Scholars Academic Journal of Biosciences (SAJB)
Abbreviated Key Title: Sch. Acad. J. Biosci.
ISSN 2347-9515 (Print)
ISSN 2321-6883 (Online)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Medicine

Antioxidant Capacity of Oils of Avocado Varieties from Cameroon and Hawaii

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Original Research Article

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Article History
Received: 28.03.2018
Accepted: 05.04.2018
Published:30.04.2018

DOI:
10.36347/sajb.2018.v06i04.006

Abstract: Avocados are medicinal foods rich in monounsaturated fatty acids which can exert many cardiovascular effects. The phytochemical content of each variety is influenced by its natural habitat. The present study compared the antioxidant potential of the oil of avocado varieties grown in Cameroon and Hawaii. Ferric reducing antioxidant power (FRAP), Total phenolic content (TPC), DPPH and ABTS radical scavenging activities characterized the antioxidant potential. The oil obtained from Serpa (Hawaiian) had the highest TPC followed by Pollock (Cameroonian) oil while those with the least TPC were Nishikawa (Hawaiian) and Fuer Florida (Cameroonian). Serpa equally topped the FRAP followed by Peterson (Cameroonian). Similarly Serpa and Peterson oil samples topped the list for DPPH and ABTS radical scavenging activities with lowest IC_{50} values. Overall, the Hawaiian avocado was richer in antioxidant capacity compared to the Cameroonian species.
Keywords: Antioxidant Capacity, Avocado, Total phenolic content.

INTRODUCTION

Persea americana Mill. Commonly known as avocado is a plant native of Central America belonging to the Lauraceae family. Avocado has traditional use as food and medicine and hence can be classified as medicinal food. Avocado is a highly caloric fruit rich in vitamins, minerals, folates, potassium, and fiber, with a unique lipid composition [1]. As a medicinal food the oil of avocado is rich in monounsaturated fatty acids (especially oleic and palmitoleic acids), and is low in saturated fats compared with other vegetable oils [2].

Consumption of oleic and palmitoleic acid has an inverse relationship with cardiovascular disease because they preserve the level of high-density lipoproteins and possess antioxidant behavior [3, 4]. Avocado is also rich in β-sitosterol, shown in clinical trials to reduce blood levels of low-density cholesterol by blocking cholesterol absorption in the intestine [5]. Avocado fruits are also rich in lipophilic bioactive components such as vitamin E, carotenoids, and sterols, which possess antioxidant and radical scavenging activities [6]. Hypotensive, hypoglycemic anti-viral, and treatment of ulcers and cardiovascular diseases have been attributed to avocado fruits [7-11]. Also attributed to avocado are the analgesic and anti-inflammatory properties [12]; and various dermatological formulations namely, emulsions for the treatment of dry skin, protective agents against ultraviolet radiation, and anti-aging agents [13].

Factors that affect the antioxidant capacity of fruit include cultivar, agronomic conditions, postharvest conditions, and the stage of fruit ripeness [14]. Avocado oil is very appreciated in the cosmetic field for its high content in fatty acids and vitamins A, D and E. Because of its chemical composition, avocado oil nourishes deeply, smooths the epidermis and relieves the skin desquamation. Avocado oil is easily absorbed by the skin where it helps to maintain its barrier function. Fatty acids deficiency produces skin lesions and deterioration, which result in desquamation, dry skin and reduced skin flexibility and smoothness. Keratinization becomes disorganized, mitosis and DNA synthesis decrease, the skin loses most of its protective functions and perspiration increases. Fatty acids deficiency-related lesions improve after percutaneous applications of fatty acids-rich oils. Therefore, such oils are extensively used in dermatology and cosmetics to treat dry skin and wrinkles and to improve wound healing, through their tissue stimulating and regenerating actions [15].

Vegetable oils and fats are good emollients, due to their lipophilic nature. These compounds efficiently prevent Tran's epidermal water loss, because of their excellent occlusive properties. A number of studies have demonstrated that the higher the saturation

degree of a certain oil, the lower its viscosity and the better its skin penetration [16]. Emollients are mainly lipids and oils, which give the skin improved moisture, smoothness and flexibility. These compounds repair the skin and influence skin permeability, improving the barrier function. Stearic, linoleic, oleic, linolenic and lauric acids are emollient compounds habitually used in cosmetics and dermopharmacy [17].

The present study was designed to compare the antioxidant capacity of the oils of avocado varieties from Cameroon and Hawaii.

MATERIALS AND METHODS

Preparation of the avocado oil.

The avocado fruits were obtained from two different locations: Cameroon (4 varieties) and Hawaii (6 varieties). The fruits (freshly ripened) were cut opened and the peels and seed were removed. The flesh (pulp) was chopped into small tiny beats and freeze dried. The weight of the dry matter was determined following which the freeze-dried samples were ground to powder. Twenty five grams of ground pulp was homogenized with 250 ml ethyl ether and allowed to sit for 72 hours. This was then filtered under vacuum and the solvent from the lipid-containing filtrate was evaporated under vacuum. The recovered oil was weighed and kept at – 20 ^{o}C for antioxidant capacity and phenolic content analysis.

Evaluation of the antioxidant capacity of the avocado oils

The hydro soluble fraction of the oils used for antioxidant capacity and phenolic content analyses were extracted as follows. Briefly 1 mL oil was homogenized in 1 mL chloroform and shaken for 10 min. Then 2 mL methanol/water (60/40) added and shaken for another 10 min and then allowed to stand. The methanol/water which constituted hydro soluble fraction of the oils were separated and stored at – 20 ^{o}C until required.

Total phenolic content (TPC)

The total phenolic content was determined following the method described by Singleton and Rossi [18] with slight modifications. In to a test tube containing 0.2 mL of diluted avocado oil was added Folin-Ciocalteu's reagent (0.25 mL) and after 3min of incubation, 35% sodium carbonate solution (1 mL) was added. This was thoroughly mixed and incubated for 1hour. The optical density was read at 760 nm using a spectrophotometer and catechin was used as standard. The total phenolic content of the oil was expressed as mM catechin equivalent.

Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power (FRAP) of avocado oils was assayed by employing the method earlier described [19]. The FRAP method measures the absorption change that appears when the TPTZ (2, 4, 6-tri-pyridyl-s-triazine)-Fe^{3+}complex is reduced to the TPTZ-Fe^{2+} form in the presence of antioxidants at 593nm. FRAP working solution was prepared freshly each time: 0.3 M acetate buffer (pH=3.6), 0.01 M TPTZ (2, 4, 6-tripyridyl-s-triazine) in 0.04 M HCl and 0.01 M $FeCl_3$*$6H_2O$ were mixed in 10:1:1 (v/v/v) ratio and stored in an amber bottle. Then 2 mL of the FRAP working solution was mixed with 75 µL of diluted oil sample; the absorbance was recorded at 593 nm after 20 min of incubation at 37 ^{o}C. The FRAP content was expressed as mM catechin equivalent.

ABTS Antioxidant activity

The method earlier described by Arnao *et al*. [20] was modified for this assay. Stock solutions included 7.4mM $ABTS^{+}$ solution and 2.6mM potassium persulfate solution. These two solutions were then mixed at equal proportion and incubated in the dark at room temperature for 16 hours to generate the $ABTS^{+}$ radical. $ABTS^{+}$ solution (1 mL) was mixed with 60mL methanol to obtain a solution with an absorbance of 1.12 at 734 nm. The diluted $ABTS^{\bullet+}$ solution was used for the analysis. Diluted avocado oil (25 - 200 µL) or catechin standards of varying concentration (10 – 60µM) were added to diluted $ABTS^{\bullet+}$ solution to a final volume of 2000 µL and after 15 min of incubation in the dark the absorbance was read at 734nm. Results are presented as the ability of oils to scavenge 50% of the radical $ABTS^{\bullet+}$ (IC_{50}).

DPPH Radical scavenging activity

The DPPH assay was done according to the method of Brand-Williams *et al*. [21] with some modifications. The stock solution was prepared by dissolving 24 mg DPPH in 100mL methanol and then stored at -20 ^{o}C until needed. The working solution was prepared by mixing 10mL stock solution with 45mL methanol to obtain an absorbance of 1.00 units at 517 nm using the spectrophotometer. Diluted avocado oil (50 - 200 µL) or catechin standards (10 – 60µM) were added to diluted DPPH solution to a final volume of 2000 µL and the absorbance read after 30 min of incubation in the dark with aid of a spectrophotometer at 515 nm. Results are presented as the ability of oils to scavenge 50% (IC_{50}) of free radical DPPH.

STATISTICAL ANALYSIS

All the tests were carried out in triplicate. Data obtained were presented as mean ± SD. Analyzed was by one way Analysis of Variance. Significant differences between means were tested by student *t* test at $P < 0.05$.

RESULTS AND DISCUSSION

The total phenolic content (TPC) and ferric reducing antioxidant power (FRAP) are presented in Table 1. Phenolic compounds are important groups of secondary metabolites in medicinal herbs and dietary plants and are characterized by at least an aromatic ring bearing one or more hydroxyl groups. Phenolic compounds possess a diverse range of beneficial biological functions, including antioxidant activity. The high tendency of phenolic compounds for metal

chelation and their redox properties allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers which could lead to antioxidant activity [22-26]. The Folin-Ciocalteu method is the most common assay that is used in estimating the TPC contents in fruits, vegetables, foods and medicinal herbs. The Cameroonian avocado oil had TPC comparable to the Hawaiian samples with exception of the Fuer Florida (Cameroonian) that had the least TPC.

The Ferric Reducing Antioxidant Power of an extract is based on the reduction of the colorless Fe(III) -TPTZ complex to Fe(II) -TPTZ, a blue colored complex [27]. The density of the colors determines the antioxidant potency of the extract. With respect to the FRAP Booth VIII (Cameroonian), Nishikawa (Hawiian) and Fuer Florida (Cameroonian) had the least antioxidant capacity. Meanwhile Serpa, Ohata, Murashinge (all Hawaiian) occupied the first, second and third position respectively and the best Cameroonian species (Peterson) occupied the fourth position in FRAP.

Table 2 presents the radical scavenging activity of the oils of avocado samples determined by two different methods. The ABTS and DPPH assays are usually classified as SET (Single Electron Transfer) a reaction [28] which is based on measuring the antioxidant reductive capacity. Prior *et al*. [29] reviewed that these two radical indicators can be neutralized by direct reduction via electron transfer or by radical quenching via hydrogen atom transfer.

The ABTS radical (ABTS•+) is produced in the ABTS test when 2,2'-azinobis (3-ethylbenzthiazoline-6-acid) (ABTS) is added to sodium persulphate. This forms a blue-green radical cation (ABTS•+) that absorbs light at 734 nm. Most antioxidants will react with ABTS•+ because it is not affected by ionic strength, it is applicable over a wide range of pH. Hence can be useful in the analysis of both hydrophilic and hydrophobic antioxidant capacities. Antioxidants will react with the blue-green ABTS radical cation and decolorizes it to its neutral form [30-32].

The DPPH assay is based mainly on the electron transfer reaction, while hydrogen – atom abstraction is a marginal reaction pathway [29]. The interactions between antioxidants and DPPH• are also determined by the antioxidant's structural conformation. Some compounds react very rapidly with DPPH•, and they reduce the number of DPPH• molecules corresponding to the number of available hydroxyl groups. 2,2-diphenyl-1-picryhydrazyl radical (DPPH•) is one of the few stable organic nitrogen radicals. DPPH• produces an intensive deep purple color when dissolved in ethanol or methanol. However, in the presence of an antioxidant the DPPH• is converted into DPPH, and its color changed from purple to yellow [33, 34]. In both radicals, their antioxidant effect may be easily evaluated by observing the decrease in absorption.

Generally, the oils from the different avocado samples presented a weak percentage radical scavenging activities when compared to catechin the reference standard. Catechin had an IC_{50} of 30.54 µM for DPPH and 27.63 µM for $ABTS^{\cdot+}$ radical scavenging activity. The best avocado sample with radical scavenging activity was Serpa with IC_{50} value of 122.45 µM for DPPH and 66.06 µM for $ABTS^{+}$. The percentage radical scavenging activity was generally better for ABTS method as compared to the DPPH method which could explain the lower IC_{50} obtained. This was expected because the $ABTS^{\cdot+}$ operates both the single electron transfer (SET) and hydrogen atom transfer (HAT) while DPPH operates only the SET. Also $ABTS^{+}$ reacts with both hydrophilic and hydrophobic antioxidants will be exposed to a large amount of antioxidants than DPPH.

Table-1: Total phenolic content (TPC) and Ferric reducing antioxidant power (FRAP) (mM catechin equiv) of oils from ten avocado varieties

Avocados	TPC	FRAP
Hawaiian		
Nishikawa	144.34±8.53[a]	56.38±8.98[a]
Beshore	154.78±28.19[a]	113.54±20.61[b]
Linda	193.26±12.31[b]	157.50±5.71[c]
Murashige	195.48±27.31[b]	170.29±8.99[d]
Ohata	191.99±16.50[b]	172.36±18.67[d]
Serpa	242.27±31.34[c]	253.23±9.14[e]
Cameroonian		
Pollock	200.02±17.25[d]	130.13±3.84[f]
Booth VIII	169.57±6.39[e]	87.00±5.79[g]
Fuer Florida	90.98±6.49[f]	56.04±7.08[a]
Peterson	155.46±29.28[a]	169.94±2.82[d]

Analyses were done in triplicates. Means calculated and standard deviations presented**.** Values in same row that do not share same superscript letter are significantly different.

Table-2: DPPH and $ABTS^{+}$ radical scavenging activity (%) of oils from ten avocado varieties

Avocado sample oil	Conc. (µM)	DPPH·		$ABTS^{\cdot+}$	
		% Radical scavenging activity	IC50 (µM)	% Radical scavenging activity	IC50 (µM)
Hawaiian					
Nishikawa NC	50	13.21±0.26[a]		22.41±2.54[a]	
	100	26.49±1.15[b]		39.50±0.08[b]	
	200	42.85±0.83[c]	233.24 (7)	55.64±1.28[c]	167.47 (6)
Beshore	25	NA		14.25 ±1.89[d]	
	50	15.20±1.15[a]		53.57±2.77[c]	
	100	28.73±0.35[b]		73.57±0.85[e]	
	200	55.52±8.33[d]	179.36 (4)	77.26±1.53[f]	78.08 (3)
Linda	50	18.27±0.13[e]		25.91±2.42[a]	
	100	36.48±1.03[f]		43.93±3.42[g]	
	200	53.91±2.99[d]	176.97 (3)	54.25±0.68[c]	165.14 (5)
Murashinge	50	8.21±4.14[g]		17.63±2.38[h]	
	100	24.03±0.13[b]		32.83±0.58[i]	
	200	42.54±1.40[c]	229.27(6)	45.78±0.72[g]	216.60 (7)
Ohata	50	13.05±0.57[a]		38.19±0.09[b]	
	100	27.95±1.56[b]		55.37±0.78[c]	
	200	46.54±5.27[c]	212.19 (5)	64.89±1.28[j]	99.68 (4)
Serpa	25	NA		14.20±0.96[d]	
	50	27.57±0.47[b]		60.41±1.16[k]	
	100	41.93±5.35[c]		77.43±1.40[f]	
	200	74.96±0.57[h]	122.45 (2)	82.93±2.031[l]	66.06 (2)
Cameroonian					
Pollock	50	8.21±1.15[g]		25.01±0.35[a]	
	100	14.90±1.84[a]	469.05	28.45±0.78[m]	
	200	23.27±0.23[b]	(10)	34.96±3.65[i]	427.25 (10)
Booth VIII	50	6.14±0.57[g]		8.79±0.65[n]	
	100	13.36±0.46[a]		15.53±1.48[d]	
	200	24.50±1.95[b]	409.13 (9)	26.12±4.35[a]	407.21 (9)
Fuer Florida	50	10.90±0.35[i]		8.23±1.55[n]	
	100	12.44±1.21[a]	516.93	11.03±0.39[p]	1010.87
	200	23.27±1.40[b]	(11)	14.83±1.35[d]	(11)
Peterson	50	14.05±3.48[a]		17.11±13.41[h]	
	100	19.20±1.15[e]		32.88±1.014[i]	
	200	31.25±0.26[j]	363.41 (8)	44.69±0.33[g]	222.32 (8)
Catechin	10	NA		27.49±2.53[a]	
	20	35.71±2.31[k]		34.91±3.12[i]	
	40	62.44±3.14[l]		67.62±3.27[q]	
	60	91.24±3.45[m]	30.54 (1)	98.43±1.27[r]	27.63 (1)

Analyses were done in triplicates. Means calculated and standard deviations presented**.** Numbers in parenthesis indicate ranking. Values in same row that do not share same superscript letter are significantly different

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