STUDIES ON NUTRIENT QUALITY, PHYTOCHEMICAL COMPONENTS AND ASSOCIATED MYCOBIOTA OF LETTUCE

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SUMMARY

Studies on the nutrients and spoilage fungi of lettuce were carried out in the Department of Plant Science and Biotechnology, Rivers State University. Lettuce samples were subjected to proximate analysis and cultural isolation techniques for associated fungi. Proximate assessment revealed the presence of protein, carbohydrate, ash, moisture, fibre and lipid. The moisture and carbohydrate content recorded highest and lowest values (89.74±0.01%) and (0.43± 0.00%), respectively. Mineral investigation showed calcium, iron, phosphorus, potassium, magnesium and sodium to be present. Although, calcium had highest value of 19.75±0.05mg/100g while phosphorus recorded lowest value of 2.10±0.01mg/100g. Vitamin evaluation revealed vitamins C, A, thiamin and niacin. Highest vitamin (95.5±0.01mg/100g) was recorded for vitamin C and lowest vitamin (0.73±0.00mg/100g) for thaimin. Phytochemical screening showed the availability of oxalate, saponin, phytate, polyphenol, flavonoid, tannin, lignate and carotenoid in appreciable concentrations. Aspergillus niger was the only fungal organism isolated from the spoilt lettuce leaf sample with an incident of 100%. Generally, lettuce is rich in vital nutrients just like other vegetables (fluted pumpkin, okazi, uziza and many more) and concerns should be targeted towards its safe handling to avoid contamination.

Keywords: Lettuce, nutrient, plant chemical, fungal flora

LETTUCE scientifically known as *Lactuca sativa* L. is an important vegetable that belongs to the Asteraceae family amongst other sister members like *Vernonia amygdalina* (bitter leaf) (Funk *et al.*, 2005). The plant is an herbaceous annual or biennial leafy plant with dense leaf and loosed rosette. Its stems are not visible as they are short and surrounded by large leaves (Bayer and Starr, 1998). Lettuce can grow up to 30 to 100m in height and peculiar for its green or red colour of leaves (Williams, 2012).

Literature has classified the leafy vegetable into four types viz: head lettuce (*L. sativa* var capitata), stem lettuce (*L. sativa* var aspergina), leaf lettuce (*L. sativa* var crispa) and Cos or Romanian lettuce (*L. sativa* var longofolia) (Trehane, 1995; Vries, 1997). Lettuce is cool season loving plant with an optimum temperature of 18-20 ^{0}C and it is mostly grown in alkaline soil (Chin *et al.*, 2001).

Nutritionally, the plant has been reported to be a good source of nutrients including carbohydrate, protein, fibre, ash, moisture, potassium, phosphorus, calcium, magnesium, sodium, vitamins A, B, C and K (Udebuani and Iheanacho, 2009; Khodadad *et al.*, 2020). These nutrients are essential for healthy living as they support in the growth and development of body system (Murray*et al.*, 2021). Lettuce also possesses anti-nutrients which include various phytochemicals such oxalate, saponin, tannin, polyphenol and many others (Zekkori*et al.*, 2018; Ijarotimi and Akande, 2019).

The plant is faced with the challenge of diseases caused by bacterial, fungi, and viruses. Diseases suchdowny mildew, leaf spot, lettuce mosaic virus, bacterial and fungal rot have been reported to affect the productivity of lettuce (Grube and Ochoa 2005; Dangl and Jones, 2001).

Generally, lettuce has wide range of economic importance as it serves as a source of food, nutrient and income to grower and traders (USDA, 2005). It is based on this literature the current study was carried out to assess the nutrient and fungi flora of lettuce leaf sold in Port Harcourt, Rivers State.

MATERIALS AND METHODS

Sample Collection

Healthy and spoilt samples of lettuce leaves were bought from fruit garden market Port Harcourt, Rivers State. Three samples were obtained from different shops within the market and completely randomized design was adopted. The samples were immediately transported to the Department of Plant Science and Biotechnology, Rivers State University for further studies.

Determination of nutrient components of lettuce

Healthy samples of lettuce leaves were sent to the Food Science and Technology Laboratory for the determination of nutrient composition. The gavimetric alkaline precipitation method was used for phytochemical assessment. All experiments were done in three replicates in accordance to the methods of AOAC (2005).

Preparation of media

Sterilization of 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 120^{o}C for an hour after washing with soap, while other equipments were 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 121^{o}C and 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 spoilt lettuce leaves

The direct plating method of Mehrotra and Aggarwal (2003) was adopted were 0.5cm of the samples showing visible signs of spoilage by moulds was cut from the healthy portions of the leaf up to the points where rot had established and inoculated onto Sabouraud Dextrose Agar in Petri dishes onto which ampicillin was added to hinder the growth of bacteria in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25^{o} C ± 3^{o} C. The entire set up was observed for 7 days to ensure full grown organisms. Pure culture of isolates was obtained after a series of isolations**.**

Identification of fungi from cashew nut

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 objective. The fungi were identified based on their spore and colonial morphology, mycelia structure and other associated structures using the keys of (Barnett and Hunter, 1998).

Determination of percentage incidence

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

$$\frac{X}{Y} \times \frac{100}{1} = \% \text{ incidence}$$

Where, X= total number of each species in a variety; Y= total number of all identified organism in a variety

Statistical analysis

Data obtained were subjected to mean and standard deviation analysis with the aid of SPSS software version 22.

RESULTS

Result of proximate composition of lettuce presented in Table 1 shows the available of moisture, ash, lipid, fibre, carbohydrate and protein.

Table 1: Proximate composition of Lettuce

Parameter	Composition (%)
Moisture	89.74±0.01
Ash	0.54±0.00
Lipid	2.6±0.00
Fibre	0.74±0.05
Carbohydrate	0.43±0.00
Protein	5.53±0.01

Result of mineral composition of lettuce shown in Table 2 reveals calcium iron, magnesium, phosphorus, potassium and sodium to be present.

Table 2: Mineral Composition of Lettuce

Parameter	Composition (mg/100g)
Calcium	19.75±0.05
Iron	3.5±0.02
Magnesium	2.70±0.00
Phosphorus	2.10±0.01
Potassium	26.5±0.05
Sodium	3.5±0.00

The result of vitamin composition tabulated in Table 3 shows the presence of vitamins A, C, Thiamin and niacin.

Table 3: Vitamin composition of Lettuce

Parameter	Composition (mg/100g)
Vit. C	95.5±0.01
Vit. A	0.98±0.05
Thiamin	0.73±0.00
Niacin	0.95±0.05

The result of phytochemical composition of lettuce in Table 4 indicates the availability of oxalate, phytate, saponin, tannin, carotenoid, phyenol, flavonoid and lignant.

Table 4: Phytochemical Composition

Parameter	Composition (%)
Oxalate	3.0±0.05
Phytate	3.17±0.00
Saponin	6.6±0.02
Tannin	2.45±0.05
Carotenoid	22.4±0.00
Polyphenol	6.03±0.01
Flavonoids	2.25±0.00
Lignants	1.65±0.00

The result of fungal characterization and percentage incidence presented in Table 5 revealed *Aspergillus niger* to be responsible for the spoilage lettuce.

Table 5: Fungal characterization and incidence

Fungal Isolate	Macroscopic examination	Microscopic examination	Incidence (%)
 ***Aspergillus niger***	Growth rate is rapid and texture of colonies are powdery and produced a radial fissure in the agar. Surface colony colour was initially white becoming black with conidial production.	Hyphae are septate and hyaline and conidial heads are radiate and globose with metulae that supports the phialides.	100

DISCUSSION

The present study has revealed the presence of different proximate parameters in lettuce. However, the study further revealed that moisture was the highest proximate parameter (89.74±0.01) and carbohydrate (0.43±0.00) the lowest. The availability of these same parameters has been reported by early researchers as well in lettuce as well as in other edible vegetables (Iheanacho and Udebuani, 2009; Olujobi, 2015). The proximate parameters of the present study are in line with those reported by Ijarotimi and Akande (2019) for unblended and blended lettuce flower. Although, higher values were reported than those recorded in the current study. Koudela and Petrikova (2008) also reported higher fiber content (6.74) than its equivalent in the present study. The high moisture content reported in the present study agrees with the report of Murray *et al.* (2021) as they indicated the moisture accumulation of 95% in lettuce. These proximate parameters are vital for day to day living as they provide energy (Carbohydrate) and amino acid (protein) (Hohl *et al*., 2001).

The mineral composition of lettuce as outlined in the present study revealed potassium (26.5mg/100g) to be the parameter with the highest value. Although, lest parameter value 3.5mg/100g was seen for both iron and sodium. Several authors have recognized mineral demands as an important nutrient variable and the mineral parameters in the present study have also been implicated to be present in several edible vegetables including lettuce (Khodadad *et al.*, 2020; Iheanacho and Udebuani, 2009).

Mampholo *et al.* (2016) reported similar mineral contents in lettuce but the values reported are lower than those recorded in the present study. Murray *et al.,* (2021) also reported lower values of the parameters assessed in the current study. Ijarotimi and Akande (2019) and Koudda and Petrikova (2008) reported higher values of the mineral contents reported in the current study. The findings of the study are in strong agreement with the reports of Khaodadad *et al.* (2020) as they indicated highest mineral content to be potassium. Generally, the importance of mineral elements cannot be over looked as it supports various metabolic process (Magnesium and potassium), aid bone strengthening (Calcium) and remains an integral component of the blood (iron) (USDA, 2005).

The present study has shown that vitamin C is highly concentrated in lettuce that every other vitamin assessed and thiamin recording the least vitamin value. This result is in line with the report of Mampholo *et al.,* (2016) concerning vitamin C. The reports of Murray *et al.,* (2021) and USDA, (2019) revealed a variety of vitamins present in lettuce including vitamins C, A, Kl, E and B9. However, the reported vitamin A to be the highest vitamin content.

Notwithstanding, the result of the present study disagrees with the report of Koudela and Petrikova (2008) as they reported no values of vitamin C for investigated lettuce. Vitamins are essential for healthy living as do not only support proper vision (Vit. A), they also strengthen the immune system (Vit. C).

Various phytochemicals are present in lettuce in appreciable concentrations as revealed in Table 4 of the present study. Although higher value of saponin was recorded than any other. Different authors have implicated similar phytochemicals to be present in lettuce as well as other plants (Khodadad *et al.,* 2020; Zekkori *et al.,* 2018). Ijarotimi and Akande (2019) reported higher values of phytate, oxalate, tannin, saponin and flavonoid compared to their, equivalents in the present study. Studies have shown the relevance of phytochemical to plants as well as man as they do not only serve as a defense material for plant but also exhibit anti-microbial, inflammatory, antioxidant properties (Chuku *et al.,* 2017).

The present study recorded only *Aspergillus niger* to be associated with the spoilage of lettuce. However, several other authors have reported both bacterial and fungal organisms that deteriorate lettuce (Jang *et al.,* 2007, Szabo *et al.,* 2000; Yu *et al.,* 2018).

Yatetto *et al.* (2019) also profiled the microbial contamination of lettuce. The study revealed *Aspergillus* genus among other fungi and bacteria to be responsible for lettuces' spoilage.

The isolate of the present study further agrees with Magnuson *et al.* (1990) as they also implicated *Aspergillus* as one of the moulds responsible for lettuce contamination and spoilage. Sources of these microorganisms could be from the soil flora, mode of transportation and handling. In addition, the activities of these microorganisms do not only affect the appearance and quality of lettuce but also its marketability and income of farmers and traders (Khodadad *et al.*,2020; Murray *et al.*, 2021).

CONCLUSION

Lettuce plant is an edible vegetable with proximate, mineral and vitamin contents. Although the plant is also attacked by spoilage organism like *Aspergillus niger*. Therefore, safe handling measures should be adopted to protect the plant.

REFERENCES

AOAC, 2005. Official methods of analysis of AOAC international. 18thedition. Association of Official Analytical Chemists, Washington, D.C., USA.

Barnett, H. L. and Hunter, B. B. 1998. *Illustrated genera of imperfect fungi*, 4th edition. American Phytopathological Society Press, St. Paul Minnesota, 218.

Bayer, R. J. and Starr, J. R. 1998. Tribal phylogeny of the Asteraceae based on two non-coding chloroplast sequences, the trnl intron and tranl/trnEIntergenic spacer. *Ann Misouri Bot Gard*, 85, 242 – 256.

Cheesebrough, M. 2000.*Distinct laboratory practice in tropical countries* part 2. Cambridge University Press London, 143-156.

Chin, D. B., Arroyo-Garcia, R., Ochoa, O. E., Kesseli, R. V., Lavelle, D. O. and Michelmore, R. W. 2001. Recombination and spontaneous mutation at the major cluster of resistance genes in lettuce (*lactuca sativa*). *Genetics*, 157, 831 – 849.

Chuku, E. C. 2009. Fungi responsible for the spoilage of plantain (*Musa paradisiaca*) at various ripening stage. *Acta Agronomical Nigeriana*, 9 (1 & 2), 35-45.

Chuku, E. C., Agbagwa, S. S. and Worlu, C. 2019. Nutrient quality and associated spoilage fungi of English pear (*Pyrus communis* L.). *International Journal of Agriculture, Environment & Bioresearch*, 4(6), 317-325.

Chuku, E. C., Chuku, O. S. and Agbagwa, S. S. 2017. Antifungal potentials of butter extracts from *Vitellaria paradox* Gaertn. *Niger. J. Mycol.*, 9, 105 – 114.

Dangl, J. L. and Jones, J. D. 2001. Plant Pathogen and integrated defence responses to infection. *Nature,* 411: 826 – 833.

Funk, V. A., Bayer, R. J., Keeley, S., Chan, R., Watson, L., Gemeinholzer, B., rcia-Jacas, N., Susanna, A. and Jansen, R. K., 2005. Everywhere but Antarctica.Using a super tree to understand the diversity and distribution of the compositae. *Biologiske Skrifter,* 55, 343 – 374.

Grube, R. C. and Ochoa, O. E. 2005. Comparative genetic analysis of field resistance to downy mildew in the lettuce cultivars "Grand Rapids" and "Ice berg". *Euphytica*, 142, 205 – 215.

Hohl, U., Neubert, B., Holger, P., Schonhof, I. and Bohm, H. 2001. Flavonoid concentrations in the inner leaves of head lettuce genotypes. *Eur. Food Res. Technology,* 213, 205 – 211.

Iheanacho, K. M. E. and Udebuani, A. C. 2009. Nutritional composition of some leafy vegetables consumed in Imo State, Nigeria. *J. Appl. Sci. Environ. Manage,* 13(3), 35 – 38.

Ijarotimi, O. S. andAkande, O. A. 2019. Phytochemical composition, antioxidant activity, biochemical properties and blood glucose lowering potential of unblanched and blanched wild lettuce leaf. *Nigerian Food Journal*, 37 (2), 34 – 43.

Jang, S., Lee, E. and Kim, W. 2007. Analysis of research and development papers on lettuce in Korea. *J. Hortic. Sci. Technol.,* 25, 292 – 303.

Khodadad, C. L. M., Hummerick, M. E., Spence, L. E., Dixt, A. R. Richards, J. T. T., Romeyn, M. W., Smith, T. M., Wheeler, R. M. and Massa, G. D. 2020. Microbiological and nutritional analysis of lettuce crops grown on the international space station. *Frontiers Plant Sci*., 11, 199.

Koudela, M. and Petrikova, K. 2008. Nutrients content and yield lettuce (*Lactuca sativa* L. Var Crispa). *Hord Sci.,* (Prague), 35(3), 99 – 106.

Magnuson, J. A., King, A. D. and Dorok, T. 1990. Microflora of partially processed lettuce. *Appl. & Environ. Microbio.,*56, 3851 – 3854.

Mampholo, B. M., Maboko, M. M., Soundy, P. andSivakumar, D. 2016. hytochemicalss and overall quality of leafy lettuce (*Lactuca sativa* L.) varieties grown in closed hydroponic system.*J. Food Quality,* 39, 805 – 815.

Mehrotra, R. S. and Aggarwal, A. 2003. Pythopathological techniques in plant pathology. **In:** *Plant pathology* 2nd edition. Tata McGraw-Hill publishing company limited, 821.

Murray, J. J., Basset, G. and Sandoya, G. 2021. Nutritional benefits of lettuce consumed at recommended portion sizes. UF/IFAS Extension University of Florida (HIS1416). https://edis.ifas.ufl.edu. Retrieved 27th Dec. 2021.

Olujobi, O. U. 2015. Evaluation of the nutritive composition of five indigenous tree leaves used as vegetable in Ekiti State.*J. Agric. & Environ. Sci.*, 4(1), 184 – 197.

Szabo, E., Scurrah, K. and Burrows, J. 2000. Survey for psychrotrophic bacterial pathogens in minimally processed lettuce. *Lett. Appl. Microbiol.*, 30: 456 – 460.

Trehane, P. 1995. International code of Nomenclature for cultivated plants in 1995. Regnum Vegetable. 133.

UDSA, 2019. Food data central. https://fdc.nal.usda.govindex.html.Retrieved 27th Dec. 2021.

USDA, 2005. Vegetables: Annual summary 01, 27.06. National Agricultural Statistics.Services. http://usda.mannlib. Cornell. Edu/reports/nassr/fruit/Pug-bban/cited.Retrieved 27th Dec. 2021.

Vries, I. M. 1997. Origin and domestication of *Lactucca sativa L.* Genetic Resources and Crop Evolution, 44, 165 – 174.

Williams, M. 2012. Organic lettuce and leaf greens. University of Kentucky Cooperative Extension Service.

Yatetto, L., Ekloh, E., Sarsah, B., Amenuney, E. K. and Adator, E. H. 2019. Microbiological contamination of some fresh leafy vegetables sold in cape coast, *Ghana J. Sci.,* 60(2), 11 – 23.

Yu, Y., Yum, S., Jeon, D. and Jeong. H. 2018. Analysis of microbiota on lettuce (*Lactucasativa* L.) cultivated in South Korea to identify foodborne pathogens.*J. Microbiol. Biotechnol.*, 28(8), 1318 – 1331.

Zekkori, B., Khallouki, F., Bentayeb, A., Fiorito, S., Preziuso, F., Taddeo, V. A., Epifano, F. and Genovese, S. 2018. A new phytochemical and antioxidant and anti-inflammatory activities of different *Lactuca, sativa* L. varcrispaextracts. *Natural Product Communication,* 13(9), 1139 – 1142.