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Research Article

Protein analysis and characterization of selected non-conventional vegetables

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Abstract

Africa boasts a vegetation with many undiscovered vegetables. Nigeria houses over 27 vegetables, of which only a few are eaten or known as conventional. A huge percentage of these vegetables are not known or completely ignored due to the scarcity of literature available on them. The essence of this study is to highlight the nutritional and scientific relevance of some of these 'unknown' vegetables. The tender green leaves of non-conventional vegetables were evaluated for protein estimation and structural elucidation using fourier transforms infrared spectroscopy (FTIR). Protein content was determined in the vegetables using Lowry's method. Spectral results showed that all the vegetables contained amines and carboxylic acid functional groups inferring the presence of amino acids (the building blocks of proteins), aromatic compounds, aliphatic amines, alkyl halides, alcohols, and phenols. *Manihot glaziovii* (ewe ege) had the highest protein content, followed by *Solanum americanum* (efo odu), *Bombax costatum* (ewe araba), *Launae ataraxacifolia* (efo yanrin), *Xanthosoma mafaffa* (omunu koko) *and Struchium sparganophora* (efo ewuro odo), which had the lowest protein content among the vegetables. The results obtained from the FTIR spectroscopy confirmed the presence of functional groups of amino acids in these unusual vegetables, buttressing their suitable use as non-conventional sources of protein.

1. Introduction

Proteins are large biomolecules or macromolecules consisting of one or more long chains of amino acid residues. Proteins perform a vast array of functions within organisms, including catalyzing metabolic reactions, DNA replication, responding to stimuli, and transporting molecules from one location to another [1]. Proteins differ from one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes and usually results in protein folding into a specific three-dimensional structure that determines its activity [2]. The muscles, skin, bones, and many other parts of the human body contain significant amounts of protein, accounting for 20% of the total body weight [3-4].

Protein content can be estimated by the Kjeldal method, Enhanced Dumas, UV-visible spectroscopy, Biuret, Lowry, Dye binding, and trimetric methods. Fourier Transform Infrared Spectroscopy (FTIR), is a technique used to obtain an infrared spectrum of the absorption or emission of a solid, liquid, or gas. An FTIR spectrophotometer simultaneously collects high spectral resolution data over a wide spectral range. Fourier transform infrared (FTIR) spectroscopy is an established tool for the structural characterization of proteins [5-9]. One of the major challenges of the post-genomic era is the rapid characterization of protein structures. Fourier transform infrared (FTIR) spectroscopy is a technique that has gained popularity

Table 1. List of Non-conventional vegetables used with their botanical and local names.

S/N	Common name	Scientific name	Local name
1	Cocoyam	*Xanthosoma mafaffa*	Omunu koko
2	African lettuce	*Launaea taraxacifolia*	Efo yanrin
3	Water bitter leaf	*Struchium sparganophora*	Efo ewuro odo
4	Manicoba	*Manihot glaziovii*	Ewe ege
5	Small- flower nightshade	*Solanum americanum*	Efo odu
6	Red flower silk cotton plant	*Bombax costatum*	Ewe araba

in this area because measurements on small quantities of proteins can be carried out very rapidly in various environments, and vegetables as a whole are said to contain small amounts of protein [8, 10-13]. In today's world, Dali (Distance Matrix Alignment) is one of the most recent techniques/methods used to identify and compare protein structures, although it is most effectively used as a computer program [14]. Some vegetables known to date to with appreciable protein concentrations include cauliflower, broccoli, cabbage and beetroot [15]. Green agricultural biomass is currently considered as a possible plant protein source [16].

According to the worldwide web, there are 27 vegetables grown in Nigeria, and more than half are said to be unusual. Leafy vegetables are important dietary items in many Nigerian homes. Apart from the variety, they add to the menu [17-19], which are valuable sources of nutrients especially in rural areas, where they contribute substantially to protein, minerals, vitamins, fibers and other nutrients that are usually in short supply in daily diets [13, 20-25]. Recently, scientists have shown interest in identifying unconventional sources of protein to meet the nutritional needs of an ever-growing population and provide valuable information about the proteinous benefits of plants (vegetables, fruit leaves and roots), especially plants growing in our vicinities [26]. These proteins are known to date as UVP (Unconventional Vegetable Protein), another addition are leaf protein concentrates (LPC) obtained from green leafy biomass, which are currently being explored as possible sustainable protein sources [27]. Vegetable proteins are welcome nutrient in the most Nigerian diets, as vegetables are cheap and also easily grown in our surroundings. Unfortunately, we either ignore them or even treat them as weeds due to lack of information on them. A possible informational gap to be filled is the proper identification of their content and protein quality. Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of the absorption or emission of a solid, liquid, or gas. It is a good technique used regularly to structurally elucidate macromolecules, including proteins. It is also known as a physicochemical analytical technique that provides a clear picture of the metabolic composition of leaves at a given time [11]. This study aimed to estimate and structurally elucidate the protein content of some unconventional vegetables.

2. Materials and methods

2.1. Sample collection

Six different leafy uncommon edible vegetables (Table 1) were obtained from Alajue farm Ede, Osun state and the authentication was carried out at the Department of Botany, Obafemi Awolowo University Ile–Ife, Osun State, Nigeria. The leafy vegetables were thoroughly washed with distilled water to remove debris and air-dried at room temperature (25 ± 1°C), until the weight remained constant. The dried leaves were ground into a powder using a pestle and mortar. The ground portion was kept in a plastic bottle in a refrigerator prior to analysis.

2.2. Reagents and chemicals

Lowry Reagent (2% sodium carbonate, 0.1N NaOH, 0.5% copper sulfate solution, 1% sodium potassium tartarate solution, Folin-ciocalteau reagent), standard protein solution, 200 mg of Bovine serum albumin, (BSA).

2.3. Preparation of leaf extract

The shade-dried leaves of each plant were pulverized in a mechanical grinder. 20 g. of leaves were pulverized (of each plant) and weighed and soaked in a 150 mL of ethanol for 3 days. The extract was filtered using Whatman No.1 filter paper and the supernatant

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was collected. The residue was re-extracted twice (with 3 days of interval between extraction) and the supernatants were collected separately. The supernatant was pooled and evaporated (at room temperature, 28 ± 1°C) until the volume was reduced to 150 mL. This process was carried out for all vegetables.

2.4. Estimation of protein

The protein content in the extracted leaf samples was determined according to the Lowry method of protein estimation [28].

2.5. Fourier transforms infrared spectrophotometer [FTIR] determination

The method by Hering and Harris [10] was used in this study. Dried powders of different solvent extracts of each plant material were used for FTIR analysis. The dried extract powder (10 mg) was encapsulated in 100 mg of KBr pellets to prepare translucent sample discs. The powdered sample of each plant specimen was loaded into an FTIR spectroscope (Shimadzu, IR Affinity, Japan), with a scan range of 45 cm^{-1} with the resolution of 4 cm^{-1}. The FTIR analysis was carried out at Redeemer's University Ede, Osun State Nigeria.

3. Results and discussion

The results of the protein estimation of the analysed vegetables are shown in Table 2, which showed that the vegetables locally known as Ewe ege and scientifically called (*Manihot glaziovii)* had the highest protein content followed by Efo odu (*Solanum americanum)*, Ewe araba (*Bombax costatum*), Efo yanrin (*Launaea taraxacifolia*), Omunukoko (*Xanthosoma mafaffa*) and Ewuro odo (*Struchium sparganophora*) having the least protein content from the vegetables.

Table 2. Protein concentration of the unconventional vegetables.

Vegetables	Concentration ± S.D (mol/cm^3)
Xanthosoma mafaffa (omunu koko)	0.33±0.01
Struchium sparganophora (Efo ewuro odo)	0.27±0.02
Manihot glaziovii (Ewe ege)	0.82±0.01
Solanum americanum (Efo odu)	0.77±0.03
Launaea taraxacifolia(Efo yanrin)	0.50±0.02
Bombax costatum (Ewe araba)	1.46±0.01

Values are means of triplicate determinations.

Protein quality is one of the factors used to determine the protein content in any food sample [29]. Aletor et al. [22], worked on determination of protein content of 4 famous vegetables eaten in Nigeria namely; *Vernonia amygdalina* (Bitter leaf), *Solanum Africana (African Night shade)*, *Amaranthus hybridus* (Green tete) and *Telfaria occidentalis* (Fluted pumpkins) and found their protein content to range between 31.5 to 54.5/100g. These values were quite high for vegetables. According to Butnariu and Butu [30], the protein content of most vegetables ranges from 0.5% to 1.5%, with the exception of legumes, which are generally known to be rich in protein and eventually classified as proteinous foods. As early as 1992, Oshodi [31] determined the protein content of 12 vegetables and found the range of 21.15 to 37.21 g/100g. The beauty of this work is that the determination was carried out on dried samples. Also the study of Arowora et al. [32] found that the protein content of leafy vegetables grown in Wukari, Taraba State, ranged from 11.17 to 25.04% with the highest protein content obtained from pumpkin leaves (*Telfairia occidentalis*). The work of Abukutsa-Onyango et al. [33], found that the protein content in priority vegetables grown along the Lake Victoria Basin was between 18-54% in value. All these studies point out the relevance of these naturally growing vegetables in the diet of the rural folk or general populace in these areas. The relevance of these vegetables cannot be ignored. The results of the FTIR was used to identify the functional groups of the active components based on the wave-band peak values in the infrared spectrum (Figs. 1-6).

The pulverized leaf and ethanolic extracts of *Strachum sparganophora* (ewuro odo), *Xanthosoma mafaffa* (omunukoko), *Bombax costatum* (Ewe araba), *Celosialaxa* (Ewe ajefawo), *Launaea taraxacifolia* (Efo yanrin) and *Manihot glaziovii* (Ewe ege) were passed into the FTIR and the functional groups of the components were separated based on the peak ratio [9, 33-34]. The Fourier transforms of these vegetables are shown in Table 3-8 and Figs. 1-6. The lack of other structural analysis like UV, H-NMR, mass spectrophotometer hindered the complete elucidation of the structures of the samples, but we can infer the presence of some essential amino acids (proteins) through the appearance of the following functional

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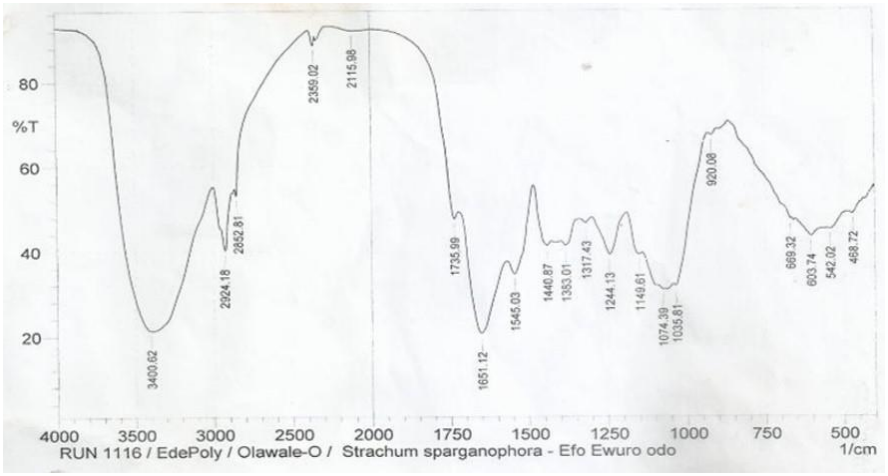

Figure 1. FT-IR Spectrogram of *Strachum sparganophora (*Efo ewuro odo).

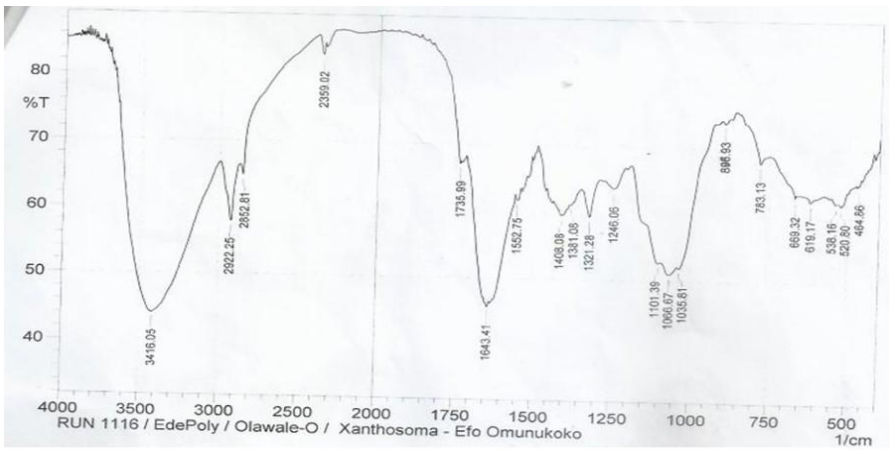

Figure 2. FT-IR Spectrogram of *Xanthosoma mafaffa* (Efo Omunukoko).

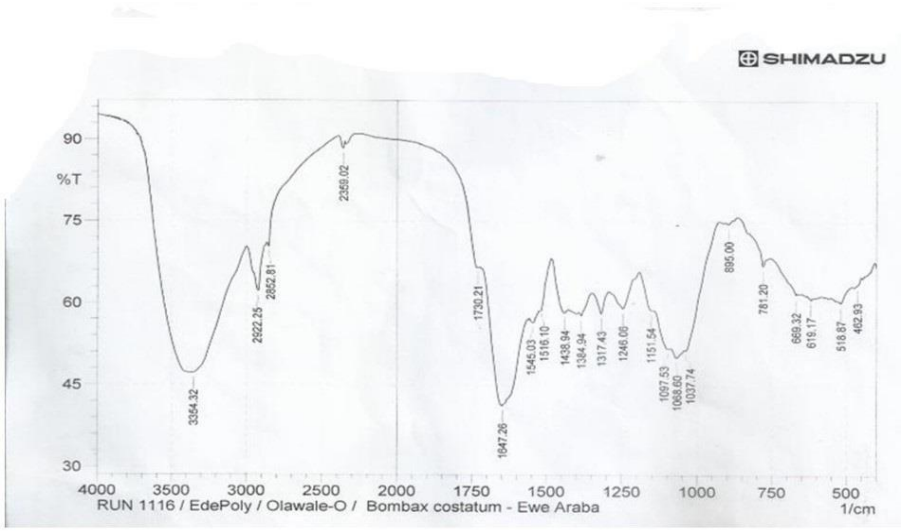

Figure 3. FT-IR Spectrogram of*Bombax costatum* (Ewe araba).

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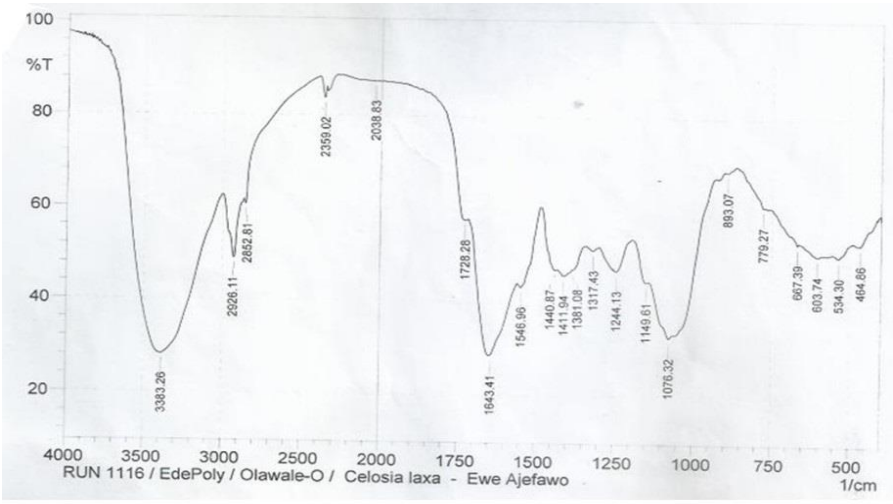

Figure 4. FT-IR Spectrogram of *Celosia laxa* (Efo ajefawo).

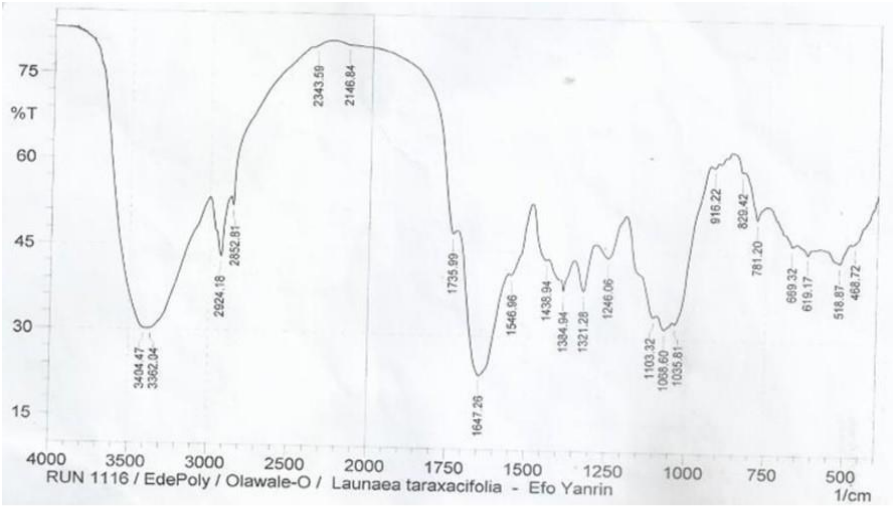

Figure 5. FT-IR Spectrogram of *Launaea taraxacifolia* (Efo yanrin).

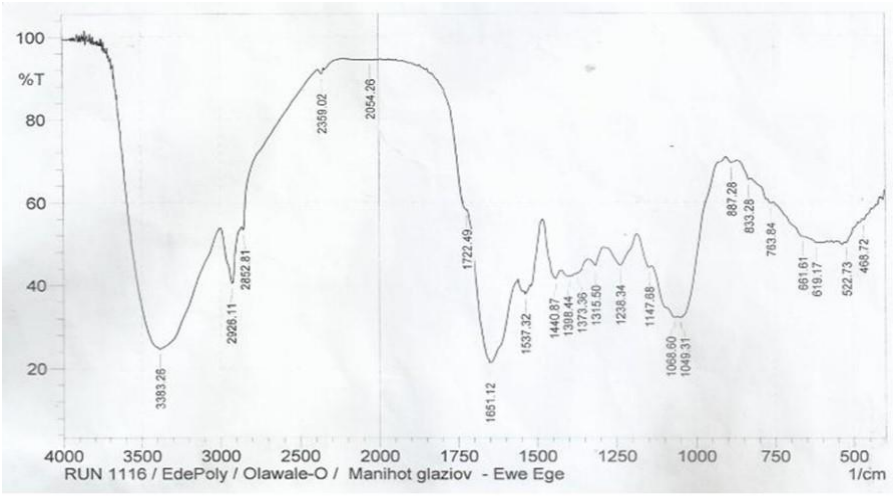

Figure 6. FT-IR Spectrogram of Manihot *glazovii* (Ewe Ege).

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Table 3. FT-IR peak values and functional groups of *Strachum sparganophora* (Efo ewuro odo).

Peak values	Functional group	Peak values	Functional group
468.72	Unknown	1383.01	Aromatics
542.02	Alkyl halides	1440.87	Unknown
603.74	Unknown	1545.03	Aromatics
669.32	Alkyl halides	1651.12	Alkenes
920.08	Alkyl halides	1735.99	Ketones
1035.81	Unknown	2115.98	Alkenes
1074.39	Unknown	2359.02	Aromatics
1149.61	Aliphatic amines	2852.81	Carboxylic acids
1244.13	Aliphatic amines	2924.18	Alkanes
1317.43	Unknown	3400.62	Alcohols,Phenols

Table 4. FT-IR peak values and functional groups of *Xanthosoma mafaffa* (Efo Omunukoko) Leaves powder.

Peak values	Functional group	Peak values	Functional group
464.86	Unknown	1321.28	Unknown
520.80	Alkyl halides	1381.28	Unknown
538.16	Unknown	1408.08	Aromatics.
619.17	Unknown	1552.75	Aromatics
669.32	Unknown	1643.41	Alkenes
783.13	Aliphatic amines	1735.99	Ketones
896.93	Aliphatic amines	2359.02	Carboxylic acids
1035.81	Unknown	2852.81	carboxylic acids
1066.67	Unknown	2922.25	Alkanes
1101.39	Aliphatic amines	3416.05	Alcohol, phenols
1246.06	Unknown		

Table 5. FT-IR peak values and functional groups of *Bombax costatum* (Ewe araba) leaves powder.

Peak values	Functional group	Peak values	Functional group
462.93	Unknown	1317.43	Unknown
518.87	Alkyl halides	1384.94	Unknown
619.17	Alkyl halides	1438.94	Aromatics
669.32	Unknown	1516.10	Aromatics
781.20	Unknown	1545.03	Alkenes
895.00	Aliphatic amine	1647.26	Alkenes
1037.74	Aliphatic amine	1730.21	Ketones
1068.60	Aliphatic amine	2359.02	Unknown
1097.53	Aliphatic amine	2852.81	Carboxylic acids
1151.54	Unknown	2922.25	Alkanes
1246.06	Unknown	3354.32	Alcohols Phenols.

groups –OH hydroxyl, C=O carbonyl of a carboxylic acid, aromatic (benzene, phenol, alkyl benzene), alkyl halides and aliphatic amines which could suggest the presence of amino acids like phenylalanine, tyrosine, tryptophan, arginine etc. We can infer that the structures of the vegetable extracts contain the basic amino acid structure [2, 10, 36]. Further analysis of the results revealed the interpretation of some major wave bands of the vegetable extract in the FTIR spectrum. According to the study of Suresh et al., [35], the (FTIR) analysis showed the most prominent peaks at ~2923 cm^{-1}, ~1636 cm^{-1} and ~1033 cm^{-1}, which correspond to lipids, protein and carbohydrate content in leaf samples, respectively, which also agreed with is study, which had maximum absorption bands ranging from ~781cm^{-1} to ~3380 cm^{-1}. The FTIR

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Table 6. FT-IR peak values and functional groups of *Celosia laxa* (Efo ajefawo) leaves powder.

Peak values	Functional group	Peak values	Functional group
464.86	Unknown	1411.94	Aromatics
534.30	Alkyl halides	1440.87	Aromatics
603.74	Alkyl halides	1546.96	Alkenes
667.39	Alkyl halides	1643.41	Alkenes
779.27	Aliphatic amines	1728.28	Ketones
893.07	Aliphatic amines	2038.83	Unknown
1076.32	Aliphatic amines	2359.02	Unknown
1149.61	Unknown	2852.81	Carboxylic acids
1244.13	Unknown	2926.11	Alkanes
1317.43	Aromatics	3383.26	Alcohols, Phenols
1381.08	Aromatics		

Table 7. FT-IR peak values and functional groups of *Launaea taraxacifolia* (Efo yanrin) leaves powder.

Peak values	Functional group	Peak values	Functional group
468.72	Unknown	1438.94	Aromatics
518.87	Alkyl halides	1546.96	Aromatics
619.17	Unknown	1647.26	Alkenes
669.32	Unknown	1735.99	Ketones
781.20	Aliphatic amines	2146.84	Unknown
829.42	Aliphatic amines	2343.59	Unknown
916.22	Aliphatic amines	2852.81	Carboxylic acids
1035.81	Aliphatic amines	2924.18	Alkanes
1068.60	Unknown	3362.04	Alcohols, Phenols
1103.32	Unknown	3404.47	Hydroxyl
1246.06	Unknown		
1321.28	Aromatics		

Table 8. FT-IR peak values and functional groups of Manihot *glazovii (*Ewe Ege) leaves powder.

Peak values	Functional group	Peak values	Functional group
468.72	Unknown	1373.36	Aromatics
522.73	Alkyl halide	1398.44	Unknown
619.17	Unknown	1440.87	Aromatics
661.61	Unknown	1537.32	Alkenes
763.84	Unknown	1651.12	Alkenes
833.28	Aliphatic amines	1722.49	Ketones
887.28	Aliphatic amines	2054.26	Unknown
1049.31	Aliphatic amines	2359.02	Unknown
1068.60	Aliphatic amines	2852.81	Carboxylic acids
1147.68	Unknown	2926.11	Alkanes
1238.34	Unknown	3383.26	Alcohols, phenols
1315.50	Aromatics		

analysis in this study confirmed the presence of alcohols, aliphatic amines, aldehydes, ketones, and aromatics in these vegetables. This was also in agreement with the work of Sravan Kumar et al., [34], who looked at the FTIR analysis of 5 selected vegetables namely; *Hibiscus cannabinus* L., (kenaf), *H. sabdariffa* L., (roselle), *Basella alba* L., (vine spinach), *B. rubra* L. (malabar spinach) and *Rumex vesicarius* L., (sorrel). FTIR spectroscopy was also used to identify the typical functional groups in freeze-dried materials

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in the study by Malghani et al. [37]. Data from the study revealed strong absorption bands at ~3600-3200 cm^{-1} due to the O-H stretching vibrations and C-H stretching vibration at 3000-2800 cm^{-1}. The C=O and C-O stretching vibrations appeared at 1700-1750 cm^{-1} and 1200-1000 cm^{-1}. The C-N stretching vibration was observed at 1300-1200 cm^{-1}. These absorption bands stated here were also observed in our study, indicating the presence of proteins in which the building block amino acids are made up of distinct amino acids with the said functional groups.

4. Conclusions

The protein estimation carried out in this study showed that *Manihot glazovii* (ewe ege) contained the highest protein content, while *Stuchium sparganophora* (efo ewuro odo) had the lowest protein in vegetable. One can infer from this study suggests that *Manihot glazovii* (ewe ege) is a good protein supplement. The FTIR results also indicate that the functional groups of active components based on the peak values of all the vegetables contain amines and carboxylic acids, inferring the presence of amino acids (the building blocks of protein), aromatic compounds, aliphatic amines, alkyl halides, alcohols and phenols. These results imply that these vegetables if consumed in sufficient amounts would contribute greatly towards meeting human nutritional requirements for normal growth and adequate protection against diseases arising from malnutrition. The vegetable (ewe ege), being the most proteinous of the vegetables used in this study could be recommended for inclusion in dietary foods to cater for protein deficiencies in the body, increase the protein content in the body and promote rapid development of the body and protection against malnutrition.

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, Copilot, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Conceptualization, methodology, writing-review and editing, Supervision, O.D.; formal analyses, investigation, writing-original draft preparation, O.B.; resources, O.D., O.B.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare that there are no conflicts of interest as regards the publication of this article.

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