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Original Paper

Optimized method for processing avocado seeds to improve selected nutrients and functional values

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The objective of this study was to develop an optimal processing technique capable of reducing antinutrients to acceptable levels, retain nutrients and functional values of avocado seeds for human consumption. Different processing conditions for probiotic fermentation, boiling and soaking techniques were studied to establish optimal processing conditions for the seeds. The antinutrients, antioxidant activity, total phenolics and selected nutrients of avocado seeds were analyzed using analytical standard methods. All processing techniques significantly ($p < 0.05$) reduced over 50 % of antinutrients. The highest total phenolics and antioxidant activity (IC_{50}) were 33.3 mgGAE/g and 0.8 mg/mL respectively which were observed at a fermentation temperature of 37 ^oC. Soaking and boiling reduced the analyzed minerals to about 30 % whereas probiotic fermentation retained 100 % of minerals analyzed, ascorbic acid and α-tocopherol. Moreover, probiotic fermentation demonstrated the best results in comparison to boiling and soaking thus, considered as an optimal processing method for improving nutritional and functional values of avocado seeds.

Keywords: probiotic fermentation, boiling, soaking, anti-nutrients, vitamins, antioxidant activity, total phenols

Introduction

Malnutrition affects more than half of the world population, especially in developing countries where plants are the major source of nutrients (Hendek and Bektaş, 2018). The cause of this problem is partly due to complexes of micronutrients such as minerals, vitamins and other essential nutrients hence reducing their bioavailability. Antinutrients are among the substances that complexes potential nutrients or product hence reduce the availability of one or more nutrients upon intake, digestion and absorption, (Yacout, 2016). The negative effects of these antinutrients rely on their concentration, chemical structure, time of exposure and interaction with dietary component (Popova and Mihaylova, 2019). Improving the nutritional value of foods by inactivating or reducing the anti-nutrients to suitable levels can improve the nutritional status of consumers (Gupta and Gangoliya, 2013). This can be achieved by developing a processing technique for reducing antinutritional compounds to an acceptable levels and yet retain the nutrients and improve sensory attributes in the final products.

Recent studies have shown that avocado seeds are the potential sources of essential nutrients such as protein, fat, vitamins and mineral that can improve nutrition in areas where malnutrition is pervasive (Talabi *et al*., 2016). These seeds are also a good source of phytochemical compounds which have a wide range of physiological functions including anti-tumoral, anti-viral, anti-bacterial, anti-diabetic and cardio-protective (Dabas *et al.,* 2013; Mahawan *et al.,* 2015; Tremocoldi *et al.*, 2018). Despite their usefulness, these seeds are always regarded as wastes due to their poor taste, difficulty in

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processing and little scientific information about their functional and nutritional values and hence thrown away into the environment which usually represents a problem that is further aggravated by legal restrictions (Dabas *et al*., 2013; Henly *et al.,* 2015).

Processing these seeds into a consumable product could be a way out to this problem. However, so far, there is no adequate processing method which has shown to reduce the antinutritional compounds to acceptable levels yet retaining the nutrients and sensory attributes of the final products from these seeds. Most of the existing methods such as thermal, non-thermal, flying and autoclaving aim only at reducing or removing the anti-nutritional compounds without regarding their effects on other potential nutrients such as water soluble and heat labile nutrients (Hendek and Bektaş, 2018). This underscores the need for developing a proper processing method that will help practitioners to tape health opportunities that are packed in these seeds. Through proper processing method, these seeds can be developed into flour, which can then be incorporated into various dried products like biscuits, bread, and other related products. It is also the best way of adding value to these seeds and generating income for all stakeholders who will be engaged in this subsector. Furthermore, the products from these seeds can eventually increase the choice space of products in the population. Therefore, this study aims at developing an optimized technique that can reduce the antinutrients, retain nutrients, improve sensory attributes and extend the shelf life of the final products.

Materials and Methods

Sample preparation Collected avocado fruits of fuerte variety were first washed with clean water to remove debris on the upper layer (skin) followed by separating seeds from the edible parts using a knife. The separated seeds were immediately chopped crossectionally at 0.2 cm thickness for each slice. This thickness was within the range as reported by other scholars in the processing and drying of seeds. Forexample, the study conducted by Hau *et al.* (2019) on the optimization for extraction of polyphenols from avocado seeds used the thickness of 0.25 cm. Also Dorta *et al.* (2012) used the thickness of 0.5 cm in drying treatments for stabilizing mango seed kernel on the effect of antioxidant activity . The chopped peaces were then placed into open containers ready for the probiotic fermentation, boiling and soaking.

Processing techniques

Fermentation The overall process of fermentation involved the three key steps. The first step was the preparation of the starter cultures and samples. Lactic acid bacterial (LAB) namely *Lactobacillus plantarum* (B-3058), *Lactobacillus johnsonii* (B-2178) and *Lactobacillus rhammnosus* (B-58149) were obtained from the Agricultural Research Service Culture Collection (NRRL) United States, Department of Agriculture, Peoria, Illinois, USA. The selection of these probiotics was due to their superior properties of working better in reducing of antinutrients, retaining of essential nutrients and functional values of various foods during fermentation (Filannino *et al.,* 2020; Moroni *et al.,* 2015). Preferably, the selected probiotics are food grade with all components appropriate for use in the preparation of food ingredients (Moroni *et al.,* 2015). All the strains received were in a lyophilized state, for this case activation process was needed in order for them to work as required. Therefore activation of the lyophilized strains were achieved following the procedures as reported by Nyamete *et al.* (2016).

The chopped pieces of the avocado seeds were firstly dipped in ethanol followed by several times of washing with deionized water prior to inoculation and this aimed at killing any indigenous microorganism that in one way or another could interfere with the controlled fermentation. The second step encompassed inoculation of the samples, this was done by mixing the prepared sample with portable water in the ratio of 1:1 in the sterile capped containers followed by inoculation with 1 ml of lactic acid bacteria culture. The inoculated samples were mixed well and capped aseptically, the samples well put into the incubators at a temperature of 30 ºC, 37 ºC and 42 ºC this was in accordance to the instructions of the manufacture of the starter culture (NRRL). The third step involved monitoring of probiotic fermentation by measuring parameters which included the pH and lactic acid prior to fermentation and at an interval of 6 hours to completion of fermentation within 24 hours. The pH was measured by using the digital pH meter and the changes in pH in culture solutions during lactic acid fermentation were; *Lactobacillus plantarum* (5.7–5.45 at 30 ºC, 5.7–4.6 at 37 ºC and 5.7–5.15 at 42 ºC) , *Lactobacillus johnsonii* (5.7–5.57 at 30 ºC, 5.7–4.8 ºC and 5.7–5.23 ºC) and *Lactobacillus rhammnosus* (5.7–5.6 at 30 ºC, 5.7–4.99 at 37 ºC and 5.7–5.32 at 42 ºC). The lactitic acid was measured by calorimetric method in accordance to Taylor (1996). After fermentation, all samples were removed from the incubators and dried into the solar tunnel dryer till the required moisture content was obtained.

Boiling Approximately 2 liters of portable water was poured into the water bath and then switched on till boiling was attained. The chopped pieces of avocado seeds were put into the boiled water in the water bath set at 100 ºC. The samples put in the water bath were monitored at a constant boiling temperature with different time interval being 5 minutes, 10 minutes, 15 minutes and 20 minutes as reported by Talabi *et al.* (2016). There after the samples were removed from the water bath and dried with the solar dryer tunnel until the required moisture content was obtained.

Soaking Small seized pieces of avocado seeds were soaked in the portable water with varying time of 6 hours, 12 hours, 18 hours and 24 hours according to the methods portrayed by Adeleke *et al.* (2017) with some modifications. The seeds were soaked in water at the ratio of each 100 g of sliced avocado seeds in 6 litres of portable water (100 g:6 L). This ratio was adapted from previsiouly published work by Oboh *et al.* (2016) with some modifications. After soaking, all the samples where removed from the water and dried into the solar dryer tunnel until the required moisture content was attained.

Drying procedures and pulverization All samples after being treated in each processing method, they were then dried into a solar dryer tunnel while monitoring moisture content until it reached the steady state. The dried samples were grounded using an electric blender into fine powder. The grounded powder was packed into the polyethylene bags and stored at room temperature for further analysis.

Analysis of antinutrients, nutritional and functional values of processed avocado seeds Antinutrient analysis In this study, the anti-nutrients analyzed included oxalates, phytates, tannins and saponin. Oxalates and phytates were analyzed by using the HPLC as described by Vũ *et al.* (2013). Tannin content was determined by using UV-Vis spectrophotometer (Shimadzu model UV-1601 PC, Kyoto, Japan) as described by Arslan *et al*., (2016), while Saponin was determine by using the screening method as reported by Ejikeme *et al.* (2014).

Total phenolic Compounds (TPC) Total phenolic compounds were determined by using the method described by Molyneux (2004) and Arslan *et al.* (2016) with some modifications. Briefly, 10 milligrams of the sample was extracted with 20 mL of 50 % aqueous methanol at 80 ºC for 1 hour, followed by filtration and volume made to 50 mL. 1 mL of the solution was put into 50 mL volumetric flask and 20 mL of distilled water added followed by 2.5 mL of folin-ciocalteu reagent and 10 mL of 17 % sodium carbonate. The mixture was homogenized and made to 50 mL with distilled water and after 20 minutes, absorbance was read on UV spectrophotometer at 760 nm using gallic acid as a standard. The results of total phenolic contents were calculated using the standard calibration curve of gallic acid and expressed as gallic acid equivalents (GAE) per 100 g.

Free radical scavenging activity The radical scavenging activities of the extracts was determined in accordance with the Molyneux (2004) method with slight modifications, which is based on the principle of scavenging the DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical. Briefly 0.5 mL DPPH was added to the solutions prepared with 2.5 mL of the plant extracts and 3 mL of methanol. The obtained mixture was vigorously shaked for 20 minutes and left to stand in the dark room for 1 hour. The same procedure was performed for the control sample. The UV-Vis spectrophotometer (Shimadzu model UV-1601 PC, Kyoto, Japan) was used to read the absorbance at 517 nm. Vitamin C was used as the antioxidant standard. The radical scavenging activity was calculated using the following formula:

$$\text{Percentage inhibition of DPPH} = \{(A_B - A_A)/A_B\} \times 100 \quad \cdots\cdots \text{ Eq. 1}$$

Where A_B is the absorption of blank sample and A_A is the absorption of tested extract solution. The results were expressed as percentage inhibition of DPPH and mean inhibitory concentrations (IC_{50}) was determined from a plot of percentage inhibition of DPPH versus concentration of the plant extract.

Mineral determination The minerals that were determined in this study were potassium, sodium, calcium, iron and zinc. Minerals were analyzed using atomic absorption spectrophotometer (AAS) according to the method of Nanda *et al.* (2003). Shortly, 5.0 g of weighed sample was put into a clean dry crucible. The weighed crucible containing the sample was put into the muffle furnace whose temperature was set at 550 ºC. After complete ashing the crucible was removed from the muffle furnace and cooled at room temperature. The ash was transferred quantitatively to 100 mL beaker using 20 mL of 1N HCL, then heated at 80–90 ºC on a hot plate for 5 minutes. This was then transferred to 100 mL volumetric flask and filled to the mark using 1N HCL. Insoluble matters were filtered and the filtrate kept in a labeled prepared containers. The absorbance of the extract was read by atomic absorption Spectrophotometer. Mineral standards were prepared to make the calibration curve.

Determination of ascorbic acid Analysis of vitamin C (ascorbic acid) content was performed by high performance liquid chromatography (HPLC) using a Shimadzu UV-VIS detector of 254 nm as reported by Vikram *et al.* (2005) with slight modifications. Shortly, triplicate samples of ≥ 2.0 g were weighed and extracted with 0.8 % metaphosphoric acid as a mobile phase. The mixture was made to 20 mL followed by agitation at 10000 rpm for 15 min. The supernatant was filtered and diluted with 10 mL of 0.8 % metaphosphoric acid. This was passed through 0.45 μ filter and the isolate supernatant was injected into the HPLC. Various concentrations of ascorbic acid standards were made and used to draw the calibration curve.

Determination of α-tocopherol content Vitamin E (α-tocopherol) was analyzed using a modification of the method of Barker *et al.* (1998) using the HPLC. About 2.0 g of the homogenized sample was mixed with 4 mL of 95 % ethanol and 1mL of 50 % KOH. The mixtures were saponified by heating at 70 °C in the water bath for 15 minutes and followed by cooling in an ice bath container. Fat-soluble vitamins were extracted with 1ml hexane containing 0.2 % BHT, and a 1mL aliquot of the hexane layer was evaporated under nitrogen. Saponification, extraction and evaporation procedures were performed under yellow light. There after the samples were reconstituted with 0.25 mL ethanol containing 0.1 % BHT. Quantification of α-tocopherol as a measure of vitamin E was done by a Shimadzu 20A Series liquid chromatograph equipped with a 250 × 4.0 mm stainless steel ODS reversed-phase column. The mobile phase used was in the ratio of 96:4 (methanol:water) for α-tocopherol detection. Also the α-tocopherol was monitored at 285 nm (Shimadzu SPD 20A) and the external standard was compared to sample extracts for determination of vitamin concentrations.

Statistical data analysis The data were analyzed using R statistical package (R Development Core Team, Version 3.6.2 Vienna, Austria) for Analysis of variance to determine the significant ($p < 0.05$) variations between the processing techniques. The means of analyzed vitamins, total phenols and minerals were separated by Tukey Honest Significant difference at $p < 0.05$.

Results and Discussion

Effects of probiotic fermentation, soaking and boiling on the antinutrients reduction in avocado seeds The high antinutritional factors present in the raw seed of avocado are a potential threat in the use of this seed in animal and human nutrition, in spite of its nutritional composition (Talabi *et al.,* 2016). The negative effects of these antinutrients rely on their concentration, chemical structure, time of exposure and interaction with another dietary component (Popova and Mihaylova, 2019). Processing methods employed in this study namely probiotic fermentation, boiling and soaking as shown in table 1, reduced greatly these natural toxicants. However, all the methods had a significant reduction of the antinutritional compounds in the samples at various set conditions. Boiling decreased significantly ($p < 0.05$) the contents of antinutritional compounds with respect to increase with time. It was also observed that boiling the samples for 10 minutes or less the effect of reducing tannins, phytates and oxalates was less than 50 % and increase of time above 10 minutes the total reduction was more than 55 % while saponin was not detected at all. However, the highest reduction of anti-nutrients with boiling were noted at 20 minutes and this agreed with the results reported by Talabi *et al.* (2016). A decrease level of antinutritional compounds was also observed in soaking as time increased due to the diffusion speed of components exchanging between the soaking water and the the sliced avocado seeds and this was in agreement with the results published by Adeleke *et al.* (2017) and Moroni *et al.* (2015). For example, it was reported by Handa *et al.* (2017) that there were a decrease in tannins and oxalates upon soaking of underutilized pulse (horsegram) as soaking time increased. Samples soaked for more than 12 hours no oxalate content was observed and similarly at above 18 hours saponin contents were not detected. Tannin level on soaking for 24 hours was significant reduced to more than 90 % this was due to leaching of tannin from the cells into water since tannins are water soluble phenolic compounds. As observed above, probiotic fermentation also reduced the tannins, phytates and oxalates to the significant levels. The significant variations in the reduction of oxalate, phytates, tannins and saponin by probiotic fermentation was due to the action and capacity of lactic acid bacteria in producing metabolites which could remove antinutrients at different temperatures. This is not a new fact, because similar results were described in other studies and were justified by Moroni *et al.* (2015) in fermented grains. All the set temperatures for probiotic fermentation managed to reduce the anti-nutrients for more than 50 % in all treatments. Upon screening of saponin, no detection was observed for both samples fermented by *Lactobacillus plantarum* and *Lactobacillus johnsonii*. in all set temperatures. Similarly samples fermented by *Lactobacillus plantarum* also showed no detection of tannin upon analysis in all set temperatures

From the results, it was observed that all samples analyzed had moderately higher values of calcium, sodium and potassium, but low values of iron and zinc as shown in table 2. Probiotic fermented samples showed no significance differences of all mineral analyzed in this experiment among the treatments while boiled samples had a significant ($p < 0.05$) lower values than the raw samples for calcium, sodium, potassium and iron except for zinc which was insignificant at $p < 0.05$. On the other hand, soaked samples showed a significant lower variations among the treatments for calcium, sodium and iron than unsoaked samples accept for potassium and zinc which were also insignificant at $p < 0.05$. The low values observed in some of the minerals in boiled and soaked samples were also reported with other published results. For example, the study conducted by Lestienne *et al.* (2005) on the effects of soaking whole cereals and legume seeds showed also a significant reduction of iron in millet, rice and soybean. Similarly, a significant decrease of calcium in undercorticated castor oil seeds on boiling and soaking was reported by Nsa *et al.* (2011). However, the maximum significant decrease in percentage for minerals in soaked samples were Ca (9 %), Na (19.8 %) and Fe (23.6 %) while for boiled samples were Ca (26.6 %), Na (21.3 %), K (14.6 %) and Fe (27.7 %). Nevertheless, values of Ca, Na and K for the raw samples obtained in this experiment were observed to be lower compared to the data published by Talabi *et al*. (2016). This

Table 1. Effects of boiling, soaking and probiotic fermentation on the antinutritional reduction in avocado seeds

	Treatment	Tannins (mg/100 g)	Oxalates (mg/100 g)	Phytates (mg/100 g)	Saponin
Soaking	Raw	0.45±0.03^{a}	0.44±0.02^{a}	2.72±0.14^{a}	+
	T_6	0.20±0.02^{b}	0.37±0.07^{b}	2.67±0.07^{a}	+
	T_{12}	0.19±0.02^{b}	0.14±0.01^{c}	1.97±0.01^{b}	+
	T_{18}	0.17±0.02^{b}	nd	0.75±0.07^{c}	+
	T_{24}	0.14±0.01^{c}	nd	0.25±0.00^{d}	nd
Boiling	Raw	0.45±0.03^{a}	0.44±0.02^{a}	2.72±0.14^{a}	+
	T_5	0.33±0.02^{b}	0.42±0.04^{a}	1.51±0.04^{b}	+
	T_{10}	0.30±0.02^{b}	0.40±0.04^{a}	1.01±0.02^{c}	nd
	T_{15}	0.20±0.01^{c}	0.11±0.00^{b}	0.54±0.01^{d}	nd
	T_{20}	0.06±0.00^{d}	0.074±0.00^{b}	0.50±0.04^{d}	nd
Fermentation (30 °C)	Raw	0.45±0.03^{a}	0.44±0.02^{a}	2.72±0.14^{a}	+
	B-3058	nd	nd	0.17±0.07^{b}	nd
	B-2178	0.09±0.01^{c}	0.04±0.00^{c}	0.20±0.00^{b}	nd
	B-59149	0.22±0.00^{b}	0.12±0.01^{b}	0.26±0.01^{b}	+
Fermentation (37 °C)	Raw	0.45±0.03^{a}	0.44±0.02^{a}	2.72±0.14^{a}	+
	B-3058	nd	0.04±0.00^{c}	0.22±0.00^{b}	nd
	B-2178	0.07±0.01c	0.07±0.00^{b}	0.09±0.00^{b}	nd
	B-59149	0.13±0.01^{b}	0.04±0.00^{c}	0.21±0.01^{b}	nd
Fermentation (42 °C)	Raw	0.45±0.03^{a}	0.44±0.02^{a}	2.71±0.14^{a}	+
	B-3058	nd	0.19±0.00^{b}	0.65±0.12^{b}	nd
	B-2178	0.01±0.00^{b}	0.03±0.00^{c}	0.35±0.27^{c}	nd
	B-59149	nd	0.41±0.04^{a}	0.50±0.02^{b}	+

Data presented as means ± SD (n = 3). The means in columns with different superscript letters are significantly different ($p < 0.05$); nd = not detected, B-3058 = *Lactobacillus plantarum*, B-2178 = *Lactobacillus johnsonii*, 59149 = *Lactobacillus rhammnosus*, T_5 = five minutes, T_{10} = ten minutes, T_{15} = fifteen minutes, T_{20} = twenty minutes, T_6 = six hours, T_{12} = twelve hours, T_{18} = eighteen hours and T_{24} = twenty four hours.

may be due to a number of factors including climate, genotype, cultivation location and difference in varieties as well as method of preparation and determination.

The results of ascorbic acid, α-tocopherol, total phenol and antioxidant activity are shown in Table 3. For vitamin C (ascorbic acid), it was noted that boiling and soaking had a tremendous decrease for all the treatments. Ascorbic acid contents of the raw avocado seeds was 9.18 mgAAE/100 g. The decrease was observed to be above 51 % in all treatments after soaking and boiling. As time increased boiled and soaked samples had a more noticeable decrease of ascorbic acid which accounted for 83 % with boiling at a maximum of 20 minutes. Similar results were reported by Han *et al.* (2004), who observed the loss of boiled potatoes to be 77–88 %. This big loss of ascorbic acid agrees with other previous reports of (Shintani, 2013), due to being sensitive with temperature and a water soluble vitamin. On the other hand, probiotic fermentation had contrary results of ascorbic acid in comparison with boiling and soaking. Probiotic fermented avocado seeds caused a significant ($p < 0.05$) increase of ascorbic acid content than unfermented samples. This increase and variations in ascorbic acid content on probiotic fermentation might be due to increased activities and ability of fermenting microorganisms that led to the disrupt of ester bond linkages thus releasing the ascorbic acic that resulted into its increase as observed by other investigators. Forexample, It has been observed that vitamin C content of red beans, citrus peels and white cabbage increased with fermentation (Jhan *et al.,*

Table 2. Effects of boiling, soaking and probiotic fermentation on the analyzed minerals in avocado seeds (mg/100g)

	Treatment	Calcium	Sodium	Potassium	Iron	Zinc
Soaking	Raw	77.73±1.87^{a}	212.70±0.36^{a}	232.90±0.40^{a}	3.61±0.08^{a}	0.18±0.01^{a}
	T_6	77.29±1.59^{a}	191.61±1.17^{b}	229.41±8.81^{a}	3.36±0.02^{ad}	0.19±0.02^{a}
	T_{12}	73.47±1.48^{ab}	187.32±3.85^{b}	227.43±2.26^{a}	3.14±0.02^{bc}	0.18±0.01^{a}
	T_{18}	70.73±2.49^{b}	182.15±0.20^{c}	225.80±1.16^{a}	2.81±0.27^{cd}	0.18±0.01^{a}
	T_{24}	71.13±1.79^{b}	170.05±0.93^{d}	225.22±3.10^{a}	2.75±0.14^{d}	0.18±0.01^{a}
Boiling	Raw	77.73±1.87^{a}	212.70±0.36^{a}	232.90±0.40^{a}	3.61±0.08^{a}	0.18±0.01^{a}
	T_5	76.28±3.56^{a}	201.70±4.76^{b}	222.28±2.11^{b}	3.36±0.01^{a}	0.20±0.02^{a}
	T_{10}	74.66±2.43^{ab}	183.42±2.24^{c}	218.87±0.21^{c}	3.19±0.05^{ab}	0.18±0.01^{a}
	T_{15}	68.68±1.19^{b}	178.81±1.33^{c}	210.41±2.95^{c}	2.73±0.39^{bc}	0.17±0.01^{a}
	T_{20}	57.08±3.56^{c}	167.32±2.13^{d}	198.97±1.11^{d}	2.61±0.04^{c}	0.17±0.01^{a}
Fermentation (30 °C)	Raw	77.73±1.87^{a}	212.70±0.36^{a}	232.90±0.40^{a}	3.61±0.08^{a}	0.18±0.01^{a}
	B-3058	78.61±7.91^{a}	235.76±4.44^{a}	237.21±4.63^{a}	3.69±0.40^{a}	0.19±0.08^{a}
	B-2178	89.73±3.25^{a}	214.05±1.24^{a}	235.44±4.51^{a}	3.74±0.08^{a}	0.19±0.01^{a}
	B-59149	84.11±1.27^{a}	222.40±4.63^{a}	233.25±3.44^{a}	3.65±0.14^{a}	0.19±0.05^{a}
Fermentation (37 °C)	Raw	77.73±1.87^{a}	212.70±0.36^{a}	232.90±0.40^{a}	3.61±0.08^{a}	0.18±0.01^{a}
	B-3058	77.10±1.34^{a}	210.72±10.9^{a}	229.82±6.91^{a}	3.72±0.37^{a}	0.18±0.01^{a}
	B-2178	78.79±5.34^{a}	212.58±8.60^{a}	229.82±6.91^{a}	3.91±0.01^{a}	0.21±0.01^{a}
	B-59149	80.38±1.10^{a}	212.99±4.80^{a}	233.73±9.25^{a}	3.67±0.01^{a}	0.18±0.01^{a}
Fermentation (42 °C)	Raw	77.73±1.87^{a}	212.70±0.36^{a}	232.90±0.40^{a}	3.61±0.08^{a}	0.18±0.01^{a}
	B-3058	77.73±0.15^{a}	213.71±1.07^{a}	233.55±6.91^{a}	3.66±0.08^{a}	0.18±0.01$^{a`}$
	B-2178	78.97±4.36^{a}	212.34±3.19^{a}	230.10±2.38^{a}	3.68±0.04^{a}	0.18±0.02^{a}
	B-59149	77.45±5.66^{a}	213.76±0.73^{a}	233.17±9.28^{a}	4.01±0.28^{a}	0.18±0.01^{a}

Data presented as means ± SD (n = 3). The means in columns with different superscript letters are significantly different ($p < 0.05$); B-3058 = *Lactobacillus plantarum*, B-2178 = *Lactobacillus johnsonii*, 59149 = *Lactobacillus rhammnosus*, T_5 = five minutes, T_{10} = ten minutes, T_{15} = fifteen minutes, T_{20} = twenty minutes, T_6 = six hours, T_{12} = twelve hours, T_{18} = eighteen hours and T_{24} = twenty four hours.

2015; Adetuyi and Ibrahim, 2014). However, the highest content of ascorbic acid (13.5 mgAAE/100 g) in this study was observed at an optimal temperature of 37 °C.

In this experiment, no significant differences were found in vitamin E (α-tocopherol) contents in all the treatments. Among the reasons for these insignificances includes inactivation of lipoxygenase enzyme responsible for lipid peroxidation upon boiling and the nature of vitamin E being fat soluble hence not affected by soak time (Sistrunk, 1977; Kansson and Jagerstad, 1990). However the results of vitamin E ranged from 0.63–0.89 mg/100 g in all processing techniques.

For the total phenolics, soaked samples revealed that there were a significant decrease from 18.22- 15.84 mgGAE/g and the highest loss was at 24 hours which accounted for 13.1 %. The decrease in total phenolic contents when samples are soaked in water is due to leaching of the total phenols as reported by Oghbaei and Prakash (2017). Boiling and probiotic fermentation retained the total phenolic contents in a sense that boiling did not show any significant differences while fermentation had a significant increase. Insignificances of the total phenolic contents observed during boiling of the sample might be attributed by opening of the cell matrix, which facilitates the extractability and bio-availability of total phytochemicals which promotes the release of bound phytochemicals as reported by Tian *et al.* (2016). The increase

Table 3. Effects of boiling, soaking and probiotic fermentation on the analyzed vitamins, total phenol and antioxidant activity in avocado seeds

	Treatment	Ascorbic acid (mgAAE/100 g)	α-tocopherol (mg/100 g)	Total phenol (mgGAE/g)	DPPH radical IC$_{50}$ (mg/mL)
Soaking	Raw	9.18±0.54^a	0.78±0.03^a	18.22±0.10^a	1.5
	T_6	3.85±0.04^b	0.76±0.05^a	17.96±0.06^a	1.7
	T_{12}	2.34±0.02^c	0.74±0.04^a	16.80±0.08^b	1.61
	T_{18}	1.70±0.01^c	0.73±0.02^a	16.07±0.09^c	1.75
	T_{24}	0.51±0.01^d	0.71±0.02^a	15.84±0.04^c	1.8
Boiling	Raw	9.18±0.54^a	0.78±0.03^a	18.22±0.10^c	1.5
	T_5	4.42±0.03^b	0.66±0.07^a	19..68±0.13^{bc}	1.8
	T_{10}	3.91±0.09^b	0.63±0.02^a	21.34±0.20^{ab}	1.75
	T_{15}	2.08±0.02^c	0.70±0.02^a	21.56±1.60^a	1.85
	T_{20}	1.70±0.04^c	0.77±0.07^a	22.07±0.90^a	2
Fermentation(30 °C)	Raw	9.18±0.54b^c	0.78±0.03^a	18.22±0.10^d	1.5
	B-3058	12.81±0.74^a	0.80±0.06^a	23.21±0.14^a	1.2
	B-2178	9.93±0.17^b	0.67±0.04^a	21.10±0.24^b	1.4
	B-59149	9.45±0.17^c	0.89±0.05^a	20.32±1.5^c	1.1
Fermentation(37 °C)	Raw	9.18±0.56^c	0.78±0.03^a	18.22±0.10^d	1.5
	B-3058	13.50±0.12^a	0.93±0.09^a	33.30±0.71^a	0.8
	B-2178	12.19±0.03^{ab}	0.89±0.06^a	28.65±0.56^b	0.9
	B-59149	9.28±0.68^c	0.83±0.02^a	24.73±0.46^c	1.0
Fermentation(42 °C)	Raw	9.18±0.56^a	0.78±0.03^a	18.22±0.10^b	1.5
	B-3058	9.95±0.54^b	0.82±0.06^a	18.38±0.71^{ab}	0.9
	B-2178	9.80±0.32^b	0.75±0.00^a	18.48±1.30^a	1.4
	B-59149	9.61±0.41^b	0.75±0.05^a	17.11±0.20^c	1..3

Data presented as means ± SD (n = 3). The means in columns with different superscript letters are significantly different ($p < 0.05$); B-3058 = *Lactobacillus plantarum*, B-2178 = *Lactobacillus johnsonii*, 59149 = *lactobacillus rhammnosus*, GAE = Gallic acid equivalents, AAE = Ascorbic acid equivalents, T_5 = five minutes, T_{10} = ten minutes, T_{15} = fifteen minutes, T_{20} = twenty minutes, T_6 = six hours, T_{12} = twelve hours, T_{18} = eighteen hours and T_{24} = twenty four hours.

of total phenolic contents upon probiotic fermentation ranged from 18.22–33.3 mgGAE/g. The increase variations of total phenol observed at different fermentation conditions were influenced by the ability of a specific microganism at different temperatures for enzymatic processes as explained by Moroni *et al.* (2015). Thus the highest increment was observed at an optimal temperature of 37 °C and it was 45.3 %. Also the increase of total phenolic contents with probiotic fermentation is being attributed by microoganisms capable of synthesizing enzymes which break the ester linkages and as result release the phenolic bonds as explained by Acosta-Estrada *et al.* (2014).

1,1-diphenyl-2-picryl-hydrazyl (DPPH) is a stable free radical that has an unpaired valence electron on one atom of the nitrogen bridge and has been often used to evaluate the antioxidant activity of foodstuff (Oghbaei and Prakash, 2017). The DPPH of the radical scavenging activity (IC$_{50}$) of avocado seeds ranged from 0.8 to 2 mg/mL. IC$_{50}$ value is defined as the concentration of the sample extracts causing 50 percent inhibition of absorbance and therefore a lower IC$_{50}$ value reflects a greater antioxidant activity of the sample (Ananas *et al.,* 2010). Moderately reduction of DPPH radical scavenging activity was observed after boiling and soaking compared with the analyzed raw material whereas probiotic fermentation had a significant increase of the activity. Similar results were reported with other previous scholars. For example, during boiling and soaking there is a significant degradation of the phytochemicals and leaching of the essential antioxidants which all contribute to loss of antioxidant activity (Perla *et al.,* 2012). The higher antioxidant activity with probiotic fermentation was signified by lower values of IC_{50} in comparison with the raw sample analyzed. Sample fermented with *Lactobacillus plantarum* had the highest antioxidant activity.The observed highest antioxidant activity in sample fermenteted with *Lactobacillus plantarum* was contributed by its exceptional characters (genetic makeup and ability to produce varieties of enzymes) in comparison with *Lactobacillus johnsonii* and *Lactobacillus rhammnosus* as explained by other researchers. Forexample, a study conducted by Filannino *et al.* (2020) on the capability of selected lactic acid bacteria to enrich the portfolio of bioactive compounds of avocado fruit, *Lactobacillus plantarum* showed the highest antioxidant activity compared to other used lactic acid bacteria. Also a study conducted by Adeleke *et al.* (2017) using various lactic acid bacteria on fermented cassava peels, Lactobacillus plantarum demonstrated the best result in the production of linamarase enzyme Further more, the pronounced increase in the antioxidant activity as a result of probiotic fermentation observed in the present experiment is in agreement with the data given by Jamro and Starzyn (2008) who found that fermentation of grass pea seeds by starter culture resulted in an increase of its antiradical effect against DPPH.

Generally, with these findings probiotic fermentation technique presented better results in both reducing antinutritional compounds to acceptable levels, retaining nutrients and functional values of processed avocado seeds compared to boiling and soaking methods. This was justified by the highest antioxidant activity, total phenolics, ascorbic acid, minerals and reduction of the antinutrients to acceptable levels as summarized in Tables 1, 2 and 3. These facts could be explained by the ability of the used microorganisms to synthesize and break the ester linkages bonds, availability of suitable substrate and utilization of the substrate by microorganisms at set condition and reduced pH on fermentation which optimizes and inactivates the activity of the enzymes such as phytase. Similar information has been published by other researchers (Acosta-Estrada *et al.,* 2014; Adeleke *et al.,* 2017; Moroni *et al.,* 2015). Therefore, probiotic fermentation technique qualifies to be an optimized method for processing avocado seeds to be used as materials in functional foods.

Conclusion

The findings from this study show that probiotic fermentation can significantly reduce the levels of antinutritional compounds and improve the nutritional and functional values of the avocado seeds. Unlike other processing methods (i.e soaking and boiling), probiotic fermentation retained all the analyzed nutrients, phenolic compounds, improved antioxidant activity of processed avocado seeds. Probiotic fermentation is therefore a processing technique for developing functional foods from avocado seeds in developing countries where malnutrition is pervasive.

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Conflict of Interest No conflict of interest was declared by the authors.

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