flaxseed Flour. *Cogent Engineering*, 3 (1): 1145566. Pp 1 – 14.
- Kiin-Kabari, D. B. and Akusu, O. M. (2014). Effects of Processing on the Proximate Composition, Functional Properties and Storage Stability of Watermelon

- (*Crullus lanatus*) seed flour. *International Journal of Biotechnology and Food Science*, 2 (7): 143 – 149.
- Kojima, Y. Parcell, J. and Cain, J. (2016). A global demand analysis of vegetable oils for food and industrial uses: A Cross-Country Panel Data Analysis with Spatial Econometrics. Agricultural and Applied Economics Association Annual Meeting Conference Paper, Boston, Massachusetts, USA.
- Kotecka-Majchrzak, K., Sumara, A., Fornal, E. and Montowska, M. (2020). Oilseed proteins – Properties and application as a food ingredient. *Trends in Food Science & Technology* 106 (2020) 160–170.
- Kowalska, G., Kowalski, R. Hawlena, J. and Rowinski, R. (2020). Seeds of Oilseed Rape as an Alternative Source of Protein and Minerals. *Journal of Elementary*. 25 (2): 513 – 522.
- Loveday, S. M. (2019). Food Proteins: technological nutritional and sustainability attributes of traditional and emerging proteins. *Annual Review of Food Science and Technology*. 10: 311 – 339.
- Maria, M.Y., Justo, P., Julio, G., Javier, V., Francisco, M. and Manuel, A. (2004). Determination of tryptophan by high-performance liquid chromatography of alkaline hydrolysates with spectrophotometric detection. *Food Chemistry* 85(2): 317-320.
- McKevith, B. (2005). Nutritional Aspects of Oilseeds. *British Nutrition Foundation, Nutrition Bulletin*, 30: 13 – 26.
- Millward, D. J. (2011). Plant Based Sources of Proteins and Amino Acids in Relation to Human Health: Impact of Agriculture on Human Health and Nutrition. Vol 1. [www.eolss.net>sample.chapters](http://www.eolss.net/sample.chapters).
- Monnard, C. R. and Dulloo, A. G. (2021). Polyunsaturated fatty acids as modulators of fat mass and in human body composition regulation and cardiometabolic health. *Obesity Review* 22:e13197.
- Ndahi, T. E., Kasum, A. L. and Badau, M. H., Mshelia, L. P. and Peter, D. (2023). Phytochemical Contents of *Guna* parts; whole fruit, rinds and seeds samples. *Arid zone journal of engineering, technology & environment*. 19(3): 477 – 492.
- Ndahi, T. E., Ofeogbu, C. C., Igwegbe, A. O., Hadiza, K. L., Elizabeth, C. C., Jibril, A. and Amina, I. M. (2019). Effect of *Guna* seed Supplements on Yield and Chemical Composition of milk from Nigerian Ewes. *Health Environment and Sustainable Development. A Book of Readings: in Honour of Professor Ibrahim Ngodi, edited by Prof. Jecinta A. Oppara*. Published by University of Maiduguri.
- Nitrayova, S. Brestensky, M., Heger, J., Patras, P., Rafay, J. and Sirotkin, A. (2014). Amino acids and fatty acids profile of Chia (*Salvia hispanica L.*) and Flax (*Linum Usitassimum L.*) seeds. *Potravinarstvo Scientific Journal for Food Industry*. 8(1): 72 – 76.
- Nguyen, D. V., Malau-Aduli, B. S., Cavalier, J., Nicholas, P. D., Malau-Aduli, A. E. (2018). Supplementation with plant-derived oil rich in omega-3 polyunsaturated fatty acids for lamb production. *Veterinary Animal Sciences*. 6: 29-40.
- Nwosu, D.J., Olubiyi, M.R., Aladele, S.E., Apuyor, B., Okere, A.U., Lawal, A.I. Afolayan, G., Ojo, A.O., Nwadike, C., Lee, M. and Nwosu, E.C. (2019). Proximate and mineral composition of selected soybean genotypes in Nigeria. *Journal of Plant Development*. 26: 67 – 76.
- OECD/FAO (2021). OECD-FAO Agricultural Outlook 2021 – 2030: Chapter 4, pp 138 – 149.
- Oguntoyinbo, O.O., Oyinloye, A.M., Adesina, O.A., Adekanmbi, F.S. and Enujiugha, V.N. (2023). Variation in chemical composition, fatty acids, amino acids, oil quality and functional properties of groundnut (*Arachis hypogaea*) as influenced by high and low dose gamma irradiation using cobalt-60 wet storage source. <https://doi.org/10.21203/rs.3.rs-2778609/v1>
- Olayinka, B.U., Abdulkaki, A.S., Alsamadany, H., Alzahrani, Y., Omorinoye, O.A.,

- Olagunju, G.R. and Sulyman, A. (2022). Proximate Composition, Amino Acid and Fatty Acid Profiles of Eight Cultivars of Groundnut Grown in Nigeria. *Legume Research*. Pp 1 – 5.
- Orsavova, J., Misurcova, L., Ambrozova, J.V., Vicha, R. and Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International Journal of Molecular Science*. 16(6):12871–90.
- Ramadan M. F., (2015). Oxidation of β -sitosterol and campesterol in sunflower oil upon deep- and pan-frying of French fries. *Journal of Food Science and Technology*, 52(10): pp. 6301-6311.
- Sá, A.G.A., Cristina da Silva, D., Pacheco, M.T.B., Morenoc, Y.M.F. and Carciofia, B.A.M. (2021). Oilseed by-products as plant-based protein sources: Amino acid profile and digestibility. *Future Foods* 3: 100023.
- Sarwar, M.F., Sarwar, M.H., Sarwar, M., Qadri, N.A. and Moghai, S. (2013). The role of oilseeds nutrition in human health: A critical review. *Journal of cereals and Oilseeds*. 4(8): 97 – 100.
- U.S. Food and Drug Administration. (2013). Tentative Determination Regarding Partially Hydrogenated Oils; Request for Comments and for Scientific Data and Information. Federal Register 78 (217): 67169-7175
- Wakshama, A. S. (2016). Processing and nutritional evaluation of “guna” seed (*Citrullus vulgaris*). B. Sci Food Science and Technology Project, Submitted to the Department of Food Science and Technology, University of Maiduguri.
- WHO/FAO/UNU Expert Consultation, (2007). Protein and amino acid requirements in human nutrition. World Health Organisation. *Technical Report Series*. 1–265.
- Wu, G. (2013). Amino Acids: Biochemistry and Nutrition. Boca Raton, Florida: CRC Press.