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Quantitative Profiling of Nutraceutical Constituents in the Pulp of Four Avocado Cultivars: A Comprehensive Study from the Itasy and Vakinankaratra Regions of Madagascar

[Profilage quantitatif des constituants nutraceutiques dans la pulpe de quatre cultivars d'avocat : une étude approfondie des régions d'Itasy et de Vakinankaratra à Madagascar]

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Abstract

The study of avocado pulp samples reveals distinct mineral compositions, highlighting their potential for nutritional and industrial applications. Notably, the Fucca variety from Vakinankaratra shows the highest magnesium levels, while elements such as sulfur, calcium, and cobalt are absent. Potassium, magnesium, and aluminum are present across varieties, with other minerals detectable in trace amounts. Fucca surpasses the Fuerte and Bacon cultivars in mineral content, with water content ranging from 46% to 55%. The protein content is significantly higher in Fucca from Vakinankaratra (42.28%) and Fuerte from Itasy (46.01%) compared to other varieties, which have much lower protein levels. Organic matter is high in all varieties, especially Fucca from Itasy (69.37%). Water-extractable substances are notably higher in Fucca from Itasy, contrasting with the absence of flavonoids and leucoanthocyanins in Bacon and Fuerte pulps. All varieties contain trace amounts of tannins, with low polyphenol levels in Fucca from Itasy. Different quantities of pulp and drying methods influence oil yield, varying from 49 to 56 grams per 100 grams of dry pulp. Fucca from Vakinankaratra has the highest oil content (56.93%), followed by Fucca from Itasy (53.15%), Bacon from Vakinankaratra (52.03%), and Fuerte from Itasy (49.37%). These findings highlight the nutritional diversity of avocado varieties, suggesting potential targeted applications in the food, pharmaceutical, and cosmetic industries to optimize the use and value of this versatile fruit.

Keywords : Avocado cultivars, Nutraceuticals, Secondary metabolites, Mineral composition, Madagacar.

Résumé

L'étude des échantillons de pulpe d'avocat révèle des compositions minérales distinctes, mettant en évidence leur potentiel pour des applications nutritionnelles et industrielles. Notamment, la variété Fucca de Vakinankaratra présente les niveaux les plus élevés de magnésium, tandis que des éléments tels que le soufre, le calcium et le cobalt sont absents. Le potassium, le magnésium et l'aluminium sont présents dans toutes les variétés, avec d'autres minéraux détectables en quantités infimes. Fucca dépasse les cultivars Fuerte et Bacon en termes de teneur en minéraux, avec une teneur en eau variant de 46 % à 55 %. La teneur en protéines est significativement plus élevée dans la Fucca de Vakinankaratra (42,28 %) et la Fuerte d'Itasy (46,01 %) par rapport aux autres variétés, qui présentent des niveaux de protéines beaucoup plus bas. La matière organique est élevée dans toutes les variétés, particulièrement dans la Fucca d'Itasy (69,37 %). Les substances extractibles à l'eau sont notablement plus élevées dans la Fucca d'Itasy, contrastant avec l'absence de flavonoïdes et de leucoanthocyanines dans les pulpes de Bacon et Fuerte. Toutes les variétés contiennent des traces de tanins, avec de faibles niveaux de polyphénols dans la Fucca d'Itasy. Différentes quantités de pulpe et méthodes de séchage influencent le rendement en huile, variant de 49 à 56 grammes pour 100 grammes de pulpe sèche. La Fucca de Vakinankaratra a la teneur en huile la plus élevée (56,93 %), suivie de la Fucca d'Itasy (53,15 %), du Bacon de Vakinankaratra (52,03 %) et de la Fuerte d'Itasy (49,37 %). Ces résultats mettent en évidence la diversité nutritionnelle des variétés d'avocat, suggérant des applications ciblées potentielles dans les industries alimentaires, pharmaceutiques et cosmétiques pour optimiser l'utilisation et la valorisation de ce fruit polyvalent.

Mots clés: Cultivars d'avocat, Nutracéutiques, Métabolites secondaires, Composition minérale, Madagascar.

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1. Introduction

Urban malnutrition is a growing problem in many regions of the world, exacerbated by the fast-paced lifestyle and the effects of climate change (FAO, 2018, 2019). It is crucial to find innovative solutions to improve the nutritional quality of foods available in urban markets (Dreher & Davenport, 2013; USDA National Nutrient Database, 2020). Avocados, known for their numerous health benefits, could be a potential response to this challenge (Lu et al., 2009; Rodríguez-Carpena et al., 2011; Dreher, 2013; Dreher et al., 2021). However, it is necessary to examine in detail the nutraceutical properties of different avocado varieties to determine their possible contribution to combating urban malnutrition.

Previous studies have shown that avocados, due to their high nutrient content, can offer various health benefits (Villa-Rodríguez et al., 2011; Yahia & Woolf, 2011; Afzal et al., 2022; Iqbal & Poór, 2024). However, most research has focused on the general aspects of avocados without a precise distinction between different varieties and their geographical origins. Furthermore, although avocado vegetable oil is recognized for its cosmetic properties, few studies have deeply explored the mineral micronutrients and macronutrients of avocados from specific regions, such as the volcanic regions of Itasy and Vakinankaratra. This study aims to fill these gaps by comparing and investigating the nutraceutical properties of four avocado cultivars: Fuerte, Fucca, and Bacon, each presenting distinct shapes and colors. These avocados come from the volcanic regions of Itasy and Vakinankaratra, known for their exceptionally fertile soils. The specific objectives of the study are as follows: (1) Analyze the mineral micronutrient content: Use a portable X-ray fluorescence device to determine the levels of potassium, magnesium, iron, phosphorus, and calcium in the different avocado varieties; (2) Evaluate the macronutrient composition: Measure moisture, protein, lipids, and total ash content using the Kjeldahl method, allowing the calculation of carbohydrate content and overall energy value; (3) Examine secondary metabolites: Conduct phytochemical screening to identify the families of molecules present and understand their variation according to regional cultivation conditions; (4) Evaluate nutraceutical properties: Identify potential health benefits, such as hydration, cardiovascular protection, and improved nutrient absorption, based on the specific properties of the avocados studied.

Various scientific methods are employed to explore the avocados nutraceuticals. First, the avocados undergo pre-treatment, including sorting, washing, and separating the skin, pulp, and stone. A meticulous drying process is then performed to preserve the integrity of the pulp, which is subsequently ground into a coarse powder for analysis (Robijaona et al., 2024). It should also be noted that in a study involving 11 leafy vegetables traditionally used as nutraceuticals in the Itasy region of Madagascar, the calcium content shows a remarkably low range, from 0.01% to 0.06% of the dry weight (Robijaona, 2023).

2. Material and Methods

2.1. Material

2.1.1. The avocado tree

The avocado (Persea americana) is a fruit tree with notable variability in its growth, influenced by factors such as variety, environmental conditions, and cultivation practices. Mature avocado trees typically attain heights ranging from 12 to 20 meters and widths from 6 to 12 meters. However, through methods such as dwarf cultivation and container planting, their size can be effectively managed to accommodate limited spaces (Nagase & Lundholm, 2021). As an evergreen species, the avocado tree features a sturdy trunk and extensive branching. Its growth habit exhibits considerable diversity depending on the variety and propagation techniques employed. Grafted trees can exhibit various forms, including the upright shape of the 'Lula' variety, the spherical form of the 'Peterson' variety, the goblet shape of the 'Collinson' variety, and the pyramidal structure of the 'Zutano' variety. Seedling trees, in contrast, generally maintain an upright, orthotropic growth pattern (Gaillard & Godefroy, 1994).

2.1.2. The avocado leaves

The leaves of the avocado (*Persea americana*) are evergreen, leathery, entire, and acuminate. In their juvenile stage, they exhibit a light green coloration, maturing to a shiny dark green. When crushed, the leaves of Mexican varieties and some hybrids emit a characteristic aniseed scent. Avocado leaves contain abacatine, a bitter compound with diuretic properties (Gaillard & Godefroy, 1994).

Adeyemi et al. (2002) demonstrated that an aqueous extract of avocado leaves exhibits analgesic and anti-inflammatory properties. Additionally, this extract contains bioactive molecules with vasodilatory and hypotensive effects, which could be beneficial for managing hypertension. Ojewole et al. (2007) further explored these cardiovascular benefits in a study published in the Cardiovascular Journal of South Africa, where they investigated the effects of *Persea americana* Mill (Lauraceae) aqueous leaf extract on experimental animals. A study conducted by Assoumou et al. (2021) on isolated rat aorta demonstrated moderate vasorelaxation and confirmed the absence of toxicity associated with the aqueous extract of *Persea americana* leaves.

2.1.3. The flowers

The avocado tree (*Persea americana*) exhibits dichogamy, a form of hermaphroditism where differentiation between male and female sexual characteristics occurs over time. The flowers, measuring 5 to 10 mm and yellow-green in color, open twice: initially as female and subsequently as male. Unlike typical flowers, they lack petals and instead have six sepals arranged in two whorls. The flowers contain both male and female reproductive organs and are clustered in panicles of 200 to 300 at the branch tips. Despite the abundance of flowers, only a small percentage of these panicles yield fruit, with approximately 5% of floral clusters being sterile (Fayet, 2017). There are two types of dichogamous flowering patterns in avocados: Type A (protogynous cultivars): Female flowers are receptive to pollen in the morning, while male flowers release pollen in the afternoon, meaning the individual is first female and then male. And type B (protandrous cultivars): Female flowers are receptive to pollen in the afternoon, while male flowers release pollen in the morning, meaning the individual is first male and then female.

2.1.4. The fruit of the avocado tree: the avocado

The avocado (*Persea americana*) consists of several distinct parts: - The exocarp: a thin, dark green rind that can be removed from the fruit; - The mesocarp: the outer green layer of the pulp; - The endocarp: forming the bulk of the pulp; - The seed or kernel: located centrally, sometimes encased in a hard shell (Biale & Young, 1971; Valmayorb, 1967). Avocados grow on trees and can remain attached for several months before picking. Postharvest ripening begins once the fruit is harvested. This phenomenon, known as the "tree factor," is due to inhibitory substances transmitted to the fruit by the leaves of the tree (Hernández et al., 2016).

2.1.5. Plant materials used

Avocados (*Persea americana*) are cultivated in various regions, with a preference for volcanic soils that provide optimal growing conditions. For this study, avocados were collected exclusively from the Itasy and Vakinankaratra regions. The three varieties examined were *Fucca*, *Fuerte* and *Bacon*.

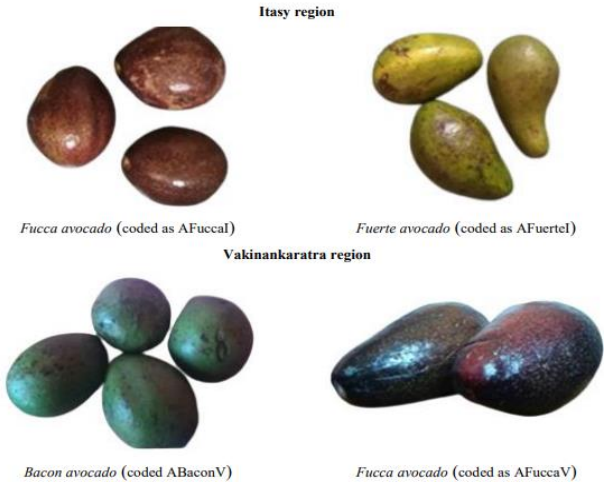

Figure 1. Avocado varieties studied

2.2. Methods

2.2.1. Drying operation

The fruits were carefully washed and pitted, and their pulp was extracted. Drying involves determining the water content or moisture of the plant material, in this case, the avocado pulp. Various methods exist for drying avocados; however, in this study, we employed a dehydrator or an electric oven set at 60 °C to preserve the integrity of the avocado's molecular composition during the process.

2.2.2. Determination of micronutrients

Micronutrients, including vitamins, minerals, and trace elements, are essential for optimal bodily function despite not providing energy. Operating in small quantities, they play crucial roles in various physiological processes. In X-ray fluorescence (XRF) analysis, a solid or liquid specimen is irradiated with high-energy X-rays emitted from a precisely controlled X-ray tube.

When these X-rays interact with the sample, they can eject an electron from an atom's K or L shell if the X-ray photon's energy exceeds the binding energy of the shell. The atom then stabilizes by filling the vacant orbital with an electron from a higher-energy shell. As this electron transitions to its lower energy state, it emits a fluorescent X-ray with energy corresponding to the specific difference in energy between the electron's quantum states.

The measurement of this energy differential forms the basis of XRF analysis (Zhou et al., 2019). In the analytical setup, a pristine, unprinted polyester film is carefully folded in half and placed between cylinders. Two grams of powdered sample are then carefully introduced into the hollow cylinders, filling the cylinder pot to 80-90% of its capacity. The apparatus is then set to mineral mode for the analysis of a pre-designated sample, with the analytical process typically completed within a minute. Calculations are performed promptly using dedicated software tools once the analysis is initiated.

2.2.3. Determining macronutrients

Macronutrients constitute the primary constituents of dietary intake, serving as vital sources of energy in the form of calories. This category encompasses proteins, lipids, and carbohydrates, among other essential nutrients (Robijaona, 2023).

a) Determining the protein content

Proteins serve as essential structural components in the human body's cellular framework, characterized primarily by their nitrogen content. The analytical technique used in this study was the Kjeldahl method, performed at the FOFIFA laboratory. This method involves treating the sample with concentrated sulfuric acid, which leads to thermal degradation, breaking down nitrogenous organic compounds. During this process, carbon and hydrogen atoms are released as CO_2 and H_2O, respectively, while nitrogen is converted to ammonia, which then forms $(NH_4)_2SO_4$ when complexed with sulfuric acid. Potassium sulfate (K_2SO_4) is added to raise the boiling point of sulfuric acid to 430 °C, and copper sulfate ($CuSO_4$) acts as a catalyst in the reaction. The ammonia generated is converted to the borate form by treating it with a concentrated sodium hydroxide solution and steam. The resulting borate compound is quantified through titration with a standard sulfuric acid solution. The Kjeldahl method involves three main stages for protein determination: organic nitrogen mineralization, nitrogen distillation, and subsequent titration.

1° Mineralization

This process entails the conversion of organic matter into mineral constituents, with protein nitrogen being converted into ammoniacal nitrogen. Initially, the samples are pulverized, followed by the precise weighing of 0.3 g, 2.7 g of potassium sulphate, and 0.3 g of copper sulphate. These components are then combined in a digestion tube and treated with 10 mL of concentrated sulfuric acid. Vigorous heating is applied, typically around 430°C, until the solution assumes a green hue and ceases boiling, after which it is allowed to cool. The resulting white solution represents the ammonium salt or mineralisate. The mineralization process is represented by the following balanced equation:

$$\underset{\text{Aminoacid}}{H_2N\text{-}CHR\text{-}COOH} + H_2SO_4 \longrightarrow CO_2 + H_2O + \underset{\text{Ammonium sulphate}}{(NH_4)_2SO_4}$$

Following the cooling process, transfer the contents of the digestion tube to a 50 mL volumetric flask and adjust to the mark with distilled water. Subsequently, nitrogen is distilled from the solution.

2° Distillation

Dispense 10 mL of the test sample and an equal volume of sodium hydroxide solution into the distillation apparatus. Direct the resultant distillate into a 125 mL Erlenmeyer flask pre-filled with 20 mL of boric acid solution. Conduct the determination using the sulfuric acid solution. Concurrently, prepare a control solution under identical conditions, allowing it to react for 10 minutes until the appearance of a green hue, a characteristic indicative of the presence of nitrogen and ammonium. This resulting green solution, recognized as the distillate obtained, is referred to as ammonium borate.

The distillation process is succinctly described by the following balanced equations:

$$NH_4^+ + NaOH \longrightarrow Na^+ + NH_3 + H_2O$$

$$NH_3 + H^+ \longrightarrow NH_4^+$$

3° Titration

For this final step, the apparatus comprises a magnetic stirrer, a 25 mL burette, and 0.1N sulfuric acid employed as the titrant. The solution subject to titration is the distillate, also referred to as ammonium borate. Positioned on the magnetic stirrer, it undergoes agitation before the gradual addition of the titrant (H_2SO_4). Upon stirring, the green solution transitions to a pink hue, with the darkness of the pink indicative of the presence or absence of nitrogen. The protein content of the pulp of each variety is quantified through the utilization of the ensuing formula:

$$\%\,N = \frac{0{,}07 \times (V_f - V_i)}{m}$$

V_f: Final titration volume
V_i: Initial titration volume
m: Mass of sample

$$\%Protein = \%N \times 6{,}25$$

0.07: Conversion factor used to calculate the nitrogen content (% N) in the sample 6.25: Conversion factor used to convert nitrogen content (% N) to protein content (Krul, 2019).

4° Determination of moisture or water content

Water constitutes a predominant component in food, exerting significant influence on various processes such as oil extraction, plant material preservation, and oil composition. Consequently, its content necessitates determination. The analysis was conducted at the organic chemistry laboratory of the Ecole Supérieure Polytechnique in Antananarivo. To ascertain the moisture content of the pulp, samples were subjected to drying in an oven set at 105°C for approximately one hour. Each variety's flesh was represented by a 10 g test sample, prepared following requisite pre-treatment procedures prior to drying. Utilizing a capsule to contain the test sample, measurements were obtained using a balance to weigh both the empty and filled capsules. The water content is computed using the following formula:

$$M\% = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

$M\%$: Moisture content in percentage

W_0 : Weight of empty capsule in grams

W_1 : Weight of the capsule with the test sample before steaming in grams

W_2 : Weight of the capsule with the test sample before steaming in grams

Table 1. Water content of the pulp of each variety

Sample	Empty capsule	Test sample Ts	Capsule + Ts after drying	Différence
AFuertel-1	33.7350	10.4558	38.8276	5.3632
AFuertel-2	7.2314	10.2071	12.1054	5.3331
AFuertel-3	8.8862	10.7650	12.6480	7.0032
AFuccaI-1	8.8862	10.2653	14.1564	4.9951
AFuccaI -2	33.7350	10.8325	39.7864	4.7811
AFuccaI -3	6.9041	10.2597	12.3894	4.7744
ABaconV-1	6.8551	10.7976	12.6515	5.0012
ABaconV-2	7.2314	10.0978	12.4253	4.9039
ABaconV-3	8.2121	10.6237	12.7557	6.0801
AFuccaV-1	8.2121	10.5686	12.7213	6.0594
AFuccaV-2	6.8191	10.2799	11.2968	5.8022
AFuccaV-3	7.5157	10.0610	12.1561	5.4206

2.3.2. Determination of organic carbon

Carbon analysis is essential for quantifying the organic matter in plant material, with a specific focus on avocado pulp. The analysis was conducted at the FOFIFA laboratory in Ampandrianomby. Organic carbon was oxidized using an excess of potassium dichromate solution in an acidic medium. The residual dichromate was subsequently quantified through titration with ferrous sulfate solution. The corresponding chemical reactions are as follows:

Oxidation of organic C:

$$C\ (org) \rightleftharpoons C^{+4}\ (CO_2) + 4\ e^-$$

$$Cr_2O_7^{2-} + 14\ H^+ + 6\ e^- \rightleftharpoons 2\ Cr^{3+} + 7\ H_20$$

Titration of the excess dichromate:

$$Fe^{2+} \rightleftharpoons Fe^{3+} + e^-$$

$$Cr_2O_7^{2-} + 7H^+ \rightleftharpoons 2\ Cr^{3+} + 7\ H_20$$

After preparing the samples, accurately weigh 0.01 g and transfer it to a beaker. Add 10 mL of 1N potassium dichromate and gently swirl the beaker to disperse the plant material in the solution. Rapidly add 20 mL of concentrated sulfuric acid ($H_2\ SO_4$). Swirl the beaker and shake vigorously for 1 minute, ensuring all handling is conducted under a fume hood for safety. Allow the mixture to stand for 30 minutes. Subsequently, add 200 mL of distilled water. Introduce 4 drops of ortho-phenanthroline and titrate the solution with 0.5 N ferrous sulfate ($FeSO_4$). The endpoint of the titration is indicated by a color change from intense green to purplish red. A blank test should be conducted under identical conditions to ensure accuracy.

2.3.3. Phytochemical screening

Phytochemical screening involves a variety of methods and techniques for extracting and analyzing natural organic substances from plants. Phytochemicals are naturally occurring organic compounds found in plant tissues, and they can also be present in plant-derived foods. These compounds may have biochemical importance or contribute to the color and other sensory properties of the plant material (Robijaona, 2024). Phytochemical screening of dried powder from three varieties of avocado is presently underway at the Chemistry and Microbiology Laboratory (CML) in Antananarivo.

2.3.4. Preparation of the extracts

The preparation of extracts is a crucial step in phytochemical screening. The following extracts will be processed from the powdered sample: aqueous extract, alcoholic extract, and acidic extract.

a) Alcoholic extract

The preparation involves introducing 2 g of the dry sample into 20 mL of alcohol at 96°C for maceration over 24 hours. The mixture is then filtered using filter paper, and the resulting filtrate constitutes the alcoholic extract.

b) Acid extract

To prepare the acid extract, 1 g of dry sample powder is added to 10 mL of 2N hydrochloric acid (HCl). The mixture is macerated overnight for approximately 8 hours at 4°C, then shaken and filtered through filter paper. The resulting filtrate is the acid extract.

c) Mechanism of action

Phytochemical screening employs chemical reactions to identify specific chemical groups within various aqueous, alcoholic, and acidic extracts. These tests provide insights into the chemical composition of the plant material. The screening is primarily conducted using tube reactions, and the results are categorized as follows:

Definitely positive test: +++
Moderately positive test: ++
Weakly positive test: +
Negative test: -

d) Flavonoids and leucoanthocyanins screening

For this procedure, the alcoholic extract is divided into three test tubes. Each tube is used to perform a different test: the first tube is for the Wilstätter test, the second for the modified Wilstätter test, and the third for the Bate-Smith test.

1° Wilstater test :

The test involves mixing concentrated hydrochloric acid with 3 g of magnesium turnings, followed by the addition of 1 mL of the alcoholic extract to the mixture. The resulting color change is observed: a red color indicates the presence of flavones, a purple color signifies flavonols, and a purplish-red color denotes flavanones and flavanols.

2° Modified Wilstater test :

In this experiment, a mixture containing 0.5 mL of hydrochloric acid (HCl), 3 g of magnesium turnings, and 1 mL of distilled water is added to 1 mL of isoamyl alcohol. The presence of flavones is indicated by a red color in the upper phase, flavonols by a purple-red color, and flavonones and flavanols by a purplish-red color.

3° Bate-Smith test :

To detect leucoanthocyanins, add 0.5 mL of concentrated hydrochloric acid to 3 mL of an alcoholic extract. Heat the resulting solution in a water bath at 100°C for 30 minutes, then allow it to cool. The presence of leucoanthocyanins is indicated by a purplish-red coloration.

e) Tannins and polyphenols screening

The detection of tannins and polyphenols was carried out through three distinct assays utilizing aqueous extracts: the 1% gelatin assay, the salted gelatin assay, and the $FeCl_3$ ferric chloride assay conducted in methanol.

1° 1% gelatine test: Polyphenols can be identified by adding 5 drops of 1% gelatin to 0.5 mL of an aqueous extract. The formation of a precipitate indicates the presence of polyphenols.

2° Salted gelatine test: To detect tannins, add 5 drops of salted gelatin to 0.5 mL of an aqueous extract. The presence of a precipitate indicates the presence of tannins.

3° $FeCl_3$ test in MeOH: To detect the presence of tannins, a solution containing 5 drops of 10% ferric chloride ($FeCl_3$) in methanol is added to 0.5 mL of an aqueous extract. The appearance of a blue-green or green-black color indicates the presence of condensed tannins, while a change to a bluish-black color indicates the presence of hydrolysable tannins.

f) Saponin screening

The test for saponin presence, known as the foam test, involves adding 1 g of dried sample powder to 5 mL of distilled water. The resulting solution is vigorously shaken for 30 seconds and then allowed to rest for 10 minutes. The formation of foam measuring 3 cm or more in height indicates the presence of saponins. Before this test, each dried sample of avocado pulp and seed from the three varieties was weighed. Distilled water was then added to each test tube to facilitate the agitation of the resulting solution.

g) Deoxyoses screening

The Keller-Killiani test is used to detect deoxyhexoses. It involves adding 0.5 mL of 10% aqueous ferric chloride ($FeCl_3$) solution and 0.5 mL of glacial acetic acid to 0.5 mL of aqueous extract, followed by agitation and addition of 0.5 mL of 36.76 N sulfuric acid (H_2SO_4). The expected result is the formation of a purple-red separation ring. Another test, the hydrochloric acid-acetone test, identifies the presence of 6-deoxyhexoses in the sample. This test involves adding 10 mL of hydrochloric acid and 1.5 mL of acetone to 10 mg of sample powder. The resulting solution is then heated in a boiling water bath for 10 minutes. A positive result is indicated by the appearance of a red color.

h) Quinone screening

The Bornträgen test is performed to detect the presence of quinones. In this test, 5 drops of 25% NH4OH and 1 mL of benzene are added to 0.5 mL of aqueous extract, and the mixture is stirred thoroughly. The presence of a red color in the upper or alkaline phase indicates the presence of anthraquinones.

i) Water extractable substances (WES)

To evaluate the water-soluble components in the sample, the following procedure is followed: Initially, 1 g of sample powder is boiled in 20 mL of distilled water on a hot plate for 15 minutes, followed by a cooling period of approximately 20 minutes. The resulting mixture is then filtered, and the filtrate is transferred into a pre-weighed glass petri dish. This dish is placed in an oven set to 60°C for 72 hours. The outcomes obtained after the oven drying process reveal the components extracted by water.

Before boiling, each type of avocado pulp and seed is placed in 20 mL of distilled water. The samples are then agitated on a vibrating hot plate for 15 minutes. After boiling, the solutions containing each type of avocado pulp are allowed to cool to room temperature before filtration. Water-extractable substances are obtained using the following formula:

$$WES = (W - W_0) \times 100$$

W : Weight of petri dish + residue

W_0 : Weight of empty petri dish

j) Avocado oil extraction

The extraction process remains consistent across all varieties, employing hexane as the solvent in a Soxhlet extraction setup. Before extraction, the dry pulp powder is weighed to ensure it does not exceed 30 g, maintaining compatibility with the apparatus. The empty flask is also weighed prior to starting the extraction process. Post-extraction, the flask containing the oil undergoes heating in an oven to remove any remaining hexane. Once dried, the oil is transferred to a desiccator and weighed for additional analysis.

3. Results and Discussion

3.1. Micronutrient results

The micronutrient analysis outcomes for the pulp of each respective variety are presented in the table provided herein:

Table 2. Micronutrients content

Element	AFuccaI	AFuerteI	AFuccaV	ABaconV
Mg (%)	1.68	1.34	1.38	1.86
Si (%)	0.00	1.03	1.10	0.22
P (%)	0.38	0.29	0.24	0.31
S (%)	0.00	0.00	0.00	0.00
K (%)	3.43	3.52	2.09	0.83
Ca (%)	0.00	0.00	0.00	0.00
Ti (%)	0.12	0.12	0.12	0.12
V (%)	0.01	0.02	0.01	0.01
Cr (%)	0.04	0.06	0.02	0.03
Mn (%)	0.00	0.04	0.00	0.02
Fe (%)	0.38	0.44	0.61	0.45
Co (%)	0.00	0.00	0.00	0.00
Ni (%)	0.04	0.05	0.04	0.05
Cu (%)	0.01	0.02	0.02	0.02
Zn (%)	0.02	0.01	0.01	0.01
As (%)	0.01	0.01	0.01	0.01
Se (%)	0.00	0.00	0.01	0.01
Sn (%)	0.00	0.00	0.00	0.00
Sb (%)	0.01	0.00	0.00	0.00
Ag (%)	0.01	0.02	0.02	0.02
Mo (%)	0.03	0.00	0.00	0.003
Zr (%)	0.07	0.09	0.08	0.09
Rb (%)	0.05	0.05	0.03	0.05
Sr (%)	0.04	0.06	0.03	0.06
Ba (%)	0.03	0.01	0.05	0.02
W (%)	0.03	0.05	0.00	0.05
Ta (%)	0.00	0.00	0.00	0.00
Au (PPM)	0.00	0.00	0.00	0.00
Hg (PPM)	0.00	0.00	0.00	0.00
Pb (%)	0.00	0.00	0.00	0.00
Cd (%)	0.00	0.00	0.00	0.00

Upon examination of the tabulated values, it becomes evident that certain elements such as Sulfur (S), Calcium (Ca), Manganese (Mn), Cobalt (Co), Selenium (Se), Tin (Sn), Tantalum (Ta), Gold (Au), Mercury (Hg), Niobium (Cb), and Cadmium (Cd) are notably absent in the pulp samples, contrasting with the presence of Potassium (K), Magnesium (Mg), and Aluminum (Al). Notably, the Fucca variety from the Vakinankaratra region exhibits the highest concentration of magnesium, while specimens from the Itasy region demonstrate elevated levels of both magnesium and aluminum. Consequently, it can be inferred that the Fucca cultivar predominates over its counterparts, Fuerte and Bacon, in terms of mineral content within the pulp. Other mineral constituents are discernible but present in marginal quantities.

3.2. Macronutrient results

3.2.1. Moisture content

Upon adherence to the prescribed protocol for water content determination, the resultant values for each respective variety are delineated in the subsequent table:

Table 3. Pulp moisture content

Sample	Mean difference	Average of Ts	Moisture	Standard deviation
AFuccaI	4.8502	10.4525	46.4022	0.1024
AFuerteI	5.8998	10.4759	55.3178	0.7805
ABaconV	0.3284	10.5063	50.7162	0.5330
AFuccaV	5.7607	10.3031	55.9122	0.2630

Ts : test drive

According to the data presented in the aforementioned table, the water content of the investigated varieties falls within the range of 46% to 55%.

3.2.2. Protein content results

The outcomes regarding protein content are depicted in the ensuing table 4.

Table 4. Proteins content

Sample	AFuccaI	AFuerteI	ABaconV	AFuccaV
% N	0.10	7.36	0.07	6.76
% Proteins	0.63	46.01	0.44	42.28

The protein content of Fuerte from Itasy and Fucca from Vakinankaratra significantly surpasses that of the remaining varieties.

3.3. Organic matter content

Upon completion of the experimental analyses, the ensuing table delineates the organic matter content observed in each variety:

Table 5. Organic matter content

Sample	AFuccaI	AFuerteI	ABaconV	AFuccaV
Mass of sample (g)	0.0126	0.0118	0.0128	0.0187
Initial titration volume	0.0	22.1	16.2	15.0
Final titration volume	16.2	39.8	32.6	28.6
Carbon (%)	40.24	32.86	38.22	39.48
Organic matter (%)	69.37	56.66	65.89	68.06

From these findings, it is evident that the pulps of all four avocado varieties exhibit notable levels of organic matter. Specifically, in the specimens originating from the Itasy region, Fucca exhibits a pronounced dominance over Fuerte, with a disparity of 12.71%. Conversely, within the Vakinankaratra region, the organic matter content of the Bacon

variety does not significantly differ from that of the Fucca variety, with a marginal difference of 2.17%.

Notably, the Fucca variety demonstrates a heightened organic matter content across both the Itasy and Vakinankaratra regions.

3.4. Phytochemical screening

3.4.1. Water extractable substances (WES)

The obtained values for the Itasy region are categorized in the following table:

Table 6. Water extractable substances (WES)

Sample	W_0 (g)	W (g)	WES (g per 1 g of dry pulp)
AFuccaI	42.303	43.284	0.981
AFuerteI	43.823	43.894	0.071

Based on the values presented in the table for the two avocado varieties sourced from the Itasy region, it is evident that the Fucca variety exhibits a higher concentration of water-extractable substances.

3.4.2. Identified chemical groups

The chemical groups found in avocado pulp were identified using a series of phytochemical screening assays. The results of these assays are presented in the following table:

Table 7. Identified chemical groups

Test	Chemical families		AFuccaI	AFuerteI	ABaconV	AFuccaV
Wilstater						
Wilstater modified	Flavonoids	Flavones			–	–
		Flavonols			–	–
Bate Smith	Leucoanthocyanins				–	–
Foam test	Saponins		+	+ +	–	–
Bornträgen test	Quinones				+	–
1% Gelatin test	Tannins		+	–	+	+
	Polyphenols		+	–	–	–
Salted gelatin test	Tannins		–	–	+ +	+
	Polyphenols		–	–	–	–
$FeCl_3$ in MeOH test	Tannins		–	–	+	+
	Pyrogallic tannins		–	–	–	–
	Phenolic compounds		–	–	–	–

Observations reveal the absence of flavonoids and leucoanthocyanins in the avocado pulp of both the Bacon and Fuerte varieties. Conversely, each variety exhibits traces of tannins. Notably, the Fucca d'Itasy variety manifests small quantities of polyphenols, whereas the other varieties do not exhibit this presence.

3.4.3. Avocado oil yields

The employed technique involves solvent extraction utilizing the Soxhlet apparatus. The resulting oil extraction yield is documented and presented in the subsequent table.

Table 8. Avocado oil yield for the four varieties

Sample	AFuccaI	AFuerteI	ABaconV	AFuccaV
Pulp (g)	46.459	52.912	101.022	60.357
Oil (g)	24.694	26.126	52.571	34.365
Yield (%)	53.15	49.37	52.03	56.93

It is important to note that the quantity of pulp varies among different avocado varieties due to their individual weights and pulp consistencies. Additionally, the drying methods used varied: the Fucca variety from the Vakinankaratra region (ABaconV) was dried in an oven at 60 °C, while the Itasy variety (AFuccaI) underwent dehydration. Despite these differences, the oil content for both varieties remains consistent, representing nearly half the mass of the dry pulp from which the extraction was conducted. According to the data in the table, the oil yield ranges from 49 to 56 g per 100 g of dry pulp. The Bacon variety from the Vakinankaratra region exhibits the highest oil content per 100 g of pulp, followed by the Fucca variety from the Itasy region, the Vakinankaratra variety, and finally, the Fuerte variety from the Itasy region.

4. Conclusion and Applications

The conclusion of the comprehensive analysis of avocado pulp samples (Fuerte, Bacon, and Fucca) reveals distinct mineral profiles, with Fucca displaying the highest magnesium concentration, particularly in the Vakinankaratra region, and elevated levels of magnesium and aluminum in specimens from the Itasy region. The water content of avocado pulp ranged from 46% to 55%, while the protein content of Fuerte from Itasy and Fucca from Vakinankaratra exceeded that of other varieties. Organic matter content was notably high for all varieties, with Fucca d'Itasy dominating over Fuerte. Water-extractable substances were more concentrated in Fucca d'Itasy, while flavonoids and leucoanthocyanidins were absent in Bacon and Fuerte, and traces of tannins were present in all varieties. Fucca d'Itasy exhibited small quantities of polyphenols, distinguishing it from others. Oil yield ranged from 49 to 56 g per 100 g of dry pulp, with Fucca from Vakinankaratra having the highest oil content. Avocado is a nutraceutical-rich fruit with significant potential for combating urban malnutrition. Increase awareness of avocado's nutritional benefits, especially its high mineral and protein content, to combat malnutrition. Encourage cultivation of Fucca varieties, particularly those from Vakinankaratra and Itasy regions, for their superior mineral and oil content. Explore innovative uses of avocado oil and organic matter in the food and cosmetic industries.

Conduct further research on avocado varieties to explore additional health benefits and potential applications in functional foods and pharmaceuticals.

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