

QUANTITATIVE ANALYSIS OF TOTAL PHENOLIC CONTENT IN AVOCADO (*PERSIA AMERICANA*) SEEDS IN EASTERN PROVINCE OF KENYA

P. Githinji¹, L. Gitu¹, E. Marete², M. Githua², M. Mugo², and E. N. Mbaka³

¹Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

²Meru University of Science and Technology, Meru, Kenya

³Kenya Methodist University, Meru, Kenya

E-mail: 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. In addition to the nutritional value of its fruit, the seeds and other parts of the plant have been used widely as medicinal agents to treat various ailments this being one of the initial points for conducting a thorough phytochemical investigation on avocado seeds with the focus on analysis of extractable natural products in respect to their potential use for pharmaceutical and food applications. The aim of this study was to conduct a phytochemical analysis on avocado seed extract. The avocado seeds were dried using various methods in order to determine the optimum drying method, which will maximize the active ingredients. Extraction of the bioactive constituents was then optimized to minimize the concentration of the undesirable constituents like the tannins. Various cultivars of avocado planted in Kenya were analyzed for their levels of bioactive constituents (quercetin and apigenin). Drying at higher temperature shortened the drying time and increased the drying rate. The total phenolic content (TPC) in the extract was not significantly affected by the oven temperatures. It was observed that there was a significant variation in phenol concentration among drying methods ($p < 0.001$) and varieties ($p < 0.001$). However the variation among methods of extractions was not significant ($p = 0.303$). The interactions among them were not significant.

Key words: Avocado seeds, *Persia Americana*, Total phenolics

1.0 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 (Figure 1) . The metabolic study of the aqueous leaf extract of *P. americana* in rat model showed the presence of phenolic acids (Owolabi *et al.*, 2007) which were metabolites of flavonol degradation by intestinal microflora (Havstee *et al.*, 2002).

Extracts from the epicarp of the immature avocado fruit have demonstrated to have both antifungal and antibacterial properties (Jacob *et al.*, 1971). 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 (Platt & Thomson, *et al.* 1992), persin, and a group of 2-alkylfurans (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.

2.0 Material and Methods

2.1 Sampling and Sample Preparation

An ethno medicinal survey was conducted to determine the medicinal use of the avocado plant parts in the Eastern Province of Kenya. Five variety of avocado fruits were collected from various avocado cultivars.. They include hass, fuerte, pinto, grafted and local. 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.

2.2 Extraction

2.2.1 Preparation of Extracts

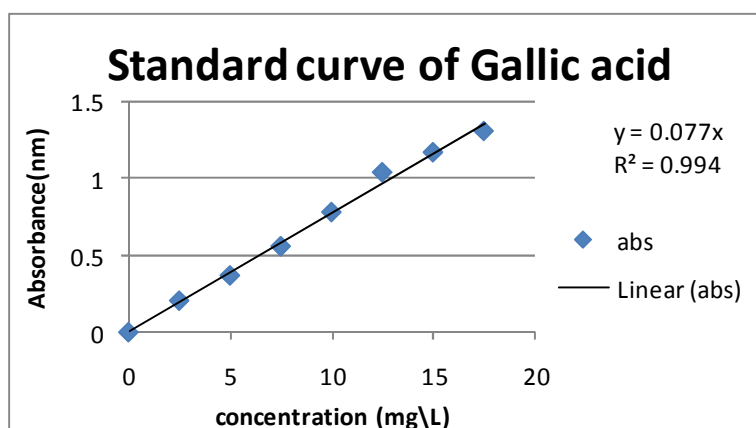
A sample of 3g of powdered avocado seeds was extracted with 100 mL of distilled water in a conical flask. The conical flask was covered with aluminium foil to prevent light exposure. The samples were heated at various temperatures of 50 °C, 70 °C and 100 °C using a stirring hot plate. After the extraction, 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).

2.2.2 Determination of the Total Phenolic Content

The TPC of the extracts was determined spectrophotometrically using the Folin-Ciocalteu method (reference). 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.

2.3 Statistical Analysis

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



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

Figure 1: Standard curve of gallic acid

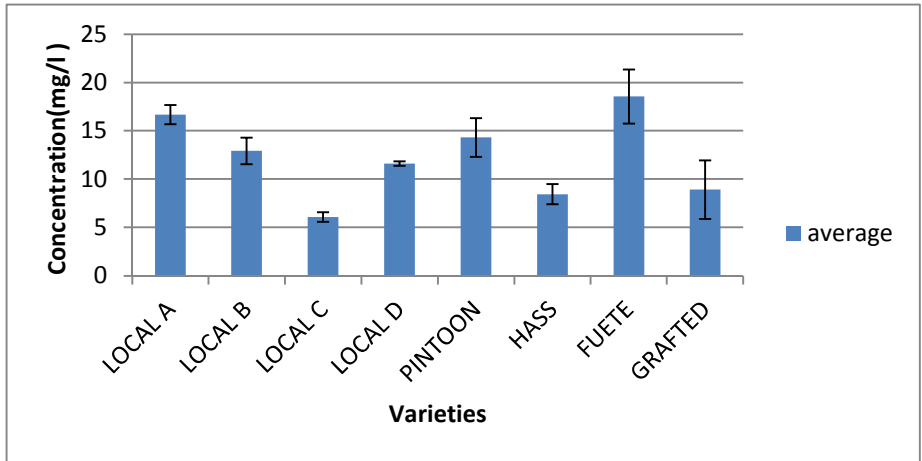


Figure 2: Total Phenolic Content of Avocado (*Persia americana*) seeds extracted at 50 °C

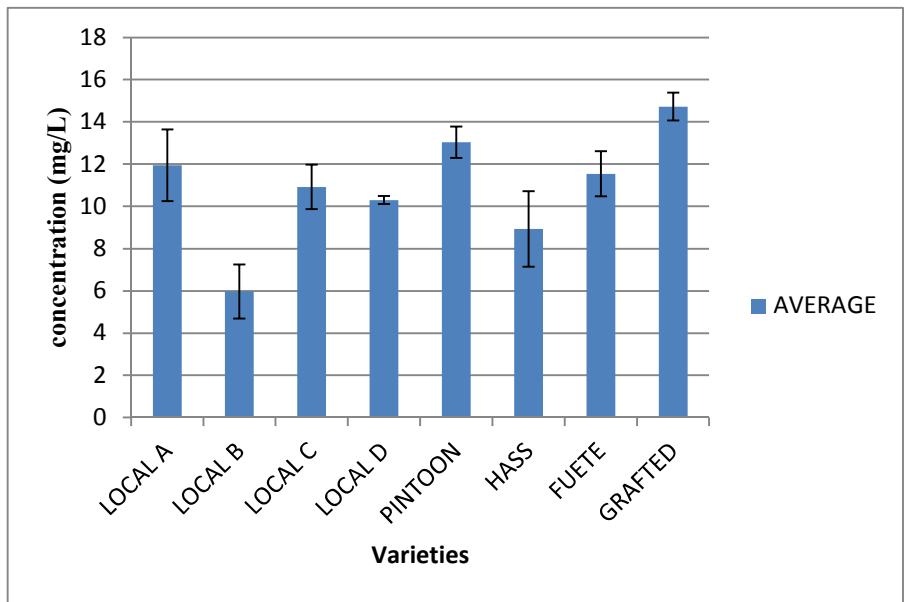


Figure 3: Total phenolic content of avocado (*Persia Americana*) seeds extracted at 70 °C

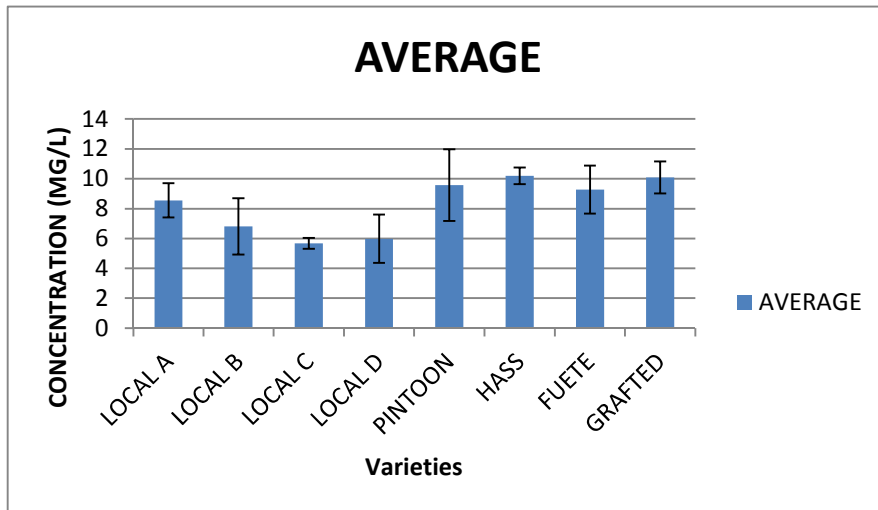


Figure 4: Total phenolic content of avocado (*Persia Americana*) seeds extracted at 100 °C

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

3.0 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 calibration equation for gallic acid was $y = 0.4623x + 0.00392$ ($R^2 = 0.989$). All the results in this study were computed from the above calibration curve and expressed as gallic acid equivalent (GAE) in mg per 100 g dry 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 by Folin Ciocalteu reagent in terms of gallic acid equivalent. The total phenol varied from 5.63 ± 2.1 to 10.4 ± 0.14 mg/g in the extracts at 100 °C.

The maximum phenolic content was found in the extract. 18.546 ± 2.7854 mg/g) of Fuete 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 are still remained in the seed matrix. It can be seen from the response surface shown in Figure 2 and Figure 3 that TPC decreased with increasing temperature from 50-100 °C. In the case of the treatment interaction of temperature, TPC of avocado Seeds the extracts 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 (Figures 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.

A smaller 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.

4.0 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 antioxidant activity. The high scavenging property of Fuete may be due to hydroxyl groups existing in the

phenolic compounds. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The data suggest that 50°C may be the more suitable temperature for the extraction of phenolic compounds.

Acknowledgements

The authors are grateful to National Council of Science And Technology funding the project through Meru University.

References

- Havsteen, B. H. (2002). The biochemistry and medical significance of the flavonoids. *Pharmacol. Ther.*, **96**, pp 67-202.
- Kawagishi, H., Fukumoto, Y., Hatakeyama, M., He, P. and Arimoto, H. (2001). Liver injury suppressing compounds from avocado (*Persea americana*). *Journal Agric Food Chem*; **49**, pp 221-521.
- Larrey, D. (1994). Liver involvement in the course of phytotherapy. *Presse Med.*, **23**, pp691-693.
- Lu, Q. Y., Arteaga, J. R., Zhang, Q., Huerta, S., Go, V. L. and Heber, D. (2005). Inhibition of prostate cancer cell growth by an avocado extract: role of lipid- soluble bioactive substances. *J. Nutr. Biochem.*, **16**, pp 23-30.
- Nayak, B. S., Raju, S. S. and Chalapathi Rao, A. V. (2008). Wound healing activity of *Persea americana* (avocado) fruit: a preclinical study on rats. *J. Wound Care*, **17**, pp 123-126.
- Ojewole, J. A., and Amabeoku, G. J. (2006). Anticonvulsant effect of *Persea americana* Mill (Lauraceae) (Avocado) leaf aqueous extract in mice. *Phytother Res.*, **20**, pp 696-700.
- Ortiz, M. A., Dorantes, A. L., Gallindez, M. J. and Cardenas, S. E. (2004). Effect of a novel oil extraction method on avocado (*Persea americana* Mill) pulp microstructure. *Plant Foods Hum. Nutr.*, **59**, pp 11-14.
- Olaeta, J. A., Schwartz, M., Undurraga, P. and Contreas, S. (2007). Use of Hass avocado (*Persea americana* Mill) seed as a processed product. Proceedings of the VI World Avocado Congress, pp 1-8.
- Salazar, M. J., El Hafidi, M., Pastelin, G., Ramírez-Ortega, M. C. and Sánchez-Mendoza, M. A. (2005). Effect of an avocado oil-rich diet over an angiotensin II-induced blood pressure response. *J. Ethnopharmacol.*, **98**, pp 335-338.