

Variation in Nutrient Contents between Early Growth and Mature Leaves Of *Spinacia Oleracea* (Spinach) and *Lactuca Sativa* (Lettuce)

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Abstract: Vegetable quality can be influenced by various factors such as genotypic differences, pre-harvest conditions, cultural practices, stage of maturity, harvesting methods and postharvest handling procedures as well as the interactions among these factors. The research was aimed at determining variation in nutrient content of the spinach and lettuce during growth and maturation period. The Seeds were planted and analyzed for proximate, mineral and vitamin content at the age of 3, 5 and 7 weeks to observe the variation. Proximate composition for the spinach and lettuce samples shows a decrease with an increase in age of the leaves (3wks -7wks) except for carbohydrate content which increase with increase in age, The minerals analysis of the leaves showed zinc, iron and copper decrease with increase in age while calcium and magnesium increase with increase in age. There is high amount of retinol (vitamin A), and ascorbic acid (vitamin C) in both leaves at week 3 while the content decrease with increase in age. It is concluded that most of the proximate, mineral and vitamin contents used to decrease with an increase in growth period, therefore young leaves are more nutritious than fully matured leaves.

Keywords: Leaf, Lettuce, Nutrient content, Spinach

INTRODUCTION

Vegetable quality can be influenced by various factors such as genotypic differences, pre-harvest conditions, cultural practices, and stages of maturity, harvesting methods and postharvest handling procedures as well as the interactions among these factors [1]. Although vegetable quality has conventionally been determined according to sensory properties like appearance, texture, taste and aroma, there is an emerging trend among consumers who are demanding for internal quality attributes such as the composition of vitamins, minerals and anti-nutritional constituents like nitrates and oxalates [2]. The nutrient quality of plant leaves generally declines with advancing maturity resulting in a decrease in protein levels and a concomitant increase in the amount of indigestible structural carbohydrates. Generally, vegetables are widely designated as "protective foods" in human diet due to their varied health benefits attributable to the richness in vitamins, essential fatty acids, minerals, amino acids and dietary fiber and various essential

bioactive compounds [3]. These include health-promoting plant secondary metabolites composed of antioxidants and phenolic compounds. It is well acknowledged that to meet recommended daily allowance of nutrition, the World Health Organization (WHO) recommendation at least 400g of fruits and non-starchy vegetables is used. Spinach (*Spinacia oleracea* L.) belongs to the family Brassicaceae and is an annual cool season, green leafy vegetable which is eaten raw. Spinach is thought to be native to Southwest Asia where it was first cultivated by the Persians. It is now cultivated throughout the world, with the exception of the tropics [4]. Depending on the cultivar, the leaves are small (usually no longer than 6 cm), sweet tender and smoother than semi-Savoy or Savoy spinach [5]. Like other leafy greens, baby spinach has a high water content and is harvested 35 days after planting (DAP) instead of the 40 days or more required for normal spinach. *Spinacia oleracea* L. crop is high in human nutrients and has relatively high levels of bioactive compounds like vitamins A and C and minerals [6].

Lettuce (*Lactuca sativa* L.) is the most popular, commercially grown leafy vegetable in many countries around the world [7]. Lettuce leaves, which are mainly consumed raw, contain dietary fiber, several important dietary minerals, vitamins (e.g., vitamin B9 and vitamin C), and bioactive compounds (e.g., carotenoids and phenolic compounds that contribute to human nutritional benefits. Baby leaf lettuce is the primary component of spring mix salads that are popular type in packaged salads. Lettuce for baby leaf production is harvested when the first four true leaves reach the length of circa 5–13 cm, approximately 30 days after planting. Plants are an important source of phytochemicals [8], and vitamins needed for proper functioning of human organisms and prevention of vitamin-related deficiencies, such as blindness (vitamin A), beriberi (vitamin B1), pellagra (vitamin B3), anemia (vitamin B6), scurvy (vitamin C), and rickets (vitamin D). Vitamin C is also required for biosynthesis of collagen and certain hormones, and has a therapeutic potential in cancer and heart disease. It is one of the most demanded salad crops in both fresh and ready-to-use markets around the globe [9]. It is a good source of mineral and various health-beneficial (anti-inflammatory, sedative, cholesterol-lowering, and anti-diabetic) bioactive compounds [10]. The study was aimed to determine the variation in nutrient contents between early growth and mature leaves of *Spinacia oleracea* (Spinach) and *Lactuca sativa* (Lettuce).

MATERIALS AND METHODS

Seeds Collection and Identification

The seeds of the spinach and lettuce were purchased from Sharada Market in Kano Municipal area of Kano State Northwestern Nigeria. The seeds were identified at Kano State Agricultural Supply Company (KASCO) Kano. Seeds were planted in a garden at School of Technology behind Department of Pharmaceutical Technology.

Methods of Proximate Analysis

The proximate composition of the spinach and lettuce were determined using standard analytical methods [11].

Moisture Content

About 5g of the spinach and lettuce samples were measured and dried in an oven at 120° C for 2hrs. The dried samples were cooled in a desiccator for 15 minutes and reweighed [11].

Ash content

Ash content was determined by incineration of 5g dried sample in a muffle furnace at 500°C for 2hrs. The ashes sample was cooled in a desiccator and reweighed. The percentage residual sample was expressed as ash content [11].

Crude lipid content

A total of 5g spinach and lettuce samples were extracted using n-hexane for 2 hours by Soxhlet method for the crude lipid content determination [11].

Crude Fiber Content

Crude fiber (CF) content was analyzed by treating 1g dried sample with 10ml of ethanol (w/v) 10ml Tetraoxosulphate (vi) acid (w/v) and distilled water [11].

Crude Protein Content

Kjeldahl method was used to determined crude protein. About 1g of the spinach/lettuce samples were transferred in to a filter paper and placed inside a Kjeldahl flask, 10cm³ of concentrated H₂SO₄ were added and digested in a conical flask until the solution becomes colorless. The distillation was carried out with 15ml of 50% of NaOH. The tip of the condenser was dipped in to a conical flask containing 6cm³ of 4% boric acid in a mixed indicator until a green coloration was observed. Titration was done in the receiver flask with 0.5ml of HCL until the solution turned red [11].

Carbohydrate Content

The carbohydrate content of the samples were determined by estimation using the arithmetic difference method [11]

% Carbohydrate = 100 - (%Moisture + % Fat + % Ash + % Fiber + % Protein).

Mineral Analysis

The minerals in the leafy vegetables were analyzed from solution obtained when 5g of the samples were digested with 10mls of 5N concentrated hydrochloride. The mixtures were placed on a water bath and evaporated almost to dryness. The solution was cooled and filtered into 100ml standard flask and diluted to volume with distilled water. Atomic absorption spectrophotometer was used to analyze the minerals separately after acid digestion of the sample, as described in the official method of the Association of Official Analytical Chemists [11].

Determination of Calcium (Ca)

About 1ml of the sample was drawn with a pipette into a test tube in duplicate. And 3ml of the solution working reagent was added and absorbance at 512 nm was read against the blank [11].

Determination of Magnesium (Mg)

About 5ml of the sample was pipette into a test tube in duplicate. Then 1ml of 0.67N Tetraoxosulphate (vi) acid (H₂SO₄) was added and 1ml of 0.05% titan yellow was added, followed by addition of 1ml of 0.01% gum acacia and 2mls of 10% sodium hydroxide (NaOH) respectively in both spinach and lettuce samples. The solution was mixed and the absorbance was taken at 520nm against the blank [11].

Determination of Iron (Fe)

About 2.5ml of the sample was drawn with a pipette into a test tube in duplicate and 0.4ml of 5N sodium hydroxide (NaOH) was added to bring the pH between 4.0-4.5. Soon 0.75ml of acetate buffer at pH 4.5, 0.5ml of 25% hydroquinone was, 0.5ml of 0.1% I,I di-pridyl and 0.35ml

of distilled water added al added to make it up to 5ml. The absorbance was taken at 520nm against the blank [11].

Analysis of the Vitamins

The vitamins in the leafy vegetables were determined by the official methods of the Association of official analytical chemist [11].

Determination of Vitamin A (Retinol)

About 1g of the sample was weighed and macerated with 20ml of n-hexane in a test tube for 10mins. Then 3ml of the upper hexane extract was transferred in to a dry test tube in duplicates and evaporated to dryness. Following this, 0.2ml of acetic anhydride chloroform reagent was added and 2ml of 50% trichloroacetic acid (TCA) in chloroform was also added. The absorbance was taken at 15secs and 30secs intervals at 620nm.

Determination of Vitamin C (Ascorbic acid)

About 0.5g of the sample was weighed, macerated with 10ml of 0.4% oxalic acid in a test tube for 10mins, centrifuged for 5mins and the solution filtered. 1ml of the filtered was transferred in to dry test tube in duplicates, 9ml of 2, 6-dichlorophenol indophenol was added and absorbance was taken at 15secs and 30secs interval at 520nm.

RESULTS

Proximate Composition of Spinach leaves

The proximate composition of spinach leaf at different ages is presented in Table 1. The result shows that higher carbohydrate content was found in 7 weeks leaf (66.6%), crude protein, ash and lipid content were more pronounced in 3 weeks leaf with 8.59%, 5.8% and 18.40% respectively while higher proportion of moisture content was recorded by 5 weeks leaf 3.9%.

Table 1: Proximate Composition of Spinach Leaves

Age of Leaf (weeks)/composition (%)				
S/N	Parameters	3 week	5 weeks	7 weeks
1	Carbohydrate (%)	56.11±9.12 ^b	59.15±7.77 ^b	66.60±1.41 ^b
2	Crude Protein (%)	8.59±0.49 ^b	7.90±0.21 ^b	7.16±0.07 ^a
3	Lipid Content (%)	18.40±0.70 ^a	18.08±0.56 ^a	17.60±4.03 ^a
4	Moisture Content (%)	3.67±0.00 ^b	3.90±0.01 ^b	2.15±0.77 ^b
5	Ash Content (%)	5.8±1.41 ^b	3.83±0.70 ^{b,a}	3.72±0.77 ^{b,a}
6	Crude fibre (%)	7.88±1.41 ^b	6.93±0.21 ^b	3.23±0.41 ^b

Table 2: Mineral Composition of Spinach Leaves

Age of Leaf (weeks)/composition (mg/kg)				
S/N	Element	3 week	5 weeks	7 weeks
1	Zinc	1.14±0.00 ^b	0.75±0.01 ^b	0.3±0.07 ^b
2	Iron	9.71±0.06 ^b	3.69±0.03 ^b	2.86±0.14 ^b
3	Copper	3.06±0.07 ^{a,b}	2.96±0.02 ^{a,b}	1.68±0.01 ^b
4	Magnesium	86.50±0.70 ^b	111.5±0.70 ^b	125.5±0.70 ^b
5	Calcium	39.55±0.07 ^b	124.5±0.70 ^b	136.2±0.28 ^b

Table 3: Retinol (Vitamin A) and Ascorbic Acid (vitamin C) Content of Spinach Leaves

S/N	Age (weeks)	Vitamin A (Ascorbic) (µg/g)	Vitamin B (Retinol) (µg/g)
1	3wks	18.77±0.12 ^b	9.70±0.14 ^b
2	5wks	13.10±0.14 ^b	4.60±0.14 ^b
3	7wks	10.93±0.14 ^b	2.60±0.4 ^b

Mineral Composition of Spinach Leaves

The mineral composition of spinach leaves at different ages is presented in the table below Table 2. The result showed that higher concentration of zinc, iron and copper was found in the 3 weeks leaf with concentration of 1.14, 9.71mg/kg and 3.06 mg/kg respectively while the 7 weeks leaf has the higher concentration of Magnesium and calcium with 125.5 mg/kg and 136.2 mg/kg respectively.

Retinol (Vitamin A) and Ascorbic Acid (vitamin C) Content of Spinach Leaves

The retinol (Vitamin A) and ascorbic acid (vitamin C) content of the spinach leaves is presented in Table 3 below. The result showed that 3 weeks old leaves had the highest content of both retinol (Vitamin A) and ascorbic acid (vitamin C) each having 18.77µg/g and 9.7µg/g respectively. On the other hand least concentration was recorded by 7 week leaves with retinol (Vitamin A) and ascorbic acid (vitamin C) content of 10.93 µg/g and 2.60 µg/g respectively.

Proximate Composition of Lettuce leaves

The proximate composition of lettuce leaf at different ages is presented in Table 1. The result shows that higher carbohydrate content was found in 7 weeks leaf (56.2%), lipid, moisture, ash and crude fibre contents were more pronounced in 3 weeks leaf with 16.67%, 6.3%, 20.50% and 4.60% respectively while higher proportion of protein was recorded by 5 weeks leaf 23.65%. Table 4

Mineral Composition of Lettuce Leaves

The mineral composition of lettuce leaves at different ages is presented in the table below Table 5. The result showed that higher concentration of zinc, iron and copper was found in the 3 weeks leaf with concentration of 0.66 mg/kg and 0.28 mg/kg

Table 5: Mineral Composition of Lettuce Leaves

Age of Leaf (weeks)/concentration (mg/kg)				
S/N	Element	3 week	5 weeks	7 weeks
1	Zinc	0.66±0.01 ^b	0.35±0.07 ^b	0.28±0.00 ^b
2	Iron	4.33±0.14 ^{a,b}	3.95±0.00 ^{a,b}	3.09±0.31 ^b
3	Copper	7.42±0.11 ^b	5.28±0.07 ^b	3.31±0.15 ^b
4	Magnesium	96.70±0.42 ^b	98.57±0.17 ^b	133.5±0.70 ^b
5	Calcium	1.15±0.00 ^{a,b}	1.15±0.70 ^{a,b}	0.00±0.00 ^b

Table 6: Retinol (Vitamin A) and Ascorbic Acid (vitamin C) Content of Lettuce Leaves

S/N	Age (weeks)	Vitamin A (Ascorbic) (µg/g)	Vitamin B (Retinol) (µg/g)
1	3wks	13.33±0.00 ^b	6.45±0.00 ^b
2	5wks	5.93±0.00 ^b	4.47±0.14 ^b
3	7wks	2.23±0.00 ^b	2.46±0.14 ^b

respectively while the 7 weeks leaf has the higher concentration of Magnesium with 133.5 mg/kg but higher concentration of calcium was recorded by 3 weeks and 5 weeks old leaves each having a concentration of 1.15 mg/kg respectively

Retinol (Vitamin A) and Ascorbic Acid (vitamin C) Content of Lettuce Leaves

The retinol (Vitamin A) and ascorbic acid (vitamin C) content of the leaves leaves is presented in Table 6. The result showed that 3 weeks old leaves had the highest content of both retinol (Vitamin A) and ascorbic acid (vitamin C) each having 13.33µg/g and 6.45µg/g respectively. On the other hand least concentration was recorded by 7 week leaves with retinol (Vitamin A) and ascorbic acid (vitamin C) content of 2.23µg/g and 2.46µg/g respectively.

DISCUSSION

High intake of fruit and vegetables is known to have a positive effect on human health and play a role in alleviating the progressive symptoms of most degenerative diseases. These benefits have been attributed to the high nutritional value and high concentrations of bioactive compounds (ascorbic acid, flavonoids and carotenoids) found in the fruits and vegetables [12]. In the present study, the variations in nutrient contents between early growth and mature leaves of *Spinacia oleracea* (Spinach) and *Lactuca sativa* (Lettuce) were determined. The result of proximate analysis (%) of the spinach and lettuce in this study showed that the Proximate composition for the spinach and lettuce decrease with an increase in age of the leaves (3wks -7wks), This is seen in Moisture contents, protein contents, lipid, Ash contents, and crude fiber while an increase is observed in Carbohydrate content. This result is similar to the findings of Odhav *et al.* [13] who found that the nutrient quality of plant leaves generally declines with advancing maturity resulting in a decrease in proteins levels and a concomitant increase in the amount of carbohydrates.

Finding of this study demonstrated that the mean values for mineral content of the selected leaves had decreased from week 3-7 in case of Fe, Zn and Cu while an increase was noticed in magnesium and calcium. These leafy vegetables could cover recommended dietary allowance (RDA) and contribute substantially for

improving human diet [14]. It's worth underlining that calcium and iron are associated with growth and maintenance of bones, teeth and muscles [15]. However, the Ca/Fe ratio higher than 1 might be considered advantageous for consumption because diet is considered good if the ratio Ca/Fe is > 1 and as poor if < 0.5 (Adeyeye and Aye, 2005). In addition, consumption of Cu. would probably reduce high blood pressure diseases because its ratio Zn/Ca is less than one [14]. Calcium and iron are important intracellular and extracellular cations respectively, which are involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction [16]. There is high concentration of both retinol (Vitamin A) and ascorbic acid (vitamin C) in younger leaves than mature leaves. The vitamin A is vital in helping body natural defence against illness, it helps vision in dim light and it also keeps skin and other lining of some parts of the body such as nose healthy. Vitamin C also helps to protect body cells, it is important in maintaining healthy skin, blood vessels, bones and cartilage, it also helps wound healing and aid in absorption of iron in the body.

CONCLUSION

From the result of this research, it can be concluded that most of the proximate, mineral and vitamin contents decreases with an increase in leaf age therefore; young leaves are more nutritious than fully matured leaves. It is recommended that young age spinach/lettuce should be used in making dishes and salad due to its higher nutritional value and also it should be given to the pregnant woman and children so as to meet up the body requirements for minerals and vitamins. Edible vegetables should be harvested before reaching matured stage of development.

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