Proximate Analysis and Antioxidant Properties of Selected Fruits in Batangas

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Abstract *- Five selected fruits in Batangas namely banana (Musa lacatan), chico (Manilkarasapota), papaya (Carica papaya L.), pomelo (Citrus grandis) and tamarind (Tamarindicusindica) were analyzed for their proximate analysis, antioxidant properties and antioxidant activity. The study aimed to make a baseline database using the information obtained which will be useful for further studies and improvement of existing products and food processes and for development of new ones. Among the five selected fruits, tamarind got the highest ash content of 12.51%, crude protein of 4.68%, crude fat of 3.39% and total carbohydrates of 46.67% but the lowest moisture content of 33.45%. Pomelo got the highest moisture content of 91.74% while chico got the highest crude fiber content of 2.64%. Papaya had the lowest total carbohydrates of 7.54% while banana had the lowest crude fiber content of 0.51%. For the antioxidant properties, tamarind had the highest TFC of 0.007 mg quercetin / L, TPC of 0.223 mg gallic acid / L, and Vitamin C of 1024.41 mg/100cm³ while papaya had the highest Beta-carotene content of 286µg/100g. The selected fruits exerted remarkable antioxidant properties. Pomelowith 94.4% hadthe strongest antioxidant activity followed by chico with 91.96%, then banana with 90.84%, papaya with 88.72% and lastly by tamarind with only 70.69%. Based on the statistical treatment of results, there were significant differences in the antioxidant properties and antioxidant activity of selected fruits in Batangas.*

Keywords *– antioxidant activity, antioxidant properties, proximate analysis*

INTRODUCTION

Batangas is one of the eleven provinces of Southern Luzon with a total land area of 316,581 hectares. Rich fertile land, good weather and assorted landscape justified for the huge agricultural base. According to Food and Agricultural Organization, fruit trees in the province were grown on almost 32, 000 hectares having an average production of around 13 metric tons per hectare [1].This was an indication that Batangas is rich in different kinds of fruits which were health beneficial.

In the past few years, interests in the composition of fruits intensified because of its possible health benefits.The researchers were convinced that nutrients found in fruits can do more than just prevent deficiency diseases. Some of the epidemiological studies indicate that antioxidants present in fruits may be important in prevention of many degenerative diseases such as cancer and cardiovascular diseases [2].

These antioxidants are substances which protect human cells from damage that are caused by free radicals such as superoxides, peroxyl, and hydroxyl radicals. These can be naturally obtained from fruits, vegetables, nuts, legumes, grains and cereals. Fruits exhibit different antioxidant activities due to the presence of different antioxidant properties. These antioxidant properties were present in foods in the form of carotenoids and polyphenols such as flavonoids, and phenolics. In some they are in the form of vitamins like ascorbic acid, vitamin A and vitamin E. They can fight not only illnesses but also prevent infection and aging process of cells. They can also be used for food preservation as food additives [3].

Polyphenols are chemical substances that can act as antioxidants. They are found in plants and has more than one phenol unit. There are subdivisions such as flavonoids and phenolics. Another antioxidant property is flavonoids which are metabolites that enhance vitamin C's functions by protecting it from

oxidation and improving how vitamin C is absorbed. They are important in plant pigmentation producing the yellow, red or blue color seen in plant petals. On the other hand, Vitamin C as an antioxidant is an essential nutrient for humans and animal species which protect the body against oxidative stress while Vitamin A acts as an antioxidant protects the cells of retina of the eye.

Based on the Bureau of Agricultural Statistics, five of the most abundant locally grown fruits in the province were banana, chico, papaya, pomelo and tamarind [4]. These fruits were frequently consumed by the localities however there was limited information on their composition. Like berries, these tropical fruits may give high amount of antioxidant activity and antioxidant properties. Thus, an understanding of the proximate analysis and some nutrient composition of these fruits is important.

Proximate analysis is the determination of the different macronutrients such as moisture, ash, crude fiber, crude fat, crude protein and carbohydrates. These macronutrients can be determined using oven drying method, dry ashing method, Weende method, Sohxlet extraction method, Kjeldhal method and chromatographic method respectively based on the Official Methods of Analysis of the AOAC International [5].

Moisture content is one of the properties that is important for nutritional labeling, food quality, and microbial stability while ash content is a measure of the total amounts of minerals present within a food [6]. While, proteins are polymers of amino acids that are major source of energy [6]. Fat content in food plays a major role in determining the overall sensory characteristics, such as flavor, texture, mouth feel and appearance [6]. Lastly carbohydrate content is the amount of sugars and polysaccharides in food while the crude fiber content is the estimate of the indigestible fiber in food [6].

The data gathered in this study can be used to make a baseline database for composition values and methodologies which will be useful for further studies. The data can provide useful information to nutritionists, health officers, and consumers. Furthermore, it can be used for the development of new products from these fruits and improvement of existing processes.

OBJECTIVES OF THE STUDY

The main focus of the study was to obtain information about the proximate analysis, antioxidant properties and antioxidant activity of the selected fruits in Batangas namely banana *(Musa lacatan)*, chico *(Manilkarasapota)*, papaya *(Carica papaya L.)*, pomelo *(Citrus grandis)* and tamarind *(Tamarindicusindica)*.

MATERIALS AND METHODS

Chemicals

The standards used for determination of antioxidant properties such as Gallic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), quercetin and Folin-Ciocalteau Phenol Reagent were ordered from Sigma-Aldrich company. All other chemicals and reagents used were analytical grade.

Material Preparation

Five locally grown fruits from Batangas namely banana (*Musa lacatan*), chico (*Manilkarasapota*), papaya (*Carica papaya L.*), pomelo (*Citrus grandis*) and tamarind (*Tamarindicusindica*) were availed from local fruit growers from Bauan, San Jose, Mataas na Kahoy and Libjo, Batangas. These fruits were selected according to their abundance in the province based from the Bureau of Agricultural Statistics [4]. The fruits were bought at ripening stage and waited for the right ripeness prior to the experiment. Selected fruits were washed and peeled and edible portion were cut to small slices. In some analysis, the samples were blended and extracted to the required procedure.

Preparation of Extract

About 45g of fruit samples were blended with 70% methanol and 30% water. The blended mixture was placed in a beaker and shaked for 30 minutes in a shaker. Then it was filtered using Whatman Filter Paper no.4 and placed in a rotary evaporator for 10 minutes.

Sample Preparation for Total Ascorbic Acid

100 g of fruit sample was blended with about 50 mL of distilled water using a food processor. After blending, it was strained through cheese cloth and washed with 10 mL portions of distilled water. Then the extracted fruit juice was completed up to 100 mL in a volumetric flask [7].

Proximate Analysis

All procedures for the determination of moisture, ash, crude protein, crude fat, total carbohydrates and crude fiber were based on AOAC (2011) [5].

Determination of Total Flavonoid Content (TFC)

The total flavonoid content was determined using Aluminum Chloride Colorimetric method and calculated as mg quercetin / L [8]. A standard calibration curve for the quercetin standard was made using different quercetin concentrations in methanol. Then, the fruit extract sample was prepared by mixing 0.5ml of fruit extract in methanol with 0.1 ml of 10% aluminum chloride ($AlCl_3$) , 0.1 ml of 1 M potassium acetate (KCH_3COO), 2.8 ml of distilled water and an additional 1.5 ml of methanol. After 30 minutes at room temperature, the absorbance of the sample was measured using UV Visible spectrophotometer at 415 nm [8].

Determination of Total Phenolics Content (TPC)

Total phenolics contents were determined by the Folin-Ciocalteau Colorimetric Method and expressed in mg gallic acid / L. A standard curve for the gallic acid was prepared using six different concentrations from 0 to 250 mg/ml. The fruit extract samples were mixed with 5ml of 10%Folin-Ciocalteau reagent and 4ml aqueous sodium carbonate (Na_2CO_3). After 15 minutes, the absorbance of the sample was read using spectrophotometer at 765 nm [8].

Determination of Total Ascorbic Acid (TAA)

A twenty ml aliquot of fruit juice sample was mixed with one hundred fifty (150) ml distilled water, 5 ml of 1 M hydrochloric acid, 5ml of 0.6 M potassium iodide and as indicator, 1 ml of starch solution. Then the sample was titrated with 0.002 M potassium iodate solution. The endpoint was identified as the permanent trace of blue-black color [7].

Determination of Beta-Carotene

Beta-Carotene of fruits was determined by Food and Nutrition Research Institute.

Free Radical Scavenging Activity (DPPH Method)

Free radical Scavenging Activity was determined using the DPPH method [9]. The blank standard used was of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution. It was prepared by mixing1 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with 3 ml of 95% methanol. Then the sample was prepared by mixing 3 ml of methanolic fruit extract with five different concentrations of DPPH solution ranging between 0.1 to 3.5µg /ml. Then after 1 hour of storage in the dark at room temperature, its absorbance was read at 517 nm using the methanol solution as reference. The remaining DPPH radical and antioxidant activity of fruit extract was calculated from the sample (As) and the blank (A0) using the equations [9]:

$$\%DPPHrem = (As / A0) \cdot 100$$
$$\%AA = 100 - \%DPPHrem$$

Statistical Analysis

The data gathered were computed, tabulated and reported as mean. The data were analyzedusing the analysis of variance (ANOVA) one way process and the significance was determined by Tukey's HSD Test ($p < 0.01$).

RESULTS AND DISCUSSION

Table 1. Proximate Analysis of Selected Fruits in Batangas

Proximate Analysis %	Banana	Chico	Papaya	Pomelo	Tamarind
Moisture	72.59	73.53	87.35	91.74	33.45
Ash	1.70	1.70	0.55	0.43	12.51
Crude Protein	1.62	0.77	1.47	1.04	4.68
Crude Fat	2.07	2.75	3.09	1.22	3.39
Total Carbo-hydrates	22.02	22.59	7.54	5.58	46.67
Crude Fiber	0.51	2.64	0.69	0.60	1.84

In Table 1, the proximate analysis of selected fruits was presented. Proximate analysis included the moisture content, ash content, crude protein content, crude fat content, total carbohydrates, and crude fiber content. These analyses are important for determination of food quality, microbial stability and can be used for nutritional labeling.

Based on the results of proximate analyses, pomelo had the highest moisture content but lowest ash content, crude fat content and total carbohydrates. This may indicate that pomelo was most perishable and with highest water activity decreasing keeping quality and stability compared to other fruit samples especially tamarind. Tamarind had the highest ash content, crude protein, crude fat and total carbohydrates however, got the lowest moisture content. Chico had the highest fiber content but had the lowest crude protein content. Banana had the lowest fiber content. The results of proximate analysis obtained can indicate that tamarind had the ability to give more of the nutritional requirements needed compared to other selected fruits.

The results for the moisture content of banana, chico, papaya, pomelo and tamarind were comparable to the data based on FNRI The Philippines Food Composition Tables. For the ash content, crude protein, crude fat and crude fiber of selected fruits, results obtained were higher compared to the Philippines Food Composition Tables while total carbohydrates of fruits were lesser in quantity.

Table 2. Antioxidant properties of Selected Fruits

Antioxidant Properties	TFC (mg Quercetin/L)	TPC (mg gallic acid / L)	TAA (mg/100cm^3)	Total Beta-Carotene Content, (µg/100g)
Banana	0.005	0.052	1018.60	19.00
Chico	0.003	0.045	203.75	30.00
Papaya	0.002	0.054	201.980	286.00
Pomelo	0.002	0.079	203.16	0
Tamarind	0.007	0.223	1024.41	31.00

Total Flavonoid Content, Total Phenolic Content, Total Ascorbic Acid (Vitamin C) and Beta-Carotene (Vitamin A) were some of the substances that are commonly found in fruits that can act as antioxidants.

Based on the analyses conducted, tamarind had the highest TFC, followed by banana then chico, and lastly by papaya and pomelo both having. The results of banana and papaya were comparable to the USDA Database for the flavonoid content of selected foods [10].

For the Total Phenolics Content of fruits, tamarind had the highest content, followed by pomelo, papaya then banana and lastly by chico with. The results were also comparable with the study of L. Fu et al [11].

The Total Ascorbic Acid (TAA) in fruits also shown in the Table 2. Based on the results, tamarind yielded the highest TAA among selected fruits while papaya had the lowest. This indicates that among the selected fruits tamarind and banana have the ability to give more of the vitamin C requirement compared with other fruits. Thus tamarind and banana were also more capable of regenerating Vitamin E based on the vitamin C content.

The Beta-Carotene of banana, chico, papaya, pomelo and tamarind as shown also in Table 2. Based on the results, papaya had the highest Beta-Carotene content while pomelo has no beta-carotene. Pomelo was not analyzed as recommended by the Food and Nutrition Research Institute (FNRI). Based on the results the color of the edible part of the fruit can be an initial indication of the amount of Beta-carotene, papaya having red-orange color had the highest Beta-Carotene and pomelo having a whitish color had negligible amount of Beta Carotene.

Antioxidant Activity Based on Free Radical Scavenging Activity

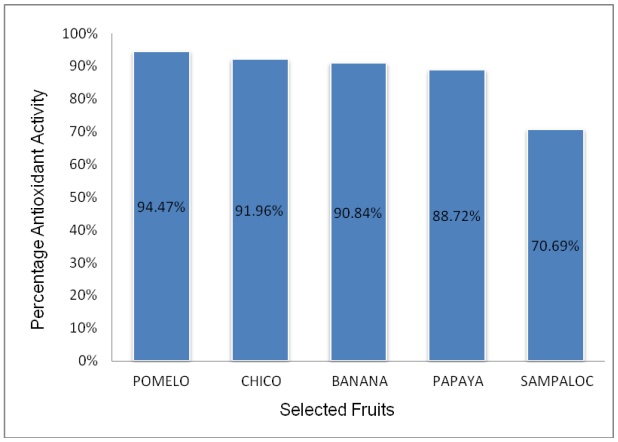

Fig. 1. Percentage antioxidant activity of banana (M. lacatan), chico (M. sapota), papaya (C. papaya), pomelo (C. grandis), and tamarind (T. indica).

Figure 1 shows the antioxidant activity of selected fruits based on the DPPH method. Antioxidant activity of fruits represents the strength of antioxidants present in fruits as well as the capability to trap free radicals. And among the fruit samples, pomelo had the highest strength of antioxidants followed by chico, banana, papaya and lastly by tamarind. This means that among the five selected fruits, pomelo can inhibit more free radicals compared to other fruit samples.

Table 3. Differences in the antioxidant activity and antioxidant properties among selected fruits

Source of Variance	F-Value	p-Value	Decision
Antioxidant Activity	643.9	0.00*	Reject Ho
% Flavonoid	81.61	0.00*	Reject Ho
% Phenolic	11469.92	0.00*	Reject Ho
Beta-Carotene	3327.27	0.00*	Reject Ho
Vitamin C	114502.00	0.00*	Reject Ho

**Significant*

Based on Table 3, the statistical treatment of the results of antioxidant activity of fruits, there was significant difference among the antioxidant activity of Banana (*M. lacatan*), Chico (*M. sapota*), Papaya (*C. papaya*), Pomelo (*C. maxima*) and (*T. indica*). This may indicate that each fruit had its own potential to reduce the free radicals and cannot be an alternative to each other.

For the antioxidant properties such as Total Flavonoid Content, Total Phenolic Content, Total Ascorbic Acid and Beta-Carotene of banana, chico, papaya, pomelo and tamarind there was also significant difference in their quantities per fruit.

CONCLUSION AND RECOMMENDATION

This study provided information that can be used for a baseline database of the some nutrient component of locally grown fruits in Batangas.

Based on the results, tamarind (*Tamarindusindica*) had the highest ash content, crude protein, crude fat and total carbohydrates however got the lowest moisture content while other fruits have fair results. Furthermore, Pomelo (*Citrus maxima*) had the highest moisture content while chico (*Manikerasapota*) had the highest crude fiber. Moreover, Papaya (*Carica papaya L.*) had the lowest total carbohydrates while banana (*Musa lacatan*) has the lowest crude fiber.

For the antioxidant properties of selected fruits, tamarind had the highest Total Flavonoid Content, Total Phenolics Content and Total Ascorbic Acid while on the other hand papaya had the highest Beta-Carotene which was obvious with the color of it edible part.

Antioxidant activity indicates the strength of antioxidant present in fruits. The selected fruits showed remarkable antioxidant acitivity. Pomelo had the highest percentage of antioxidant activity followed by chico, banana, papaya and lastly by tamarind.

Based on statistical treatment of data, there were significant differences on the antioxidant activity and antioxidant properties of selected fruits.

For further studies, it is recommended to determine the total carbohydrates of selected fruits by experimental method for more accurate results.

A better extraction procedure for determination of antioxidant properties of selected fruits can be used for more comparable results.

A parallel study using other locally grown fruits in Batangas as an additional to the baseline database is also recommended.

Also, determination of other antioxidant properties of the locally grown fruits in Batangas can be conducted for the database.

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