Science World Journal Vol. 18(No 1) 2023
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

EVALUATION OF THE PROXIMATE COMPOSITION AND METAL CONTENT OF SPINACH (*SPINACIA OLERACEA*) FROM SELECTED TOWNS IN NASARAWA STATE, NIGERIA

*Amos Idzi Ambo, Ombugadu Patience and Edward Bebe Ayakeme

Department of Chemistry, Federal University of Lafia, PMB 146 Lafia, Nasarawa State, Nigeria

*Corresponding Author Email Address: amboamosidzi@yahoo.com

ABSTRACT
A study was carried out to evaluate the metal and proximate content of spinach (*Spinacia oleracea*) commonly consumed in Nasarawa State, Nigeria in order to ascertain its suitability for consumption. The metal concentration was determined using Atomic Absorption Spectrophotometer (AAS) and the proximate content was determined according to the method described by Association of Analytical Chemist (AOAC). The results obtained varied with the sampling locations. The mean concentration of the metals ranged from: Zn (2.09-2.48 mg/kg), Pb (0.24-0.73 mg/kg), Mn (3.20-8.52 mg/kg), Cu (0.21-0.31 mg/kg) and Ni (0.01-0.23 mg/kg). The values for proximate content showed that moisture content ranged from (1.00-1.45 %), ash content (8.63-8.74 %), crude protein (14.13-14.44%), crude fibre (2.52-2.63%), crude fat (23.02-23.11%) and carbohydrate (50.10-50.59%). The result obtained does not reveal any significant level of contamination by heavy metals as most of the values were within the permissible limits of consumption of vegetables. However, the growing of the plant in locations near pollution sites should be discouraged. As for the proximate content the values obtained are indication that all the samples are rich sources of protein, fibre, and carbohydrates with a high probability of storage due to their significant moisture content. This underscores their nutritive value as a viable option for plant base nutrients for consumption.

Keywords: Analysis, spinach, proximate, metals, Nasarawa

INTRODUCTION
Food safety is a worldwide concern because food in many cases is contaminated or poisoned thereby rendered unwholesome from the sources which are numerous. The sources vary with the contaminants themselves (Agbenin *et al.,* 2009). Toxins, pesticides and heavy metals are some of the contaminants that accumulate in foods causing poisoning. Vegetables for instance, contained some essential components needed for a healthy lifestyle such as vitamins, carbohydrates, proteins, minerals, trace elements and fibers required by the human body to fight against diseases (Itanna, 2002). The essentiality of some heavy metals that include; Cu, Zn and Mn cannot be overlooked because they help in effectively regulating and maintaining the proper functioning and balancing of the human membrane. Their deficiencies may cause immune system breakdown; however, their excessive concentrations could be toxic. Manganese deficiency for instance, causes reproduction impairment with high infant mortality (Mahurpawar, 2015). Parkinson's disease is credited with the intake of excess trace metal ions. To a large extent, the potential toxicity of heavy metals is crucial because they are widespread and toxic even at low concentrations, though their toxicity may depend on the form in which they exist in the environment (Dube *et al.,* 2001). Most heavy metals are not biodegradable, have long biological half-lives and have the potential for accumulation in the different body organs leading to unwanted side effects (Jarup, 2003; Sathawara *et al.,* 2004; Singh *et al.,* 2010; Nabulo *et al.,* 2011; Neha *et al.,* 2022). Furthermore, heavy metals disrupt important biochemical processes in human organs such as kidneys, liver, and bone causing chronic inflammatory disease and in most cases cancer (Jarup, 2003; Alsafran *et al.,* 2021). The biochemical processes of cell and tissue functions are interrupted by these metals through multiple pathways including interactions with proteins and other biomolecules (Varsha *et al.,* 2010). Consequently, their bioaccumulation at higher trophic levels of human occupant in the ecological food chain occurs, creating heavy metal induced problems (Varsha *et al.,* 2010).
For example, As, Pb and Cd metals have no beneficial effects in humans, and there is no known homeostasis mechanism for them in the system (Draghici *et al.,* 2010). Thus, their accumulation in human's body is a threat. Lacatuşu and Anca-Rovena (2008) observed that the life span of humans in Baia -Mare and Copsa -Mica in Romania significantly decreased by an average of 9-10 years due to industrial activities taken place in the area which discharges excessive concentrations of Pd and Cd resulting in the pollution of soils and vegetables within these locations. A similar incidence was also witnessed in Zamfara state, Nigeria caused by excessive Pd poisoning leading to the loss of lives. According to Itanna (2002) high levels of Pb in the blood put children at risk of neurobehavioral-cognitive deficits, such as IQ deficiency, behavioral disorders and impaired hearing. Other forms of ailments such as skin lesions, lung cancer and dermatitis allergies have been linked to Cr intake (Lacatusu and Anca-Rovena, 2008).
Food is the main route of exposure for heavy metal ingestion and it is believed that heavy metal contaminants in foods are far higher than any other exposure routes including air and drinking water (Aiyesanmi *et al.,* 2012). Activities such as urbanization, industrialization, agriculture and mining processes are factors that have greatly accelerated heavy metal concentrations, although these metals are natural occupants in soils (Facchinelli *et al.,* 2001).
Spinach and other vegetables have edible parts that are consumed wholly or in parts, raw or cooked as part of main dish or salad. They include leaves, stems, roots, flowers, seeds, fruits (Asaolu *et al.,* 2012). Spinach is made up of chiefly cellulose, hemi cellulose and pectin substances that give them their structures and firmness (Mohammed and Sharif, 2011). They are good sources of oil, carbohydrate, protein, minerals and vitamins depending on the part consumed (Mepba *et al.,* 2007). It is an important item in the diet of many Nigerian homes because of the presence of vitamin and

mineral elements (Mohammed and Sharif, 2011).The plants are valuable sources of nutrients especially in rural areas where they contribute substantial amount of protein, minerals, vitamins, fibres and other nutrients which are usually in short supply in daily diet (Emurotu and Onianwu, 2017). The consumption of numerous types of edible vegetable as food supplement is beneficial especially in nutritionally marginalized population in developing countries like Nigeria, where poverty and access to quality food is a challenge. This is more so that most rural dwellers lack basic necessities of life. Asaolu *et al.* (2012) reported that in many developing countries, the mineral intake is inadequate to meet the nutrient requirements of the rapidly growing population. The required minerals cannot be synthesized by the human body and must be provided by plants through dietary means. Sobukola *et al. (*2007) described leafy vegetables as very important protective foods that are useful for health maintenance of the body and also in fighting and prevention of various diseases. Orech *et al.* (2005) reported that vegetable, fats and oil lower lipid level in blood thereby reducing the occurrence of coronary artery diseases which can damage the heart. In Nigeria, leafy vegetables are relatively available and affordable particularly during raining season but are found to be among the least consumed foods due to the ignorance of their nutrient's composition (Orech *et al*., 2005). WHO (2003) recommended individuals to consume 400g or more of vegetables per day to protect against none communicable diseases such as, obesity and cardiovascular diseases.

Excessive concentration of certain metal in varying locations such as dump sites, industrial sites has been observed. The tendency of contamination usually arises in the environment from such locations. Vegetables pick up the heavy metals from dump sites, cultivated soil, irrigated water or when exposed to air containing the metals. The plant then bio-accumulate the contaminants in them and when consumed the heavy metals are transferred to humans (Ashraf *et al.,* 2021). Vegetables are very essential to man and necessary attention is being given to them in most part of Nigeria today. The World Health Organization (WHO) indicated that food consumption by man should contain at least 30% of vegetables depending on the weight of the individual (Sawathara *et al.,* 2004). Nonetheless, because vegetables are consumed raw or semi-processed, there is the general belief that they could contain elevated levels of heavy metals compared to other foodstuffs. Metal accumulation in vegetables may however be attributed mainly to the use of wastewater for irrigation and application of agrochemicals by farmers for diseases and pest control which is on the increase without compliance to good agricultural management practices (Letshwenyo and Mokokwe, 2020; Gupta *et al.,* 2021a; Haque *et al.,* 2021). This, in the long run has been known to affect both animals and humans who consume them. Thus, measures for heavy metal monitoring in vegetables and the environment, accompanied by continuous regular surveys and monitoring, are very imperative. Nonetheless, such programs are not common or well explored in the Nasarawa State.

This study on the proximate and heavy metals content of vegetable grown in selected areas within the state will help create awareness and the development of policy and regulation towards mitigating the environmental impact of heavy metals consumption. This is part of an ongoing broad-based coordinated effort of monitoring program by government in food supply at the state level for sustainable development.

MATERIALS AND METHOD

Sample Collection and Preparation

The vegetable samples (*Spinacia oleracea*) were randomly collected from four Local Government Areas (Akwanga, Lafia, Nasarawa Eggon and Wamba) by picking using gloves hands to avoid contamination (Fig.1). Sizable quantities collected were taken to the laboratory. The vegetables collected from the different locations were thoroughly washed with deionized water to remove debris and other particulate matter. The samples were then air dried at room temperature for 7 days and then oven dried at 60 ˚C until a constant weight was obtained. The dried samples were then pulverized using morter and pestle, sieved to pass through 1mm mesh sieve and stored in a clean air tight plastic container. The content was then advanced for digestion and analysis.

Sample Digestion and Analyses

To each powdered sample, 1g was weight into a 100 cm^3 beaker and 10cm^3 of aqua regia (mixture of HCl and HNO_3) in ratio 2:1 were added to the content in the beaker and covered with a watch glass and left to stand for 12 h. The digestion of the sample was enhanced using a thermostatically electric heating mantle at a temperature of 85˚C in a fume cupboard until the volume of the content reduced to 5 mL. A further addition of 15cm^3 of the aqua regia solution was then made and the content evaporated to obtain a clear solution of 5 cm^3. The content was then cooled to room temperature and the solution filtered using filter paper (Whatman No. 540) to remove any impurities such as waxy solids. A further dilution was made up to volume of 50 cm^3 using deionized water and the solution run on an SP 1900 Pye Unicam Atomic Absorption Spectrophotometer equipped with an air-acetylene burner. For every set of analyses a blank solution was run in duplicate to assess the precision of the data. The instrument setting and operating conditions were carried out according to the manufacturer's specifications.

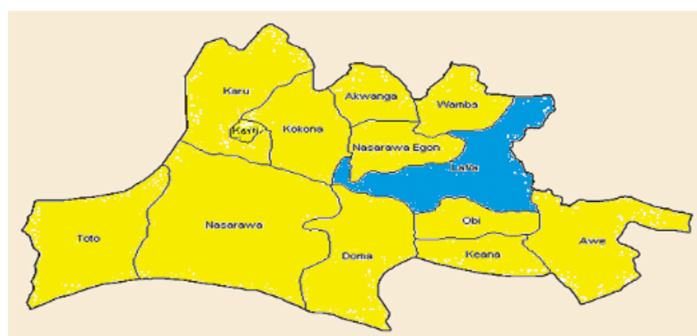

Fig. 1: Map of Nasarawa state showing the sampling towns

Determination of Proximate Composition

The moisture content, crude protein, fat, crude fiber, ash content and calorific content were determined according to the methods described by Standard Method of Association of Official Analytical Chemist (AOAC, 2010) while carbohydrate was determined by difference according the method by Aiyesanmi and Idowu (2012).

Data Analyses

The data obtained from the analyses were in triplicate. The data were all subjected to statistical tests of significance using analysis of variance (ANOVA) and the Student t-test at $p<0.05$ to evaluate the metal concentration and proximate content. This was imperative in order to establish whether there is significant variation in the metals and proximate content in the samples from the sampling locations. The decision is that for the set of data analyzed where probabilities are less than 0.05 ($p < 0.05$) is it considered statistically significant. In each case, all the statistical analyses were done using SPSS software for windows.

RESULTS AND DISCUSSION

Elemental Composition of Sampled Spinach

Table 1 shows the results of the levels of metal concentration in all the spinach samples. The values obtained for these metals (Zn, Pb, Mn and Cu) indicated that their concentrations in the spinach are not significant to pose any threat to consumers when compared to their acceptable standard limits for consumption. From the Table, it is apparent that the most predominant metal is Mn with a concentration that ranged between 3.20-8.52 mg/kg. The sample from Akwanga recorded the highest mean concentration of 8.52 mg/kg while Lafia has the least, with a value of 3.20 mg/kg. Low values from Nasarawa Eggon and Wamba were recorded as 5.51 mg/kg and 5.24 mg/kg, respectively. The results revealed that a significant difference existed in the entire samples ($p<0.05$). As for Zn the values obtained ranged from 2.09-2.48 mg/kg which are relatively lower than those reported by Tasrina *et al.* (2015). Although these values falls within the World Health Organization (WHO, 2001) permissible limit, which suggests that the consumers of spinach from the sampled areas do not face any danger of Zn toxicity as at the time of study. The values of Pb in the samples analyzed ranges from 0.24-0.73 mg/kg. The highest concentration (0.73 mg/kg) was recorded in Lafia and the least (0.24 mg/kg) in Wamba. Values of 0.59 mg/kg and 0.40 mg/kg were recorded in Akwanga and Nasarawa Eggon, respectively. These values are higher than the permissible level of 0.3mg/kg stipulated by WHO/FAO (2001), and compares well with 0.36mg/k reported by Adefemi *et al.* (2012) in Bauchi, northern Nigeria. The result of copper varied from 0.21-0.31mg/kg. The most significant concentrations of 0.31mg/kg were recorded in the samples from Wamba and Lafia, respectively while the least concentration of 0.21mg/kg was recorded in Nasarawa Eggon. Akwanga had a concentration of 0.23mg/kg which is in between the former and the later. The values obtained are all within the permissible level of 0.3mg/kg stipulated by WHO/FAO (2001).This however, is an indication that the spinach in the sampled areas does not contain heavy metals in concentrations that could be considered toxic except for Pb (Table 1). Cr and Cd were not detected in all the samples while Ni was only detected in Akwanga with a mean concentration of 0.23mg/kg.

Table 1: Result of Metal Concentration of Spinach (*Spinacia Oleracea)*

Metal Concentration (ppm)	**Sampling locations**				**FAO/WHO Limit (Mg/kg)**
	Akwanga	Lafia	N/Eggon	Wamba	
Zn	2.48±0.01	2.21±0.01	2.09±0.01	2.36±0.01	99.4
Pb	0.59±0.00	0.73±0.00	0.40±0.00	0.24±0.00	0.3
Mn	8.52±0.01	3.20±0.01	5.51±0.06	5.24±0.01	5.0
Cu	0.23±0.01	0.31±0.02	0.21±0.01	0.31±0.03	73.3
Ni	0.23±0.00	0.02±0.00	0.01±0.00	0.02±0.00	67.9

Proximate analysis

Table 2 shows the results of proximate analysis of the spinach samples. The ash content varied from 8.63-8.74 % in all the samples analysed. A significant value of 8.74% was recorded in the sample from Nasarawa Eggon while the value of 8.63% which is the lowest was recorded from the sample from Wamba. Akwanga and Lafia have ash contents of 8.64% and 8.69%, respectively. The results revealed that the ash contents exhibited no significant difference in all samples analysed. The values obtained also indicated that the spinach analysed in this study are of high nutritional quality. The moisture content varied from 1.00-1.45%. The sample from Wamba had the highest moisture content (1.45%) while that of N/Eggon is the least (1.00%). Lafia and Akwanga had values of 1.05% and 1.04% which are very close. The moisture content obtain for the spinach in this study suggests that all the samples are relatively dry when compared to the permissible level of 2%, and can therefore be stored for a long period of time without undue microbial and biochemical spoilage. The crude protein content in this study ranges from14.13-14.44% (Table 2). The result is an indication that the sampled spinach could serve as excellent sources of protein in diets if consumed regularly. The results of the crude fat and oils ranges from 23.02-23.11% with no significance difference ($p<0.05$) recorded in all samples analysed. One of the samples showed a more significant content of crude fat content of 23.11% and 23.02% (Table 2). The high values recorded are an indication of good source of plant fat from the samples. High fat contents in food imply high caloric value and possible source of fat-soluble vitamins (Adeyeye *et al*., 2021).

The crude fiber contents from the spinach ranged from 2.52-2.63%. The values obtained as shown (Table 2) are within recommended daily allowance for children and for lactating mothers. However, the values obtained in this study are comparatively lower than 9.5-12.12% when compared for some medicinal plants by Shagal *et al*. (2012) , but are in line with that for water leaves and okra reported by Oche *et al.* (2019). The carbohydrate content in this study ranged from 50.10-50.59%. The values suggest significant carbohydrate content in the samples. For high consumption the spinach from these locations could contribute significantly to the carbohydrate content of the body. Although, the results revealed no significance difference ($p<0.05$) in all the samples analyzed the plant are good source of nutrient.

Table 2: Proximate content of Spinach

Sampling location	Parameters					
	Moisture content	**Ash content**	**Crude protein**	**Crude fibre**	**Crude fat**	**Carbohydrate**
Akwanga	1.04±0.01	8.64±0.01	14.35±0.01	2.57±0.02	23.02±0.08	50.39±0.14
Lafia	1.05±0.02	8.69±0.02	14.13±0.01	2.52±0.02	23.04±0.08	50.59±0.11
N/Eggon	1.00±0.69	8.74±0.01	14.44±0.01	2.63±0.04	23.11±0.13	50.10±0.17
Wamba	1.45±0.01	8.63±0.04	14.28±0.01	2.59±0.01	23.03±0.04	50.39±0.04

CONCLUSION

The investigation of the mineral and proximate content of spinach a popularly consumed plant in Nasarawa state indicated that the plant is rich in certain minerals, crude fat, crude fibre and carbohydrate content. These metals investigated (Zn, Cd, Cr, Ni, Cu, Pb and Mn) are in trace amount, and falls within the permissible level in the vegetables except for Pb. The proximate analysis revealed high probability of storage of the plant over a long period of time without spoilage due to their moderate moisture content. The spinach is highly nutritious due to their significant crude protein and carbohydrate contents. However, it is important that close monitoring and periodic evaluation of the plant is carried out as a precautionary measure to ensure compliance to dietary intake and safety.

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