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Original article

Fermented Hass avocado kernel: Nutritional properties and use in the manufacture of biscuits

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ABSTRACT

In this study, the use of fermented Hass avocado kernel (FHK) with *Lactobacillus plantarum* to produce functional biscuits was examined. The chemical composition and antinutrient factors were evaluated for raw and fermented Hass avocado kernels. Fatty acids were separated by gas liquid chromatography. The physical properties, color, and sensory attributes of the biscuits were assessed using professional methods. The protein increased by 54% after fermentation to become (7.93%) in FHK while it was 5.15% in raw Hass avocado kernel (RHK). The crude fiber and ash decreased after fermentation by 18% and 8%, respectively. A significant ($p < 0.05$) increase was recorded in total phenol content, antiradical effect against DPPH and flavonoid content of FHK compared with RHK. After fermentation, reduction of tannins content was 80.76%, oxalates content 89.95%, alkaloids 70%, while traces of phytates and saponin were detected. The relative density, saponification value and iodine value of FHK oil were 0.917 g/ml, 212.26 mg KOH/g oil and 72.74 g Iodine/100 g oil, respectively. FHK oil had the following sequence: PUFA (51.54%) > SFA (26.72%) > MUFA (21.83%). The highest spread ratio (6.17) was recorded in biscuits produced by replacing 10% of FHK. Difference between the biscuit samples in the color from all treatments was completely compatible with the sensory evaluation results. Substituting 5% and 10% of FHK flour significantly improved both the brittleness and the total percentage of replacement.

1. Introduction

Avocado (*Persea americana* Mill) is an evergreen eatable fruit belonging to the family Lauraceae cultivated all over the world (Talabi et al., 2016). The pulp of the fruit is thick; rich in vital compounds especially vitamin A, B, and E; folic acid; fatty acids such as linoleic, oleic, stearic, and myristic acids (Emelike et al., 2020). Hass avocado is becoming the main avocado cultivated in the world with a high acceptance due to its high pulp content, late maturity, and the ability to withstand relatively long storage (García Villacreses, and Guerrero Chica, 2021). The size of Hass fruit is medium, 8 to 10 cm long, weight varies from 150 to 400 g, it is rough and corky in texture, with a rough and grainy surface, and its skin has a green color that darkens from purple to black when mature. Many studies are underway to determine how we may take advantage of the nonedible parts of plant materials and to explore their nutritional benefits, which may soon prove to be profitable. To preserve the environment, methods must be taken to make use of waste especially since the inedible parts of many fruits and vegetables such as avocados contain bioactive compounds in high amounts (Le et al., 2021). Chemically, avocado seeds contain phytosterols, fatty acids, triterpene, and glucosides of abscisic acid (Espinosa-Garza et al., 2019), which give seeds many biological activities including antioxidant, antihypertensive, fungicidal, larvicidal, and hypolipidemic activities (Suvanto et al., 2017). The beneficial effects of avocado kernels, which account for 13–18% of the fruit (Rozan et al., 2021) provide an interesting alternative for future study. The avocado kernels contain 70% of the antioxidants contained in the whole avocado, as well as polyphenols, and soluble fiber which reduces cholesterol and aids in disease prevention (Dabas et al., 2019). However, high concentrations of antinutritional factors mainly tannins, saponins, oxalates, phytates, and alkaloids are found in avocado kernel flour (Tanwar et al., 2018), it is necessary to minimize the level of antinutritional compounds, which can be achieved by many technolog-

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ical treatments, including soaking, roasting, germination, and fermentation (Ojha et al., 2020; Le et al., 2021). According to Kumari and Amarakoon (2021), fermentation using *Lactobacillus plantarum* as starter culture at 37 °C for 72 h are the best conditions for reducing the antinutrient content of avocado kernels. These bacteria are popular and widely used as a simple, high safety, ease of handling, cost-effective, and enhancing nutritional and biologically active compounds compared to other treatments (Mechmeche et al., 2017; Teng et al., 2021). The fermentation by lactic acid bacteria can improve the antioxidant and textural properties of breads (Sidari et al., 2020) And the fermentation decreased tannins and phytic acid contents in wheat bran (Eiman et al., 2008). Globally, there is a growing trend to increase the use of vegetable protein, and to increase the use of sources of healthy oils, fiber, and antioxidants in the production of bakery products, while reducing carbo-derived products in the diet (Al-Saab, and Gadallah, 2021; Mecha et al, 2021; Giuffrè et al., 2022). No adequate studies have been conducted on the effect of fermenting Hass avocado kernel on the bioactive contents and nutritional applications of the resulting fermented kernel. So, this study was designed to estimate the effects of fermentation on the nutrient and antinutrient content of Hass avocado kernels, which use as functional food sources in the biscuit industry and to improve environmental protection by reducing food waste.

2. Materials and methods

2.1. Materials

Hass avocado fruits were taken from orchards of PICO Modern Agriculture Compony (latitude: 30.53°, longitude: 30.794°) in El-Beheira governorate, Egypt. The soil of orchards is light clay with good drainage and ventilation, the local climate is moderate to hot and humid, the wind speed is light, and the temperature often ranges between 13 and 35 °C, the distance between plants on the line is 4 m, and the distance between the lines is 6 m, the trees are 12 years old, sixty fruits were randomly collected from 2 ha of the middle of the orchard. All fruits were harvested at the maturity stage after 190–200 days from full bloom, a non-damaged, absence of visible decay, and fresh avocado fruits were collected during November 2020.

Wheat flour (72% extraction) and other materials for biscuit preparation were collected from the local market in Damanhour city, Egypt. The *Lactobacillus plantarum* strain was obtained from the Microbiology Laboratory, Department of Food Sciences, Alexandria University. Chemicals and reagents were bought from Sigma-Aldrich in the United States.

2.2. Methods

2.2.1. Preparation of fermented Hass avocado kernel flour

The fruits were processed to obtain 5 kg of kernels, which were collected, washed, cut into 1–3 mm thick slices, and then subjected to steam for 10 min. Steamed slices were dried at 60 °C using an oven to a constant weight. The *L. plantarum* strain was used to ferment avocado kernel slices. The fermentation procedure illustrated by Kumari and Amarakoon (2021) was used with few alterations. Fermented avocado kernels were oven-dried at 60 °C for 24 h. A multi-speed electric grinder (Model No.: MB-355, China) at speed 2 used to allow the fermented avocado kernel flour to pass through 35 mesh. The fermented flour was kept refrigerated in airtight plastic containers.

2.2.2. Preparation of biscuits

Biscuits were manufactured by partially replacing soft wheat flour (72% extraction) with 5%, 10%, 15% or 20% of FHK flour. Table 1 shows the modified recipe for the standard procedure for semihard sweet biscuits produced by Senyorita, El-Sadat, Egypt, which was adapted after preliminary experimentation. An electric mixer (LFO60546, China) used to obtain smooth mixture form sugar, fat, eggs and powdered milk. Then, flour, baking powder, and salt were added to form a soft dough. Then kneaded, rolled out into sheets, cut into the desired shape and transferred to bake at 180 °C for 17 min.

2.2.3. Proximate chemical analysis

Following the AOAC Method (2000), the moisture, protein (N × 6.25), ash, and crude fiber contents were determined. A Soxhlet apparatus was used to extract Hass avocado kernel oil using hexane at 70 °C for 8 h. Extraction was carried out on dried powder. Carbohydrate content was obtained using the following equation:

$$\begin{aligned}\%\,\text{carbohydrates} = 100 - (\% \;\text{moisture content} \\ + \text{protein content} + \text{ash content} \\ + \text{oil content} + \text{crude fiber content})\end{aligned}$$

2.2.4. Antinutrient determination

Total tannins were quantified by the Folin and Ciocalteu method as reported by Makkar et al. (1993), in which tannic acid is used as the standard. The total saponins were quantified using the spectrophotometric methodology reported by Brunner (1984). Oxalate concentration was detected by Nwinnuka et al. (2005) procedure. Phytate content was determined following the methodology reported by Vaintraub and Lapteva (1988), in which the color reading is recorded by a spectrophotometer at 500 nm. The alkaloids content was estimated in accordance with Mulder-Krieger et al. (1982). All determinations were achieved in triplicate and presented as mg/100 g sample.

2.2.5. Physicochemical properties of Hass avocado kernel oil

Relative density, refractive index, relative viscosity, saponification, iodine value, and acid value were determined using the methods of the American Oil Chemists' Society.

2.2.6. Fatty acid analysis

The fatty acid profile for Hass avocado kernel oil was determined using methyl ester and gas liquid chromatography based on Radwan (1978) technique.

2.2.7. Extraction and analysis of total phenolic content and total flavonoids

The total phenolic compounds and total flavonoids in avocado kernels were extracted according to Di Stefano et al. (2017), then follow the technique described by Zilic et al., (2012) to assess the total phenolic and total flavonoid content.

2.2.8. Determination of radical DPPH scavenging activity

Stable DPPH, as developed by Hwang and Do-Thi (2014), was used to evaluate the avocado kernel's antioxidative ability. Trolox was used to create the standard curve. The following equation was used to measure the DPPH free radical inhibition:

$$\text{Inhibition}\,(\%) = 100 \times [(\text{A control} - \text{A sample})/\text{A control}]$$

where A control is the absorbance of the blank and A sample is the absorbance of the test samples.

Table 1
Recipe of biscuits prepared using different proportions of Hass avocado kernel flour as a substitute for wheat flour.

Ingredient (g)	Control	5-FHK	10- FHK	15- FHK	20- FHK
Wheat flour (72% extraction)	100	95	90	85	80
FHK	0	5	10	15	20
Shortening	25	25	25	25	25
Sugar	25	25	25	25	25
Fresh whole egg	8	8	8	8	8
powdered milk	2	2	2	2	2
baking powder	1	1	1	1	1
salt	0.5	0.5	0.5	0.5	0.5
Vanilla	0.3	0.3	0.3	0.3	0.3

DPPH free radical inhibition data are reported as μmol Trolox equivalents (TE)/100 g sample.

2.2.9. Determination of biscuits' physical properties

The physical properties of the biscuits were established following the procedure explained by Adeola and Ohizua (2018) with little alteration. Biscuit thickness and diameter were determined using digital Vernier calipers for an average of six biscuits, with the value reported in millimeters. Then, calculated the spread ratio (diameter/thickness). The average weight of biscuits was measured using six individual biscuits with laboratory weighting balance. Hardness (N) expresses the maximum load (N) determined by using a Textile analyzer (Stable Micro Systems Serial No. 5014 England) TA-XT Plus.

2.2.10. Color measurements of biscuits

The L*, a*, and b* values for biscuits color were evaluated using a Konica Minolta CR-410 Chroma meter (Konica Minolta, Sensing, Inc., Japan). The following equation was used for calculating the total color difference (ΔE):

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5}$$

where $\Delta L = L_{sample} - L_{standard}$, $\Delta a = a_{sample} - a_{standard}$, and $\Delta b = b_{sample} - b_{standard}$.

2.2.11. Sensory evaluation of biscuits

Appearance (symmetry), color, aroma, taste, hardness, crunchiness, and overall acceptability of the coded biscuit samples were evaluated for by a 36-member panel using a 9-point hedonic scale (1 indicates very poor, and 9 indicates excellent). Hardness is the force required to fully chew through the sample when put between molars.

2.2.12. Statistical analysis

All the trials were run in triplicate. The data were studied using SPSS 16 software and are reported as mean ± SD and compared by one-way ANOVA. The results were considered significant at $p < 0.05$.

3. Results

The chemical composition of raw and fermented Hass avocado kernel flour is shown in Table 1. The mean moisture and protein percentages of the raw and fermented Hass avocado kernel flour significantly increased after fermentation ($p < 0.05$); the opposite occurred for crude fiber and ash contents, which significantly decreased after fermentation. The oil extract and carbohydrate contents were not significantly affected by fermentation. The moisture content increased after fermentation by 17%: moisture content was 12.41% in RHK and 14.55% in FHK after fermentation. The protein content increased by 54% after fermentation to 7.93% in FHK and 5.15% in RHK. The crude fiber and ash contents decreased by 18% and 8%, respectively. The crude fiber content was 6.07% in FHK and 7.37% in RHK before the fermentation. The ash content decreased from 2.11% to 1.93% in the unfermented and fermented Hass avocado kernel flour, respectively.

Regarding to minerals, a significant increase ($p < 0.05$) was noticed after avocado kernel fermentation in the contents of Ca, Mg, Fe, P, and Zn, as shown in Table 2. Calcium content was 17.23 and 18.73 mg/100 g for RHK and FHK, respectively. Magnesium content was 0.21 and 0.40 mg/100 g for raw and fermented Hass avocado kernel flour, respectively; Fe and Zn contents doubled from 1.23 to 2.46 mg/100 g and 0.24 to 0.49 mg/100 g after 72 h of fermentation, respectively. Phosphorus content was 27.25 and 29.67 mg/100 g for RHK and FHK, respectively. Potassium was the highest mineral element found in raw kernel flour (78.32 mg/100 g), which decreased to 67.88 mg/100 g after fermentation with Lactobacillus plantarum for 72 h. Sodium content was 2.37 and 2.14 for RHK and FHK, respectively. Mn content did not significantly change after fermentation.

The compositions of the most important phytochemicals and antinutrients of raw and fermented Hass avocado kernels are recorded in Table 3. The total phenolic content increased twofold from 23.01 to 49.36 mg GAE/g in both raw and fermented Hass kernel. The flavonoid content was observed to be high in fermented kernel (1.52 mg quercetin/g) compared to raw kernel (1.75 mg quercetin/g). The data presented in Table 3 demonstrate that the tannin content decreased by 80.76% after fermentation to 1.26 mg/100 g, oxalates content decreased by 89.95% to 0.43 mg/100 g in FHK, and the number of alkaloids decreased by 70% to 0.66 mg/100 g in FHK. The major parameters that characterize the fermented Hass avocado kernel oil quality presented in Table 4. FHK oils were very light yellow in color. The relative den-

Table 2
The chemical composition of raw Hass avocado kernel (RHK) flour and fermented Hass avocado kernel (FHK) flour.

Component	RHK	FHK
Moisture %	12.41 ± 1.63b	14.55 ± 1.68a
protein %	5.15 ± 0.33b	7.93 ± 0.41a
Oil extract %	3.93 ± 0.28a	3.79 ± 0.69a
Crude fiber %	7.37 ± 0.63a	6.07 ± 0.17b
Carbohydrates %	69.03 ± 1.05a	65.72 ± 2.06a
Ash %	2.11 ± 0.17a	1.93 ± 0.35b
Ca (mg / 100 g)	17.23 ± 0.01b	18.73 ± 0.42a
Mg	0.21 ± 0.001b	0.40 ± 0.06a
Fe	1.23 ± 0.007b	2.46 ± 0.11a
P	27.25 ± 0.07b	29.67 ± 0.76a
K	78.32 ± 0.17a	67.88 ± 0.40b
Na	2.37 ± 0.05a	2.14 ± 0.25b
Zn	0.24 ± 0.01b	0.48 ± 0.00a
Mn	0.19 ± 0.001a	0.21 ± 0.06a

The data are expressed as the mean ± standard deviation (n = 6). Values followed by different letters in rows are significantly different at $p < 0.05$.

Table 3
Phytochemical and antinutrients analysis of raw Hass avocado kernel flour and fermented Hass avocado kernel flour.

Components	RHK	FHK
Total phenolics (mg GAE/ g)	23.01 ± 0.86b	49.36 ± 1.25a
Total flavonoids (mg quercitine /g)	1.52 ± 0.73b	1.75 ± 0.03a
DPPH (μ mol TE/g)	148.33 ± 3.69b	105.58 ± 3.69a
Tannin (mg/ 100 g)	6.55 ± 0.61a	1.26 ± 0.07b
Saponin (mg/ 100 g)	5.32 ± 0.37a	0.08 ± 0.00b
Oxalates (mg/ 100 g)	4.28 ± 0.17a	0.43 ± 0.13b
Phytates (mg/ 100 g)	1.19 ± 0.05a	0.15 ± 0.06b
Alkaloids (mg/ 100 g)	2.14 ± 0.11a	0.66 ± 0.10b

The data are expressed as the mean ± standard deviation (n = 6). * RHK: raw Hass avocado kernel flour, * FHK: fermented Hass avocado kernel. Values followed by the different letters in rows are significantly different at $p < 0.05$.

Table 4
Physical and chemical properties of fermented Hass avocado Kernel oil.

Relative density (20 °C/20 °C)	0.917 ± 0.002
Refractive index (40 °C)	1.465 ± 0.001
Saponification value (mg KOH/g oil)	212.257 ± 0.36
Iodine value (g I_2 /100 g oil)	72.74 ± 1.18
Acid value (mg KOH/g oil)	2.13 ± 0.15
Free fatty acids (% as oleic acid)	0.47 ± 0.02
Peroxide value (m Eq/ kg oil)	4.61 ± 0.11

The data are expressed as the mean ± standard deviation (n = 6).

sity of FHK oil was 0.917 g/ml. The refractive index value obtained for FHK oil (1.465).

The saponification value of fermented Hass avocado kernel oil was 212.26 mg KOH/g oil. The iodine value of FHK oil was 72.74 g/100 g. The acid value of FHK oil was found to be 2.13 mg KOH/g oil. The free fatty acid of fermented avocado kernel oil was 0.47%, as oleic acid. The peroxide value of FHK oil was determined to be 4.61 meq/kg.

The fatty acid lipid profile of FHK illustrated a prevalence of unsaturated fatty acids (51.51%), especially linoleic acid, which accounted for 39.76% of the total fatty acids. Palmitic (21.75%) and stearic (1.33%) acids were the saturated fatty acids found in the largest quantities in FHK oil.

The physical properties of biscuits produced by substituting different percentages of avocado kernel flour, including diameter, weight, thickness, spread ratio, and hardness, are shown in Table 5. The results show that replacing 20% of wheat flour with avocado kernel flour did not significantly affect both the weight and thickness of the produced biscuits, and the diameter of the produced biscuit samples ranged between 39.4 mm in the control samples to 40.7 mm in the produced samples with the replacement of 10% of wheat flour with avocado kernel flour. The highest spread ratio of 6.17 was recorded in the samples produced by replacing 10% of the wheat flour with avocado kernel flour, and the lowest value (5.95) was recorded in the control sample ($p < 0.05$).

Table 5
Fatty acid profile of fermented Hass avocado Kernel oil.

Fatty acid	Fatty acid methyl ester content (%)
10:0	0.4 ± 0.001
12:0	0.73 ± 0.007
14:0	0.22 ± 0.001
16:0	21.75 ± 0.054
16:1	3.88 ± 0.004
17:0	0.85 ± 0.01
18:0	1.33 ± 0.08
18:1	17.43 ± 0.12
18:2	39.76 ± 0.71
18:3	11.75 ± 0.12
20:0	0.75 ± 0.007
20:1	0.48 ± 0.005
22:0	0.26 ± 0.004
24.0	0.41 ± 0.01
Σ SFA	26.70
Σ MUFA	21.79
Σ PUFA	51.51
PUFA/SFA ratio	1.93

The data are expressed as the mean ± standard deviation (n = 6).

The hardness of the produced biscuits ranged from 96 N in the samples produced by replacing 20% of the wheat flour with avocado kernel flour to 103 N in the samples produced by substituting 5% and 10% of the wheat flour with avocado kernel flour.

Table 6 presents the results of the color evaluation of the biscuit samples produced by substituting wheat flour with different proportions of fermented Hass avocado kernel flour. The results show that increasing the proportion of avocado kernel flour substitution resulted in a gradual significant increase in the a* (redness) and b* (yellowness) values, and a gradually significant decrease in lightness. Table 7 shows the results of the sensory evaluation of biscuits produced by substituting of different levels of wheat flour with avocado kernel flour. The results show that the odor of the produced biscuits was not significantly affected ($p < 0.05$) by substitution up to 20%, and neither appearance nor color were significantly affected ($p < 0.05$) by substituting wheat with avocado flour to a level of 15%, but by raising the percentage of substitution to 20%, these properties decreased significantly ($p < 0.05$). The hardness was significantly negatively affected by raising the level of substitution to 15%, and the samples produced by 20% substitution were significantly softer ($p < 0.05$) than the other samples (see Table 8).

4. Discussion

During fermentation, the ability of microorganisms to enzymatically degrade cell walls increases, microbial nitrogen increases and the production of single-celled proteins by microorganisms are the main reasons behind the increase in crude protein content (Munishamanna et al., 2017). The decrease in crude fiber after fermentation may be due to the conversion of part of the crude fiber and lignocellulosic compounds into protein through microbial fermentation, as reported by Igbabul et al. (2012), or to the enzymatic breakdown of fibers by fermented microorganisms, which approves with the conclusions of Ojokoh and Eromosele (2015) who stated a reduction in crude fiber from corn and pumpkin mix after fermentation. The decrease in the ash content may be because of the leaching of soluble substances to the treatment water or were used by microorganisms during the metabolism process through the fermentation period (Ogbonnaya et al., 2010; Igbabul et al., 2012). The same trend in moisture, protein, ash, fiber, and carbohydrate in was reported by Emelike et al., (2020); Kumari and Amarakoon (2021). Whereas Ejiofor et al., (2018) found a higher contents in avocado seeds than in the current study: 49.03% carbohydrate, 17.90% lipid, 15.5% protein, 15.10% moisture, and 2.26% ash, which may be due to the different variety and cultivation conditions. Avocado seeds are characterized by their high carbohydrate content and low ether extract content. Therefore, the avocado kernel flour can be used as a healthy raw material that can be used as a good substitute to wheat flour and starch in food applications (Araújo et al., 2018).

The observed increases and decreases in mineral concentrations after incubation with *Lactobacillus plantarum* for 72 h are likely due to microbial degradation of carbohydrates and proteins and the consequent reduction in dry matter (Day and Morawicki, 2016; Nkhata et al., 2018) and metabolic activities of fermented microorganisms associated with the decomposition of oxalate and phytate to release free minerals (Nnam and Obiakor 2003; Pranoto et al.,

Table 6
Physical properties of biscuits produced with different rates of fermented Hass avocado kernel flour.

	Control	5-FHK	10- FHK	15- FHK	20- FHK
Diameter (mm)	39.4 ± 0.1c	40.1 ± 0.15b	40.7 ± 0.11a	40.2 ± 0.08b	39.6 ± 0.09c
Thickness (mm)	6.6 ± 0.05a	6.6 ± 0.1a	6.6 ± 0.1a	6.6 ± 0.1a	6.6 ± 0.1a
Spread ratio	5.95 ± 0.7bc	6.08 ± 0.65ab	6.17 ± 0.54a	6.08 ± 0.72ab	6.01 ± 0.51b
Weight (g)	13.53 ± 0.14a	13.58 ± 0.15a	13.61 ± 0.1a	13.59 ± 0.07a	13.51 ± 0.11a
Hardness (N)	97c	103a	103a	100b	96d

The data are expressed as the mean ± standard deviation (n = 6). * FHK: fermented Hass avocado kernel, where 5-, 10-, 15-, and 20-FHK indicate the ratio of fermented Hass avocado kernel flour replacement. Values followed by different letters in rows are significantly different at $p < 0.05$.

Table 7
Color attributes of biscuits prepared from wheat flour blends with Hass avocado kernel flour.

samples	L*	a *	b *	ΔE
Control	82.58 ± 0.04a	2.57 ± 0.06e	33.62 ± 0.05e	0
5-FHK	79.14 ± 0.05b	5.28 ± 0.03d	35.61 ± 0.04d	4.81 ± 0.22d
10-FHK	78.94 ± 0.04b	6.05 ± 0.03c	37.18 ± 0.04c	6.17 ± 0.13c
15-FHK	77.12 ± 0.03c	6.88 ± 0.04b	38.47 ± 0.05b	8.48 ± 0.17b
20-FHK	76.49 ± 0.05d	7.56 ± 0.05a	39.71 ± 0.05a	9.95 ± 0.24a

The data are expressed as the mean ± standard deviation (n = 6). * FHK: fermented Hass avocado kernel, where 5-, 10-, 15-, and 20-FHK express the ratio of fermented Hass avocado kernel flour replacement. Values followed by different letters in the same column are significantly different at $p < 0.05$. Lightness (L): L = 0 for darkness, L = 100 for lightness; a*: chromaticity on a scale of green (−) to red (+); b: chromaticity on scale of blue (−) to yellow (+), 90° = yellow; 180° = bluish to green and 270° = blue scale.

Table 8
Organoleptic properties of biscuits produced with different amounts of fermented Hass avocado kernel flour.

	Control	5-FHK	10- FHK	15- FHK	20- FHK
Appearance	7.15 ± 0.52a	7.22 ± 0.47a	7.11 ± 0.39a	7.04 ± 0.45a	6.75 ± 0.13b
Color	7.27 ± 0.23a	7.35 ± 0.17a	7.24 ± 0.19a	7.11 ± 0.14a	6.63 ± 0.36b
Odor	7.16 ± 0.33a	7.22 ± 0.18a	7.24 ± 0.21a	7.19 ± 0.14a	7.15 ± 0.28a
Taste	7.04 ± 0.11b	7.42 ± 0.56a	7.44 ± 0.43a	7.39 ± 0.46a	7.13 ± 0.12b
Hardness	7.72 ± 0.41a	7.74 ± 0.35a	7.69 ± 0.47a	7.59 ± 0.12b	7.45 ± 0.16c
Crunchiness	7.16 ± 0.11b	7.36 ± 0.16a	7.30 ± 0.13a	7.19 ± 0.09b	7.05 ± 0.12c
Overall acceptability	7.21 ± 0.18b	7.41 ± 0.22a	7.35 ± 0.13a	7.23 ± 0.24b	7.03 ± 0.16c

The data are expressed as the mean ± standard deviation (n = 36). * FHK: fermented Hass avocado kernel, where 5-, 10-, 15-, and 20-FHK express the amount of fermented Hass avocado kernel flour replacement. Values followed by different letters in rows are significantly different (LSD) at $p < 0.05$.

2013; Nkhata et al., 2018). Helen and Okhonlaye (2019) reported increases in Ca, Fe, and Mg contents in soy milk with the increase in the natural fermentation period. Ekundayo et al. (2013) also reported increases in the Mg, Ca, Na, and P contents of African bush mango seeds after fermentation. As shown in Table 2, the contents of protein, calcium, phosphorus, potassium, iron, and zinc in Hass kernels, which are usually disposed of in landfills, are high and increase with fermentation, indicating that this inedible part of the avocado requires more attention. According to a study published by the California Avocado Association, avocado seeds contain lower concentrations of magnesium and calcium, which are necessary for blood clotting, muscle contraction, and strengthening teeth and bones. Avocado seeds are high in potassium and phosphorous. Potassium maintains a steady heartbeat and fluid balance in the body, and phosphorous is necessary for strong bones and the synthesis of DNA and phospholipids, which carry fats through the bloodstream (Mahawan et al., 2015). Runyogote (2021) found that the increase in total phenolic content in fermented avocado seeds varied from 18.22 to 33.3 mg GAE/g and the highest increase (45.3%) was detected at a temperature of 37 °C within 24 h. The increase in the total phenolic content can be explained by the effect of proteolytic enzymes from the *Lactobacillus plantarum* bacteria, which usually hydrolyze phenolic complexes into soluble free phenols during fermentation (Adetuyi and Ibrahim, 2014). As a result, the increased or retained total phenolic contents observed during avocado kernel fermentation can be attributed to the opening of the cell matrix, which assists total phytochemical bioavailability, as well as breakdown of the ester linkages by enzymes during probiotic fermentation (Tian et al., 2016). About antioxidant effect against DPPH, fermented Hass avocado kernels a significant ($p < 0.05$) increase showed after fermentation in agreement with that decided by Wang et al., (2006) for fermented soy milk and Jamro and Starzyn (2008) for fermented grass pea seeds. The enhanced antioxidant activity reported for fermented avocado kernels might be attributed to an increase in hydroxyl groups or amino groups in antioxidant compounds as well as phenolic production (Olawoye et al., 2017). Significant increase in the total flavonoids was also noticed by Dulf et al., (2017) in fermented pomaces with *Aspergillus niger* after 3 days. Yepes-Betancur et al., (2021) used the solid state to ferment Hass avocado seed with *Aspergillus niger* to release bioactive compounds with antioxidant capacity. The increase in flavonoid content after fermentation may be due to the increase in the acid value, which occurs due to the release of bound flavonoid components, making them more bioavailable. There are few studies that illustrate the effect of fermentation processes on the active compounds in avocado kernel. A significant reduction in antinutrients ($p < 0.05$) were found in Hass kernel after fermentation by starter cultures of *L. plantarum* for 72 h at 37 °C compared with raw Hass avocado kernel. The highest antinutrients content in fermented Hass avocado kernel was identified for tannin followed by alkaloids and oxalate, and traces of phytates and saponin were detected after fermentation. The statistically significant reduction in the level antinutrients after fermentation may be due to the action of lactic acid bacteria to produce different levels of metabolites, which can remove or reduce antinutrients, in addition to the solubility of some antinutrients in water. Similar trends were described in other studies by Runyogote (2021) in fermented avocado seed. In compare with current study, high content of antinutrients reported by Ibhaze (2017) after heat treatment for 60 min in pear avocado seeds: the contents of saponin, alkaloids, oxalate, phytate, flavonoids, and tannin were 10.83, 1.6 1.44, 10.34, 6.16, and 0.02 mg/100 g, respectively. The differences may be attributed to the different treatments, cultivars, climatic conditions, harvesting time, nature of the seeds (unripe or ripe), and soil properties. The relative density of FHK oil is close to the 0.9032 g/ml reported by Orhevba and Jinadu (2011) for avocado oil seed, the 0.905 g/ml for melon seed oil reported by Giwa et al. (2010), and the 0.918 g/ml reported for groundnut, and less dense than the 0.939 g/ml reported for neem seed (Akpan, 1999). The refractive index value obtained for FHK oil (1.465) is in the acceptable range for edible oil and is in close agreement with values reported for conventional oils from avocado seed (1.457) determined by Otaigbe et al. (2016), soybean (1.466–1.470) and palm kernel (1.449–1.451) reported by Bello et al., (2011). The high refractive index of fermented Hass avocado kernel oil seems to confirm the carbon chain length and double bond increases in their fatty acids (Eromosele and Paschal 2003). The saponification value of fermented Hass avocado kernel oil is significantly higher compared with the values for common oils, such, palm oil (196–205 mg KOH/g oil), groundnut oil (188–196 mg KOH/g oil), and corn oil (187–196 mg KOH/g oil) (Eromosele and Paschal 2003). The iodine value is an indicator of the number of double bonds of the fatty acids that make up the oil, which reflects the oxidation susceptibility of the oil. The iodine value of FHK oil is greater than the 38.35 and 43.86 g/100 g for avocado seed oil reported by Otaigbe et al., (2016) and Gidigbi et al., (2019), respectively. A low iodine value implies the stability of the oil with oxidation, so the oil can be considered a non-drying oil. The acid value of FHK oil is very close to the 2.06 mg KOH/g reported by Bora et al., (2001), while is lower than 3.01 mg KOH/g reported by Otaigbe et al., (2016) for avocado seed oil. According to Peace and Aladesanmi (2008) FHK oil suitable for consumption purposes because the acid value is<4 mg KOH/g. Free fatty acid is an important variable when considering the quality of oil because the lower the free fatty acids, the better the quality of the oil. Our data indicate that avocado seed oil has low levels of hydrolysis and lipolytic activities. The free fatty acid of fermented avocado kernel oil value (0.47%) is close to the 0.51% determined by Gidigbi et al., (2019) in unfermented avocado kernel oil. This value obtained shows that avocado seed oil is suitable for human consumption according to Gerhard Knothe and Krahl (2004), who concluded that refined edible oil usually contains<0.5% free fatty acids. The acid and free fatty acid values are two parameters determine if the oil can be applied for either consumption or industrial use. The peroxide value is the measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation and can be defined as a reaction between fatty acids and oxygen, which results in an oxidative degradation of lipids (Rossi-Márquez et al., 2021). Otaigbe et al. (2016) reported a peroxide value of avocado seed oil of 45 meq/kg.

The general proportions of fatty acids (Table 4) of fermented Hass avocado kernel had the following sequence: PUFA (51.51%) > SFA (26.70%) > MUFA (21.79%). In the current study, the PUFA/SFA ratio (1.93) is higher than the minimum advised by the British department of health (1994), which is 0.45. Galvão et al., (2014) and Ge et al., (2018) computed the UFA/SFA ratios in avocado kernel, which ranged from 1.81 to 2.62, which agrees with the current study results. The results of current study agree with those of Aliakbarzadeh et al., (2016), who reported that the major fatty acids in avocado kernel obtained from GC–MS data were linoleic acid (51.9%), palmitic acid (22.5%), oleic acid (15.7%), and linolenic acid (5.8%), and the quantified minor fatty acids were palmitoleic acid (1.9%), myristic acid (0.7%), cis11-eicosenoic acid, (0.5%), erucic acid (0.4%), behenic acid (0.3%), and myristoleic acid (0.2%). Jaen (2005) explained that high levels of unsaturation are important because they play a vital function in lowering blood cholesterol levels and treating atherosclerosis.

Color is one of the most important quality parameters of baking products. It is usually described using the Hunter color parameters, which include lightness (L*), redness/greenness (a*), yellowness/blueness (b*), and total color difference (ΔE), the last of which is used to describe the color differences between different samples (Rozan, 2017). When replacing some components of a food product with alternatives of economic or nutritional value, it is desirable to ensure the color is as close as possible to the original to maintain the level of consumer acceptance.

Theoretically, when the total color difference between two samples is one, the human eye can perceive the color difference between them (Vervoort et al., 2012). The calculated values for the color difference between the samples in Table 6 show that it would be theoretically possible to perceive the color difference between the biscuit samples resulting from all the treatments. However, by linking this result with the results of the sensory evaluation, this difference in color was not sufficient for consumers to reject the color of the biscuit samples produced by substituting different amounts of wheat flour with fermented Hass avocado kernel flour until the substitution level reached 20%. These results can be explained by the breadth of consumers' color preference for biscuits. These results may be due to fermented Hass avocado kernel containing polyphenol oxidases activities, which are responsible for the production of brown and yellow–red pigments (Hatzakis et al., 2019). Therefore, due to enzymatic browning, the lightness of the biscuits may have decreased. Dabas et al. (2011) found that when avocado kernels are crushed and exposed to oxygen, a vibrant and stable orange pigment develops in a polyphenol-oxidase-dependent reaction.

The substitution of 15% of wheat flour with fermented Hass avocado kernel flour improved the taste of the produced biscuit compared to the control samples, and 20% substitution significantly reduced this improvement ($p < 0.05$). The substitution of 5% and 10% of wheat flour of fermented Hass avocado kernel flour significantly improved the crunchiness as well. In summary, substituting 10% of the wheat flour with avocado kernel flour improved the acceptability of biscuits produced compared to biscuits produced from 100% wheat flour.

Organoleptic properties results of biscuits can be attributed to the fact that biscuit dough is a product that does not need to form a cohesive gluten network, as it was not affected by substituting wheat flour with fermented Hass avocado kernel flour. Some of the soluble proteins and peptides in avocado kernel can also improve the odor and color of the produced biscuit without affecting the texture parameters. Increasing the percentage of replacement to high levels (15% and 20%), the perceived decrease in the amount of wheat flour starch played a role in the decline in this improvement in texture characteristics in addition to the color, appearance, and overall acceptability.

5. Conclusions

In this study, the ability to use fermented Hass avocado kernel flour with *Lactobacillus plantarum* to produce functional biscuits was examined. The protein, calcium, phosphorus, potassium, iron, and zinc contents in Hass avocado kernels, which are usually disposed in landfills, are high and increase with fermentation, so more attention should be paid to this inedible part of the avocado. Th total phenolic content, antioxidant effect against DPPH, and flavonoids content significantly increased after fermentation. After fermentation, the reduction in the tannins, oxalates, and alkaloids contents was 80.76%, 89.95%, and 70%, respectively, and traces of phytates and saponin were detected. FHK had the following order of contents: PUFAs (51.54%) > SFAs (26.72%) > MUFAs (21.83%). Replacing 20% of wheat flour with FHK flour did not significantly affect either the weight or thickness of the produced biscuits. The highest spread ratio value (6.17) was recorded in the samples produced by replacing 10% of the wheat flour with of FHK. Theoretically, we found a color difference between biscuit samples from all treatments, but consistency in the sensory evaluation results. This color difference was not sufficient for the panel of evaluators to reject the color of the biscuit samples produced by substituting different levels of wheat flour with FHK flour until the substitution level reached 20%. Replacing 10% of wheat flour with FHK flour improved the acceptability of the biscuits produced compared to biscuits produced from 100% wheat flour.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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