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Evaluation of Chemical Compositions and Thickening Properties of *Irvingia gabonensis* and *Pleurotus tuber-regium*

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Abstract

The chemical compositions and thickening properties of *Irvingia gabonensis* (ọgbọnọ) and *Pleurotus tuber-regium* (ọsụ) were investigated. *I. gabonensis* had higher lipid content than *P. tuber-regium* with values of 42.44% and 0.63% respectively; ash, protein and crude fiber contents were also higher in *I. gabonensis* than *P. tuber-regium* except for carbohydrate composition that had values of 27.91% and 58.3% respectively. The result of phytochemical composition revealed that flavonoid was present in both samples likewise, tannins, saponin, alkaloids, phenol and cyanogenic glycosides. Oxalate and phytate contents of *I. gabonensis* were 7.68% and 3.56% respectively, which were absent in *P. tuber-regium.* Both samples showed a variation in their solubility at different temperatures using vegetable oil, palm oil and water as solvents. Gelatinization of *I. gabonensis* started at a temperature of 68°C and ended at 92°C while that of *P. tuber-regium* did not gelatinize but coagulated which promote the thickening properties of the samples. The aromatic amino acids identified in the samples were tryptophan and histidine at a wavelength of 280 nm and 362 nm for *P. tuber-regium* and *I. gabonensis* respectively. The IR analysis revealed the presence of the OH stretch of alcohols, C-H stretch of alkane in both samples.

Keywords

Gelatinization, *Irvingia gabonensis, Pleurotus tuber-regium*, Phytochemicals, Proximate and Solubility

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1. Introduction

The ignorance of nutritive and non-nutritive compositions of some food substances has resulted to their wastage in terms of economic returns or post harvest loses as well as their consumption. Seeds are among the predominant plants products in the present dietary. A lot of researches have been done on some Nigerian seeds used in soup thickening [1, 2, 3, 4] including *Irvingia gabonensis* (ọgbọnọ) seed [5, 6] and *Pleurotus tuber-regium* (ọsọ) [7, 8]. In Nigeria, it is a common practice among the South-Eastern states people to use the plant species in soup preparations. These are mostly used due to their thickening, flavouring and binding properties [9, 10]. Thickening agents are used in cooking and there are different types of thickening agents. Selecting the right type of food thickener can make or mar dishes. Most of the thickening agents are starch based. Starch becomes gelatinous when it is cooked thereby producing a thickening property that blends well into the foods. When cooked properly, a gelatinized starch has a neutral flavour which can be used in moderation with most foods to produce a thick product. Starches that are not properly cooked have "starchy" flavour that can come through prominently in food.

Irvingia gabonensis (Aubry-Lecomte ex O'Rorke) Baill; Fig. 1) common names include African Mango, ọgbọnọ, bread tree, bush mango, dika nut, dikka, duiker nut, etima, barteri, kaka, and wild Mango. The pounded seed is added to meat and different vegetable dishes as a sauce as well as soup

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(commonly known as draw soup). However, among other uses of the seed probably due to its thickening properties and slippery nature, ọgbọnọ can also be used when medicinal condition such as dysphasia causes difficulty in swallowing. The thickened liquid plays a vital role in reducing risks of aspiration for dysphasia patients [11].

Pleurotus tuber-regium (Fr.) Singer; Fig. 2, asclerotial) is an edible king tuber oyster mushroom that is wildly consumed in Nigeria. The skin of sclerotium when peeled off after soaking in water, the inner whitish tissue is used to substitute in part or whole for the melon seeds in soup preparation due to its thickening properties. Generally, *P. tuber-regium* (ọsụ) soup is a delicacy in the South Eastern Nigeria and many states in Nigeria. The plant has been found useful in variety of agroforestry products for the production of feeds, enzymes and as medicinal products [12]. The medicinal properties that have been reported include the use of the extracts of *Pleurotus* species due to its antigenotoxic, bioantimutagenic [13]; antiinflamatory, antilipidaemic, antihypertensive, and antihyperglycaemic [14]; antibacterial and antifungal [15] activities.

Figure 1. Image of Irvingia gabonensis.

Figure 2. Image of Pleurotus tuber-regiu.

The study aimed at investigating on both plant species their chemical compositions and thickening properties by determining the following:

a) proximate and phytochemical compositions

b) aromatic amino acid present as well as protein contents using UV-visible spectroscopy.

c) functional groups present in the plant samples as well as in the palm oil and vegetable used as solvent for their dissolution by IR spectroscopy.

   solubility in palm oil, vegetable oil and water at different temperatures i.e. 40°C, 60°C, 80°C, 100°C and room temperature and also the

d) gelatinization properties in water

2. Materials and Methods

The samples of *Irvingia gabonesis* and *Pleurotus tuber-regium* were bought from Nkwa-Achara Market in Uturu, Isuikwuato Local Government Area, Abia State Nigeria. The samples were air-dried after which the *P. tuber regium* was cut into smaller bits to facilitate smooth grinding. The samples were then ground and then stored in different airtight sample plastic plates which were properly labelled.

2.1. Analysis of Phytochemical Composition

The determination of alkaloid, phenol, phytate, tannin, cyanogenic glycoside and oxalate were done according to the method of [16] and that of Saponin by [17]

2.2. Analysis of Proximate Composition

2.2.1. Determination of Moisture Content

A washed crucible was dried in an oven for one hour. The crucible was placed in a dessicator and allowed to cool, it was weighted afterward and a measured amount of the ground samples placed in an oven for 24 hours at 60°C was measured into the crucible and was placed in an oven for 3 hours at 105°C. The weight loss of the moisture content was calculated as follows [17].

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1} \quad (1)$$

where:

W_1 = Weight of empty crucible

W_2 = Weight of crucible + sample before drying

W_3 = Weight of crucible + sample after drying.

2.2.2. Determination of Crude Fat

Five (5) g of the sample was weighed and then oil was extracted for 24 hours using a Soxhlet extractor with petroleum ether. The extract was distilled out over a steam bath and the oil was dried in a dessicator and was weighed [17].

$$\% \text{ Crude fat } = \frac{W_3 - W_2}{W_2 - W_1} \times \frac{100}{1} \quad (2)$$

where:

W_1 = Initial samples weight in grams

W_2 = Weight of beaker in grams

W_3 = Weight of beaker and fat residue in grams.

2.2.3. Determination of Ash Content

The crucible with its cover which has been dried for 2 hours at 100^0C from oven was removed to cool in a dessicator. The weight of the crucible with cover, were recorded to the nearest 0.1mg (W_1). 5.0 g of sample was weighed with the crucible and weight of crucible with the cover and sample recorded to the nearest (W_2) the sample were ashed in furnace at 600°C for 2 hours after the furnace reaches temperature. The crucible were allowed to cool in furnace to less than 200°C and then placed in dessicator with reined top. The crucible with cover and ash was cooled and weighted to the nearest 0.9 mg (W_3) [17]

$$\% \text{ ASH } = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1} \quad (3)$$

where:

W_1 = Weight of empty crucible

W_2 = Weight of crucible + sample before ashing

W_3 = Weight of crucible + sample after ashing

2.2.4. Determination of Crude Fiber

The sample was oven dried at 105°C for 8-10 hours. 5.0g of the powdered dried sample was put in a beaker. The beaker was placed on a hot plate and heated for 3 minutes with occasional rotation of the beaker. The beaker was mixed with 250ml portion of boiling water. The residue was carefully transferred into a beaker and 200ml of 1.25% NaOH was added, it was boiled for 3 minutes, cooled filtered and was twice with 50ml of boiling water. Finally, the sample was washed with 25ml of 95% alcohol. The residue was oven dried for 2 hours at 130°C and cooled in a dessicator and weighed [18].

$$\% \text{ o Crude fibre } = \frac{\text{loss in weight in ignition}}{\text{Weight of sample (g)}} \times \frac{100}{1}$$

$$\% \text{ Crude fibre } = \frac{W_3 - W_2}{W_1} \times \frac{100}{1} \quad (4)$$

where:

W_1 = Initial sample weight in grams

W_2 = weight of crucible and sample after boiling washing and drying

W_3 = weight of crucible and sample as ash.

2.2.5. Determination of Protein (Kjeldahl Method)

The total nitrogen (N) was determined and multiplied with factors 6.25 to obtain the protein contents. 2.0 g of the sample was mixed 40ml of H_2SO_4 in a digestion flask of tablets of selenium catalyst was added to it before it was heated under a fume cupboard until a clear solution was obtained (i.e. the digested). The digest was diluted to 200ml in a volumetric flask and used for the analysis. 50 ml of the digest was mixed with equal volume of 40% NaOH solution in a kjeldahl distillation apparatus. The mixture was distilled into 50ml of boric acid containing 3 drops of methyl red indicator; a total 50 ml of distilled was collected and total against 0.02N EDTA from green to deep red point. A reagents blank was also digested and distilled. The N_2 contents and hence the protein contents was calculated using the formula below [17].

$$\% \text{ Protein } = \frac{\text{T.V} \times 0.0014 \times 6.25 \times 100}{\text{Weight of sample used}} \quad (5)$$

where:

T.V = Titre value

0.0014 = Nitrogen Constant

6.25 = Kjeldahl Constant

2.2.6. Determination of Total Carbohydrate Content

The total carbohydrate was estimated as the remainder after accounting for ash, crude fibre, protein, fats and oils and moisture content [17].

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

2.3. Determination of Gelatinization Property

Three (3) grams of the sample was put into a beaker containing 20mls of water which was heated. A thermometer was used to check the temperature at which gelatinization started and when it stops respectively which were recorded.

2.4. Determination of Solubility, Spectroscopic Analysis Using UV-Visible and IR Spectrophotometer

Standard methods were also used to determine the Solubility of the samples in different solvents which include water, palm oil and vegetable oil. The spectroscopic analysis of the samples using the UV-Visible and IR spectrophotometer were also carried out. The UV-Visible Spectrophotometer was used to identify the aromatic amino acids present in the samples while the IR Spectrophotometer were carried out on the samples as well as on the palm oil and vegetable oil used

as solvent to determine the functional groups present in them.

3. Results and Discussion

The results of analyses *of I. gabonensis*and *P. tuber-regium* are presented in Tables 1 - 6 below. The results of quantitative analysis of phytochemical compositions in 'ọgbọnọ' *(I. gabenensis)* and 'ọsụ' *(P. tuber-regium}* are shown in Table 1 and the proximate compositions in Table 2. Table 3 shows their solubility test while Tables 4, 5 and 6 show the identified amino acids, gelatinization test in water of the samples and Infrared wavelength of absorptions from *P. tuber-regium, I. gabonensis, p*alm oil and vegetable oil respectively.

The quantitative estimation of *I*, *gabonensis* and *P. tuber-regium*in Table 1 shows that *I. gabonensis* and *P. tube-regium* contain alkaloids, flavonoids, saponins, phenols, tannins, cyanogenic glycosides, also present were oxalates and phytate in *I. gabonensis* but were absent in *P. tuber-regium.* The result revealed that the percentage composition of *I*, *gabonensis* was higher than that *of P. tuber-regium* in its alkaloids (14.0 ± 1.0 against 1.5 + 0.14), phenolic content (9.33 ± 0.33 against 1.11 ± 0.51), tannin (10.8 ± 1.76 against 6.17 ± 1.26), tannin (4.15 ± 0.22 against 3.33±0.3) and cyanogenic glycoside (5.15±0.1 against 4.43±0.33) except for saponin. *I. gabonensis* also had 7.68 ± 0.55%of oxalate and 3.56 ± *0.22%*ofphylate. Oxalate and phytate were absent in *P. tuber-regium*.

Table 1. The results of quantitative estimations of phytochemicalin 'ọgbọnọ'*(I. gabonensis)* and 'ọsụ'*(P. tuber-region).*

Sample	Cyanogenic glycoside (%)	Flavonoid (%)	Oxalates (%)	Saponin(%)	Alkaloid (%)	Phenol ml/mg	Tannin ml/mg	Phytate (%)
Irvingigabonensis	5.15±0.1	10.8± 1.76	7.68 ±0.55	0.5 ±0.2	14.0 ± 1.0	9.33 ± 0.33	4.15 ±0.2	3.56 ±0.2
Pleurotus tuber-region	4.43 ±0.33	6.17± 1.26	ND	1.0±0.5	1.5±0.14	1.11±0.51	3.33 ±0.3	ND

Phytochemicals are known to have antimicrobial activities [19] and also aid in providing protection against insect attacks and plant diseases. Phenols and its compounds are mainly used in disinfection. The presence of flavonoids indicates the medicinal value of the samples. Flavonoids are antioxidants and free radical scavengers which prevent oxidative cell damage, have anticancer activity and protect the cell from any form of possible carcinogenesis [19, 20]. Flavonoid also lowers the risk of heart diseases [19]. Alkaloids are known to exhibit marked physiological activity. Alkaloids are generally used as basic medicinal agents for analgesic, antispasmodic, and bacterial activities [21, 22]. Saponins are useful in medicine and pharmaceutical industries due to its forming ability. The presence of tannins has been found to possess astringent property which hasten the healing of wounds and inflamed mucus membranes [21]. Cyanogenic glycosides act as a defensive mechanism to plant against bacterial and herbivorous attack [23]. Oxalate in large amount is considered poisonous and harmful. Phytate has been reported to decrease bioavailability of minerals in monogastric animals [24]. The presence of phytate in food can bind some essential minerals in the digestive tract which may result in mineral deficiencies. Phytochemicals generally are biologically active compounds found in plants in small amounts. They are not established nutrients but seem to contribute significantly to protect against degenerative disease. However, the term does not apply to compounds used in the treatment of established acute diseases, but rather are substances that have protective effect at low levels against the development of degenerative diseases over a lifetime [25].

The Proximate composition of *I. gabonensis* and *P. tuber-regium* shown in Table 2 indicates that *I. gabonensis* was higher than that of *P. tuber-regium* in its lipid, ash, protein, and crude fiber contents except for moisture and carbohydrate

Table 2. Result of Proximate Compositions of Ogbọnọ (I. gabonensis) and Ọsụ (P. tuber-regium).

Sample	Moisture (%)	Lipids (%)	Ash (%)	Protein (%)	Crude fiber (%)	Carbohydrate
Irvingigabonensis	7.67 ±0.76	42.44 ±0.84	6.53 ±0.25	8.05' ±0.49	7.4 ±0.17	27.91 ±2.07
Pleurotus tuber-region	27.02 ±0.82	0.63 ±0.09	2.98 ±0.28	7.94 ±0.79	3.4 ±0.17	58.3±0.32

The proximate composition of *I. gabonensis* and *P. tuber-regium* shown in table indicate that *I. gabonensis* had higher percentage composition than *P. tuber-regium* in its lipid content (42.44 0.84 against 0.63 ± 0.09), ash content (6.53 ± 0.25 against 2.98 ± 0.28), protein content (8.05 0.49 against 7.94 ± 0.79), and crude fiber content (7.4 ± 0.17 against 3.4 ± 0.17) except for moisture (7.67 ± 0.76 against27.02 ± 0.82) and carbohydrate (27.91 ± 2.07 against58.3 ± 0.32).

The presence of protein in the sample indicates its nutritional value [26]. The high content of lipid in *I.gabonensis* indicate good aroma [27]. *Irvingia gabonensis* is an oil seed and can be used as a source of oil. The ash contents of fresh foods rarely exceed 5%, although some processed foods can have ash content up to 12% [28]. The ash content of *I. gabonensis* was 6.53% which is a bit higher than 5% while that of *P. tuber-regium* was 2.98%. A level much higher than expected may indicate the presence of other material that may contain

higher inorganic materials. The percentage moisture content of *I. gabonensis* was lower than that of *P. tuber regium* Moisture content analysis is important in determining the shelf life of foods and products. Moisture rich foods are easily susceptible to the microbial attack but low moisture foods usually slow down growth of microorganism. Moisture content of food is a determination factor of the quality and the stability of the processed food products. Crude fiber is a measure of the quantity of indigestive cellulose, potassium lignin and other components resembling it that is present in foods. Fiber offers a variety of health benefits and is essential in reducing the risk in chronic diseases such s diabetes, obesity, cardiovascular disease and diverticulitis [29].

The result of solubility test shown Table 3 revealed that the solubility test for *I. gabonensis* in the various solvents ranged from insoluble to completely soluble at increasing temperature of the different solvents used but, the solubility test of *P. tuber-regium* in all the solvent were insoluble at various temperature used.

Table 3. Solubility test of Ọgbọnọ *(I. gabonensis)* and Ọsụ *(P. tuber-regium)* in different solvents at different Temperatures.

Temperature (°C)	Ọgbọnọ *(Irvingia gabonensis)*			Ọsụ *(Pleutus tuber-regium)*		
	Water	Palm oil	Vegetable oil	Water	Palm oil	Vegetable oil
Room temperature	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
40°C	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
60°C	Partially soluble	Insoluble	Partially soluble	Insoluble	Insoluble	Insoluble
80°C	Soluble	Partially soluble	Soluble	Insoluble	Insoluble	Insoluble
100°C	Soluble		Soluble	Insoluble	Insoluble	Insoluble

Solubility is the property of a solid, liquid or gaseous chemical substance to dissolve in a solid, liquid or gaseous solvent to form a solution of the solute in the solvent. The solubility of a substance fundamentally depends on the physical and chemical properties of the solute and solvent as well as on temperature; pressure and the pH of the solution [30]. The variation in the solubility test for *I. gabonensis* could be as a result of the physical and chemical properties of solvent and the solute. The composition of the solvents use in the solubility test may alter the solubility. Depending on the nature of the solute the solubility may increase or decrease with temperature. Generally, the overall solvation capacity of a solvent depends primarily on its polar nature [31]. The IR spectroscopy result showed some level of similarity in the energy absorb by *I.gabonensis* and the solvents used especially the OH stretch group present at wavelengths (cm^{-1}) of 3786, 3747 and 3717 for *I. gabonensis*, palm oil and vegetable oil (Table 4) which was absent in which was absent in *P. tuber-regium*

The frequencies of absorption (Table 4) were observed for *I. gabonensis,* palm oil, and vegetable oil at the peaks 3786, 3747 and 3717(cm^{-1}) which indicate the O-H stretch functional group of the compound alcohol and phenols while that of OH stretch of carboxylic acids and amines were observed for *P. tuber-regium,* palm oil, and vegetable oil at the peaks 3294, 3472 and 3479, 2577 (cm^{-1}) respectively. The C=O stretch of carbonyl compounds for aldehyde, ketone and carboxylic acid were also observed from the samples. The functional groups of the protein composition present in the samples were also identified by the frequencies of the absorbed peaks that corresponded to it.

Table 4. Infrared Absorptions Frequencies (cm^{-1}) from *P. tuber-regium, I. gabonensis,* Palm Oil and Vegetable Oil.

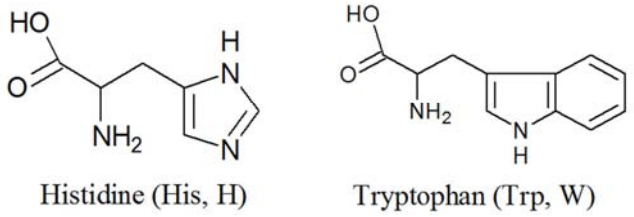
Pleurotus tuber-regium	Irvingia gabonensis	Palm Oil	Vegetable Oil	Type of bond
	3786	3747	3717	OH stretch of Alcohols and phenols
3294		3472	3479 2577	OH stretch of carboxylic acids, amines
2901	2910 2857	2916	2929	-C-H stretch of alkanes
2352 2122	2352	2355 2060 2032	2351 2023	
1652	1738 1650	1733	1741	C=O stretch of aldehyde, ketone and carboxylic acid
1546	1541			C=C stretch of aromatic stretch
1432	1450	1448	1451	-C-H bending methyl and methylene
1362 1320	1392 1241	1370	1367	C-O stretch of carboxylic acids
1061	1174			C-N stretch of amine
		1086		C-O stretch of esters
902 591	839 722	980 701 584	717	=C-H Bend of alkene and substituted aromatic compounds

The protein absorption maximum (λmax) shown in Table 4 for *I. gabonensis was at* 362nm while that of *P. tuber-regium* was 280 nm which is caused by the aromatic amino acid identified as Histidine and Tryptophan respectively. The IR spectra also revealed the functional group of a protein molecule which signifies the presence of protein in both samples analyzed.

The Amino acids identified at each of the wavelength for *I. gabonensis* and *P. tuber-regium* by UV-Visible Spectrophotometer is shown in Table 5. Histidine was identified in ọgbọnọ *(Irvingia gabonensis)* at a wavelength of 362nm while the amino acid observed in ọsụ *(Pleurotus Tuber-region)* was tryptophan at a wavelength of 280 nm. The structures of these amino acids are shown in Fig. 3.

Table 5. Amino acids identified at each of the wavelength for *I. gabonensis*and *P. tuber-regium* by UV-Visible Spectrophotometer.

Sample	Wavelength Observed (nm)	Identified Amino acid
Ọgbọnọ (Irvingia gabonensis)	362	Histidine
Ọsụ (Pleurotus Tuber-region).	280	Tryptophan



Histidine (His, H) Tryptophan (Trp, W)

Fig. 3. Structures of identified Aromatic Amino acids.

Table 6 shows the test result of gelatinization *of the samples. It* was observed that *P. tuber regium* does not gelatinize but rather coagulates and forms no gel. *I. gabonensis* started gelatinizing at 68°C and ended at 92°C of which could be why its solubility test at 60°C was partially soluble. More so, it requires heating to form gel.

Table 6. Result for gelatinization Test in Water.

Sample	Initial temperature	Final temperature
Ọsụ (Pleurotus Tuber-region).	No gelatinization	No gelatinization
Ọgbọnọ (Irvingiagabonensis)	68°C	92°C

4. Conclusion

The results obtained from the chemical compositions of *Irvingia gabonensis* and *Pleurotus tuber-regium* revealed that they are good sources of phytochemicals and nutrients needed for the maintenance of good health. They also contain aromatic amino acids as seen in the UV-visible protein analysis and can also be exploited for other uses. Their application in soup as a thickening, flavouring and binding agents play vital role in the sauce and soup thickening as well as aroma especially for the *Irvingia gabonensis*.

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