

Quantitative Analysis of Total Phenolic Content in Avocado (*Persia Americana*) Seeds in Eastern Province of Kenya

P.Githinji^{1*}, L. Gitu¹, E. Marete², M. Githua², M. Mugo², and E. N.Mbaka³

1. Jomo Kenyatta University of Agriculture and Technology, P.O Box 62000-00200, Nairobi, Kenya.

2. Meru University of Science and Technology, P.O Box 972-60200, Meru, Kenya.

3. Kenya Methodist University, P.O Box 267-60200, Meru, Kenya.

* Email of the corresponding author: githinji12@gmail.com

Abstract

Phytochemical rich plants have played a significant role in diet based therapies to prevent and cure various ailments. The avocado (*Persia Americana* Mill,) fruits are much sought after for their high nutritional and sensory value. Avocado (*Persia Americana*) seeds were analysed for total phenolic content. This phenolic component is responsible for antioxidant activity. The amount of phenols was analysed using Folin-Ciocalteu method. The maximum phenolic content was found in the Fuerte seed extract (18.55 ± 2.8 mg/g) prepared at 50°C. The phenolic content decreased by 10.3% at an extraction temperature of 50 °C to 70 °C and 32.1% at an extraction temperature of 50 °C to 100 °C for a duration of 30 minutes.

Keywords: Avocado seeds, *Persia Americana*, Total phenolics

Introduction

The fruit of *Persea americana* Mill of family Lauraceae is eaten in many parts of the world. In recent years, research has focused on various parts of the plants. The fruit in particular has been shown to possess various medicinal properties. The edible fruit pulp contains up to 33% oil rich in monounsaturated fatty acids (Ortiz *et al.*, 2004) that are believed to modify the fatty acid contents in cardiac and renal membranes and enhance the absorption of α/β carotene and lutein (Salazar *et al.*, 2005). The carotenoid content has been reported to play significant role in cancer risk reduction (Lu *et al.*, 2005). Other properties of the oil include wound healing (Nayak *et al.*, 2008) and hepatoprotection (Kawagishi *et al.*, 2001).

Phytochemical screening of the leaf extract of *P. americana* revealed the presence of flavonoids which were powerful antioxidants capable of scavenging free radicals (Owolabi *et al.*, 2007) by donating a hydrogen atom or electron to stabilize the radical species (The figure 1 shown below is a standard curve for Gallic acid. The metabolic study of the aqueous leaf extract of *P. americana* in rat model showed the presence of phenolic acids which were metabolites of flavonol degradation by intestinal microflora (Havsteen *et al.*, 2002).

Extracts from the epicarp of the immature avocado fruit have demonstrated to have both antifungal and antibacterial properties. The seed of the immature fruit was also found to have antibacterial properties (Jacob *et al.*, 1971). The antifungal properties of the immature avocado were established to be due to the idioblast oil cells, which are made up of alkaloids, sesquiterpene hydro peroxides, other terpenes (Roginsky *et al.* 2005) persin, and a group of 2-alkylfuran (Rodriguez-Saona *et al.*, 1998) Tannins, catechin flavones, and polyphenolic compounds are often found in the tissues and seed of the avocado fruit. These chemicals are all antimicrobial in nature and could have contributed to the antibacterial activity of the immature fruit (Jacob *et al.* 1971). The objective of the study was to optimize the extraction for maximum active ingredient and minimum interfering content.

Material and methods

Sampling and sample preparation

Sampling was done in Meru region where five variety of avocado fruits were collected from various avocado cultivars. They include hass, fuerte, pinto, grafted and local varieties. The fruits were deseeded and the seeds cut into small pieces and air-dried at ambient temperature. The dried seeds were then pulverized into powder using a Warring blender.

EXTRACTION

Preparation of extracts

A sample of 3g of powdered avocado seeds was extracted with 100 mL of distilled water in a conical flask. The conical flask was covered with aluminium foil to prevent light exposure. The samples were heated at various temperatures of 50 °C, 70 °C and 100 °C using a stirring hot plate. After the extraction, the extracts were then cooled in ice and then filtered under vacuum. The filtrates were filled in storage containers and stored at about -18°C before analysis. The filtrate was subsequently used for the determination of total phenolic content (TPC).

Determination of the Total Phenolic Content

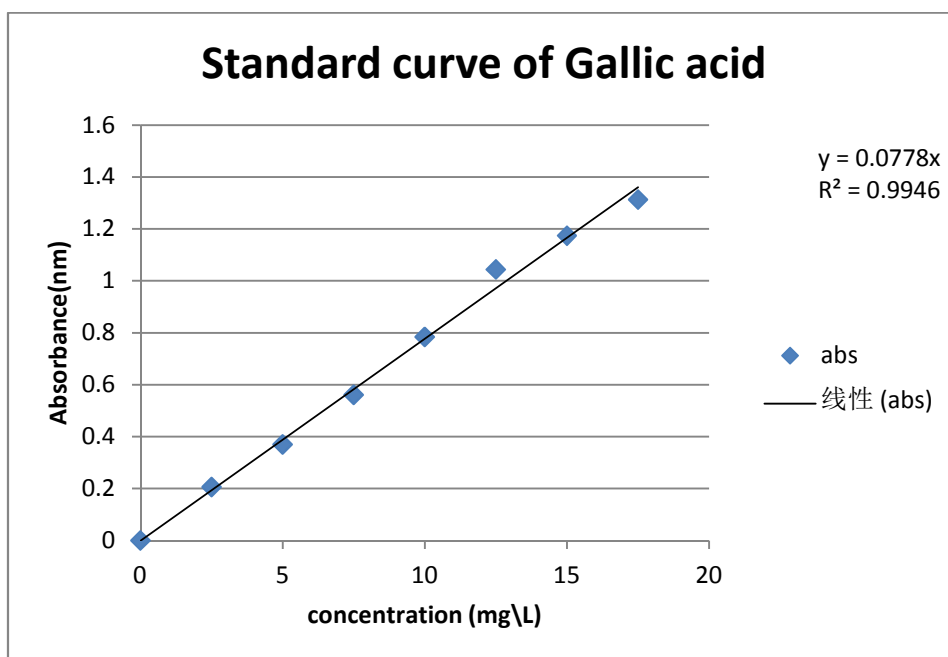
The TPC of the extracts was determined spectrophotometrically using the Folin-Ciocalteu method. (Singleton and Rossi, 1965). Gallic acid standard solutions were prepared at 0.0, 2.5, 5.0, 7.5 10.0, 12.5, 15 and 17.5 mg/ml.

The extracts/standards (0.2 ml) and the gallic acid standards was mixed with 0.5 ml Folin-Ciocalteu reagent, 1.5 ml of 20% sodium carbonate and 7.8 ml of distilled water and allowed to stand for 2 hours after which the absorbance was read at 760 nm. The concentration of total phenolic compounds in the extracts was determined by comparing the absorbance of the extract samples to that of the gallic acid standard solutions. All samples were run in triplicate. The total phenolic content of the extract was then calculated as mg of Gallic acid equivalents (GAE) /g of dry weight of the avocado seed powder.

Statistical analysis

The experimental results in single factor experiments were analyzed using Microsoft excel. All data were expressed as means ± standard deviations of triplicate measurement.

Figure 1: Standard curve of Gallic acid



$Y=0.07734+0.00529$
 $R^2=0.99186$

Figure 2 : Total Phenolic Content of Avocado (*Persia americana*) Seeds Extracted at 50

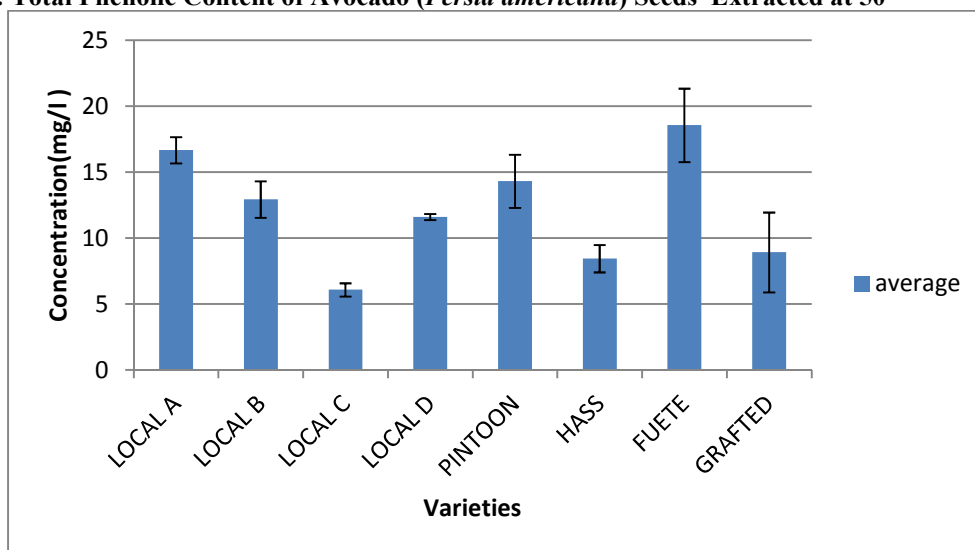


FIG 2: Total Phenolic Content of Avocado (*Persia Americana*) Seeds Extracted at 70 °c

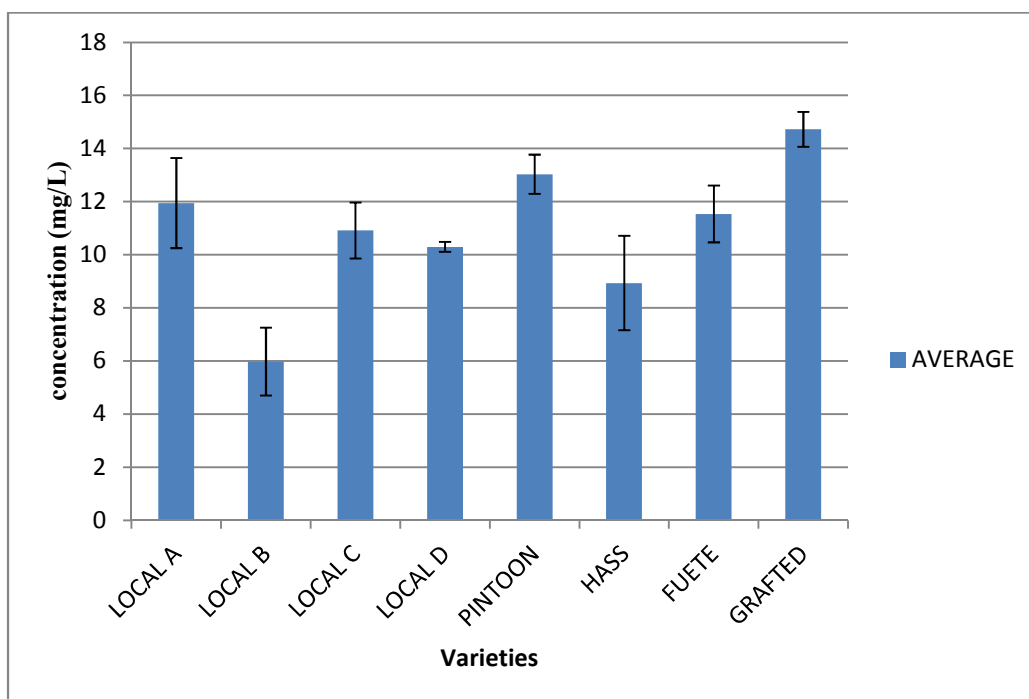
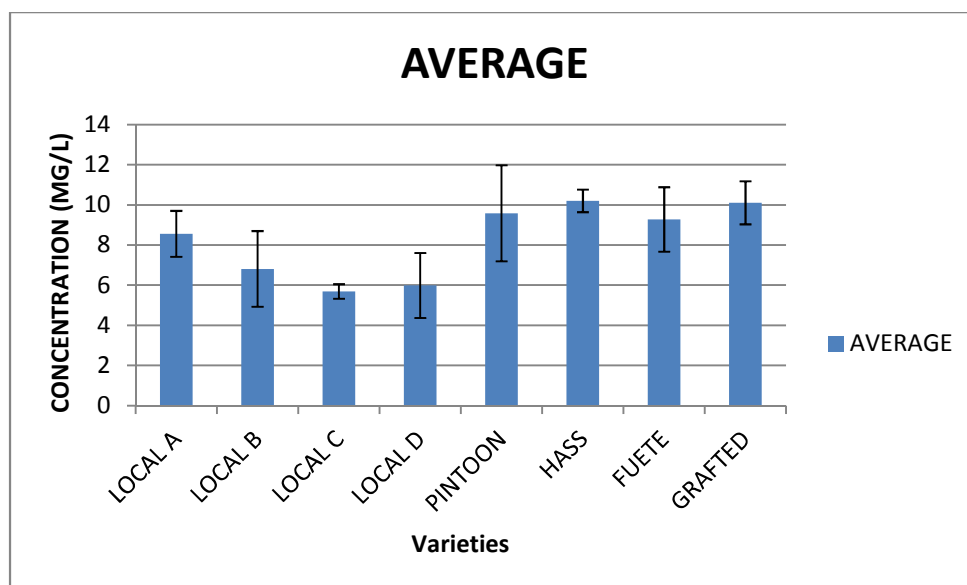


Figure 3: Total Phenolic Content of Avocado (*Persia Americana*) Seeds Extracted at 100 °c



Each value in the graph was obtained by calculating the average of three experiments \pm standard deviation

Results and Discussion

Phenolic compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates, which prevent various food ingredients from oxidation (Cuvelier *at al* a 1996). A calibration curve of gallic acid was constructed to measure the amount of phenolic compounds in the avocado seeds. The weight. Table.1 shows the variation of mean absorbance with concentration of Gallic acid. Figure 2 and Figure 3 shows the contents of total phenols in avocado seeds samples extracted at 50° C, 70° C and 100° C respectively that were measured using Folin Ciocalteu reagent in terms of gallic acid equivalent. The total phenol varied from 5.63 ± 2.1 to 10.4 ± 0.14 mg/L in the extracts at 100° C. The maximum phenolic content was found in the extract. 18.55 ± 2.8 mg/g) of Fuede Seeds extracted at 50° C. In general, increasing the temperature beyond certain values may encourage possible concurrent decomposition of

phenolic compounds which were already mobilized at lower temperature or even the breakdown of phenolic that have still remained in the seed matrix. It can be seen from the shown in Fig 2 and Fig 3 that TPC decreased with increasing temperature from 50-100 °C. In the case of the treatment interaction of temperature, the total phenolic decreased by 10.3% when going from 50 °C to 70 °C and 32.1% when going from 50 °C to 100 °C at an extraction time of 30 min (Fig. 1, 2 and 3). Degradation of some of the thermo labile phenolic compounds may have occurred after the optimum extraction temperature was reached, thereby leading to a lower concentration of phenolic compounds. Therefore, moderate extraction temperature of 50 °C, 70 °C and 100 °C were chosen as the upper, middle and lower levels, respectively, to be applied in extraction procedure optimization. An increase of about 3.4% in TPC occurred for hass sample when temperature increased from 50 °C to 100 °C. Increased temperature may breakdown or increase hydrolysis of the bond of some bound phenolic compound and cause them become extractable phenolic compounds.

Conclusion

The maximum phenolic content was found in the Fuerte extract (18.45 ± 2.78 mg/g). The result of the present study showed that the extract of Fuerte, which contain highest amount of phenolic, compounds exhibited the greatest. The high scavenging property of Fuete may be due to hydroxyl groups existing in the phenolic compounds. The data suggest that 50°C may be the more suitable temperature for the extraction of phenolic compounds.

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