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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

Cucurbita pepo L
natural-resources-grown-in-Ethiopia.-It-is-a-medium-sized-plant-grown-for-its-fruits,-leaves,-seeds-and-flowers-are-
C. pepo
study-was-to-investigate-the-primary-metabolite-such-as-carbohydrate,-protein,-fat,-fiber,-moisture-and-ash-in-leaf-

Kjeldahl-method-(AOAC-official-method:-920.39,-925.10,-962.09)-and-APHA-2540.-The-contents-in-the-plant-are-

Keywords: Pumpkin; *C c b a* ; 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 nutritive. 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 occurence of disease associated with damage of coronary artery. Pumpkin, one of the vegetables belongs to the Cucurbitaceous family and grows easily from either seeds or cuttings with roots [8]. Pumpkin (*C.*) is mostly used to refer to cultivars with round fruits, which are used in the mature state for baking or feeding livestock [9]. Pumpkin plant is an annual plant with leaf; it has a climbing stem of up to 12 m long and fruit with a round fibrous flesh [10]. There is wide variation in fruit size, fruit weight, shape and rind

body [12]. The pulp of ripe fruit of *C c b a* is used to relieve intestinal inflammation or enteritis, dyspepsia and stomach disorder. The seeds and oil from pumpkin seeds have been used for many years for relieving of difficulties associated with an enlarged prostate gland and micturition problems related to overactive bladder [13]. The pumpkin seeds yield approximately 50% fatty oil, (mostly linoleic and oleic acid and tocopherol (HMPC). It is also believed to help in the production of urine and healing of burns [10]. The objectives of the present study are to determine the percentage of primary metabolite such as protein, carbohydrate, moisture, fat, fiber and ash in young leaf and fruits of pumpkin.

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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Cucurbita pepo

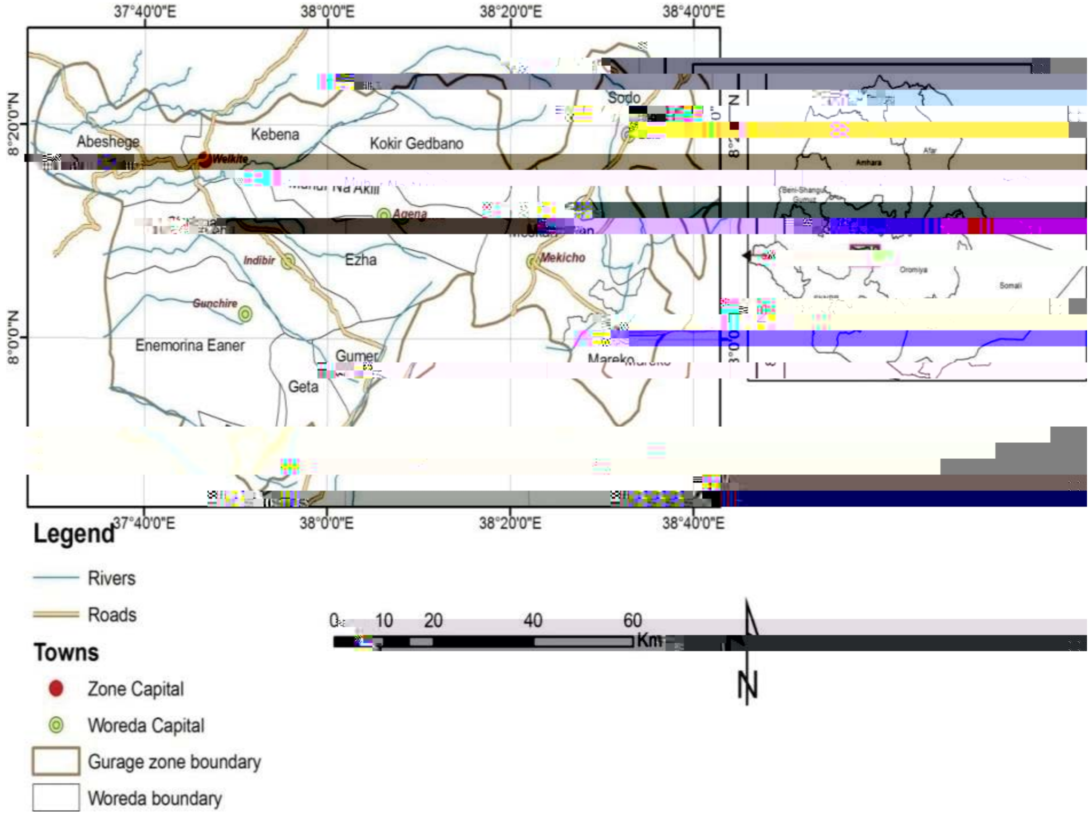

Figure 1:

Chemistry Department of Wolkite and JIJE Analytical Testing Service Laboratory at Addis Ababa.

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 varieties 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, H_2SO_4, copper sulfate, hexane, acetone, boric acid of analytical reagent grade which were purchased from JJ 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 ripped 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:

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

%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:

$$\%Ash = \frac{Wt\ of\ the\ Residue}{Dry\ Wt\ of\ the\ Sample} \times 100$$

%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. H_2SO_4 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 H_2SO_4. 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.

$$\%Nitrogen = \frac{\times \qquad \times 14}{Wt\ of\ the\ Sample}$$

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.

%Fiber=(Wt of Dried matter/Wt of ash) × 100

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

% Carbohydrate 100-(% Moisture+%Fat+%Ash+%Protein+%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 of %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 Jarrahdale, Porcelain Doll and Sugar pie respectively. It was relatively low in Jarrahdale and similar in the other two varieties. There was significance difference among the varieties for the ash content. Percentage of Fat is 3.01 ± 0.02, 3.10 ± 0.1 and 4.87 ± 0.12% in Jarrahdale, Porcelain Doll and Sugar pie respectively. There was no significant difference between Jarrahdale and Porcelain Doll whereas there was between Jarrahdale/Sugar pie and Porcelain Doll/Sugar pie fruits of pumpkin. Percentage Fiber was respectively 17.50 ± 0.5, 15.50 ± 0.2, 16.50 ± 0.44 in Jarrahdale, Porcelain Doll and Sugar pie of pumpkin. In this study, the significance of variation between samples was analyzed using one-way ANOVA, which was done using detail calculations following a statistical formula or Microsoft excel. The result of the analysis is described in the subsequent paragraphs.

Accordingly, there was no significant difference (p>0.05) in mean contents of %ash between leaves of Jarrahdale/Sugarpie however,

Primary metabolite	Contents in Present (%)		
	Jarrahdale	Porcelain Doll	Sugar Pie

Table 1:

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

Citation: *Cucurbita pepo*