ADVANCES IN FOOD TECHNOLOGY AND NUTRITIONAL SCIENCES

Openventio PUBLISHERS

ISSN 2377-8350 Open Journal http://dx.doi.org/10.17140/AFTNSOJ-SE-2-107

Special Edition
"Polyphenols for improving food quality and nutraceuticals"

Research

Corresponding author
Nonso Collins Ejiofor, MSc
Department of Medical Biochemistry
University of Nigeria
Enugu Campus, Enugu, Nigeria
E-mail: bianate@yaoo.ca

Special Edition 2
Article Ref. #: 1000AFTNSOJSE2107

Article History
Received: January 19th, 2018
Accepted: February 7th, 2018
Published: February 14th, 2018

Citation
Ejiofor NC, Ezeagu IE, Ayoola M, Umera EA. Determination of the chemical composition of avocado (*Persea americana*) seed. *Adv Food Technol Nutr Sci Open J*. 2018; SE(2): S51-S55. doi: 10.17140/AFTNSOJ-SE-2-107

Determination of the Chemical Composition of Avocado (*Persea Americana*) Seed

Nonso Collins Ejiofor, MSc[1*]; Ikechukwu Edwin Ezeagu, PhD[1]; Moses Babatunde Ayoola, MSc[2]; Emmanuel Arinze Umera, MSc[3]

[1]Department of Medical Biochemistry, University of Nigeria, Enugu Campus, Enugu, Nigeria
[2]Department of Basic Sciences, Medicine, Mississippi State University, Mississippi State, MS 39759, USA
[3]Department of Medical Bichemistry, University of Jos, Jos, Nigeria

ABSTRACT

Introduction: This research work was carried out to determine chemical components of *Persea americana* seed. *Persea americana* (avocado) is widely grown fruit in parts of South East, Nigeria, where it is used as a medicinal plant in the treatment of several ailments by alternative medical practitioners.
Materials and Methods: The chemical components of *Persea americana* seed includes the proximate and minor components. The fleshy part of the fruit was removed to obtain the seed. The dried seeds were ground to powder and dried to constant weight.
Results: The seed is high in carbohydrate (49.03±0.02 g/100 g), lipid (17.90±0.14 g/100 g), protein (15.55±0.36 g/100 g) and moisture (15.10±0.14 g/100 g). It also showed low amounts of ash (2.26±0.23 g/100 g). The seed also presented total oxalate (14.98±0.03 mg/100 g). Levels of tannin (6.98±0.04 mg/100 g) and phytic acid (3.18±0.16 mg/100 g) were also present in low amounts.
Conclusion: It seems *P. americana* seed contains substantial amount of nutrients that could warrant its utilization in animal feed or food.

KEYWORDS: Chemical components; *Persea americana*; Proximate; Nutrients.

INTRODUCTION

The fruit of *Persea americana*, commonly known as avocado, is an edible fruit from Central America which is easily adaptable in tropical regions including Nigeria.[1] The species belongs to the family Lauraceae. *P. americana* is one of the 150 varieties of avocado and pear. The fruit tree can attain a height up to 20 m, with large spreading and flat topped crown. The avocado is classified as an evergreen, although some varieties lose their leaves for a short time before flowering. The tree canopy ranges from low, dense, and symmetrical to upright and asymmetrical. Leaves are 7-41 cm in length and variable in shape (elliptic, oval, and lanceolate). They are often pubescent and reddish when young, becoming smooth, leathery, and dark green when mature. Fruits are large, 5-15 cm long, ovate to spherical, shining green and fleshy.[2] In addition, the fruit is a large fleshy berry with a single seed. It is pyriform, green, with a high oil content rich in vitamins A, B and E.[3,4] The fruits are edible and the bark; leaves, stem and roots are used as local medicine against diseases.[5-7]

The avocado has an olive-green peel and thick pale yellow pulp that is rich in fatty acids such as linoleic, oleic, palmitic, stearic, linolenic, capric, and myristic acids. This fruit is normally used for human consumption, but it also has been used as a medicinal plant in Mexico and elsewhere in the world.[8] The avocado seed represents 13-18% of the fruit, and it is a by-product generally not utilized. Usually, the seed is discarded during the processing of the pulp. The seed waste may represent a severe ecological problem.[9] But it may be of interest to industry as a source of bioactive compounds. It is reported to contain phytosterols, triterpenes, fatty ac-

ADVANCES IN FOOD TECHNOLOGY AND NUTRITIONAL SCIENCES
Open Journal
Openventio PUBLISHERS
ISSN 2377-8350
http://dx.doi.org/10.17140/AFTNSOJ-SE-2-107

ids, and two new glucosides of abscisic acid.[10] Several biological activities of the avocado seed have been reported such as antioxidant, antihypertensive, larvicidal, fungicidal, hypolipidemic, and recently amoebicidal and giardicidal activities.[11] Additionally, several studies have also focused on the evaluation of acute toxicity of the fruit and leaves.[12]

The seed of *P. americana* has a diverse application in ethnomedicine, ranging from treatment for diarrhea, dysentery, toothache, intestinal parasites to the area of skin treatment and beautification.[13] The seeds are rich in tannins and carotenoids and tocopherols from the fruit were shown to inhibit the *in vitro* growth of prostate cancer cell lines.[14]

Currently, the seed represents an under-utilized resource and a waste issue for avocado processors. The avocado seed is discarded in the majority of countries, although in some countries such as Niger Republic, it is consumed.[15,16] This waste may represent an ecological or human contaminant. On the other hand data on its chemical composition could qualify it for use in food or animal feed. The avocado seeds are rich in phenolic compounds, and these may play a role in the putative health effects.[17] This study is therefore seeks to evaluate the nutrients and select minor components of the avocado seed.

MATERIALS AND METHODS

Sample Collection and Preparation

Samples of ripe avocado pear (*P. americana*) were purchased from the New market, Enugu, Nigeria. The succulent fleshy part of the fruit is removed to obtain the seed. The seeds were minced by means of a grater and dried to a constant weight in an oven at 50 °C. It was then ground to powder and then stored in a container.

Proximate Analysis

Proximate analysis for protein, crude fat, moisture, ash and carbohydrate were analyzed using the methods as described by AOAC (Table 1).[18]

Tannin content determination was determined using the method as described by Van Burden and Robinson.[19]

Total oxalate determination was done using the titration method of Day and Underwood,[20] as described by Rathod and Valvi.[21]

Phytate was determined using the method as described by Reddy and Love.[21]

RESULTS AND DISCUSSION

The results of the proximate composition of *P. americana* seed presented in Table 1 shows that the carbohydrate in the seed is 49.03 g/100 g. This value was slightly higher than 48.11±4.13 g/100 g, reported by Arukwe et al[23], but was found to be quite higher than 17.32±0.09 g/100 g and 19.02±0.30 mg/100 g, as reported by Ayoola et al[24] and Okolo et al[25] respectively for both groundnut and soya beans. Carbohydrates are related to energy generation.[23] Observed carbohydrates in the investigated samples may be an indication that the samples could produce energy to power the cells and tissues of the body on consumption. The seed also presented high lipid content. The lipid content presented by the sample was higher than 12.24±0.07g/100 g and 16.54±2.10 g/100 g reported by both Oluwole et al[26] and Arukwe et al[23] respectively. The lipid content presented by the seed is lower than those of groundnut (48.53±0.40 g/100 g) and soya beans (23.92±0.51 g/100 g).[24,25] However, it was higher than the 11.68±0.59 g/100 g reported for maize by Okolo et al.[25] It also showed a favorable amount of fat content as against popular beans variety consumed in Nigeria.[25,27,28] This is an indication that it could be an oil fruit. Generally, fats have many functions. Aside insulation and conservation of body temperature in organisms, their fatty acid components such as lauric acid, etc, have been reported to improve health.[29] The sample presented a protein content of 15.55±0.36 g/100 g. This value is lower than the 17.94±1.40 g/100 g and 4.44±0.06 g/100 g presented by both Arukwe et al[23] and Oluwole et al[26] respectively. The protein content of 7.05±0.01 g/100 g reported for maize by Okolo et al[25] was quite lower than that of the present study. However, groundnut and soybeans presented a much higher values of 24.50±0.20 g/100 g and 37.51±0.15 g/100 g respectively.[24,25] A study by Okolo et al[25] showed that avocado seed contained more protein than both sorghum and millet (13.09±1.23 g/100 g and 12.09±0.05 g/100 g) respectively; however, the same report revealed that both staple food samples contained more carbohydrate than avocado seed (72.00±1.44 g/100 g and 67.80±0.01 g/100 g). Aside contributing to diets, the relative impact of pro-

Table 1: A Comparative Table of the Proximate Composition between Avocado seed and Major Food Items in Nigeria.

Parameter (g/100 g)	Avocado Seed	*Groundnut	Maize**	**Soybeans
Protein	15.55±0.36	24.50±0.20	7.05±0.01	37.51±0.15
Lipid	17.90±0.4	48.53±0.40	11.68± 0.59	23.92±0.51
Carbohydrate	49.03±0.02	17.32±0.09	71.21±0.05	19.02±0.30
Ash	2.26±0.23	1.45±0.03	1.50±0.10	9.01±0.01
Moisture	15.10±0.14	7.31±0.31	9.05±1.20	8.30±0.29

Values are mean±S.D (n=4) *Ayoola et al[24]; **Okolo et al[25].

ADVANCES IN FOOD TECHNOLOGY AND NUTRITIONAL SCIENCES

Open Journal

Openventio PUBLISHERS

ISSN 2377-8350 http://dx.doi.org/10.17140/AFTNSOJ-SE-2-107

Table 2: Anti-Nutritional Constituents of *P. americana Seed* (mg/100 g).

Parameter	Value (mg/100 g)
Tannin	6.98±0.04
Total oxalate	14.98±0.03
Phytic acid	3.18±0.16

Values are mean±S.D (n=4).

teins in body system should not be over looked. As chemical compounds, they repair and replace worn out cells, form structural and globular materials that holds the body, form blood proteins, boost immune system, etc.[30]

The ash content is the measure of the mineral content present in a plant. The ash content of *P. americana* in the present study was slightly lower than the 2.40±0.19 mg/100 g reported by Arukwe et al.[23] However, the value was higher than the 1.15±0.03 g/100 g recorded by Oluwole et al.[26] The ash content (2.26±0.23 g/100 g) of *P. americana* indicates higher mineral content present in the seed when compared to both groundnut (1.45±0.03 g/100 g) and maize (1.50±0.10 g/100 g).[24,25] Soybeans on the other hand presented a much higher mineral content having presented an ash content of 9.01±0.01 g/100 g as reported by Okolo et al.[25] The moisture content reported in the present study showed that the seed is rich in moisture. The moisture content however, is higher than the 2.40±0.19 g/100 g reported by Arukwe et al.[23] Avocado seed presented higher moisture content than groundnut (7.31±0.31 g/100 g), maize (9.05±1.20 g/100 g) and soybeans (8.30±0.29 g/100 g).[24,25] Moisture content helps in maintaining the protoplasmic content of cells. However, when compared with results of proximate analysis from other climes, there was evident difference. These differences were seen in the results reported by both Oluwole et al.[26] and Bora et al.[31] These differences could be attributable to environmental conditions.

Anti-nutritional properties also showed presence of phytate, tannin and oxalate. The tannin content is higher than the value reported (0.24±0.12) by Arukwe et al.[23] Tannin has been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals. Therefore, foods rich in tannins are considered to be of low nutritional value. However, recent findings[32] indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances. Incidences of certain cancers, such as esophageal cancer, have been reported to be related to consumption of tannins-rich foods such as betel nuts and herbal teas, suggesting that tannins might be carcinogenic.[32] However, recent reports have shown there plant tannins are related to polyphenols. Polyphenols are a diverse group of plant secondary metabolites found throughout the plant kingdom, encompassing such subgroups as tannins and flavonoids, among others.[33] Polyphenolic compounds have shown to be bioactive, due to their antioxidative, antimicrobial, antiviral, and antitumor activities.[34,35] The tannin being polyphenols have been shown to have antioxidant property.[36] This antioxidant property of tannins is responsible for its anticarcinogenic and antimutagenic potentials by protecting cellular oxidative damage, including lipid peroxidation.[37] The generation of superoxide radicals was reported to be inhibited by tannins and related compounds. The antimicrobial property of tannic acid can also be used in food processing to increase the shelf-life of certain foods, such as catfish fillets.[32,37] Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, and modulate immune-responses (Table 2).[38]

Oxalate content of avocado seed is also at same level with soursop fruit (2.79±0.48 mg/100 g) as reported by Degnon et al.[39] Phytate content of soursop fruit is slightly different from that of avocado seed. Oxalate binds with calcium to form calcium-oxalate crystals which are deposited as urinary calcium (stones) that are associated with blockage of renal tubules.[40] Proper food processing would reduce these anti-nutrients. The phytate content of avocado seed is lower than that of locust bean pulp as reported (6.28±0.06 mg/100 g) by Dahouenon-Ahoussi et al.[41] Adegoke et al[42] reported higher values for tannin, oxalate and phytic acid (11.29 g/100 g, 4.07 g/100 g and 12.87/100 g) respectively for avocado seed; however, boiling for 25 min and soaking for 24 hrs effectively reduced the anti-nutritional factors without any adverse effect on the nutritional quality. The anti nutritional components of *Terminalia catappa* (almond) seeds shows higher values when compared with those of avocado seed.[43] However, the anti-nutrient parameters tannin, phytate and oxalate reported in the present study were lower than the lethal doses (30 mg/kg, 50-60 mg/kg and 2-5 g/kg) reported by Inuwa et al[44] respectively. It can be concluded that the levels of oxalate, phytate and tannin values of avocado seed is considered low and would not be unsafe for animal consumption as most of the values are below the lethal doses of these phytochemicals. It contains substantial contents of nutrients that can warrant its trial and utilization in feed formulation.

The variations in the results from this study and other reported works may be due to geographical factors of the study area, sampling period and other factors.

CONCLUSION

It was observed that *P. americana* seed contains substantial nutrients that could meet the needs and requirements of the body, thus it is good for human and animal consumption. It can be rec-

ISSN 2377-8350 http://dx.doi.org/10.17140/AFTNSOJ-SE-2-107

ommended for inclusion in animal feeds formulation. It would be important to assess the toxicity in greater detail, however.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Leite JJG, Brito ÉHS, Cordeiro RA. Chemical composition, toxicity and larvicidal and antifungal activities of *Persea americana* (avocado) seed extracts. *Revista da Sociedade Brasileira de Medicina Tropical*. 2009; 42(2): 110-113. doi: 10.1590/S0037-86822009000200003

2. Keay RWJ. *Tree in Nigeria*. Oxford, UK: Oxford Science Publications Clarendon Press; 1989.

3. Hill DS, Waller JM. *Pest and Diseases of Tropical Crops*. Hong Kong: ELBS edition; 1994: 431.

4. Ozolua RI, Anaka ON, Okpo SO, Idogun SE. Acute and subacute toxicological assessment of the aqueous seed extract of *Persea Americana* Mill (Lauraceae) in Rats. *Afri J Tradit Comple Altern Med*. 2009; 6(4): 573-578.

5. Neuwinger HD. *African Traditional Medicine*. Stuttgart Germany: Medpharm; 2000.

6. Jirovetz L, Buchbauer G, Stoyanova AS, Georgiou EV, Damianova ST. Composition, quality control and antimicrobial activity of the essential oil of long time stored dill (*Anethumgraveolens L.)* seeds from bulgaria. *J Agric Food Chem.* 2003; 18(51): 3854-3857. doi: 10.1021/jf030004y

7. Waruhiu AN, Joseph K, Alain AR, Zac T, Rodger RBL. Domestication of Dacryodesedulis. 2. Phenotypic variation of fruit traits in 200 trees from four populations in the humid lowlands of Cameroon. *Food Agric Environ.* 2004; 2(1): 340-346.

8. Dreher ML, Davenport AJ. Hass avocado composition and potential health effects. *Crit Rev Food Sci Nutr*. 2013; 53(7): 738-750. doi: 10.1080/10408398.2011.556759

9. Ortiz MA, Dorantes AL, Gallndez MJ, Cárdenas SE. Effect of a novel oil extraction method on avocado (*Persea americana* Mill) pulp microstructure. *Plant Foods Hum Nutr*. 2004; 59(1): 11-14. doi: 10.1007/s11130-004-0032-3

10. Ramos MR, Jerz G, Villanueva S, López-Dellamary F, Waibel RP, Winterhalter P. Two glucosylatedabscisic acid derivates from avocado seeds; (*Persea americana* Mill. Lauraceae cv. Hass), *Phytochemistry*. 2004; 65(7): 955-962. doi: 10.1016/j.phytochem.2003.12.007

11. Rodríguez-Carpena G, Morcuende D. Andrade MJ, Kylli P, Estevez M. Avocado (*Persea americana Mill*.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. *J Agric Food Chem*. 2011; 59(10): 5625-5635. doi: 10.1021/jf1048832

12. Anaka ON, Ozolua RI, Okpo SO. Effect of the aqueous seed extract of *Persea americana* Mill (Lauraceae) on the blood pressure of Sprague-Dawley rats. *Afr J Pharm Pharmacol*. 2009; 3(10): 485-490.

13. Pamplora GD, Roger MD. *Encyclopedia of Medicinal Plants*. 1999: 719-720.

14. Lu QY, Arteaga JR, Zhang Q, Huerta S, Go VL, Heber D. Inhibition of prostate cancer cell growth by an avocado extract: Role of lipid-soluble bioactive substances. *J Nutr Biochem*. 2005; 16: 23-30. doi: 10.1016/j.jnutbio.2004.08.003

15. Owolabi MA, Jaja SI, Coker HAB. Vasorelaxant action of aqueous extract of the leaves of *Persea americana* on isolated thoracic rat aorta. *Fito.* 2005; 76(6): 567-573. doi: 10.1016/j.fitote.2005.04.020

16. Ojewole J, Kamadyaapa DR, Gondwe MM, Moodley K, Musabayane CT. Cardiovascular effects of *Persea americana* Mill (Lauraceae) (avocado) aqueous leaf extract in experimental animals. *Cardiovasc J S Afr.* 2007; 18(2): 69-76.

17. Dabas D, Shegog RM, Ziegler GR, Lambert JD. Avocado (*Persea americana*) seed as a source of bioactive phytochemicals.*Curr Pharm Des.* 2013; 19(34): 6133-6140.

18. AOAC. Official Methods of Analysis of the Association of Analytical Chemists international. 18th ed. Washington DC, USA: Official methods; 2005: 8.

19. Van Burden TP, Robinson EC. Formation of complexes between protein and tannin acid. *J Agric Food Chem.* 1981; 1: 77-82.

20. Day RA, Underwood AL. *Quantitative Analysis*. 5th ed. Upper Saddle River, NJ, USA: Prentice-Hall publication; 1986: 701.

21. Rathod VS, Valvi SR. Antinutritional factors of some wild edible fruits from kolhapur district. *Recent Research in Science and Technology*. 2011; 3(5): 68-72.

22. Reddy MB, Love M. The impacts of food processing on the nutritional quality of vitamins and minerals. *Adv Exp Med Biol*. 1999; 459: 99-106. doi: 10.1007/978-1-4615-4853-9_7

23. Arukwe U, Amadi BA, Duru MKC, et al. Chemical composition of *Persea americana* leaf, fruit and seed. *IJRRAS.* 2012; 11(2): 346-349.

ISSN 2377-8350 http://dx.doi.org/10.17140/AFTNSOJ-SE-2-107

24. Ayoola PB, Adeyeye A, Onawumi OO. Chemical evaluation of food value of groundnut (*Arachi hypogaea*) seeds. *Am J Food Nutr.* 2012; 2(3): 55-57.

25. Okolo SC, Olutayo O, Doyinsola I, Adedayo A, Ikokoh PP. Orishadipe AT. Comparative proximate studies on some Nigerian food supplements. *Ann Biol Res.* 2012; 3(2):773-779.

26. Oluwole S, Yusuf K, Fajana O, Olaniyan D. Qualitative studies on proximate analysis and characterization of Oil From *Persea americana* (Avocado Pear). *J Nat Sci Res*. 2013; 3(2): 68-73.

27. Nwodo SC, Nwinyi CO. Proximate analysis of *Sphenostylis stenocarpa* and *Voadzeia subterranean* consumed in South –Eastern Nigeria. *J Agric Ext Rural Dev.* 2012; 4(3): 57-62.

28. Adamu AS, Oyetunde JG. Comparison of dietary proximate and mineral values of two varieties of bean. *Asian Journal of Natural & Applied Sciences*. 2013; 2(2): 103-106.

29. Fite B. *The Healing Miracle of Coconut Oil*. Colorado Springs, CO, USA: Piccadilly Books Ltd, Healthwise Publications; 2000: 1-4.

30. Olusanya JO. Proteins. In: *Essentials of Food and Nutrition*. Lagos, Nigeria: Apex Books Limited; 2008: 13-21.

31. Bora PS, Narain N, Rocha RVM, Paulo QM. Characterization of the oils from the pulp and seeds of avocado (cultivar: Fuerte) fruits. *Grasas y Aceites.* 2001; 52(3-4): 171-174. doi: 10.3989/gya.2001.v52.i3-4.353

32. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: A review. *Crit Rev Food Sci Nutr*. 1998; 38(6): 421-464.

33. Haukioja E. Induction of defenses in trees. *Annu Rev Entomol.* 1991; 36: 25-42. doi: 10.1146/annurev.en.36.010191.000325

34. Okuda T, Yoshida T, Hatano T, Ito H. Ellagitannins renewed the concept of tannins. In: Quideau S, ed. *Chemistry and Biology of Ellagitannins: An Underestimated Class of Bioactive Plant Polyphenols*. Singapore: World Scientific Publishing Co. Pte. Ltd.; 2009: 1-54. doi: 10.1142/9789812797414_0001

35. Serrano J, Puupponen-Pimiä R, Dauer A, Aura AM, Saura CF. Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. *Mol Nutr Food Res*. 2009; 53: S310-S329. doi: 10.1002/mnfr.200900039

36. Zhao S, Liu JY, Chen SY, Shi LL, Liu YJ, Ma C. Antioxidant potential of polyphenols and tannins from burs of Castanea mollissima Blume. *Molecules.* 2011; 16(10): 8590-600. doi: 10.3390/molecules16108590

37. Suvanto J, Nohynek L, Seppänen-Laakso T, Rischer H, Salminen JP, Puupponen-Pimiä R. Variability in the production of tannins and other polyphenols in cell cultures of 12 Nordic plant species. *Planta*, 2017; 246(2): 227-241. doi: 10.1007/s00425-017-2686-8

38. Weisburger JHJ. Antimutagens, anticarcinogens, and effective worldwide cancer prevention. *J Environ Pathol Toxicol Oncol*. 1999; 18(2): 85-93.

39. Degnon MRG, Adjou ES, Noudogbessi JP, et al. Investigation on nutritional potential of soursop (Annonamuricata L.) from Benin for its use as food supplement against protein-energy deficiency. *IJB.* 2013; 3(6): 135-144.

40. Blood DC, Radostits OM. *Veterinary Medicine*. 7th ed. London, UK: BalliereTindall; 1989: 589-630.

41. Dahouenon-Ahoussi E, Adjou ES, Lozes E, et al. Nutritional and microbiological characterization of pulp powder of locust bean (*Parkia biglobose* benth.) used as a supplement in infant feeding in northern Benin. *Afri J Food Sci.* 2012; 6(9): 232-238. doi: 10.12691/jfnr-2-6-3

42. Adegoke GO, Akinbile JT, Olapade AA, Ashaye OA. The effect of processing methods on the nutritional profile of avocado (*Persea americana* Mill) seeds. *IJRRAS.* 2012; 11(2): 186-194.

43. Akpabio UD. Evaluation of proximate composition, mineral element and anti- nutrient in almond (*Terminalia catappa)* seeds. *Adv Appl Sci Res.* 2012; 3(4): 2247-2252.

44. Inuwa HM, Aina VO, Gabi B, Aimola I, Toyin A. Comparative determination of antinutritional factors in groundnut oil and palm oil. *Adv J Food Sci Technol.* 2011; 3(4): 275-279.