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Nutritional Composition and Fungal Pathogens of Lentil (*Lens culinaris* M.) in Port Harcourt Metropolis

Chuku, E.C[1]., Emiri U.N[2]. & Wekhe, O. E[3].
[1&3]Department of Plant Science and Biotechnology, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria. [2]Department of Crop Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.
***Corresponding Author:** *ucheemiri@gmail.com*

ABSTRACT

Lentil, of the legumes family contains proteins, carbohydrates, oils, and ash in appreciable proportions. It also provides iron, calcium, phosphorus, magnesium, vitamin A, and vitamin B. A study on the nutritional composition and fungal pathogen of lentil was carried out in the Department of Plant Science and Biotechnology, Rivers State University. Nutrient and anti-nutrient composition were obtained using the methods of the Association of Official Analytical Chemist (AOAC) while standard mycological technique was used for fungi isolation. Proximate evaluation showed moisture, ash, fibre, lipid, carbohydrate and protein to be present in lentil. However, highest (55.85 ± 2.35%) and lowest (0.81± 0.01%) values were observed for carbohydrate and lipid respectively. Mineral assessment revealed the availability of calcium, iron, phosphorus, magnesium, potassium and sodium. However, phosphorus recorded highest value of 27.5± 65.5mg/100g while iron had lowest value of 5.45± 0.05mg/100g. Anti-nutrient screening indicated the presence of phytochemicals, such as glycoside, oxalate, saponin, tannin, carotenoid, polyphenol, flavonoid and lignan. Lignan had the highest value of 12.55± 0.05% while the lowest value of 0.01 ± 0.00% was recorded for glycoside. The two fungi isolated from Lentil (*Lens culinaris* M.) were *Candida sp* and *Microsporum sp. Candida sp* had highest incidence of 86.35%, while lowest incidence of 13.63% was recorded for *Microsporum sp*. Generally, Lentil though rich in valuable nutrients and minerals, is also susceptible to fungal spoilage. *Lens culinary* M. is recommended for consumption on account of its rich valuable nutrients and minerals. However, proper care and good phytosanitary measures should be given to the crop, to guard against fungal infection

Keywords: Lentil (*Lens culinaris* M.), Fungi, Pathogen, Nutrient, Mineral, Anti-nutrient, Phytochemicals.

Introduction

The lentil (*Lens culinaris* Medik.) is a diploid legume (2n = 14) with a large genome of 4,063 Mbp, is phylogenetically nested within the tribe Vicieae, and belongs to the Papilionoideae subfamily of the Fabaceae family (Andrews *et al*, 2005). Legumes are known for their ability to fix atmospheric nitrogen (N_2) through symbiosis with bacteria called rhizobia through the formation of specialized structures called nodules, which is advantageous for growth in soils with low N_2 content (Dhull *et al*., 2021). The importance of lentil cultivation lies in its resistance to drought and its ability to grow in wide range of soils, from light to heavy, with a pH of 5.5 - 9; therefore, this legume production presents an encouraging panorama.

Lentils contain proteins, carbohydrates, oils, and ash in proportions of 23%, 59%, 1.8%, and 02%, respectively, and also provide iron, calcium, phosphorus, magnesium, vitamin A, and vitamin B (Dhull *et al*., 2023).

In addition to providing essential and non-essential amino acids and carbon skeletons for the metabolic needs of the human body, lentils are sources for some storage proteins that are described as biologically active proteins (Gibson *et al,* 2017).

The origin of the lentil dates back to 11,000 BC in the Fertile Crescent, with *L. culinaris subsp orientalis* (Boiss.) being the wild progenitor. This indicates that this legume was domesticated in Asia.

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However, other evidence points to the progenitor of the lentil being *L. nigricans* (M. Bieb.) whose domestication occurred in Europe (Sarker *et al*., 2005).

Therefore, the regions with the highest species richness of the genus Lens (with three and four species) are the Crimean Peninsula, southeastern Turkey, and the eastern Mediterranean countries of Syria, Jordan, Israel, and Palestine. Regions with two species of the genus Lens include Spain, the Balkans, Albania, Greece, and western Turkey (Kahraman, *et al,* 2004a). *Lens culinaris* (Medik.) is the only cultivated species for which wild species, which serve as genetic reserves, are threatened by poor competitiveness and low palatability, along with the fact that they occur in small, isolated populations. It is necessary to promote the maintenance and care of these lentil genetic reservoirs to preserve the evolutionary history of this legume and not only focus on its economic and agricultural impact worldwide.

Some researchers have reported nutritional composition of Lentil, however, there is dearth of information on anti-nutrient content and fungi pathogen associated with lentil sold in the open market, especially in Port Harcourt metropolis. Against this backdrop, this study was carried out to evaluate the nutrient and anti-nutrient content of lentil. The study also aims to isolate and identify fungal pathogen associated with spoilage of lentil.

Materials and Methods

The study area

The study was carried out at the Department of Plant Science and Biotechnology, Rivers State University Port Harcourt. Rivers State has a mean annual rain fall of 2000-3000 mm and mean annual temperature of 25 -28°C area, while relative humidity varies between 70-85%.

Collection of Samples

Healthy and partially rotted samples of lentil were bought from the Fruit Garden Market, Kaduna Street, Diobu, Port Harcourt, and immediately transported to the Department of Plant Science and Biotechnology Laboratory, Rivers State University for processing and analysis.

Determination of Proximate Composition

Standard Analytical Method AOAC, (2005) was the method used to determine the proximate composition comprising; Moisture, Ash, Crude Fiber, Lipid, Protein, and Carbohydrate.

Determination of Moisture (%)

For moisture content, a gravimetric (measurement by weight) was used. In this method, 5 grams of the sample was dried to a constant weight of 120°C in a crucible and left to stay overnight. Then crucible was transferred to oven again and weighed after 2 hours, this was repeated until constant weight was obtained.

Calculation:

Moisture Content % = $(W_2-W_1)- (W_3-W_1) \times 100\ (W_2-W_1)$.

Where:
W_1= weight of empty crucible, W_2= weight of crucible + sample, W_3= weight of crucible +dry sample.

Determination of Ash (%)

The gravimetric method was also used in the determination of ash content. 2 grams of the sample was added in a clean pre-weighed crucible for 24 hours at 550°C. An empty crucible was accurately weighed, and then 10ml of sample was weighed in it using sensitive balance. The sample in crucible was placed in muffle furnace at 550°C for more than 3 hours until white to grey ash was obtained, then crucible was removed from furnace to a desiccators to cool, then weighed.

Determination of Protein (%)

Kjeldal Method of Nitrogen Analysis was used to determine the protein content of both samples. K_2SO_4 was used as a catalyst for concentrated H_2SO_4 to digest approximately 2 grams of each sample. Ammonia produced in the digested sample was afterward distilled into boric acid and titrated with 0.1m of HCl.

To ascertain the amount of protein in each sample, the crude protein formula is used.

$$\text{Crude Protein} = \frac{\text{Titre value x 1.4 x 50 x 100 x 65}}{(1000 \text{ x } 10 \text{ x } 1)N_2}$$

Determination of crude Fiber (%)

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For crude fiber or fiber determination, Acid Alkaline Hydrolysis was used in boiling 2 grams of the samples with 0.1m of H_2SO_4 in a beaker and then filtered through a Büchner funnel.

The residue was washed with hot water till it was free from acid. The material was transferred to the same beaker and added 200ml of 1.25% NaOH solution and refluxed for 30 minutes. Again filtered and the residue was washed with hot water till it was free from alkaline. The total residue was transferred to a crucible and placed in hot air oven, allowed to dry to a constant weight at 80-110 °C and weighed. The residue was ignited in muffle furnace at 550- 600°C for 2-3hrs, cooled and weighed again. The loss of weight due to ignition was the weight of crude fiber.

Determination of lipid (%)

Lipid (total fat) content is determined through the Sochlet Solvent Extraction Technique. Gravimetrically, the residue was dried to a constant weight and calculated as:

Percentage (%) of Ether Extract = $\left[\frac{\text{Weight of extract}}{\text{Weight of Sample}}\right] \times 100$

Determination of Carbohydrate (%)

Carbohydrate content was calculated as the difference between the totalities of all other proximate content and 100.

i.e. 100- (moisture + ash + fibre + protein + lipid).

Determination of Mineral Composition

A digestion mixture containing concentrated nitric acid and concentrated tetraoxosulphate (vi) acid (H_2SO_4) in a ratio of 3:1 was used as wet digestion to be able to carry out mineral analysis. 0.2 gram of lentil samples in powdered forms were weighed and put into a conical flask and 5cm^3 of digestion mixture was added and placed in a fume cupboard for digestion at a temperature of 150ºC-200ºC for 2 hours; the mixture became digested and was allowed to cool. Distilled water (30cm^3) was added to the digest, shaken vigorously, and filtered. In a volumetric flask, the filtrate is marked up into 100 cm^3.

In the digest, the Flame photometer was used to determine sodium (Na), Potassium (K), Calcium (Ca), Iron (Fe), Magnesium (mg), and Phosphorous (P), but when EDTA complexometric fixation (ASTM, 2004) is used in the digest, it determines calcium.

Determination of Anti-nutrient (Phytochemicals)

Antinutrients can also be referred to as phytochemicals. Some anti-nutrients determined in the sample of Lintel include glycoside, oxalates, saponin, tannins, carotenoids, polyphenols, flavonoids, and lignins. Standard Analytical Method, AOAC, (2005) was used in the determination of the anti-nutrients of lentil.

Determination of Saponin

For Saponin determination, 100cm^3 of 20% aqueous ethanol was added to 5grams of each sample in a 250cm^3 conical flask; the mixture was heated over a hot water bath for 4 hours and stirred continuously at a temperature of 55ºC. The residue of the mixture was reextracted with another 100cm^3 of 20% aqueous ethanol after filtration and heated for another 4 hours at a constant temperature of 55ºC with continuous stirring.

The combined extract was evaporated to 40cm^3 over a water bath at 90ºC. 20cm3 of diethyl ether was added to the concentrate in a 250cm^3 separation funnel and agitated vigorously from which the aqueous layer was recovered while the ether layer was discarded and the purification process was repeated twice. 60cm^3 of n-butanol was added and extracted twice with 10cm^3 of 5% sodium chloride after discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes after which, the solution was transferred into a crucible and was dried in an oven to a constant weight.

Determination of Polyphenol

Determination of polyphenols took a different means as 0.5 grams of the samples were boiled for 15 minutes with 50cm^3 of ether to extract the polyphenolic components. 10cm^3 of distilled water, 2cm^3 of 0.2m of ammonium hydroxide solution, and 5cm^3 of concentrated amyl alcohol were also added to the 5cm^3 of the extract and left to react for 30 minutes for color development. Optical density was measured at 505nm.

In preparation for the phenol standard curve, 0.2 grams of tannic acid dissolved in distilled water and diluted to 200 marks (1mg/cm^3).

Determination of Flavonoid

For Flavonoid determination, 0.5ml of appropriately diluted was mixed with 0.5ml methanol, 50μl of 10% Alcl, 50μl of 1m of potassium acetate and 1.4 ml of water and allowed to incubate at room temperature for 30 minutes. The absorbance of the reaction mixture was subsequently calculated while non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

Determination of Tannin

In tannin determination, 1gram of the samples was put into a conical flask of 100cm^3 of distilled water and set to boil gently for 1 hour on an electric hot plate and filtered using 125nm Whatman filter paper into a 100cm^3 volumetric flask. For color development, 5.0cm^3 of Folin-Denis reagent and 10cm^{3}of saturated Na_2CO_3 solution were added to distilled water of 50cm^3 and 10cm^{3}of diluted extract of aliquot volume was pipetted into the 100cm^3 conical flask.

The solution was allowed to stand for 30 minutes in a water bath at a temperature of 25 after thorough agitation. With the aid of a Spectrum Lab 23A Spectrophotometer, optical density was measured at 700nm and compared on a standard tannic acid curve. To obtain the tannic standard curve, dissolution of 0.20g of tannic acid in distilled water and dilution to 2 00cm^{3}marks (1mg/cm^3) were used.

Varying concentrations (0.2-1.0mg/cm^3) of the standard tannic acid solution were pipette into 5 different test tubes of 5cm^3 of Folin-Denis reagent and 10cm^3 saturated Na_2CO_3 solutions were added and made up to 100cm^3 marks with distilled water. Optical density (absorbance) against tannic acid concentration was plotted.

Determination of Glycoside

For glycoside determination, 5g of both samples were weighed into a clean distilled flask, to which 20 ml of distilled water was added and the samples were left to stand overnight for proper hydrolysis to occur. The samples were distilled into 20ml sodium hydroxide containing 0.5g crystal.

The distillate was filtered with 0.02N silver nitrate in the presence of 0.2ml 5% potassium iodide and 1ml 6N ammonium hydroxide solution to a permanent turbidity. The formula below is used in calculating glycoside.

1ml 0.02N $AgNO_3$ = 20N or 1.08HCN.

Determination of Oxalate

In the determination of oxalate constituents, 5g of the samples were weighed and collected. In a conical flask, 100ml of distilled water was added and left to heat for an hour and allowed to cool. The samples were filtered and 25ml of the filtered samples was measured into a container. The 20ml of H_2SO_4 was added to the 25ml of filtered samples. Using a heating mantle, the sample was left to heat till it got to 70 ºC temperature (using a thermometer to check the temperature in between heating), after the flask was brought down, the sample was titrated with $KMnO_4$ till its color changed to pink.

Mycological Studies

Sterilization of the conical flask, slides, Petri dishes, and all the equipment needed for the experiment was carried out in the laboratory. The glass wares were sterilized in the oven at 120ºC for an hour after washing with soap, while other equipment was surface sterilized with 70% ethanol to reduce microbial contamination (Chuku, 2009). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used was Sabouraud Dextrose Agar prepared in a conical flask using the standard method. The mouth of the flask was plugged with non-absorbent cotton wool and wrapped with aluminum foil. The conical flask containing the mycological medium was autoclaved at 121º C and a pressure of 1.1kg cm^{-3} for 15 minutes. The molten agar was allowed to cool to about 40 º C and dispensed into Petri dishes at 15mls per plate and allowed to further cool and solidify.

Isolation of Fungi from Lentil

A three-fold serial dilution was used in accordance with the method of Mehrotra & Aggarwal (2003) where 1g of the spoilt lentil samples were transferred into the first test tube containing 9mls of normal saline. 1 ml of the solution was transferred to the second test tube and finally from the second to third.

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An aliquot (0.1ml) from the second and third dilutions were plated onto Sabouraud Dextrose Agar in Petri dishes containing ampicillin to hinder the growth of bacteria and this was done in triplicate. The inoculated plates were incubated for 5 days at an ambient temperature of 25° C ± 3° C (Chuku, 2009). The entire setup was observed for 7 days to ensure full-grown organisms. A pure culture of isolates was obtained after a series of sub culturing.

Identification of Fungal Organisms from Lentil

Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were properly teased apart to ensure proper visibility. The well-spread spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high-power objectives. The fungi were identified based on their spore and colonial morphology, mycelia structure, and other associated structures using the keys (Barnett & Hunter, 1998).

Determination of Percentage Incidence

The percentage incidence of fungal occurrence was determined by the formula stated below (Chuku *et al.,* 2019):

$$\frac{x}{Y} X \frac{100}{1} = \% \, incidence$$

Where: X = total number of each organism in a variety.
Y = total number of all identified organisms in a variety.
Data obtained were subjected to mean and standard deviation analysis with the aid of SPSS software version 22.

Results

The result of the proximate composition of lentil presented in Table1 revealed the presence of moisture, ash, lipid, fiber, carbohydrate, and protein in varying concentrations. The highest (55.58 ± 2.35%) and lowest (0.81 ± 0.01%) values were observed for carbohydrate and lipid respectively.

The result of the mineral composition of lentil shown in Table 2 revealed the availability of calcium, iron, phosphorus, magnesium, potassium, and sodium. However, phosphorus recorded the highest value (270.5 ± 65.5mg/100g) while iron had the lowest value (5.45 ± 0.05mg/100g).

Table 1: Proximate Composition of Lentil

Parameter	Composition %
Moisture	7.35 ± 0.15
Ash	2.45 ± 0.05
Lipid	0.81 ± 0.01
Fibre	2.85 ± 0.05
Carbohydrate	55.85 ± 2.35
Protein	28.55 ± 0.05

Table 2: Mineral Composition of Lentil

Parameter	Composition mg/100g
Calcium	80.15 ± 0.05
Iron	5.45± 0.05
Magnesium	94.5 ± 0.5
Phosphorus	27.5 ±65.5
Potassium	87.5 ± 0.5
Sodium	40.5 ± 0.05

The result of the antinutrient composition of lentil presented in Table 3 revealed glycoside, oxalate, saponin, tannin, carotenoid, polyphenol, flavonoid, and lignant to be available. Polyphenol had the highest value (10.25 ± 0.05%) while the lowest value (0.01± 0.00%) was recorded for glycoside.

Table 3: Antinutrient (Phytochemical) Composition of Lentil

Parameter	Composition %
Glycoside	0.01 ± 0.00
Oxalate	0.02± 0.00
Saponin	0.02 ± 0.00
Tannin	0.03± 0.00
Carotenoid	9.15 ± 0.00
Polyphenol	10.25± 0.05
Flavonoid	7.3± 0.10
Lignant	12.55 ± 0.05

The fungi isolation result in Table 4 below showed *Candida* sp and *Microsporum* sp as the two fungal organisms associated with spoilt lentil, although at different percentage incidences. *Candida* sp had 86.35% incidence while 13.63% incidence was recorded for *Micropsorum* sp.

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Table 4: Fungi Characterization and Incidence of Lentil

Fungal Isolates	Microscopic Examination	Probable Organism	(%) Incidence
Isolate A	Budding, spherical to enlongate cells, forming psuedomycelium.	*Candida* sp	86.35
Isolate B	Thick-walled, spindle-shaped, multispetate, rough walked macroconidia.	*Microsporum* sp	13.63

Discussion

Lentil, just like all other legumes, has been reported to possess vital nutrients of health importance to both man and animals (GRDC, 2018). The current study has also highlighted the presence of moisture, ash, fibre, lipid, protein and carbohydrate in lentil. Zdunczyk *et al*. (1993) implicated same proximate nutrient both in the fresh and boiled lentil. Prajapati *et al*. (2020) also indicated high carbohydrate and protein contents in lentil seeds, which is in accordance with the values of the present study. However, Liberal *et al*. (2023) reported higher values for proximate content of lentil compared to those documented in the present study. Similar situation was also observed for the report of Zai-Ul-Haq *et al*. (2011) on lentil. Grusak (2009) emphasized the health benefits of lentil consumption as it could supply energy due to its high carbohydrate content.

Lentil contains valuable mineral elements including calcium, iron, magnesium, phosphorus, potassium and sodium (Table 3). Literatures have also implicated same minerals in lentil but at varying concentrations (Sharma *et al*., 2022). Repas and Gyori (2024) reported same mineral elements but higher values than those documented in the current study. Bansal *et al*. (2023) further revealed that the mineral composition of lentil seed could be influenced by the moisture content of the growing area. El-Satter (2018) reported the impact of lentil variety on the variation of mineral composition as they reported varying concentrations of same mineral elements for the different lentil varieties. These mineral elements are essential for daily system metabolism and functionality (Faris *et al*., 2013).

Several antinutrients were observed in the assessed lentil seed and this is in agreement with early literatures (Zdunczyk *et al*., 1993; El-Satter, 2018). Hefnawy (2011) reported the influence of cooking methods on the antinutrients composition of lentil as it revealed decreasing values for cooked seeds compared to the raw seeds. These antinutrients have been implicated by early researchers to give lentil medicinal and pharmaceutical potentials (Zhang *et al*., 2018; Mustata *et al*., 2022; Li *et al*., 2023).

Like every other legume, lentil is also faced with the challenge of fungal infection leading to spoilage (Kumar *et al*., 2016). The current study revealed *Microsporum* and *Candida* to be associated with the spoilage of lentil seed. This finding differs from previous literature as they reported other fungal organisms (Rahim and Dawar, 2012). However, the findings from this work agree with the report of Sajjad *et al*. (2024) as they reported other yeast species other than *Candida* to contaminate lentil. These fungal organisms have been reported to cause several diseases to man (Padhy *et al*., 2023).

Conclusion

Lentil possesses valuable nutrients and minerals including moisture, ash, fibre, lipid, protein, carbohydrate, calcium, iron, magnesium, phosphorus, potassium and sodium. Anti-nutrients (phytochemicals) were also present but at varying quantities. The crop is also susceptible to spoilage by fungal organisms. Lentil, just like other legumes is recommended for consumption on account of it's rich valuable nutrients. However, proper care and good phytosanitary measures should be given to the crop to guard against fungal infection.

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