

Fermentation of *Persea americana* Seed Flour Using *Lactobacillus plantarum* and Investigate Its' Effect on Nutritional Quality

P.D.S.R. Kumari and R. Amarakoon

ABSTRACT

This study aims to investigate the fermentation of Avocado seed flour using *Lactobacillus plantarum*. Proximate composition and most important mineral contents of Avocado seed flour after fermenting for 24, 48 and 72 h were compared with dry unfermented Avocado seed flour. Avocado seed flour fermented for 72 h had significant increase ($p < 0.05$) of crude protein content from $6.42 \pm 0.08\%$ to $9.26 \pm 0.28\%$. Mineral contents were determined by Atomic Absorption Spectrophotometer. Ca, Mg, Fe contents of dry unfermented Avocado seed flour were $208.89 \pm 0.3 \mu\text{g/g}$, $99.05 \pm 0.01 \mu\text{g/g}$, $9.04 \pm 0.01 \mu\text{g/g}$ and fermented Avocado seed flour (72 h) were $227.06 \pm 0.050 \mu\text{g/g}$, $189.22 \pm 0.33 \mu\text{g/g}$, and $18.11 \pm 0.006 \mu\text{g/g}$ respectively. There is a significant increase ($p < 0.05$) of Ca, Mg and Fe content of fermented (72 h) Avocado seed flour. Fermentation of Avocado seed flour for 72 h enhance the nutritional quality.

Keywords: Avocado seeds, *Lactobacillus plantarum*, Mineral content, Proximate composition.

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I. INTRODUCTION

The *Persea americana*, commonly known as avocado is a local fruit that has a wide distribution around the tropical region of the globe. Avocado is a nutrient dense fruit. The smooth flavour of avocado has attracted as an oleaginous fruit crop around the world. Furthermore, ease of cultivation and management contribute towards cultivating this crop as a raw dietary supplement and for pharmaceutical preparations [1].

Avocado seeds, which represent 16% of total fruit mass on a wet basis are considered as a waste product in food industries. Therefore, it has become an underutilized product. Avocado seeds are enriched with several phytochemicals which can be nutritionally and medically important. [2] The Avocado seeds has 70% of the antioxidants found in the whole avocado, and it is full of antioxidants that can lower cholesterol and fighting many diseases [2]. Studies have shown that Avocado seeds have more soluble fibre than oatmeal and just above any other food. Vital compounds in Avocado seeds boost the immune system and is a deterrent towards debilitating diseases. The Avocado seeds seem to have a good anti-inflammatory ability which can help in arthritis and other diseases [2]. The Avocado seeds oil can increase the collagen in the skin thus helping to keep skin looking young and wrinkle-free. Further, it makes the hair shiny and helps to get rid of dry and dead skin [3].

Currently, the exploitation of non-edible parts of fruits including seeds has emerged as a trending approach to mitigate the increase of wastes in the food industry [3]. Bioactive compounds within the underutilized parts of fruits

can often be inactive. Bioactive substances produced during fermentation can have enhanced functionalities, thereby the quality of foods can be improved. Hence, microbial fermentation and fermentation using enzymes from plants or microorganisms are used to upgrade the quality. These production approaches have their advantages and disadvantages [4]. Therefore, the microbiological method of fermentation of the raw material can transform it into a processed product with added value. Lactic Acid Bacteria as a starter culture is preferred as against other starter bacterial cultures due to the high safety handling. [4]. These bacteria are very common are widely used as a simpler and cost-effective method in food and beverage production to improve nutritional quality, functional properties as well as to improve sensory characteristics [5].

There were no studies found in fermenting of Avocado seed flour by using food grade microorganism in Sri Lanka but done elsewhere. Therefore, this research was designed to investigate the fermentation of Avocado seed flour by using *Lactobacillus plantarum*. Further, it is aimed to develop an instant beverage out of fermented Avocado seed flour for consumption.

II. METHOD

A. Sample Collection and Storage

Ripen Avocado fruit of a common local variety in Sri Lanka were taken for the experiment. The seeds of Avocado fruits were collected by removing the fleshy part of the fruit. Seeds were subjected to steam blanching after slicing into 2

mm thick slices. The blanched slices were dried in an oven to obtain a constant weight which maintained at 50 °C. Dried samples were sealed and hygienically packed in an airtight container for further analysis at the laboratory of microbiology.

B. Maintenance of *L. plantarum* Cultures

L. plantarum cultures required for the fermentation process were obtained from the culture collection of the Department of Microbiology, Faculty of Science, University of Kelaniya, Sri Lanka. The *L. plantarum* cultures were stored under -80 °C refrigerator maintained in sterile MRS broth with glycerol (20%) where the working cultures of *L. plantarum* were maintained on sterile MRSA plates maintained at 37 °C and routinely sub-cultured every four-week time.

C. Preparation of *L. plantarum* starter Culture

L. plantarum starter cultures were prepared as described in [6] with some modifications. The stock cultures that were grown in MRS broth (10.0 mL) at 37 °C for 48 h were inoculated into sterile MRS broth (10% w/v, 50.0 mL) with Avocado Seed Flour (2.5 g), refined sugar (1.5 g), skim milk (1.0 g) and incubated at 37 °C for 24 h.

D. Preparation of Dry Unfermented and Fermented Avocado Seed Flour

Four different samples were prepared by adding dried Avocado seed chips (50.0 g) into conical flasks with sterile distilled water (SDW, 500.0 mL). Thereafter each flask was sterilized in an autoclave at 121 °C for 5 minutes, the prepared starter cultures of *L. plantarum* were transferred into three flasks to perform the fermentation process for different time durations such as 24 h, 48 h, and 72 h at 37 °C. After the fermentation, Avocado chips were washed with sterile distilled water and soaked in salt solution (10%, 500 mL) for 15 minutes. Thereafter wash it thrice with sterile distilled water to remove salt residues. Each fermented Avocado seed sample was oven-dried at 55 °C for 2-3 days. The dried chips were ground by using a blender and sieved with 8 mm mesh size.

E. Analysis of the Proximate Composition of Fermented and Unfermented Avocado Seed Flour

The method of analysis of the proximate composition of the Avocado seed flour is as described by [7], with slight modifications. The Proximate composition were analysed for both unfermented and fermented Avocado seed flour (24 h, 48 h, and 72 h) for the contents of moisture, crude protein, crude fibre, crude fat, ash, and total carbohydrate in triplicates.

1) Determination of moisture content

The method described in [7], with slight modifications was followed for the determination of moisture content of each avocado flour sample. Sample (3.00 g) was weighed into pre-weighed Petri dishes and dried in the oven (Philip Harris, UK) at 105±1 °C ± 1 for 2-3 h until a constant weight was maintained. Final dried samples were cooled and weighed.

2) Determination of crude protein content

The micro-Kjeldahl method of nitrogen analysis described in [7], with slight modifications was followed to determine the crude protein content of each dried avocado flour sample.

Sample (3.00 g) was added into a digesting tube that contained 25.00 mL of conc. H₂SO₄ and one catalyst tablet was heated at low temperatures for digestion. The digested Avocado seed flour samples were diluted with distilled water (100.0 mL) and NaOH (40%, 10.0 mL). In the presence of the Kjeldahl indicator, liberated NH₃ collected into a boric acid solution (4%, 10.0 mL), was titrated with HCl (0.1M). A blank was prepared without a sample.

3) Determination of crude fibre content

Crude fibre content was determined according to the method described in [7], with slight modifications. Defatted Avocado seed flour samples (3.00 g) were measured and added into a conical flask which contained H₂SO₄ acid (5%, 200.0 mL) and Sodium hydroxide (5%, 200.0 mL), respectively and the content was brought to the boiling point and kept boiling for 40 minutes while stirring with a glass rod keeping the volume constant by adding hot water time to time. The residues collected by filtering the content through the Buchner funnel into a filter paper, were dried at 105±1 °C in the oven (Philip Harris, UK) and, in the Muffle furnace (Wise Therma, South Korea) dried Avocado seed flour samples were ashed at 550±5°C.

4) Determination of ash content

Ash content was determined according to the method described in [7], with slight modifications. Sample (3.00 g) was weighed into a pre-weighed porcelain crucible and incinerated in a muffle furnace (Wise Therm, South Korea) at 550 °C for 6 h until a light grey ash was observed, and a constant weight obtained. The Avocado seed flour sample was cooled and weighed to obtain ash content.

5) Determination of crude fat content

The method described in [7], with some modifications was followed for the determination of the crude fat content of each Avocado seed flour sample. By using a Mojonnier flask, that was used to extract the fat of each dried Avocado seed flour sample with Hydrochloric acid (6N), crude fat content was determined.

6) Determination of total carbohydrate content

From the determined proximate contents, the carbohydrate content of each dry unfermented and fermented avocado seed flour sample was calculated by the following formula:

$$\text{Carbohydrate (\%)} = 100 - (\text{moisture\%} + \text{ash\%} + \text{crude protein\%} + \text{crude fat\%} + \text{crude fibre \%})$$

F. Analysis of Minerals of Unfermented and Fermented Avocado Seed Flour

The method of wet acid digestion described in [8], was employed to the determination of the concentration of sodium, potassium, calcium, magnesium, and iron of each Avocado seed flour sample by using an Atomic Absorption Spectrometer (GBC 2000, SavantAA).

G. Statistical Analysis

Data obtained in triplicates were carried out by one-way analysis of variance (ANOVA) technique. The Tukey's multiple comparison test used to identify the means that differ significantly at p<0.05. Results were expressed as mean ±

standard deviation, standard deviation of triplicate for proximate composition and minerals analysis which is important.

III. RESULTS

The results obtained for proximate composition analysis by

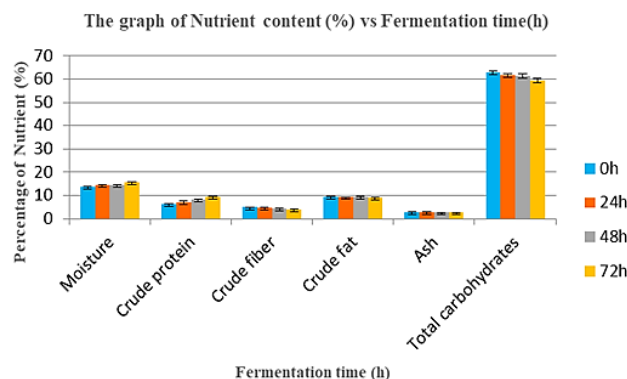


Fig. 1. Effect of fermentation of *L. plantarum* on the nutrient content of Avocado Seed flour (n= 3). Bars represent the mean ± standard deviation. Same letters mean no statistical difference between treatments at Tukey Pairwise Comparisons, $p < 0.05$.

nutritional assay of unfermented and fermented avocado seed flour samples (Table I) is shown in bar charts (Fig. 1). The composition of the most important minerals of dry unfermented and fermented Avocado seed flour samples (Table II) by the Atomic Absorption Spectroscopic analysis is shown in bar charts (Fig. 2). Those results were compared by one way ANOVA technique and Tukey's multiple comparison test.

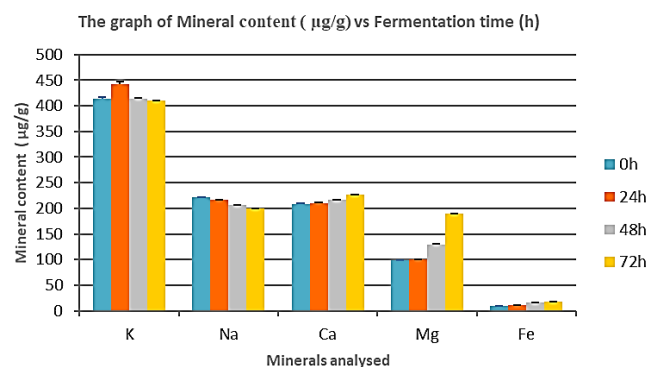


Fig. 2. Effect of fermentation of *L. plantarum* on the mineral content of Avocado Seed flour (n= 3). Bars represent the mean ± standard deviation. Same letters mean no statistical difference between treatments at Tukey Pairwise Comparisons, $p < 0.05$.

TABLE I: RESULTS OF PROXIMATE COMPOSITION OF UNFERMENTED AND FERMENTED AVOCADO SEED FLOUR

Fermentation time (h)	Nutrient content (%)					
	Moisture content*	Crude protein content*	Crude fiber content*	Crude fat content*	Ash content*	Total carbohydrates*
0	13.63 ^A ± 0.40	6.42 ^D ± 0.08	4.90 ^A ± 0.13	9.30 ^A ± 0.08	2.82 ^A ± 0.08	62.92 ^A ± 0.61
24	14.29 ^A ± 0.34	7.22 ^C ± 0.25	4.85 ^A ± 0.09	9.29 ^A ± 0.04	2.63 ^A ± 0.14	61.72 ^A ± 0.64
48	14.41 ^A ± 0.31	8.20 ^B ± 0.26	4.14 ^B ± 0.34	9.26 ^A ± 0.27	2.64 ^A ± 0.09	61.36 ^A ± 0.95
72	15.57 ^A ± 0.30	9.26 ^A ± 0.28	4.05 ^B ± 0.16	8.97 ^A ± 0.06	2.58 ^A ± 0.05	59.57 ^B ± 0.81

*The values are mean ± standard deviation of the replicates.

The values with common superscript letters in each column are not significantly different ($p < 0.05$).

TABLE II: RESULTS OF MOST IMPORTANT MINERALS CONTENT OF UNFERMENTED AND FERMENTED AVOCADO SEED FLOUR

Mineral	Mineral content (µg/g)			
	Unfermented flour	24h		72h
		fermented flour	48h fermented flour	fermented flour
Potassium (K) *	473.14 ^A ± 3.06	441.98 ^B ± 5.72	413.12 ^C ± 1.92	410.42 ^C ± 0.56
Sodium (Na) *	221.12 ^A ± 1.12	215.85 ^B ± 0.72	206.13 ^C ± 0.18	200.05 ^D ± 0.56
Calcium (Ca) *	208.89 ^D ± 0.30	210.41 ^C ± 0.52	215.83 ^B ± 0.64	227.06 ^A ± 0.05
Magnesium (Mg) *	99.05 ^C ± 0.01	100.06 ^C ± 0.05	129.81 ^B ± 1.21	189.22 ^A ± 0.33
Iron (Fe) *	9.04 ^D ± 0.01	11.40 ^C ± 0.01	17.00 ^B ± 0.01	18.11 ^A ± 0.06

*The values are mean ± standard deviation of the replicates.

The values with common superscript letters in each row are not significantly different ($p < 0.05$).

IV. DISCUSSION

A. Proximate Compositions of Unfermented Avocado Seed flour and fermented Avocado Seed Flour Samples

Moisture is the main factor that affects microbial growth and is considered as a critical factor for the shelf life of food. According to [9] the moisture content of powdered dry Avocado seeds is 15.10 ± 0.14 g/100 g. According to the results there was no significant effect ($p < 0.05$) on the moisture content of unfermented Avocado seed flour when compared with fermented Avocado seed flour for all the incubation periods. Hence, the fermentation process does not affect the moisture content of Avocado seed flour.

Minerals in our diet are very important to maintain healthy bones, muscles, heart, and brain functions. According to this study, there was no significant effect ($p < 0.05$) on the ash content of avocado seed flour upon the fermentation with *L.*

plantarum. Results obtained for the ash content for both fermented and dry unfermented seed flour agree with the results published in [10]. With an increase in the incubation period, there was a slight reduction in the total ash content in each of the samples. According to study of [11], the fermentation process that is carried out by microorganisms cause the extraction of soluble minerals into processing water, or usage of those minerals for the metabolic activities by microorganisms.

According to [12], *P. americana* is a good source of dietary protein and its high-fat content could contribute calories to man and animal ration. According to the study of [13], the protein content of seed flours increased with the fermentation. According to the results, it revealed that fermentation of avocado seed flour with *L. plantarum* shows a significant effect ($p < 0.05$) on the percentage crude protein content of seed flour. The results of this study highlight the gradual

increase in the crude protein content of fermented Avocado seed flour with the incubation period, in which there was a 44% increment at 72 h incubation period.

This increase in crude protein content was mainly due to the capability of microorganisms to degrade cellulolytic materials during the fermentation upon secretion of extracellular enzymes. Further, it may cause be caused by the increased production of single-cell proteins by microorganisms due to an increase in microbial nitrogen during their fermentation process [14].

Crude fibre is a quantity of indigestible cellulose, pentoses, lignin, and other components present in food that provides health benefits. According to the study in [9], the results show that the crude fibre content of the avocado pear seed flour was about 2.87%. This study shows a higher fibre content, and it could serve as a good dietary fibre source providing more health benefits. Crude fibre plays an important role to improve the bulkiness of food, the satisfaction of appetite, gastrointestinal motility, and improving the absorption and reabsorption of cholesterol and bile acids respectively, and thereby could lower cholesterol levels and prevent the formation of plaque [9]. According to the results of this study, there was no significant ($p < 0.05$) difference between the dry unfermented Avocado seed sample and the sample fermented for 24 h. However, there was a significant difference between the unfermented sample and those fermented for 48 h and as well as 72 h. It confirms that when carry out fermentation process for 48 h to 72 h, it may cause to significant decrease of crude fibre content of Avacado seed flour. According to the study of [11], it may cause due to fibrous tissues in seeds become soft during the microbial fermentation process and conversion of dietary fibre and lignocelluloses that present in seeds into proteins by microorganisms.

According to the results of this study, it is revealed that fermentation using *L. plantarum* does not significantly change ($p < 0.05$) the crude fat content. These results confirm the fact that fermentation using *L. plantarum* does not induce a significant effect ($p < 0.05$) on the crude fat content of avocado seed flour. According to the results of this study, it indicates that both fermented and dry unfermented avocado seed flour has a higher fat content than reported in [11]. Fermentation increases crude fat content upon forming simple fatty acids due to the breakdown of large fat molecules.

The carbohydrate content of Avocado seed flour, according to [10] is $47.35 \pm 3.24\%$ and in contrast, value stated in [9] showed $80.12 \pm 0.15\%$. The results obtained for this study show an intermediate value when compared with both fermented and dry unfermented Avocado seed flour samples. According to the results, total carbohydrate content has reduced during fermentation. According to the study in [11], it may be due to the formation of simple sugars upon the bioconversion of complex sugars during fermentation or use as an energy source for microbial growth and metabolism.

B. Analysis of the Quantity of Most Important Minerals of Unfermented and Fermented Avocado Seed Flour

In this study, Potassium (K), Sodium (Na), Magnesium (Mg), Calcium (Ca), and Iron (Fe) contents were determined in each of dry unfermented and fermented avocado seed flour sample. According to the results, Na and K contents were

significantly decreased ($p < 0.05$) with the fermentation periods. Na content was shown as 221.12 ± 1.12 , 215.85 ± 0.72 , 206.13 ± 0.18 , 200.05 ± 0.56 for 0h, 24h, 48h, and 72h respectively. K contents of each sample was shown as 473.14 ± 3.06 , 441.98 ± 5.72 , 413.12 ± 1.92 , 410.42 ± 0.56 for 0 h, 24 h, 48 h, and 72 h, respectively

Ca, Mg and Fe contents were significantly increased ($p < 0.05$) with the incubation periods. For 0 h, 24 h, 48 h and 72 h of fermented Avocado seed flour, Ca content was shown 208.89 ± 0.3 , 210.41 ± 0.52 , 215.83 ± 0.64 , 227.06 ± 0.05 and Mg content was shown 99.048 ± 0.01 , 100.06 ± 0.050 , 129.81 ± 1.21 , 189.22 ± 0.33 respectively. Fe content was shown 9.043 ± 0.01 , 11.40 ± 0.01 , 17.00 ± 0.01 , 18.11 ± 0.06 for 0 h, 24 h, 48 h and 72 h fermented Avocado seed flour samples respectively. According to the study of [15], it may cause due to the reduction of oxalate and phytate content of seeds with the fermentation which are complexed with minerals to affect the bioavailability of those minerals.

V. CONCLUSION

The percentage of moisture, crude protein, crude fat, crude fibre, and total carbohydrate content of dry unfermented Avocado seed flour is $13.63 \pm 0.40\%$, $6.42 \pm 0.08\%$, $9.30 \pm 0.08\%$, $4.90 \pm 0.13\%$ and $62.92 \pm 0.61\%$ respectively and for fermented Avocado flour (72h) are $15.57 \pm 0.30\%$, $9.26 \pm 0.28\%$, $8.97 \pm 0.06\%$, $4.05 \pm 0.16\%$ and $59.57 \pm 0.81\%$ respectively. Fermentation of Avocado seed flour using *L. plantarum* had significantly increased ($p < 0.05$) on crude protein content, crude fibre content and total carbohydrate content at the maximum incubation period of 72 hrs.

The contents of K, Na, Ca, Mg and Fe for dry unfermented avocado seed flour are $473.14 \pm 3.06 \mu\text{g/g}$, $221.12 \pm 1.12 \mu\text{g/g}$, $208.89 \pm 0.299 \mu\text{g/g}$, $99.05 \pm 0.006 \mu\text{g/g}$ and $9.04 \pm 0.012 \mu\text{g/g}$, respectively and in fermented Avocado seed flour (72 h), are $410.42 \pm 0.056 \mu\text{g/g}$, $200.050 \pm 0.56 \mu\text{g/g}$, $227.06 \pm 0.050 \mu\text{g/g}$, $189.22 \pm 0.327 \mu\text{g/g}$, and $18.11 \pm 0.06 \mu\text{g/g}$, respectively. The contents of Fe, Mg and Ca had significantly ($p < 0.05$) increased with the incubation period (72h) and K and Na contents were significantly ($p < 0.05$) reduced with fermentation period of Avocado seed flour. Fermentation of Avocado seed flour for 72hrs enhance the nutritional quality.

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