

UV - Visible Spectrophotometric Quantification of Total Polyphenol in Selected Fruits

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To cite this article:

Terefe Tafese Bezuneh, Eyob Mulugeta Kebede. UV - Visible Spectrophotometric Quantification of Total Polyphenol in Selected Fruits.

International Journal of Nutrition and Food Sciences. Vol. 4, No. 3, 2015, pp. 397-401. doi: 10.11648/j.ijfnfs.20150403.28

Abstract: Fruits are known as a richest source of bioactive compounds as polyphenols which are known to have significant health promoting properties. The present study investigates the total polyphenol content of some selected fruits extracted in: acidified 70 % ethanol, acidified 70 % methanol, acidified 70 % acetone, and 100 % water solvents. Standard gallic acid solution prepared in the range of 50-500 µg/L was used to plot a calibration graph. A good linear calibration graph ($r=0.998$, $n=3$) was obtained by plotting absorbance at 511 nm versus standard solution and all results are given as gallic acid equivalent (GAE, mg/g, dry weight). The concentration of total polyphenols varies with the solvent used and also among different samples. Higher concentration was detected in papaya fruit both in the peel and pulp (238.6 ± 3.64 , 135.2 ± 0.09 ; GAE, mg/g, dry weight) respectively and lower concentration in banana peel and pulp (43.2 ± 0.13 , 26.6 ± 0.06 ; GAE, mg/g, dry weight) respectively.

Keywords: Gallic Acid, Antioxidant, Total-Polyphenol

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) may be produced in the cell during normal aerobic metabolism in living organisms. The ROS and RNS are oxygen and nitrogen based radicals as: superoxide anion ($O_2^{\cdot-}$), alkoxyl (RO^{\cdot}), peroxy (ROO^{\cdot}), hydroxyl radical (OH^{\cdot}), peroxides (H_2O_2), peroxy nitrite ($ONOO^{\cdot}$), and nitric oxide radicals (NO) [1, 2].

The production of reactive radicals ROS and RNS in the animal cell can alter the normal function of the cell. Their highly reactive nature leads them causing much damage to the cell and cell components as: carbohydrates, proteins, lipids, DNA and RNA, which lead to cell death and tissue damage [3-5].

Accumulation of reactive radicals (ROS and RNS) in the cell results in imbalance between the production of oxidative species and the protection system by antioxidants in the cell [6]. Oxidative stress is an imbalance state between production of free radicals and that of the antioxidant defense capacity of the cell [7].

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 [8-10].

Antioxidants reduce the oxidative stress in the cell by neutralizing free radicals. They neutralize radicals by donating an electron or hydrogen to the free radicals, thus protecting cell components from potential damage [11, 12, 44].

Antioxidants prevent the formation of oxidative stress through different mechanisms. The first one is that, antioxidants are known to chelate metal ions such as copper and iron ions. In doing so, they prevent the metal-catalyzed formation of reactive radical species (ROS, RNS). Secondly, their free radical scavenging properties enable them to minimize the concentration of free reactive radicals. Thirdly, they inhibit enzymes that might activate formation of free radicals in the cell [13].

Fruits in the human diet contain bioactive compounds which are attributing these fruits for preventing various health related problems [14, 15]. Fruits are listed as the richest source of polyphenols, a class of plant-based bioactive molecules [16, 17]. 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 [18,19].

The chemical structure of polyphenols varies from that of a simple phenolic molecule, such as phenolic acid and phenolic alcohols with only one phenol ring to that of a complex high-molecular mass polymer [20,18]. Polyphenols can be subdivided into several classes based on their chemical structure. They are mainly classified as: Flavonoids, quinones, tannins, lignins, coumarins, anthocyanins, phenolic acids, phenolic alcohols, stilbenes and lignans [21,43]. Flavonoids are widely distributed in plant tissues and are responsible for the colour of some plants and plant bodies. They are classified as: flavonols, flavones, flavanones, isoflavones, anthocyanidins and flavanols (catechins and proanthocyanidins) [22-24].

Different factors including: degree of ripeness [25], variety [26], storage conditions [27], soil composition, geographic location and climate are factors responsible for the variation in the concentration of phenolic compounds in fruits. This variation in concentration of phenolic compounds attributes fruits with their difference in colour, taste and flavour [20].

The antioxidant and metal chelating capacity of polyphenols are responsible for reducing the risk of oxidative damage to the cell [11,42]. This property makes polyphenols a protective agent against so many degenerative and infectious diseases. 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) [28, 29], cancer [30,31,45], osteoporosis, diabetes mellitus, neurodegenerative disease, oral diseases, atherosclerosis, aging and other degenerative diseases [32-37].

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 matrices for the present study were: 70 % acidified ethanol in water, 70 % acidified acetone in water, 70% acidified methanol in water and 100 % water solvents.

2. Materials and Methods

2.1. Apparatus

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

2.2. 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), Ethylene diaminetetraacetic 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.

2.3. Procedure

2.3.1. 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. These peel and flesh samples were then sliced separately 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.

2.3.2. 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 [45-47].

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 total polyphenolic 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 phenolics.

2.3.3. 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 [38].

One milliliter (1 mL) of each sample extract was transferred to a different 25mL volumetric flask containing 2.5 mL of 3.54 g·L⁻¹ 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 1,10-phenanthroline hydrate (1,10-phen) and 2.5 mL of 3.72 g L⁻¹ 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.

2.3.4. Statistical Analysis

Data were expressed as mean \pm 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 $p < 0.05$ for all data analyzed.

Table 1. Total polyphenol content of fruits expressed as mg GAE/g, dry weight ($n=3$).

Fruits tissue	Fruits	Total polyphenol content (mg GAE/g, dry weight)			
		Extraction Solvents			
		Ethanol	Methanol	Acetone	Water
Pulp	Mango (<i>Mangifera indica</i> L.)	28.40 \pm 0.12 ^a	26.80 \pm 0.12 ^a	41.20 \pm 0.08 ^a	65.90 \pm 0.00 ^a
	Banana (<i>Musa acuminata</i>)	31.90 \pm 0.07 ^a	26.60 \pm 0.06 ^a	94.40 \pm 0.09 ^a	74.10 \pm 0.05 ^a
	Avocado (<i>Persea Americana</i>)	32.10 \pm 0.17 ^a	31.20 \pm 0.14 ^a	76.40 \pm 0.07 ^a	53.70 \pm 0.44 ^a
	Apple (<i>Malus Domestica</i>)	44.70 \pm 0.13 ^a	45.90 \pm 0.24 ^a	131.50 \pm 0.07 ^a	86.40 \pm 1.03 ^a
	Papaya (<i>Carica Papaya</i>)	53.00 \pm 0.18 ^a	39.10 \pm 0.08 ^a	135.20 \pm 0.09 ^a	122.30 \pm 0.13 ^a
	Mango (<i>Mangifera indica</i> L.)	150.20 \pm 0.67 ^a	122.90 \pm 0.08 ^a	183.3 \pm 1.23 ^a	90.70 \pm 0.11 ^a
Peel	Banana (<i>Musa acuminata</i>)	77.70 \pm 0.09 ^a	74.80 \pm 1.67 ^a	76.00 \pm 0.08 ^a	43.20 \pm 0.13 ^a
	Avocado (<i>Persea Americana</i>)	188.80 \pm 0.12 ^a	192.10 \pm 0.07 ^a	188.80 \pm 0.12 ^a	157.1 \pm 0.07 ^a
	Apple (<i>Malus Domestica</i>)	109.00 \pm 0.07 ^a	87.70 \pm 0.15 ^a	92.10 \pm 0.08 ^a	84.80 \pm 0.20 ^a
	Papaya (<i>Carica Papaya</i>)	238.63 \pm 3.64 ^a	204.20 \pm 0.07 ^a	227.90 \pm 0.08 ^a	147.60 \pm 0.13 ^a

All data are presented as mean \pm SD of the three replicates ($n=3$). Similar superscript alphabets within the same column indicates significant different ($p < 0.05$)

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 $P < 0.05$.

As can be seen from the results, higher total polyphenol in pulp was found in papaya 135.2 \pm 0.09 GAE (mg/g, dry weight), whereas the lowest values were found in banana 26.6 \pm 0.06 GAE (mg/g, dry weight). In peel, the highest total polyphenol content was found in papaya 238.6 \pm 3.64 GAE (mg/g, dry weight), whereas the lowest values were found in banana 43.2 \pm 0.13 GAE (mg/g, dry weight). Hence, both in pulp and peel samples a higher concentration of total polyphenols was recorded in papaya samples and lower concentration in banana samples. Significant amounts of phenolic compounds were extracted in water as the solvent both in the pulp and the peel of fruit samples.

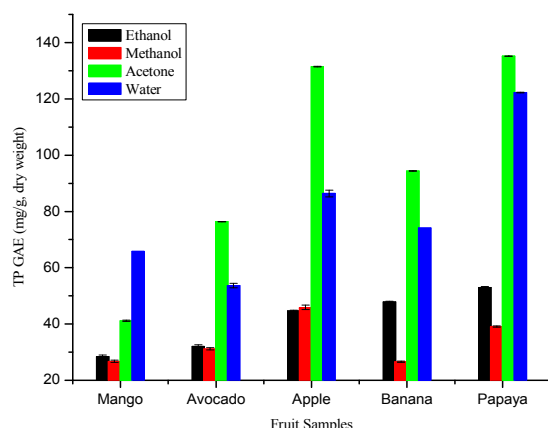


Figure 1. Concentration of total polyphenols expressed as GAE (mg/g, dry weight) in pulp of fruit samples extracted in the solvent indicated in the legend.

3. 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 and peel of different fruit samples were presented in Table 1.

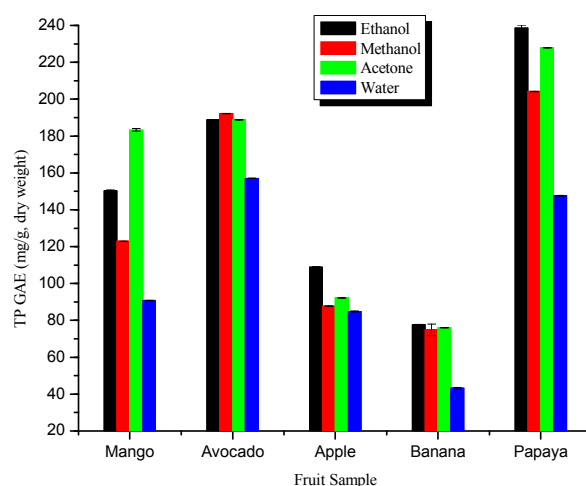


Figure 2. Concentration of total polyphenols expressed as GAE (mg/g, dry weight) in peel of fruit samples extracted in the solvent indicated in the legend.

Previous reports have revealed the presence of higher concentrations of total polyphenols in peel of fruit compared to that of the pulp. A study by Hana *et al.*, reported that mango peel exhibited a higher total polyphenol content compared to the pulp [39]. Carolina *et al.* has also reported a higher total polyphenol concentration in the peel of apple than the pulp [34]. The same was reported by Fatemeh *et al.* for banana fruit. In that report, the total polyphenol concentration in banana peel was almost 2-fold higher than in the pulp [41].

Hence, the results we obtained are in accordance with these previous reports, namely that higher concentrations of total polyphenols have been recorded in the peel of the fruit compared to other edible parts of the fruit. The total polyphenol content of fruits in peel varied significantly ($P < 0.05$) from that of the pulp. This was true for all fruit samples selected for this investigation, regardless of the solvent used.

4. 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 peel and pulp of selected fruits. This study showed that a higher concentration of total polyphenol was found in the fruit peel than in the pulp of the fruits included in the study.

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