Investigation of Primary Metabolites in Young Leaf and Fruit of Three Varieties of Pumpkin (*Cucurbita pepo*) from Gurage Zone, Ethiopia

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Abstract

Pumpkin (*Cucurbita pepo* L.), an herbaceous running plant belonging to family Cucurbitaceae, is one of the natural resources grown in Ethiopia. It is a medium sized plant grown for its fruits, leaves, seeds and flowers are edible. The leaf and fruit produced by *C. pepo* is the most palatable vegetables in the country. The purpose of the study was to investigate the primary metabolite such as carbohydrate, protein, fat, fiber, moisture and ash in leaf and fruit of three varieties of pumpkin namely; Jarrahdale, Porcelain Doll and Sugar pie. Young leaves and ripened fruits of the varieties of pumpkin were collected from selected districts of Gurage zone. Composite sample was grinded to powder size and air dried before analysis. The results obtained were: Carbohydrate (28.15%)>Protein (26.31%)>Fiber (17.55%)>Ash (10.96%)>Moisture (10.17%) in leaves and Carbohydrate (41.68%)>Moisture (17.33%)>Fiber (16.50%)>Ash (10.95%)>Protein (9.88%)>Fat (3.66%) in fruits of pumpkin were determined using Kjeldahl method (AOAC official method: 920.39, 925.10, 962.09) and APHA 2540. The contents in the plant are high enough and proportional to common vegetables. It is recommendable to enhance the consumption of leaf and fruits of plants.

Keywords: Pumpkin; *Cucurbita pepo*; Primary metabolite; Stables; AOAC.

Introduction

Vegetables are an important component in human’s diet, especially in developing countries. It is needed to complement staples in diet, supplying essential minerals and vitamins that may not be obtained solely from staples. They generally produce more nutrients per unit land area than staples such as rice [1]. Vegetables are the fresh, edible and succulent parts of herbaceous plants. They are considered as special food crops owing to their valuable food ingredients that can be effectively utilized by the body. They contain appreciable number of vitamins and minerals that are highly beneficial for the maintenance of health and prevention of diseases.

They also contain high amount of dietary fiber and a minimal amount of protein [2,3]. Knowledge of the nutritive value of local dishes, soup ingredients and local foodstuffs is necessary in order to encourage the increased cultivation and consumption of those that are highly nutritious. Consuming the local foodstuffs like pumpkin plant will help to enhance the nutrients of the poor who cannot afford enough protein foods of animal origin [4,5]. Vegetables are good sources of oil, carbohydrates, minerals and vitamins depending on the vegetable consumed [6,7] reported that vegetable fats and oil lower blood lipids thereby reducing the occurrence of disease associated with damage of coronary artery. Pumpkin, one of the vegetables belongs to the Cucurbitaceae family and grows easily from either seeds or cuttings with roots [8]. Pumpkin (*C. pepo*) is mostly used to refer to cultivars with round fruits, which are used in the mature state for baking or cooking. The pumpkin plant parts like root, stem, flower, fruit, twig exudates, and modified plant organs of pumpkin has been used for extraction of natural resources grown in Ethiopia. It is a medium sized plant grown for its fruits, leaves, seeds and flowers are edible. The leaf and fruit produced by *C. pepo* is the most palatable vegetables in the country. The purpose of the study was to investigate the primary metabolite such as carbohydrate, protein, fat, fiber, moisture and ash in leaf and fruit of three varieties of pumpkin namely; Jarrahdale, Porcelain Doll and Sugar pie. Young leaves and ripened fruits of the varieties of pumpkin were collected from selected districts of Gurage zone. Composite sample was grinded to powder size and air dried before analysis. The results obtained were: Carbohydrate (28.15%)>Protein (26.31%)>Fiber (17.55%)>Ash (10.96%)>Moisture (10.17%) in leaves and Carbohydrate (41.68%)>Moisture (17.33%)>Fiber (16.50%)>Ash (10.95%)>Protein (9.88%)>Fat (3.66%) in fruits of pumpkin were determined using Kjeldahl method (AOAC official method: 920.39, 925.10, 962.09) and APHA 2540. The contents in the plant are high enough and proportional to common vegetables. It is recommendable to enhance the consumption of leaf and fruits of plants.

Materials and Methods

Site description

The study area, Gurage Zone as stated in Figure 1 below, is located between 7.8°C-8.5°C North latitude and 37.5°C-38.7°C East longitude of the equator. It is around 180 km away from the capital city of Ethiopia, Addis Ababa to southwest direction. The zone comprises altitudes ranging from 1,001 to 3,500 meters above sea level. The mean annual temperature of the zone ranges 13-30°C and the mean annual rainfall ranges 600-1600 mm. The laboratory activities were involved at

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Sample collection

Young leaves and ripened fruits of the three pumpkin varieties (Jarrahdale, Porcelain Doll and Sugar pie) were collected from selected districts of Gurage zone where the varieties of the pumpkin are available. The districts selected from Gurage zone were Ezha, Meskan, Cheha, Enemor-ena-ener, Sodo, and Gumer. From each district, three sites were selected. The collected leaves and fruits of each variety of pumpkin obtained from the selected districts in the zone were homogenized to get composite sample. Then, they were placed in plastic materials until preparation and analysis.

Chemical and reagents

Reagents that were used in the analysis were all analytical grade. Deionized water, perchloric acid, nitric acid, sulfuric acid, sodium hydroxide, and hydrogen peroxide, H2SO4, copper sulfate, hexane, acetone, boric acid of analytical reagent grade which were purchased from JI Laboglass St. Company were used acting as reagents and solvents throughout all procedures starting from sample collection to analysis.

Characterization method

The AOAC [14,15] were used for characterization of primary metabolite contents in the sample. Accordingly, AOAC 925.10 for moisture, AOAC-920.39 for fat, AOAC-923, 03 for ash, ES ISO-1871:2013 for crude protein and AOAC -962.09 for fiber contents were used with minor modification.

Primary metabolite determination

The collected young leaves and ripened fruits of pumpkin were cut into pieces, air dried and grinded to powdered size. The methods of Association of Official Analytical Chemists (AOAC) [14] were used for determination of moisture, ash, fiber, protein, fat and carbohydrate contents of the samples.

%Moisture: Five grams of each sample was weighed into three separate petri dishes and dried in a vacuum oven at 105°C for 24 hrs to constant weight. The samples were removed from the oven, cooled in desiccators, and weighed again. The percentage moisture content (± SD) was calculated using the following equation:

\[
\text{Moisture} = \frac{\text{Original Wt.} - \text{Final wt.}}{\text{Original Wt.}} \times 100
\]

%Fat: Five grams of each dried weight was transferred to an extraction thimble and its opened was plugged with a cotton wool. The thimble was dropped into the Soxhlet extractor and sufficient organic solvent (hexane: acetone) was added until it siphoned in to a dried receiving flask which has been weighed. More solvent was poured into the Soxhlet. The flask with the extractor was placed over an electric heating medium and reflux condenser was fixed to it. The fat was extracted by dropping into the flask in a certain time and switched. The solvent was evaporated off in hot water bath. The flask and its content were dried until removed, cooled, and weighed. The percentage fat content (± SD) was calculated as:

\[
\text{%Fat} = \frac{\text{Wt. of the Fat}}{\text{Wt. of the sample}} \times 100
\]

Figure 1: Map of the study areas, Source: Reference [21].
%Ash: Ten grams of dried samples was transferred to a muffle furnace for ignition. The samples were allowed to burn for 2 hours at 550°C. They are then removed and cooled in desiccators. The percentage ash content (± SD) was calculated as:

\[
\%\text{Ash} = \frac{\text{Wt of the Residue}}{\text{Wt of the Sample}} \times 100
\]

%Protein: Kjeldahl method (ES ISO 1871:2013) was used for the determination of crude protein content. 5 g of the sample, copper sulphate catalyst, and 25 mL of Conc. H2SO4 was heated over a Bunsen flame in a fume cupboard to expel any poisonous gas and then heated with shaking at intervals for 1:30 hour until the mixture become clear. A 350 mL of distilled water was added, followed by the addition of 50 mL of 2% boric acid with 1 mL methyl red indicator. A 75 mL of 50% NaOH was added to make the solution alkaline. The ammonia was distilled into the boric acid solution. A 250 mL of the distillate was collected after washing the walls of the receiver and the condenser. The distillate was titrated with 0.1N H2SO4. Crude Nitrogen was determined based on the Kjeldhal procedure and crude protein value was obtained by multiplying the nitrogen value by a factor of 6.25.

\[
\%\text{Nitrogen} = \frac{\text{ml of Acid} \times N \times \text{Acid} \times 14}{\text{Wt of the Sample}}
\]

Therefore, %Crude Protein = %Nitrogen × 6.25

%Fiber: The method known as AOAC official Method 962.09 was used for the determination of crude fiber content. Exactly known mass (5 gm) of the samples was digested in sulfuric acid and the sample was filtered and washed with boiling water. The residue was then transferred to a beaker and boiled again. The residue was dried in a vacuum oven and weighed. The dry mass was incinerated in a muffle furnace for 2 hours at 550°C, cooled and weighed again. The percentage of crude fiber was calculated as the following formula.

\[
\%\text{Fiber} = \left(\frac{\text{Wt of Dried matter}}{\text{Wt of ash}}\right) \times 100
\]

%Carbohydrate: The percentage carbohydrate content in the samples was determined by the following formula:

\[
\%\text{Carbohydrate} = 100 - (%\text{Moisture} + %\text{Fat} + %\text{Ash} + %\text{Protein} + %\text{Fiber})
\]

Energy (kcal) = ([%CHO × 4] + (% CP × 4) + (CL × 9)]

Where, CHO, CP and CF stand for carbohydrate, crude protein and crude fat respectively.

Methods of Data Analysis

The statistical analyses of the results were done using the statistical software like excel. The statistical analyses were conducted using statistical package of microcal origin 6.1. First class ANOVA was made to check whether there is significant difference or not between means at 95% confidence interval. Calibration graphs and bar graphs were drawn using Microsoft Office Excel 2007 and microcal origin 6.1.

Results and Discussion

As indicated in Table 1, the ash contents in the studied leaves of pumpkin varieties were 16.52 ± 0.98, 15.87 ± 0.11 and 16.54 ± 0.05% in Jarrahdale, Porcelain Doll and Sugar pie respectively. The content in leaves of Sugar pie approaches the value in Jarrahdale variety. It was low in Porcelain Doll variety. These values indicate that, the Sugar pie and Jarrahdale varieties of pumpkin are rich in minerals, which are the constituents of ash. The values of carbohydrate determined in the leaves of the three varieties (21.15 ± 0.11, 29.78 ± 1.09 and 33.51 ± 0.34% in Sugar pie, Jarrahdale and Porcelain Doll) were high compared with the values of others. Porcelain Doll variety has high in carbohydrate content and the Sugar pie variety on the other hand has low values in some extent.

The percentage of crude fat is too low in all varieties having (1.83 ± 0.03, 1.60 ± 0.01 and 2.52 ± 0.04%) in leaves of Jarrahdale, Sugar pie and porcelain Doll pumpkin respectively. Porcelain Doll has more values of fat from the rest variety. Almost all variety under study has the same values of fiber (17.60 ± 0.05, 17.35 ± 0.02 and 17.70 ± 0.03%) in Jarrahdale, Porcelain Doll and Sugar pie respectively. The leaf of Sugar pie variety of pumpkin has more %protein (32.48 ± 0.09%) whereas 21.97 ± 0.03% in Porcelain Doll and 24.27 ± 0.05% in Jarrahdale. The leaf of three variety of pumpkin is good to consume because of high contents of protein. The leaves of the three varieties under study, have also the same values %moisture which are 10.00 ± 0.43, 10.00 ± 0.43 and 10.52 ± 0.04% in Jarrahdale, Porcelain Doll and Sugarpie respectively.

In fruits of the three varieties of pumpkin, the primary metabolite was also available and reported in Table 2 below. The percentage of ash was determined as 9.08 ± 0.03, 11.05 ± 0.26 and 12.73 ± 0.02% in leaves of Sugar pie, Jarrahdale and Porcelain Doll pumpkin respectively. These values indicate that, the Sugar pie and Jarrahdale varieties of pumpkin are rich in minerals, which are the constituents of ash. The values of carbohydrate determined in the leaves of the three varieties were high compared with the values of others. Porcelain Doll variety has high in carbohydrate content and the Sugar pie variety on the other hand has low values in some extent.

Accordingly, there was no significant difference (p>0.05) in mean contents of %ash between leaves of Jarrahdale/Sugar pie however,
there was significant difference between leaves of Jarrahdale/Porcelain Doll and Porcelain Doll/Sugar pie. There was a significant difference (P<0.05) in mean contents of % Fat, % Moisture, and % Carbohydrate among leaves of the varieties. Furthermore, % Protein was significantly different in leaves of Jarrahdale/Sugar pie and Porcelain Doll/Sugar pie but, there was no significant difference between Jarrahdale/Porcelain Doll. Percentage fiber value was not significantly different between Jarrahdale/Porcelain Doll and between Jarrahdale/Sugar pie, however, significantly different between Porcelain Doll and Sugar pie. The significance differences of this content among leaves of varieties indicate that the varieties are differing in proximate contents. There was significance difference among varieties with % Fiber. Percentage of Crude protein and % moisture was 13.46 ± 0.17, 6.51 ± 0.02, 9.66 ± 0.11 and 13.76 ± 0.01, 20.98 ± 0.02, 17.24 ± 0.04 respectively in Jarrahdale, Porcelain Doll and Sugar pie and these data implies that there was significance difference for both protein and moisture content among the varieties of pumpkin. Percentage of carbohydrate was the dominator one and had the values 43.19 ± 0.11, 42.86 ± 0.1, 39.00 ± 0.16 in fruits of Jarrahdale, Porcelain Doll and Sugar pie varieties of pumpkin. There was also significance difference for this metabolite among the varieties. The significance difference of the values of all primary metabolite among fruits of the three varieties of pumpkin indicates, the different contents of the metabolite are there in different varieties of pumpkin.

Comparison of primary metabolite in leaves and fruits

The ash content was 10.96% in leaves and 10.95% in Fruits of pumpkin, which was similar in both parts. Since ash content of a plant material is an index of total, mineral content it implies that pumpkin is a better mineral sources. Carbohydrate (41.68%) was high in fruits of pumpkin compared with in leaves (28.15%). Main function of carbohydrate in the body is for energy supply. Iyon and Bassir [16] observed that leafy vegetables might not be an important source of carbohydrates due to their consumption along with other carbohydrate rich food such as cereals. The calorific values of most vegetables are low. However, the values in pumpkin was not that much low as the expected vegetables. Thus, the pumpkin plant is rich with carbohydrate. Fat content was low (1.98%) in leaves and high (3.66%) in fruits of pumpkin. This data indicates the fat content in both parts of pumpkin was low compared with the other metabolite.

Fiber and moisture were also the constituents of pumpkin and they were determined as 17.55, and 10.17% respectively in leaves and 16.50 and 17.33% respectively in fruits of pumpkin. Fiber was almost the same in both parts and whereas the moisture content was relatively high compared to in leaves, which was low. Fiber cleanses the digestive tract, by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. It also adds bulk to the food, prevents the intake of excess starchy food, and may therefore guard against metabolic conditions such as hypercholesterolemia and Diabetes mellitus [17]. Dietary fiber is an important constituent in pumpkin and other vegetables, helping to reduce serum cholesterol level, risk of coronary heart disease, and contributing to prevent colon and breast cancers and hypertension [3]. The values fiber in leaves and fruits of pumpkin was somewhat high enough for contributing this activity. It is known that products that have low fat values normally have high moisture contents. The increase in moisture content in fruit could be as are salt of water absorption by the fibers and other natural chemical component of the pumpkin fruits [18]. Moisture content is a widely used parameter in the processing and testing of food. It is an index of water activity of many foods. The observed value may imply that pumpkin may have a short shelf life since microorganisms that cause spoilage thrive in foods having high moisture content and also indicative of low total solids [17]. Pumpkin is also a good source of vegetable protein having protein contents of 26.31% in leaves and 9.88% in fruits. The leave was rich in protein standing from the data. Both values were higher than (3.3%) recorded by the USDA Nutrient Database for Standard Reference [19]. Its protein content makes it suitable for consumption, as a necessity for body development. The protein values of pumpkin as observed in this study confer on it the advantage as a rich source of vegetable protein. Incorporating both leave and fruits of pumpkin in the diet can furnish it with enough amounts of protein, which provides several benefits, such as provision of imperative body constituents, maintenance of fluid balance, formation of hormones and enzymes, contribution to immune function.

Comparison of current study with the literature values

The proximate (primary metabolite) values determined in pumpkin of the present study was comparable with those in different countries reported by researchers as it can be seen in Table 3 below. The ash content determined in deferent parts of pumpkin was ranged from 5 to 15% in the reported data while the contents in leaves and fruits of the present study was 10.96% which shows the comparability with the reported data. The carbohydrate content in leaves (28.15%) and in fruits (41.68%) were, lower than those in fruits from Egypt (75.84%) and Nigeria (66.647%), Bangladesh (72.84 ± 0.11%). It was similar with seeds of pumpkin from Nigeria but higher than in pods (16.97 ± 0.21%) of pumpkin from Nigeria and seeds, which was 12.160, 14.019, and 15.63% from Zimbabwe, Korea, and Sudan respectively. The moisture content (10.17%) in leaves was the same as (10.94%) in leaves of pumpkin from Nigeria and fruits of pumpkin from Bangladesh (10.14 ± 0.12%). The protein content in leaves (26.31%) was higher than in fruits from Egypt, fruits, seeds, leaves from Nigeria, and lower than in leaves of pumpkin from Korea and Zimbabwe. %Fat in the present study was in the same range with those from Nigeria except it was on lower than in seed (38%) reported by Elinge et al. [20] and (43.460 ± 0.098%) Raphael et al. [9] from Zimbabwe. Fiber content was higher than the data in all countries reported in the table. The result of the present study implies that it was a good manner with those done in different countries by researchers.

| Primary metabolite | Jarrahdale | Porcelain Doll | Sugar Pie |
|-------------------|------------|----------------|-----------|
| %Ash              | 9.08 ± 0.03| 11.05 ± 0.26   | 12.73 ± 0.02|
| %Carbohydrate     | 43.19 ± 0.11| 42.86 ± 0.1   | 39.00 ± 0.16|
| %Fat              | 3.01 ± 0.02| 3.10 ± 0.01    | 4.87 ± 0.12|
| %Fiber            | 17.50 ± 0.5| 15.50 ± 0.2    | 16.50 ± 0.44|
| %Protein          | 13.46 ± 0.17| 6.51 ± 0.02   | 9.66 ± 0.11|
| %Moisture         | 13.76 ± 0.01| 20.98 ± 0.02  | 17.24 ± 0.04|

Table 2: Average Contents (% ± SD, n=3) of primary metabolite in Fruits of Jarrahdal, Porcelain Doll and Sugar pie varieties of pumpkin.
The plant has primary metabolites in its leaves and fruits. There is high nutritional and medicinal importance. The result of the present study implies that it was more or less with those done in different countries by researchers. In general, the content in leaves of pumpkin is comparable and has higher values of fiber. Therefore, the leaves of pumpkin are categorized under the class of edible leafy vegetable. Among the three varieties of pumpkin, Jarahdale was the dominating one by primary metabolite in both leaves and fruits.

Conclusions

Pumpkin (Cucurbita Pepo) is one of the most economically, nutritionally, and medicinally important traditional vegetable crops worldwide and can be cultivated both in temperate and tropical regions. The plant has primary metabolites in its leaves and fruits. There is high value of protein in leaves and high value of carbohydrate in fruits. The contents of primary metabolite in leaves and fruits of pumpkin are high enough and proportional to the contents of common vegetables. It is recommendable to use it as food source frequently along the lines of its importance. The result of the present study implies that it was more or less with those done in different countries by researchers. In general, the content in leaves of pumpkin is comparable and has higher values of fiber. Therefore, the leaves of pumpkin are categorized under the class of edible leafy vegetable. Among the three varieties of pumpkin, Jarahdale was the dominating one by primary metabolite in both leaves and fruits.

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