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Anthony Oluwadamilola Ogungbemi

ao.ogungbemi@oaustech.edu.ng

Olusegun Agagu University of Science and Technology

Muhydeen Olanrewaju Owooniran

Olusegun Agagu University of Science and Technology

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**PROXIMATE, MINERAL, PHYTOCHEMICAL AND VITAMIN COMPOSITION OF
PERSEA AMERICANA MILL. FRUIT PULP AT DIFFERENT RIPENING STAGES**

Anthony Oluwadamilola Ogungbemi ^{1*}, Muhydeen Olanrewaju Owooniran ²

1. Plant Ecology and Environmental Botany Research Laboratory, Department of Biological Sciences, Botany Unit, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria.

2. Plant Ecology and Environmental Botany Research Laboratory, Department of Biological Sciences, Botany Unit, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria.

*Corresponding Author Email: ao.ogungbemi@oaustech.edu.ng

ORCID: 0009-0001-4694-3595

Abstract

This study evaluated the proximate, mineral, phytochemical and vitamin composition of *Persea americana* Mill. fruit pulp at different ripening stages. Fresh avocado fruits were obtained from Bodija Market, Ibadan, Nigeria, and authenticated at the University of Ibadan Herbarium with voucher specimen number UIH-23289. The fruit samples were categorized into unripe, hard-ripe and ripe stages, with four replicates per treatment. Standard analytical procedures of the Association of Official Analytical Chemists (AOAC) were employed for the determination of proximate, mineral, phytochemical and vitamin compositions. Data obtained were subjected to one-way analysis of variance (ANOVA) using SPSS (version 16), while Duncan Multiple Range Test (DMRT) was used for mean separation at $p \leq 0.05$.

The results revealed significant variations in the nutritional composition of avocado pulp across the ripening stages. Hard-ripe pulp exhibited significantly higher fat content, while unripe pulp contained higher concentrations of phytochemicals and antioxidant-related compounds. Ripe pulp showed elevated levels of vitamin A and vitamin C. Iron was the most abundant mineral element detected among the samples. The findings demonstrate that ripening significantly influences the nutritional and phytochemical characteristics of avocado fruit pulp.

The study highlights its nutritional potential as functional food ingredients and provides useful information for its commercialization.

Keywords: *Persea americana*, avocado pulp, ripening stage, phytochemicals, proximate composition, vitamins, minerals.

Malnutrition and micronutrient deficiencies remain major public health concerns globally, particularly in developing countries where access to nutrient-rich foods and quality healthcare is limited (Gode and Pehlivan, 2015). Increasing awareness of the relationship between diet and health has stimulated interest in functional foods and plant-derived bioactive compounds capable of promoting human health and reducing the risk of chronic diseases (Gupta *et al.*, 2013). Fruits constitute an essential component of human nutrition because they supply important nutrients including carbohydrates, dietary fibre, vitamins, minerals and antioxidants necessary for proper physiological functioning (Cochrane *et al.*, 2016; Bailey *et al.*, 2019). Among tropical fruits, avocado (*Persea americana* Mill.) has gained considerable scientific and commercial importance due to its exceptional nutritional composition and health-promoting properties (Bhuyan *et al.*, 2019; Sommaruga and Eldridge, 2020).

Avocado is a nutrient-dense fruit belonging to the family Lauraceae and is widely cultivated in tropical and subtropical regions of the world (Ramos-Aguilar *et al.*, 2021). The fruit is highly valued for its rich composition of monounsaturated fatty acids, dietary fibre, proteins, vitamins and essential mineral elements (Dreher and Davenport, 2013). Avocado pulp contains appreciable quantities of vitamins A, C, E, K and B-complex vitamins, as well as important minerals such as potassium, magnesium, calcium, phosphorus, iron and zinc (Martínez-Ballesta *et al.*, 2017). In addition to its nutritional value, avocado possesses several bioactive phytochemicals including phenolics, flavonoids, tannins, alkaloids, carotenoids and saponins which exhibit antioxidant, anti-inflammatory, antimicrobial and cardioprotective activities (Kindahunsi, 2018; Wang *et al.*, 2016). These compounds contribute significantly to disease prevention and overall health maintenance. Consequently, avocado is increasingly recognized as a functional food with applications in food, pharmaceutical and nutraceutical industries (Amado *et al.*, 2019).

Ripening is an important physiological process that influences the biochemical, nutritional and sensory qualities of fruits. During ripening, significant changes occur in moisture content, carbohydrate metabolism, lipid accumulation, vitamin composition and phytochemical concentration (Manzanares *et al.*, 2020). These transformations affect fruit texture, flavour, colour, nutritional quality and shelf life. In avocado fruits, ripening is accompanied by softening of the

pulp, increased lipid biosynthesis and alterations in antioxidant compounds and mineral composition (Sellamuthu *et al.*, 2013). Previous studies have shown that fruit ripening may significantly influence the concentration of vitamins, flavonoids, phenolic compounds and antioxidant activities in fruits (Vizzotto *et al.*, 2007; Sudha *et al.*, 2011). Understanding these compositional changes at different ripening stages is important for consumers, nutritionists, food processors and researchers because the nutritional and health-promoting potentials of the fruit may vary considerably depending on the stage of maturity and ripeness.

Despite the increasing global consumption and economic value of avocado, information regarding the comparative nutritional, phytochemical and vitamin composition of avocado pulp at different ripening stages remains limited, particularly in Nigeria and other developing countries. Most previous studies have focused primarily on postharvest physiology, oil composition and general nutritional characteristics without extensive evaluation of mineral, proximate, phytochemical and vitamin changes during ripening (Ramos-Aguilar *et al.*, 2021). Therefore, there is a need for comprehensive investigation into the compositional variations associated with avocado fruit ripening in order to provide scientific information useful for nutritional recommendations, food processing, postharvest management and industrial applications.

The present study was therefore designed to evaluate the proximate, mineral, phytochemical and vitamin composition of *Persea americana* fruit pulp at different ripening stages, namely unripe, hard-ripe and ripe stages. Specifically, the study aimed to determine the mineral composition, proximate composition, phytochemical constituents and vitamin profile of avocado pulp and to compare the nutritional variations associated with the different stages of ripening.

MATERIALS AND METHODS

2.1 Collection and Identification of Samples

The freshly plucked Avocado fruits of *Persea americana* Mill. in the family Lauraceae used for the research were randomly selected from Bodija market in Ibadan and were taken to the University of Ibadan Herbarium for identification with supplied voucher specimen number UIH -23289. This was transferred to the Department of soil resources management, Faculty of Agriculture and forestry University of Ibadan where it was analyzed.

2.2 Experimental Design

A completely randomized design (CRD) was adopted for the study.

2.3 Experimental Site and Plant Material

The study was carried out at the Department of soil resources management, Faculty of Agriculture and Forestry University of Ibadan (7.45006 and 3.89198). A total samples of 36 Avocado fruits (3 x 3 x 4) were analyzed. Three (3) samples each of Hard-ripe, ripe and unripe fruits designated as Hard-ripe pulp 1, Ripe pulp 2, and unripe pulp 3 in four (4) replicates T_{1,1}, T_{1,2}, T_{1,3} and T_{1,4}; T_{2,1}, T_{2,2}, T_{2,3} and T_{2,4}; and T_{3,1}, T_{3,2}, T_{3,3} and T_{3,4} respectively were selected. The collected fruits were washed thoroughly for three to four times in tap water and later in distilled water to remove contamination. Then, cut into pieces with knife to carefully separate the peel, pulp and the seed after which they were digested and analyses were carried.

2.4 Data Collection: Quantitative Analyses of Physicochemical Parameter of Avocado fruit Pulp.

The samples were analysed chemically according to the official method of analyses described by the Association of Official Analytical Chemist (AOAC, 18th Editions, 2005). The identified plant samples were transferred to the laboratory of the Department of Soil and Agronomy, University of Ibadan where the quantitative analysis of the Physicochemical properties of plant samples were carried out in four (4) replicates. Mineral, Proximate, Non-nutritional and Vitamin compositions were determined.

2.4.1 Determination of Mineral Composition (AOAC, 975.11)

The mineral composition analyzed were Calcium(Ca), Magnesium(Mg), Potassium(K), Sodium (Na), Phosphorus (P), Zinc (Zn), Iron (Fe), and Carbon (C) following the procedure by the Association of Official Analytical Chemist (AOAC, 18th Editions, 2005).

2.4.1.1 Determination calcium, potassium and sodium

The ash of each sample obtained was digested by adding 5ml of 2 MHCL to the ash in the crucible and heat to dryness on a heating mantle. 5ml of 2 MHCL was added again, heat to boil, and filtered through a Whatman No. 1 filter paper into a 100ml volumetric flask. The filtrate was made up to mark with distilled water stoppered and made ready for reading of concentration of Calcium, Potassium and Sodium on the Jenway Digital Flame Photometer (PFP7 Model) using the filter corresponding to each mineral element.

The concentration of each of the element was calculated using the formula: %Ca or %K or

$$\% \text{ Na} = \frac{\text{Meter Reading(MR)} \times \text{Slope} \times \text{Dilution factor}}{1000} \dots\dots\dots \text{Equation 2.1}$$

Where, MR x slope x dilution factor will give you the concentration in part per million (ppm or mg/kg). Concentration will be recorded in % when you divide by 10000.

2.4.1.2 Determination of Phosphorus using Spectrophotometric method (AOAC, 975.16)

Phosphorus was determined routinely by the vanado-molybdate colorimetric or spectrophotometric method described by Association of Official Analytical Chemist (AOAC, 18th Editions, 2005).

The ash of each sample obtained was treated 2M HCl solution as described for calcium determination above. 10ml of the filtrate solution was pipetted into 50ml standard flask and 10ml of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow development. The concentration of phosphorus was obtained by taking the optical density (OD) or absorbance of the solution on a Spectronic 20 spectrophotometer or colorimeter at a wavelength of 470nm.

The percentage phosphorus was calculated from using the formula:

$$\% \text{ Phosphorus} = \frac{\text{Absorbance} \times \text{Slope} \times \text{Dilution factor}}{10000} \dots\dots\dots \text{Equation 2.2}$$

2.4.1.3 Determination of Se, Mg, Pb, Cd, Cu, Mn, Fe, Ni, Zn using Buck 200 AAS (AOAC, 975.23)

The digest of the ash of each sample above as obtained in calcium and potassium determination was washed into 100ml volumetric flask with deionised or distilled water and made up to mark. This diluent was aspirated into the Buck 200 Atomic Absorption Spectrophotometer(AAS) through the suction tube. Each of the trace mineral elements was read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination.

2.4.1.4 Determination of Nitrogen-free extract (Nfe)

175 The Nfe was determined by difference. This was done by subtracting SUM of % Moisture + %
176 Crude Protein + % Ether Extract + % Crude Fibre + % Ash from 100.

177
$$\text{Nfe} = [100 - (\% \text{ M} + \% \text{ CP} + \% \text{ EE} + \% \text{ CF} + \% \text{ Ash})] \dots\dots\dots \text{Equation 2. 3}$$

178 **2.5 The Proximate Composition**

179 The proximate composition that were determined are Moisture content, Ash content, Crude fiber,
180 Fat content, Crude protein, carbohydrate using procedure by the Association of Official Analytical
181 Chemist (AOAC, 18th Editions, 2005).

182 **2.5.1 Determination Crude Protein (AOAC Official Method 988.05)**

183 The crude protein in the sample were determined by the routine semi-micro Kjeldahl,
184 procedure/technique. This consists of three techniques of analysis namely Digestion, Distillation
185 and Titration. Apparatus used were Analytical Balance, Digestion tubes, Digestion Block Heaters,
186 50ml Burette, 5ml Pipette, 10ml Pipette, 10ml Measuring Cylinder, 100ml Beakers and Fume
187 Cupboard. The reagents used were concentrated H₂SO₄, 0.01NHCL 40% (W/V) NaOH, 2% Boric
188 Acid Solution, Methyl Red – Bromocresol green mixed indicator, Kjeldahl Catalyst tablet.

189 0.5g of each finely ground dried sample was weighed carefully into the Kjeldahl digestion tubes
190 to ensure that all sample materials got to the bottom of the tubes. To this were added 1 Kjeldahl
191 catalyst tablet and 10ml of Concentrated H₂SO₄. These were set in the appropriate hole of the
192 Digestion Block Heaters in a fume cupboard. The digestion was left on for 4 hours, after which a
193 clear colourless solution was left in the tube. The digest was cooled and carefully transferred into
194 100ml volumetric flask, thoroughly rinsing the digestion tube with distilled water and the flask
195 was made up to mark with distilled water. The distillation was done with Markham Distillation

196 Apparatus which allows volatile substances such as ammonia to be steam distilled with complete
197 collection of the distillate. The apparatus was steamed out for about ten minutes. The steam
198 generator was then removed from the heat source to allow the developing vacuum to remove
199 condensed water. The steam generator was then placed on the heat source (heating mantle) and
200 each component of the apparatus was fixed up appropriately. 5ml portion of the digest above was
201 pipetted into the body of the apparatus via the small funnel aperture. To this was added 5ml of
202 40% (W/V) NaOH through the same opening with the 5ml pipette. The mixture was steam-distilled
203 for 2 minutes into a 50ml conical flask containing 10ml of 2% Boric Acid plus mixed indicator
204 solution placed at the receiving tip of the condenser. The Boric Acid plus indicator solution
205 changes colour from red to green showing that all the ammonia liberated have been trapped.

206 The green colour solution obtained was then titrated against 0.01N HCl contained in a 50ml
207 Burette. At the end point or equivalent point, the green colour turns to wine colour which indicates
208 that all the Nitrogen trapped as Ammonium Borate $[(\text{NH}_4)_2\text{BO}_3]$ have been removed as
209 Ammonium chloride (NH_4Cl).

210 The percentage nitrogen in this analysis was calculated using the formula:

211 **% N = Titre value x Atomic mass of Nitrogen x Normality of HCl used x 4** Equation 2.4

212 **or**

213 % N = Titre value x Normality/Molarity of HCL used x Atomic mass of N x Volume of flask
214 containing the digest x 100/1 Equation 2.5

215

216 Weight of sample digested in milligram x Vol. of digest for steam distillation. The crude protein
 217 content is determined by multiplying percentage Nitrogen by a constant factor of 6.25.

218 Percentage Crude Protein = % N x 6.25 Equation 2.6

219 **2.5.2. Determination of Crude Fat or Ether Extract (AOAC Official Method 2003.06)**

220 1gm of each dried sample was weighed into fat free extraction thimble and pug lightly with cotton
 221 wool. The thimble was placed in the extractor and fitted up with reflux condenser and a 250ml
 222 soxhlet flask which has been previously dried in the oven, cooled in the desicator and weighed.
 223 The soxhlet flask was then filled to $\frac{3}{4}$ of its volume with petroleum ether (b.pt. 40° – 60°C), and
 224 the soxhlet flask Extractor plus condenser set were placed on the heater. The heater was put on for
 225 six hours with constant running water from the tap for condensation of ether vapour. The set was
 226 constantly watched for ether leaks and the heat source was adjusted appropriately for the ether to
 227 boil gently. The Ether was left to siphon over several times for over at least 10 – 12 times until it
 228 was short of siphoning. It was after this was noticed that any ether content of the extractor was
 229 carefully drained into the ether stock bottle. The thimble containing sample was then removed and
 230 dried on a clock glass on the bench top. The extractor, flask and condenser was replaced and the
 231 distillation continues until the flask was practically dried. The flask which then contained the fat
 232 or oil was detached, its exterior cleaned and dried to a constant weight in the oven.

233 Percentage fat/oil : $\frac{W_1 - W_o}{\text{Wt. of Sample}}$ x $\frac{100}{1\text{taken}}$ Equation 2.7
 234
 235

236 Where W_o is the initial weight of dry soxhlet flask and W_1 is final weight of oven dried flask +
 237 oil/fat

2.5.3 Determination Dry Matter and Moisture (AOAC Official Method 967.08)

2g of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into the oven set at 100°C to dry to a constant weight for 24 hours overnight. At the end of the 24 hours, the crucible plus sample was removed from the oven and transferred to dessicator, cooled for ten minutes and weighed.

$$\% \text{ DM} = \frac{W_3 - W_0}{W_1 - W_0} \times \frac{100}{1} \dots \text{Equation 2.8}$$

$$\% \text{ Moisture} = \frac{W_1 - W_3}{W_1 - W_0} \times \frac{100}{1} \dots \text{Equation 2.9}$$

Or

$$\% \text{ Moisture} = 100 - \% \text{ DM} \dots \text{Equation 2.10}$$

Where W_0 is the weight of empty crucible, W_1 is weight of crucible plus sample

and W_3 is the weight of crucible plus oven-dried sample.

2.5.4 Determination of Ash (AOAC Official Method 942.05)

2.0gm of the sample were weighed into a porcelain crucible. This was transferred into the muffle furnace set at 550°C and left for about 4 hours. About this time, it had turned to white ash. The crucible and its content were cooled to about 100°C in air, then room temperature in a dessicator and weighed. This was done in duplicate. The percentage ash was calculated from the formula below:

$$\text{Ash content} = \frac{\text{wt. of ash}}{\text{original wt. of sample}} \times \frac{100}{1} \dots \text{Equation 2.11}$$

2.5.5 Determination of Fibre (AOAC 958.06)

2.0gm of the sample was accurately into the fibre flask and 100ml of 0.255N H₂SO₄ added. The mixture was heated under reflux for 1 hour with the heating mantle. The hot mixture was filtered through a fibre sieve cloth. The filtrate obtained was thrown off and the residue was returned to the fibre flask to which 100ml of (0.313N NaOH) was added and heated under reflux for another 1 hour. The mixture was filtered through a fibre sieve cloth and 10ml of acetone added to dissolve any organic constituent. The residue was washed with about 50ml hot water on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue were oven-dried at 105°C overnight to drive off moisture. The oven-dried crucible containing the residue was cooled in a dessicator and later weighed to obtain the weight W₁. The crucible with weight W₁ was transferred to the muffle furnace for Ashing at 550°C for 4 hours.

The crucible containing white or grey ash (free of carbonaceous material) was cooled in the dessicator and weight to obtain W₂. The difference W₁ – W₂ gives the weight of fibre. The percentage fibre was obtained by the formula:

$$\% \text{ Fibre} = \frac{W_1 - W_2}{\text{wt. of sample}} \times \frac{100}{1} \dots\dots\dots \text{Equation 2.12}$$

2.6. Phytochemical composition of pulp in Avocado at different stage of ripening

2.6.1 Test for Tannins

Test for Tannins was carried out using the procedure described by Trease and Evans (1978). About 5.0ml of each portion of plant extract was stirred with 10ml distilled water and filtered. Ferric chloride reagent was added to the filtered. A blue-black, green or blue-green precipitate was taken as evidence for the presence of tannins.

288 **2.6.2 Test for Alkaloids**

289 Alkaloid determination was carried out using the procedure described by Trease and Evans (1978).
290 About 0.1ml of each extract was stirred in 5ml of 1% aqueous hydrochloric acid in a steam bath.
291 The solution was filtered and 1ml of the filtrate was treated with a few drops of Dragmdroff's
292 reagents. Turbidity or precipitation was taken as the primary evidence for the presence of alkaloids
293 in the extract being evaluated.

294 **2.6.3 Test for Saponins**

295 Test for Saponins was carried out using the procedure described by Wall *et al.* (1952). The ability
296 of saponins to produce frothing in aqueous solution was used as screening test for this compound.
297 About 1ml of each extract was shaken with water in a test tube. Frothing which persist on warming
298 was taken as preliminary proof for the presence of saponin.

299 **2.6.4 Test for Flavonoids**

300 Flavonoids was determined using the procedure described by Sofowora (1993). About 5ml of
301 dilute ammonia solution were added to a portion of the filtrate of each plant extract, followed by
302 addition of concentrated H₂SO₄. A yellow colouration observed in each extracted indicated the
303 presence of flavonoids. The yellow colouration disappeared on standing.

304 **2.6.5 Test of Terpenoids**

305 About 5ml of each extract was mixed with 2ml of chloroform and 3ml of each concentrated H₂SO₄
306 was carefully added to form a layer. A reddish brown colouration of the interface was formed to
307 show positive result for the presence of terpenoids (Salkowski Test).

308 **2.6.6 Estimation of Total Carotene Present**

Carotenoids was determined using the procedure described by Zarkaria *et al.* (1979). The total carotenoids in the sample were extracted in petroleum ether. The total carotenoids were estimated in UV/visible spectrometer at 645nm.

Five to ten gram of the plant sample was weighed. After extracting the sample in 12% alcoholic KOH, the sample was saponified for about 30nm minutes in a shaking water bath at 37°C. The saponified sample was transferred into a seperating funnel (packed with glass wool and calcium carbonate) containing 10-15ml of petroleum ether and mixed gently. The petroleum ether layer with the carotenoid pigment was taken up. The lower aqueous phase was transformed into another separating funnel and the petroleum ether extract containing the carotenoid pigment to an amber colour bottle. The extraction of the aqueous phase was repeated. Similarly, with petroleum ether, until it becomes colourless. The aqueous phase was discarded. To the petroleum ether extract, added a small quantity of hydrous sodium sulphates to remove turbidity. The final volume of the oetrouleum ether extract was noted and diluted if needed by a known dilution factor. The absorbance of the extracted at 450nm and 503nm was noted in a spectrophotometer using petroleum ether as a blank.

For Amount of total Carotenoid present = $P \times 4 \times V \times 100\text{ug/W}$ Equation 2.13
Where, P = optical density of the sample, V = Volume of the sample, W = Weight of the sample.

2.6.7 Statistical Analyses

Data obtained were expressed as mean $\pm$ standard error of mean (SEM). Statistical analyses were performed using one-way analysis of variance (ANOVA) with SPSS version 16. Means were separated using Duncan Multiple Range Test (DMRT) at $p \leq 0.05$

3.0

RESULTS

Table 3.1 showed the relative mineral composition of pulp such as calcium (Ca), magnesium (Mg), Potassium(P), Sodium (Na), Carbon (C), Iron (Fe) and Zinc (Zn) in Avocado fruit at different stages of ripening at $p \leq 0.05$. The comparison between the three samples showed equal level of significance in all the samples ($p \leq 0.05$).

Table 3.3 showed the mean percentage non- nutritional composition in Avocado fruit pulp at different stages of ripening at $p \leq 0.05$. The Phytochemical composition includes Phenolic, Flavonoid, Alkaloid, Tannin, Saponin, Oxalate and Carotenoid. The percentage Phytochemical composition in Hard-ripe were 92.788 ± 0.07^c , 92.758 ± 0.15^c and 92.283 ± 0.08^b which indicate equal level of significance in Hard-ripe 1 and Hard-ripe 2, while the Hard-ripe 3 has the highest level of significance ($p \leq 0.05$). The ripe pulp of Avocado fruit was observed and the results showed that the three samples were 84.38 ± 0.05^a , 84.363 ± 0.07^a and 84.538 ± 0.14^a respectively and it was denoted that ripe 1, ripe 2, ripe 3 has equal level of significance.

345 **Table 3.1: Relative mineral composition of avocado fruit pulp at different stages of ripening**

Content(%) → Sample ↙	Ca	Mg	K	Na	P	Zn	Fe	C
Hard-ripe 1	0.034±0.001 ^a	0.068±0.001 ^a	1.377±0.003 ^c	0.305±0.002 ^c	0.140±0.001 ^b	0.002±0.000 ^{ab}	0.042±0.017 ^b	42.36±0.357 ^b
Hard-ripe 2	0.034±0.001 ^a	0.07±0.001 ^{ab}	1.376±0.002 ^c	0.308±0.002 ^c	0.141±0.001 ^b	0.002±0.000 ^{ab}	0.042±0.017 ^b	42.363±0.367 ^b
Hard-ripe 3	0.034±0.001 ^a	0.072±0.001 ^{ab}	1.374±0.003 ^c	0.305±0.002 ^c	0.14±0.001 ^b	0.002±0.000 ^{ab}	0.042±0.017 ^b	42.358±0.371 ^b
Ripe pulp 1	0.038±0.001 ^b	0.071±0.001 ^{ab}	1.272±0.010 ^b	0.228±0.005 ^b	0.14±0.002 ^b	0.003±0.000 ^b	0.007±0.000 ^a	40.735±0.099 ^a
Ripe pulp 2	0.038±0.001 ^b	0.072±0.001 ^b	1.275±0.010 ^b	0.230±0.005 ^b	0.141±0.002 ^b	0.003±0.000 ^b	0.007±0.000 ^a	40.71±0.119 ^a
Ripe pulp 3	0.038±0.002 ^b	0.072±0.001 ^b	1.272±0.010 ^b	0.228±0.004 ^b	0.141±0.002 ^b	0.002±0.000 ^{ab}	0.007±0.000 ^a	40.755±0.100 ^a
Unripe pulp 1	0.051±0.001 ^c	0.129±0.001 ^c	1.126±0.001 ^a	0.193±0.001 ^a	0.089±0.002 ^a	0.003±0.000 ^b	0.013±0.000 ^{ab}	45.213±0.286 ^c
Unripe pulp 2	0.050±0.001 ^c	0.128±0.001 ^c	1.125±0.002 ^a	0.192±0.001 ^a	0.089±0.001 ^a	0.003±0.000 ^b	0.013±0.000 ^{ab}	45.215±0.280 ^c
Unripe pulp 3	0.051±0.001 ^c	0.128±0.001 ^c	1.126±0.001 ^a	0.192±0.001 ^a	0.09±0.002 ^a	0.003±0.000 ^b	0.013±0.000 ^{ab}	45.21±0.290 ^c

346 Values are mean ± SEM (n = 4). Means followed by different superscripts differ significantly at $p \leq 0.05$. Mean ± standard error of the

347 mean; % = Percentage; Ca = Calcium, M=Magnesium, P= Potassium, N=Sodium, P=Phosphorus, Z = Zinc, Fe = Iron, Carbon = C

348 **Table 3.2 Relative Proximate Composition of Avocado Fruit Pulp at Different Stages of Ripening**

349 Values are mean $\pm$ SEM (n = 4). Means followed by different superscripts differ significantly at $p \leq 0.05$. Mean $\pm$ standard error of the
 350 mean; % = Percentage; Ca = Calcium, M=Magnesium, P= Potassium, N=Sodium, P=Phosphorus, Z = Zinc, Fe = Iron, Carbon = C

Content(%)  Moisture	Ash	Crude Fibre	Fat	Crude Protein	Carbohydrate	
Sample 						
Hard-ripe 1	11.628±0.025 ^b	2.873±0.014 ^c	2.695±0.151 ^a	0.863±0.027 ^b	7.438±0.045 ^b	74.438±0.158 ^b
Hard-ripe 2	11.64±0.033 ^b	2.89±0.014 ^c	2.69±0.130 ^a	0.845±0.054 ^b	7.433±0.049 ^b	74.445±0.158 ^b
Hard-ripe 3	11.628±0.038 ^b	2.863±0.019 ^c	2.695±0.151 ^a	0.858±0.018 ^b	7.428±0.048 ^b	74.468±0.153 ^b
Ripe pulp 1	12.755±0.023 ^c	2.743±0.020 ^b	3.568±0.033 ^b	0.575±0.027 ^a	6.84±0.027 ^a	73.645±0.052 ^a
Ripe pulp 2	12.743±0.023 ^c	2.743±0.030 ^b	3.575±0.036 ^b	0.575±0.029 ^a	6.853±0.028 ^a	73.638±0.053 ^a
Ripe pulp 3	12.765±0.020 ^c	2.735±0.026 ^b	3.588±0.035 ^b	0.575±0.027 ^a	6.84±0.037 ^a	73.663±0.052 ^a
Unripe pulp 1	9.693±0.019 ^a	2.22±0.011 ^a	2.945±0.037 ^a	1.015±0.031 ^c	7.848±0.024 ^c	76.545±0.221 ^c
Unripe pulp 2	9.708±0.028 ^a	2.235±0.026 ^a	2.965±0.061 ^a	1.04±0.045 ^c	7.855±0.030 ^c	76.578±0.224 ^c
Unripe pulp 3	9.688±0.014 ^a	2.233±0.005 ^a	2.95±0.045 ^a	1.035±0.041 ^c	7.835±0.038 ^c	76.558±0.219 ^c

351

352 **Table 3.3: Relative Phytochemical composition of Avocado Fruit Pulp at Different Stage of Ripening**

353

354 Values are mean $\pm$ SEM (n = 4). Means followed by different superscripts differ significantly at $p \leq 0.05$. Mean $\pm$ standard error of the

Content(%) $\rightarrow$ Sample	Phenolic mg/100g	Flavonoid mg/100g	Alkaloid mg/100g	Tannin mg/100g	Saponin mg/100g	Oxalate mg/100g	Carotenoid mg/100g
Hard-ripe 1	92.788 $\pm$ 0.07 ^c	10.415 $\pm$ 0.029 ^a	9.025 $\pm$ 0.024 ^a	57.72 $\pm$ 0.189 ^b	39.400 $\pm$ 0.056 ^c	4.758 $\pm$ 0.037 ^d	68.51 $\pm$ 0.062 ^c
Hard-ripe 2	92.758 $\pm$ 0.15 ^c	10.42 $\pm$ 0.189 ^a	9.31 $\pm$ 0.05 ^{ab}	57.805 $\pm$ 0.289 ^b	39.57 $\pm$ 0.109 ^c	4.31 $\pm$ 0.06 ^{ab}	68.475 $\pm$ 0.061 ^c
Hard-ripe 3	92.283 $\pm$ 0.08 ^b	10.57 $\pm$ 0.167 ^a	9.55 $\pm$ 0.16 ^{ab}	57.858 $\pm$ 0.188 ^b	39.41 $\pm$ 0.147 ^c	4.515 $\pm$ 0.152 ^{bc}	68.525 $\pm$ 0.154 ^c
Ripe pulp 1	84.38 $\pm$ 0.05 ^a	14.543 $\pm$ 0.339 ^b	14.543 $\pm$ 0.339 ^c	52.59 $\pm$ 0.691 ^a	32.958 $\pm$ 2.608 ^b	4.225 $\pm$ 0.034 ^a	56.583 $\pm$ 0.122 ^a
Ripe pulp 2	84.363 $\pm$ 0.07 ^a	14.855 $\pm$ 0.390 ^{bc}	14.883 $\pm$ 0.187 ^c	52.633 $\pm$ 0.669 ^a	32.905 $\pm$ 2.441 ^b	4.55 $\pm$ 0.090 ^{cd}	56.683 $\pm$ 0.120 ^a
Ripe pulp 3	84.538 $\pm$ 0.14 ^a	14.968 $\pm$ 0.157 ^{bc}	14.85 $\pm$ 0.273 ^c	52.66 $\pm$ 0.836 ^a	32.82 $\pm$ 2.505 ^b	4.483 $\pm$ 0.088 ^{bc}	56.738 $\pm$ 0.146 ^a
Unripe pulp 1	156.768 $\pm$ 0.17 ^d	15.498 $\pm$ 0.121 ^c	9.745 $\pm$ 0.080 ^b	72.598 $\pm$ 0.062 ^c	24.685 $\pm$ 0.078 ^a	5.7 $\pm$ 0.023 ^c	63.938 $\pm$ 0.041 ^b
Unripe pulp 2	156.738 $\pm$ 0.17 ^d	15.503 $\pm$ 0.112 ^c	9.73 $\pm$ 0.079 ^b	72.585 $\pm$ 0.057 ^c	24.673 $\pm$ 0.067 ^a	5.68 $\pm$ 0.018 ^c	63.95 $\pm$ 0.043 ^b
Unripe pulp 3	156.738 $\pm$ 0.17 ^d	15.513 $\pm$ 0.126 ^c	9.745 $\pm$ 0.072 ^b	72.58 $\pm$ 0.066 ^c	24.678 $\pm$ 0.065 ^a	5.68 $\pm$ 0.030 ^e	63.94 $\pm$ 0.044 ^b

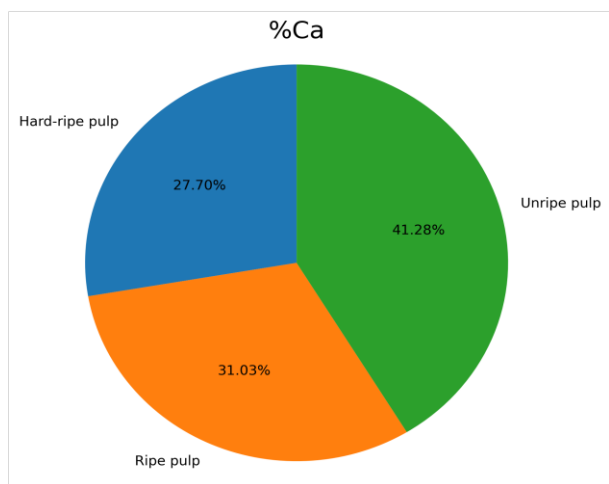
355 mean; % = Percentage; Ca = Calcium, M=Magnesium, P= Potassium, N=Sodium, P=Phosphorus, Z = Zinc, Fe = Iron, Carbon =

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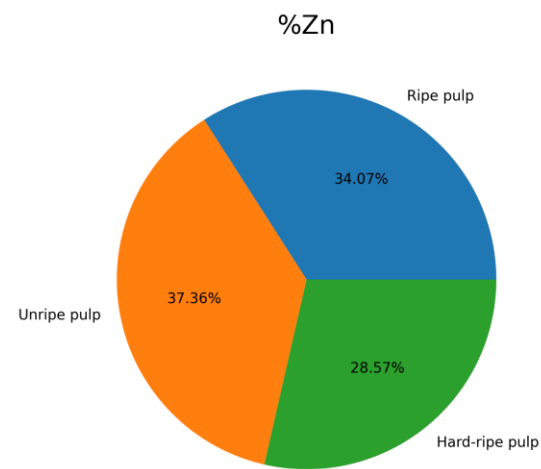
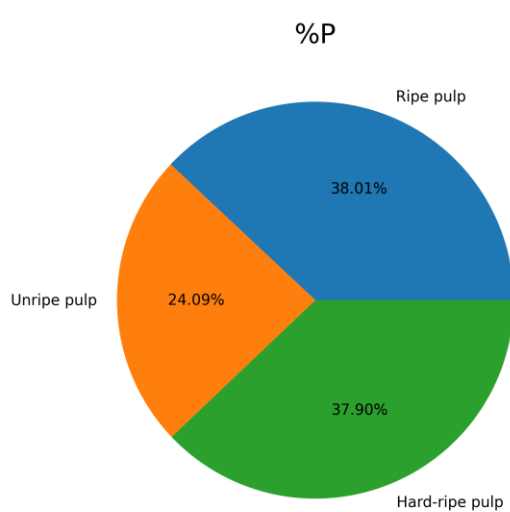
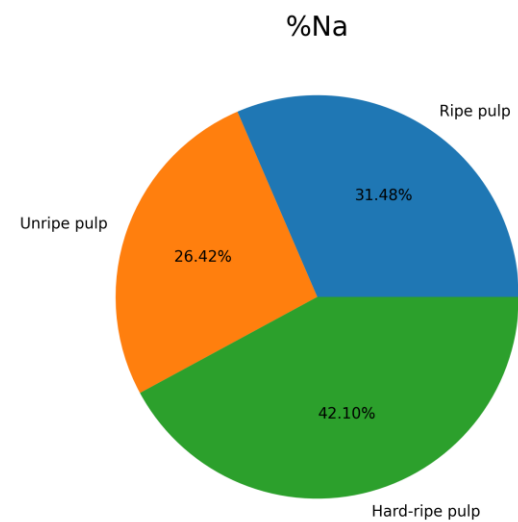
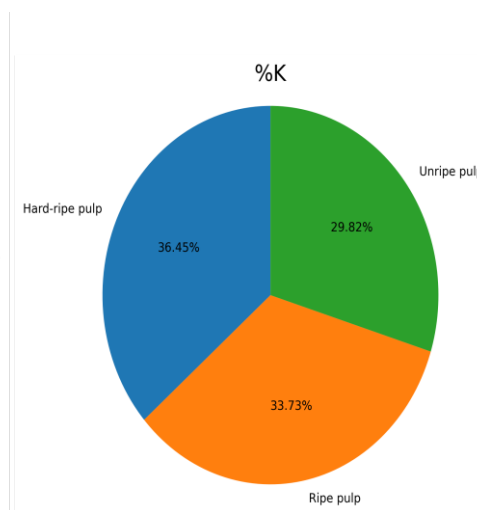
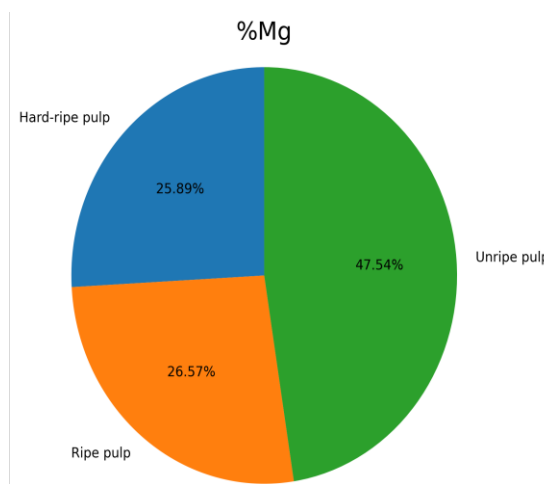
357 **3.4: Relative Vitamin Composition of Avocado Fruit Pulp at Different Stages of Ripening**

358 Values are mean $\pm$ SEM (n = 4). Means followed by different superscripts differ significantly at $p \leq 0.05$. Mean $\pm$ standard error of the
 359 mean; % = Percentage; Ca = Calcium, M=Magnesium, P= Potassium, N=Sodium, P=Phosphorus, Z = Zinc, Fe = Iron, Carbon = C

Content(%) $\rightarrow$ Sample	Vitamin A mg/100g	Vitamin C mg/100g	Riboflavin mg/100g	Thiamine mg/100g	Niacin mg/100g
Hard-ripe 1	14 $\pm$ 0.034a	6.388 $\pm$ 0.026a	1.825 $\pm$ 0.046a	1.213 $\pm$ 0.020a	3.42 $\pm$ 0.056b
Hard-ripe 2	14.023 $\pm$ 0.035a	6.408 $\pm$ 0.026a	1.835 $\pm$ 0.039a	1.223 $\pm$ 0.020a	3.44 $\pm$ 0.056b
Hard-ripe 3	14 $\pm$ 0.041a	6.368 $\pm$ 0.026a	1.795 $\pm$ 0.039a	1.213 $\pm$ 0.024a	3.41 $\pm$ 0.056b
Ripe pulp 1	24.34 $\pm$ 0.034c	8.795 $\pm$ 0.021c	2.89 $\pm$ 0.292b	1.505 $\pm$ 0.019b	3.555 $\pm$ 0.014c
Ripe pulp 2	24.36 $\pm$ 0.034c	8.815 $\pm$ 0.021c	2.91 $\pm$ 0.292b	1.525 $\pm$ 0.019b	3.575 $\pm$ 0.014c
Ripe pulp 3	24.35 $\pm$ 0.034c	8.805 $\pm$ 0.021c	2.9 $\pm$ 0.292b	1.515 $\pm$ 0.019b	3.565 $\pm$ 0.014c
Unripe pulp 1	21.413 $\pm$ 0.055b	8.648 $\pm$ 0.031b	1.295 $\pm$ 0.017a	1.47 $\pm$ 0.025b	3.073 $\pm$ 0.014a
Unripe pulp 2	21.393 $\pm$ 0.055b	8.628 $\pm$ 0.031b	1.283 $\pm$ 0.019a	1.458 $\pm$ 0.031b	3.063 $\pm$ 0.019a
Unripe pulp 3	21.413 $\pm$ 0.059b	8.643 $\pm$ 0.027b	1.293 $\pm$ 0.019a	1.468 $\pm$ 0.031b	3.075 $\pm$ 0.027a



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362 **Figure 3.1A: Mean Percentage Mineral composition in pulp of Avocado fruit at different stages of ripening**

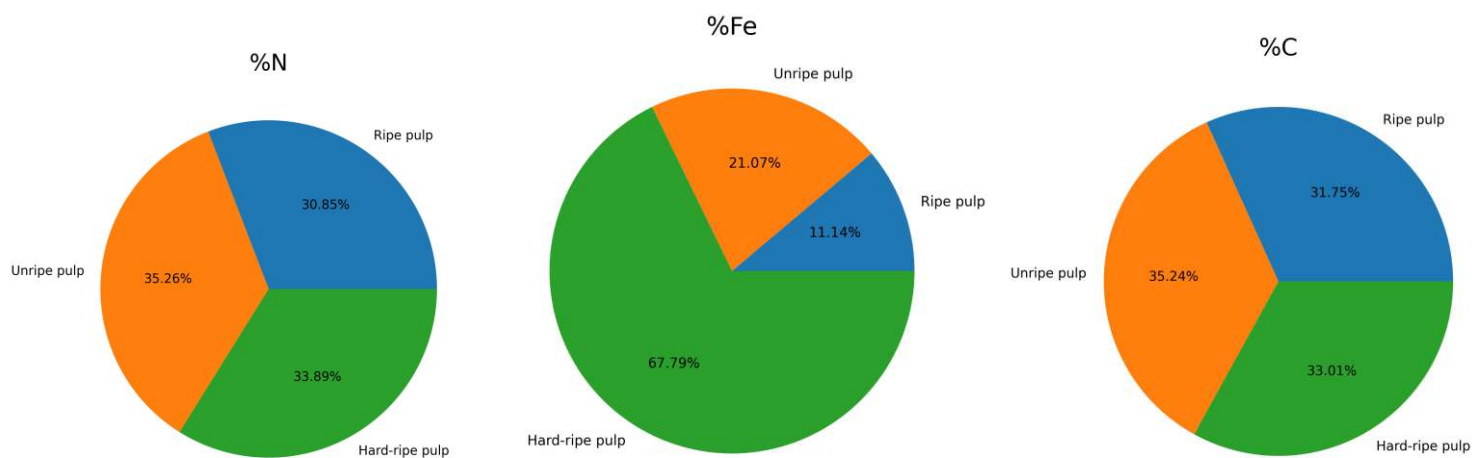
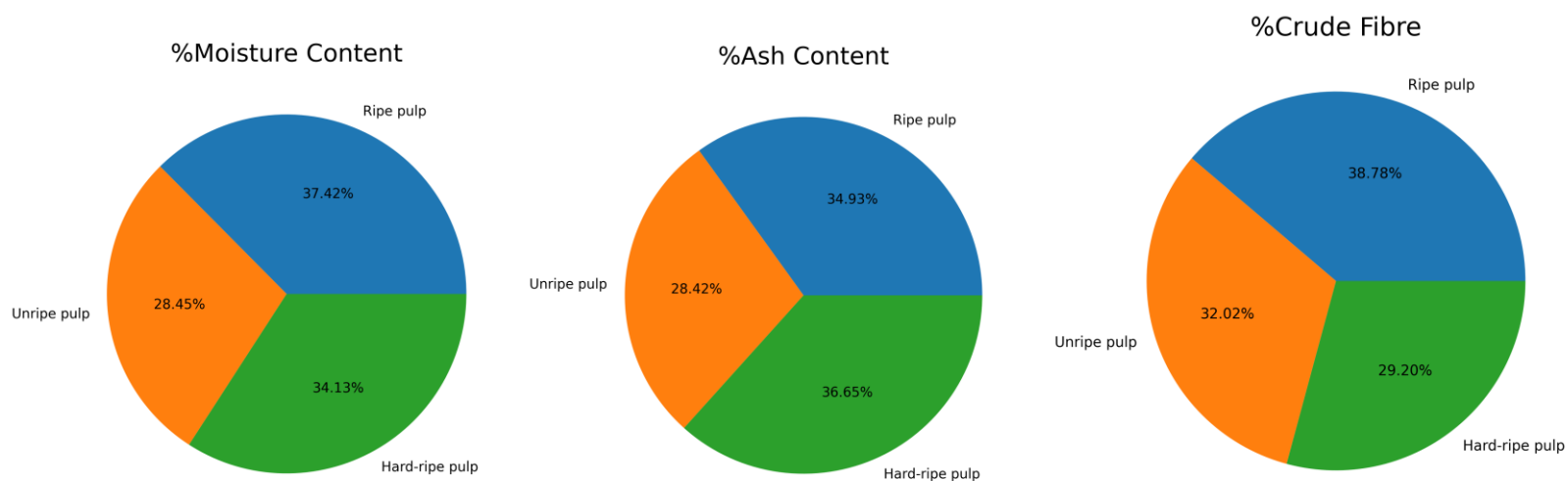
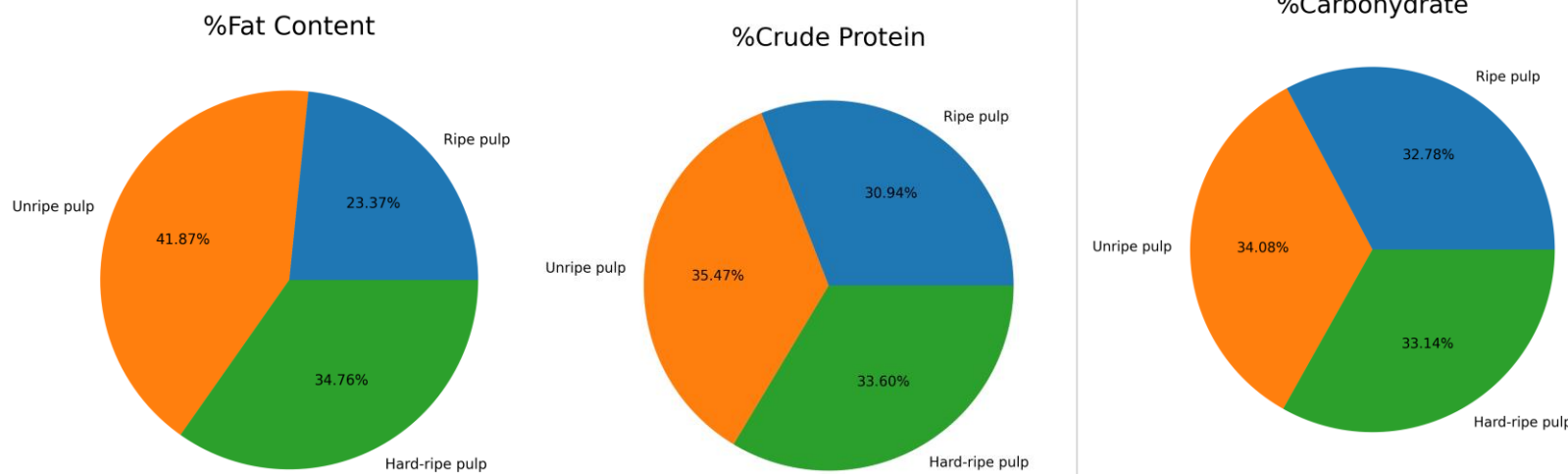


Figure 3.1B: Mean Percentage Mineral composition in pulp of Avocado fruit at different stages of ripening.



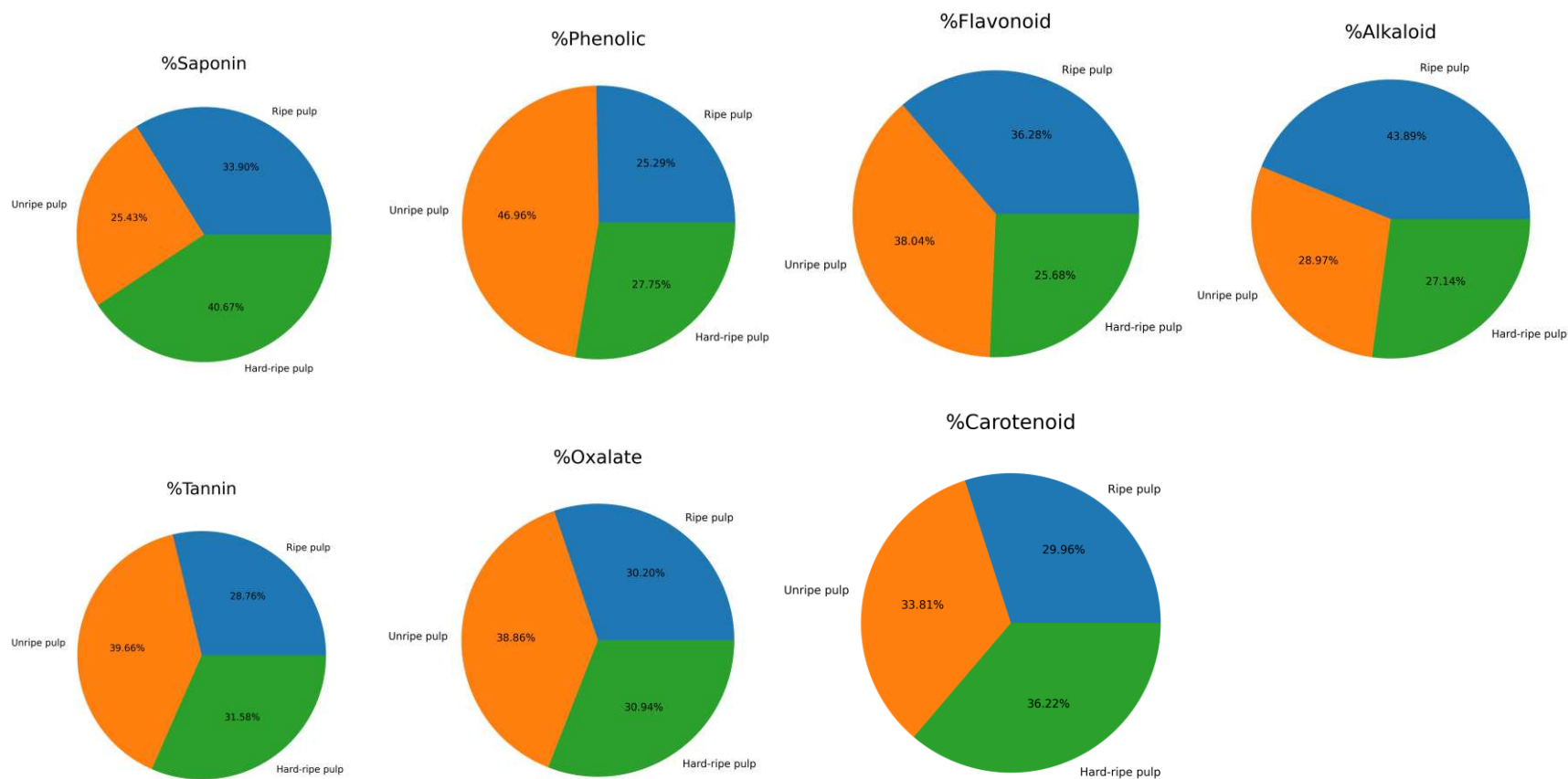
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368 **Figure 3.2: Mean Percentage proximate composition in pulp of Avocado fruit at different stages of ripening**

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371 **Figure 3.3: Mean Percentage Phytochemical compositions in pulp of Avocado fruit at different stages of ripening**

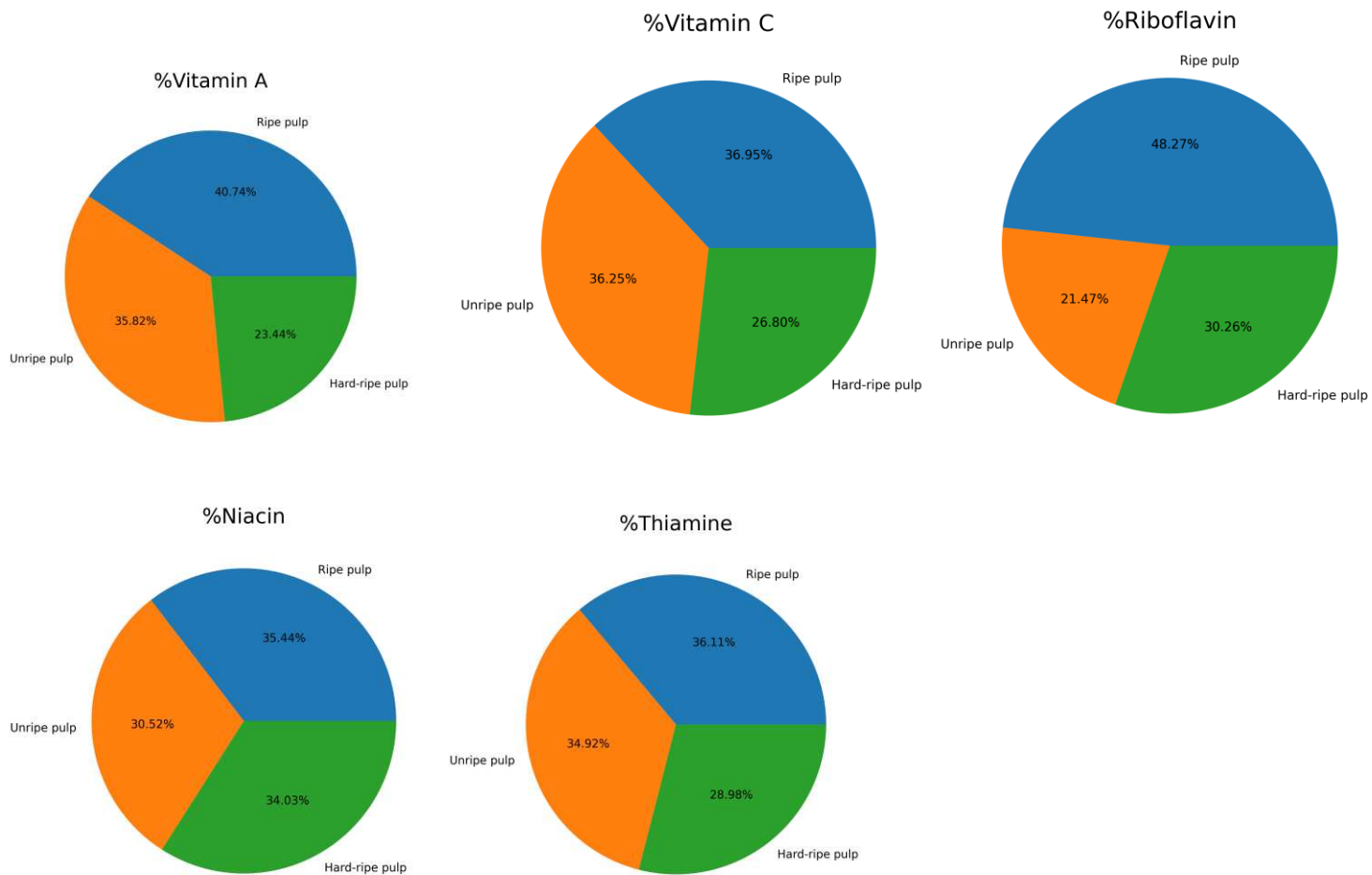


Figure 3.4: Mean Percentage Vitamin composition in pulp of Avocado fruit at different stage

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DISCUSSION

377 *Persea americana*, commonly known as avocado, has recently gained substantial popularity and
378 is often marketed as a superfood because of its unique nutritional composition, antioxidant content,
379 and biochemical profile (Bhuyan *et al.*, 2019). Avocados is among the fastest expanding markets
380 worldwide and consumption particularly in North America and Europe. It has increased during
381 recent decades due largely to a combination of socio-economic and marketing factors. Avocado
382 production, however, is associated with significant water conflicts, stresses and hot spots, as well
383 as with other negative environmental and socio-economic impacts on local communities in the
384 main production zones. It is evident that avocado demand and production are increasing, and the
385 economic market for Avocados continue to expand globally (Sommaruga and Eldridge, 2020).
386 The variety most grown around the world is Hass avocado due to its high fruit yield, resistance to
387 postharvest management, fruit availability almost year-round, and excellent fruit quality
388 characteristics Ramos-Aguilar *et al.*, 2021.

389 Studied on the composition of Avocado fruit pulp at different ripening stages is crucial for various
390 reasons. It aids in dietary planning by revealing changing nutrient levels, helps consumers make
391 healthier choices, and highlights the fruit's potential health benefits. Understanding taste and
392 texture changes is essential for both consumers and food professionals. It also guides storage
393 practices, reduces food waste, informs agricultural decisions, and fosters innovation. Recognizing
394 consumer preferences and economic implications further underscores the importance of this
395 research, making it a multidisciplinary endeavor with far-reaching impacts.

The mineral composition, proximate composition, Phytochemical composition, and vitamin composition in Avocado fruit pulp can vary depending on ripening stage.

4.1 The Mineral Composition in Pulp of Avocado Fruit at Different Stages of Ripening

Figure 3.1A showed the percentage mineral composition includes the (Ca), Magnesium (Mg), Potassium (P), Sodium (Na), Carbon (C), Iron (Fe) and Zinc (Zn) in Avocado fruit at different stages of ripening.

Calcium is an indispensable mineral for human health, it enhances the biological functions of tissues, acts as a cofactor for enzyme reactions and intervenes in physiological processes (Martínez-Ballesta, 2017). In this research work, the highest calcium content was observed in ripe pulp (41.28%). Hard-ripe pulp (27.70%) and unripe pulp (31.03%) were obtained. Magnesium has a strong presence in plant foods, and also plays an essential role in human body maintenance. It is associated with energy metabolism and prevention of chronic disease. Highest level of magnesium in unripe fruit pulp (47.54%) followed by ripe pulp (26.57%) and Hard-ripe pulp (25.89%) were observed. Potassium plays an important role in the human body and sufficient amounts of it in the diet protect against heart disease, hypoglycemia, diabetes, obesity and kidney dysfunction. Regular intakes of potassium lower blood pressure. The findings of this study revealed that the percentage mineral composition of potassium in Hard-ripe pulp (36.45%), ripe pulp (33.73%) and unripe pulp (29.82%) represented in Figure 3.1A. This is in line to the findings of Onwuka (2019) which showed high percentage of potassium content in African Avocado pulp. Sodium had 26.42% in Hard-ripe and 42.10% in ripe as well as 31.48% in unripe, the value from this study showed that the pulp of avocado fruit has high level of sodium in ripe pulp than the Hard-ripe and unripe. Guerrero *et al.* (1998) also reported the high level of mineral content in pulp of avocado fruit which

helps maintain osmotic pressure and balance between the plant cells and intercellular spaces. This regulates water movement within the avocado fruit. Hegedűs *et al.* (2017) reported that Phosphorus has a higher value of mineral composition of 35% in ripe and 25% in Hard-ripe followed by the unripe which had the lowest mineral composition. This is similar to this work in which the ripe pulp had 38.01% and Hard-ripe had the value of 37.89 followed by 24.09% of unripe. Phosphorus had been reported to help to regulate and activate various hormone receptors and hormonal balance in the body (Jones *et al.*, 2019). The zinc content observed were unripe pulp (37.36%), ripe pulp (34.07%) and Hard-ripe (28.57%). Zinc concentration varies in plant foods but fruits are a good source of this element. It is needed for enzyme structure and activity. In addition, its consumption improves the immunity system and it may be a promising option for producing immunity against COVID-19 (Nedjimi, 2020).

4.2 The Proximate Composition of Avocado Fruit Pulp at Different Stages of Ripening

Moisture is one of the most important indices evaluated in foods, especially fruits. It is a good indicator of their economic value because it reflects the fruits' solid contents and serves to assess their perishability (Vinha *et al.*, 2013). The findings of this study revealed the highest moisture content in ripe pulp (37.42%) followed by the Hard-ripe pulp (34.13%) and the unripe (28.45%).

The value of obtained from this research were (36.65%, 34.93%, 28.42%) respectively which is related to the work of (Bolarinwa, 2014) stated the Ash acts as a buffer to help maintain the optimal pH balance in the body and support immune cell function and antioxidant activity to fight infections. ash content increases gradually as Avocado fruit ripens from unripe to ripe stages.

The result obtained from this research work showed high level of crude fibre in pulp of avocado fruit ripe 38.78%, 29.20%, 32.02% respectively this is similar to (Yar Narváez, 2021) which stated

that it is certain that avocado fruits have higher results for these traits because their consumption is related to the reduced risk of diseases, such as cardiovascular issues, and improved digestion, as in the case of fiber.

Crude protein makes up a modest fraction of Avocado pulp, accounting for 20%-40% of the content in unripe fruit. Singh (2021) which is related to this research work which indicate the presence of protein in unripe 35.47%, Hard-ripe 33.60% and ripe 30.94% respectively. Fats are vital in the structural and biological functioning of the cells and help in the transport of nutritionally essential fat soluble vitamins. Fat contents of unripe pulp 43 % obtained for the sample was higher than the 0.23% of the ripe (Louko *et al.*, 2007). Similarly to the previous research the highest value of fat content was in unripe pulp followed by the Hard-ripe and ripe (41.87%, 34.76% and 23.37%) as showed in Figure 3.2. Reported by Santos et al The total carbohydrate results from the study were higher with the value (3.29 g 100 g⁻¹) also reported by (Tan *et al.*,2016 in Red Thompson avocado (3.39 g 100 g⁻¹); this is good because it is recommended that people consume calories in the form of carbohydrates. In this research work it was observed that unripe pulp has the highest value followed by the Hard-ripe and ripe.

4.3 The Phytochemical composition in Pulp of Avocado Fruit At Different Stages Of Ripening

Phenolic content decreases as pears ripen. Hard-ripe 27.75%, ripe 25.29%, unripe 46.96% respectively showed that the phenolic value decreases at different stages of ripening, this indicate the unripe pulp of avocado fruit had highest non-nutritional value of phenolic percentage. The fruit pulp content showed Hard-ripe (38.04%), ripe (36.28%) and unripe (25.68%). It can be stated that flavonoids have health-enhancing special effects like anti-

allergy, antioxidant, anti-inflammatory, anti-cancer and anti-viral, the flavonoids composition was $3.48 \pm 0.09\%$ and $3.38 \pm 0.11\%$ for the pulp (Kindahunsi, 2018).

The presence of alkaloids makes such fruit useful as a stimulant, anaesthetic, pain relieve and are known to exert antipyretic action because they are potent bioactive compounds. Alkaloid components of an investigated fruit pulp was reported by Kindahunsi (2018) to have 30% of alkaloid which is also in support of this research. The Hard-ripe pulp (27.14%), ripe pulp (43.89%) and unripe pulp (28.97%) were observed. Saponin between 30%-46% is higher than that for some fruits, though it lowers cholesterol but has good properties as cytotoxic, haemolysis and permeabilization of the intestine (Sarikahya, 2018). Similar saponin contents Hard-ripe (40.67%), ripe (33.90%) and unripe (25.43%) were observed. Tannins values of Hard-ripe pulp (31.58%), ripe pulp (28.76%) and unripe pulp (39.66%) were observed. The presence of tannins results in lowering of available protein by antagonism and can, therefore, elicit protein insufficiency syndrome such as kwashiorkor, which could be of health benefits to immediate consumers of this fruit (Gilani, 2005).

The percentage of oxalate of Hard-ripe 30.94%, ripe 30.20% and unripe 38.86% as seen in Figure3. showed the highest value of non-nutritional component pf oxalate was found in unripe pulp. It is much safer to consume the fruit with low oxalate because when these plants are eaten they may have an adverse effect because oxalates bind calcium and other minerals.

4.4 The Vitamin Composition in Pulp of Avocado Fruit At Different Stages Of Ripening

Vitamin A is important for eye health and vision, especially adapting to low-light conditions (Drogoudi, 2017). The ripe pulp (40.74%) had the highest value of Vitamin A than the unripe (35.82%) and Hard-ripe (23.44%) respectively. Avocado pulp provides beneficial vitamin C that

has important roles as an antioxidant, for collagen formation, immune health, brain function, iron absorption, and wound healing .In this research the vitamin C content was observed as Hard-ripe (26.80%), ripe (36.95%) and unripe (36.25%). Vitamin C was observed highest in the ripe and unripe pulp. Riboflavin was observed to be between 33-40% of total vitamins in avocado pulp, increasing in concentration and percentage during ripening (Dreher, 2018). The values investigated showed the Hard-ripe pulp (30.26%), ripe pulp (48.27%) and unripe pulp (21.47%). Highest Niacin, (35.44%) and Thiamine (36.11%) were observed in ripe fruit pulp. Thiamine is important for normal growth in children and during pregnancy. It deficiency impairs development (Manzanares *et al.*, 2021).

4.5 Conclusion

The present study demonstrated that ripening significantly influences the proximate, mineral, phytochemical and vitamin composition of *Persea americana* fruit pulp. Unripe pulp exhibited higher concentrations of phytochemicals and antioxidant-associated compounds, while ripe pulp contained higher vitamin levels. Hard-ripe pulp showed favorable proximate characteristics, particularly in fat composition.

The findings suggest that avocado pulp at different ripening stages possesses valuable nutritional and functional properties suitable for food, nutraceutical and health-related applications. The study contributes important compositional data that may support avocado processing, dietary recommendations and future food product development.

4.6 Recommendation

All the identified compositions in the Avocado fruit pulp are of immense economic values. Some of these elements are not only important for carrying out the normal functioning of plant but are

also essential for supporting the various biochemical reactions in human body. Potassium maintains the salt balance of cells tissues while zinc promotes the growth of immune cells. It is therefore recommended that avocado pulp in diets is a great benefits to human health, particularly, the consumption of the Hard-ripe pulp relative to the ripe and unripe.

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4.3 Disclosure Statement

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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4.5 ORCID ID

ORCID ID: <https://orcid.org/0009-0001-4694-3595>

4.6 Author Contributions

A.O.O. conceived and designed the study, supervised the laboratory analyses and prepared the manuscript. M.O.O. participated in data collection, laboratory analyses and data visualization. Both authors read and approved the final manuscript.

4.7 Data availability statement: All-important data supporting the findings of this study are available within the paper. Any further request will be provided.

4.8 Consent to Publish: Not applicable

4.9 Ethics approval and consent to participate: Not applicable, as the study does not involve human participants or animals.

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