



Research Article

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Terefe T. Bezuneh

Arbaminch University, College of Natural Science, Department of Chemistry, Arbaminch, P.O. Box, 21, Ethiopia

Correspondence:

Terefe T. Bezuneh

Arbaminch University, College of Natural Science, Department of Chemistry, Arbaminch, P.O. Box, 21, Ethiopia

Spectrophotometric quantification of total polyphenols in commercially available fruits

Terefe T. Bezuneh*

Abstract

Fruit polyphenols are a group of plant bioactive compounds which can play significant role in preventing various health related problems. The main concern of the present investigation is also to quantify the total polyphenol (TP) content of some selected commercially available fruits and also to investigate the effect of solvent type on the extraction of polyphenols. The concentration of total polyphenols varies significantly with the solvent used and also among different samples at $P < 0.05$. Higher concentration was detected in papaya fruit (pulp) (135.20 ± 1.09 ; GAE, mg/g, dry weight) and lower concentration in banana (pulp) (26.60 ± 1.06 ; GAE, mg/g, dry weight) respectively.

Keywords: Extraction, Gallic acid, Total polyphenol.

INTRODUCTION

Fruits in the human diet are listed as the richest source of polyphenols a group of plant bioactive compounds which can play significant role in preventing various health related problems^[1]. Polyphenols are secondary plant metabolites characterized by the presence of one or more hydroxyl functional group attached to a single or to multiple aromatic rings^[2-6]. The antioxidant and metal chelating capacity of polyphenols are responsible for reducing the risk of oxidative damage caused by free radicals to the cell^[7, 8]. Free radicals are highly reactive species in which, it reacts with cell and cell components (carbohydrates, proteins, lipids, DNA and RNA) which lead to cell death and tissue damage^[9-11].

Accumulation of free radicals in the cell will result in oxidative stress which is an imbalance between the production of oxidative species (free radicals) and the protection system by antioxidants in the cell^[12, 13]. Oxidative stress results in propagation of the oxidative chain through lipid peroxidation which is responsible for the development of human diseases such as: cancer, cardiovascular disease, multiple sclerosis, autoimmune disease, Parkinson's disease, eye diseases, cellular aging, coronary heart disease, diabetes, mutagenesis and neurodegenerative infections^[14-16].

Polyphenols (antioxidants) reduce the oxidative stress in the cell by neutralizing free radicals and thus protecting cell components from potential damage^[7, 17, 18]. Antioxidants prevent the formation of oxidative stress by 1) chelating metal ions such as copper and iron ions in which it prevents the metal-catalyzed formation of reactive radical species, 2) their free radical scavenging properties enable them to minimize the concentration of free reactive radicals, 3) they inhibit enzymes that might activate formation of free radicals in the cell^[19].

Different in vivo and in vitro investigations supported the connection between the antioxidant nature of polyphenols and the reduction in the risk of cardiovascular disease (CVD)^[20-21], cancer^[21-23], osteoporosis, diabetes mellitus, neurodegenerative disease, oral diseases, atherosclerosis, aging and other degenerative diseases^[24-27].

The main objectives of the present study were to determine the total polyphenol composition of selected fruits. The solvents used to extract phenolic compounds from fruit sample matrixes for the present study were: 70 % acidified ethanol in water, 70 % acidified acetone in water, 70% acidified methanol in water and 100 % water solvents.

MATERIALS AND METHODS

Apparatus

All spectrophotometric measurements were made on a SP65 UV/Visible spectrophotometer (Gallenkamp, UK) using a 1.0 cm optical path length glass cell.

Reagents and Chemicals

Gallic acid (Riedel-de Haen), Ethanol (Avonchem, UK), Acetone (Essex, UK), Methanol (Merk, Brazil), Iron(III) chloride hexahydrate (Guangdong Guanghua, China), 1,10-phenanthroline hydrate (Fisher Scientific, UK), Ethylenediaminetetraacetic acid dihydrate (Avonchem, UK), Potassium Acetate (Park, UK), Glacial acetic acid (Avonchem, UK), Hydrochloric acid, are chemicals that were used in this study. All reagents and chemicals were of analytical grade and double distilled water was used to prepare solutions.

Procedure

Sample Collection and Preparation

The fresh fruit samples, Banana (*Musa acuminata*), Mango (*Mangifera indica* L.), Papaya (*Carica Papaya*), Avocado (*Persea Americana*), and Apple (*Malus domestica*) samples were collected randomly from a local market in Arbaminch town, Ethiopia. Randomly selected fruit samples were taken to the laboratory for analysis. Only fruit samples with no apparent physical or microbial damage were selected. The samples were washed to remove dirt particles on the surface of the samples and the peel and flesh of each fruit samples were separated manually. The flesh (pulp) of the fruit samples were then sliced into pieces using scalpel and oven-dried at 60 °C for two days. The oven-dried samples were ground to a powder using a mortar and pestle and then sieved using mesh sieve of 2mm diameter and stored in polyethylene bag until required for analysis.

Extraction

The efficiency of the extraction of polyphenols from different sample matrices depends greatly on the nature of the solvent used, extraction time, extraction temperature and solvent to sample ratio. This is because of the diverse nature of phenolic compounds. Different solvents have been used for this purpose where methanol, ethanol, acetone, and their combinations with different proportions of water have been used most frequently for the extraction of phenolics from plant materials [28-29].

For the present investigation, acidified 70% acetone in water (7:3, v/v), 70% acidified ethanol in water (7:3, v/v), 70% acidified methanol in water (7:3, v/v) and 100% water were used as solvent to extract phenolic compounds from the selected fruit samples. Extraction procedures have been performed by homogenizing 1g oven dried fruit sample in 45 mL of the desired solvent at 5 °C for 2 hours on a hot plate. The extract was filtered through Whatman No.41 filter paper. One milliliter of the filtrates were transferred to 25 mL volumetric flask and made up with double distilled water to the mark and stored at 5 °C until used for analysis of the total polyphenols.

Determination of Total Polyphenol Content

The procedure developed by Mônica *et al.* have been adopted for the quantification of total polyphenols in 5 different fruit samples with slight differences, namely the standard solution used was gallic acid instead of pyrogallol acid [30]. One milliliter (1 mL) of each sample extract was transferred to a different 25 mL volumetric flask containing 2.5 mL of 3.54 g/L Iron (III) chloride hexahydrate (FeCl₃·6H₂O) solution. The volumetric flask containing the sample solution was then placed in a water bath and maintained at 80°C for 20 min. After this, 2.5 mL of acetate buffer (CH₃COOH/CH₃COOK) solution (pH 4.6), 5.0 mL of 3.28 g/L 1,10-phenanthrolinehydrate (1,10-phen) and 2.5 mL of 3.72 g/L Ethylene diaminetetraacetic acid dihydrate (EDTA) solutions were added, respectively. Finally, each flask was filled to the mark with distilled water, cooled and then the absorbance measurements were made at 511 nm.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) and evaluated by one way analysis of variance (ANOVA) using Statistical Packages for the Social Sciences (SPSS) software. Significant level used was $p \leq 0.05$ for all data analyzed.

RESULTS AND DISCUSSION

In this study, 5 different fruits were investigated for their total phenolic compounds (TP). The results obtained for the total polyphenol quantification in pulp of different fruit samples were presented in Figure 1. As it has been reported in different investigations selection of solvent has significant effect on the extraction of polyphenols from different sample matrixes. In the present investigation the extraction ability of each solvent used for the extraction purpose vary significantly at $P \leq 0.05$. Among the test solvent used for the present investigation acetone showed the maximum extraction efficiency. Generally, the efficiency of the solvent in total phenolic extraction can be ordered as: Acetone > Water > Ethanol > Methanol. In contrast to the present result, Koffi *et al.* compared the efficiency of acetone, water, ethanol and methanol solvents and TP in ethanolic extracts where higher than that of aqueous, acetic and methanolic extract [4, 31].

Figure 1 shows that, the concentration of total phenolic content (TPC) in the analyzed Papaya, Apple, Avocado, Banana and Mango pulp varied significantly from 135.2 to 39.10; 131.50 to 44.70; 76.40 to 31.20; 94.40 to 26.60 and 65.90 to 26.80 GAE (mg/g, dry weight), respectively. As it can be seen from the result different solvent has different extraction efficiency in which the concentration of total polyphenol also varies significantly with change in solvent. Generally, higher amount of total polyphenol was recorded in papaya 135.2±1.09 GAE (mg/g, dry weight), whereas the lowest values were found in banana 26.6±1.06 GAE (mg/g, dry weight). The concentration of total polyphenol in the fruits analyzed in the present investigation can be ordered as; Papaya 135.2 ± 1.09, followed by Apple, 131.50 ± 1.07, Banana 94.40 ± 0.09 Avocado 76.40 ± 1.07 and Mango 65.90 ± 1.00 GAE (mg/g, dry weight).

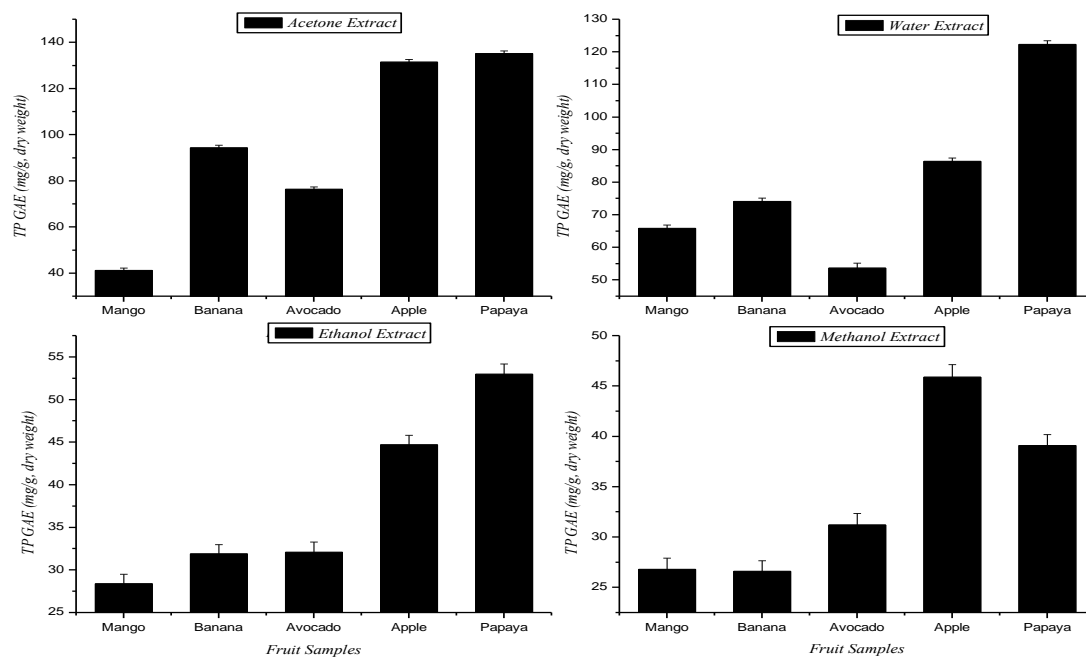


Figure 1: Concentration of total polyphenols expressed as GAE (mg/g, dry weight) in pulp of fruit samples extracted in the solvent indicated in the legend.

CONCLUSION

Fruits can be listed as a principal source of polyphenols, a group of plant-based bioactive compounds. Various *in vitro* and *in vivo* investigations revealed the significant importance of polyphenols in preventing various human degenerative diseases. The present study investigated the composition of phenolic compounds in the pulp of selected fruits. This study showed that a higher concentration of total polyphenol is found in papaya fruit compared to that of apple, banana, avocado and mango. Among the solvent used acetone is the most efficient solvent for the extraction of phenolic compounds from the selected fruits.

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