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Obtention and Evaluation of Lyophilized Pulp Powder of Different Cultivars of Avocado

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Abstract: There are many studies focused on the extraction and commercialization of lipid fractions of avocado pulp, because its integral pulp has few applications in food industry, due to many problems, such as fast enzymatic darkening that causes alterations in color and flavor. This enzymatic control is a challenge for food industry, since it depends on many interrelated chemical and biochemical factors. Drying avocado pulp may reduce the activity of some oxidative enzymes and keep stability for other compounds. Thus, this work aimed at lyophilizing pulps of different avocado cultivars and evaluating their physicochemical and biochemical characteristics. It was determined pulp coloration, moisture content, acidity, total and reducing sugars, proteins, lipids, ashes, phenolic compounds, antioxidant activity and activity of oxidative enzymes (polyphenol oxidase and peroxidase). The results characterized the avocado pulp powders, highlighting a high antioxidant activity, which may control enzymatic activity.

Keywords: *Persea Americana* Mill, drying, enzymatic darkening, phenolic compounds.

1. Introduction

Avocado cultivation in Brazil, primarily in the state of Paraná, is still not well explored, due to little commercialization of this fruit and the lack of information that help use efficient postharvest conservation methods. Such information, related to their physicochemical and biochemical composition, is scarce in literature. Introducing new products into the food sector helps move other areas, such as productive area; thus, avocado producers may amplify their cultivated areas and increase their income, since each avocado tree may produce many fruits.

Avocado is cultivated all over the Brazilian territory and, according to Tango and Turatti, it is one of the most productive fruit trees. Also, according to these authors, in average, an avocado is 70% pulp, 17% pit and 14% skin [1]. A great number of avocado varieties is found in several regions in the national territory and their chemical composition is quite variable [2].

There are many cultivars - or varieties - of avocado trees that differ in relation to their fruit characteristics, such as size, shape, skin and pulp color, chemical compounds and others. As examples of cultivars, it can cite “Hass”, “Ouro Verde”, “Quintal”, “Fortuna”, “Brenda”, “Beatriz”, “Choquete”, “Geada” and so forth.

Avocado’s nutritional value is based on their slightly high protein and lipid content, which may reach up to 30% of the pulp. Other substances that constitute the avocado pulp are minerals and vitamins A and E. Due to their chemical composition, this fruit is also suitable for industrial purposes [2,3]. According to Salgado et al., avocado is rich is oleic acid and b-sitosterol, which is an unsaturated fat used as an aid in the treatment of hyperlipidemia [4].

In literature, there are few studies that discuss the use of avocado pulp in food industry and this is due to the fruit’s high enzymatic activity. Adding avocado paste to meat byproducts was an alternative to increase their nutritional value, as suggested by Rueda-Lugo et al., who evaluated the replacement of animal fat with avocado pulp paste, which is rich in vegetable oil, aiming at improving the nutritional quality of sausages. Regarding mineral content, avocado pulp is provided with phosphor, calcium, magnesium, sulfur, copper, iron, manganese, zinc and sodium [5].

The fruit postharvest transformations may also be monitored by evaluating total phenolic compound content, since they participate in the development of flavor, scent, color, shelf life and functionality,

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notably as antioxidants [6]. Some phenolic compounds are used as substrate for oxidative enzymes, such as chlorogenic acid (natural substrate for polyphenoloxidase (PPO)) [7]. PPOs are able to oxide phenolic compounds using molecular oxygen. There are two types of PPOs: o-diphenol-oxidases, also known as catechol oxidase, tyrosinase or phenolase; and p-diphenol-oxidases, named laccase [8]. Peroxidases (PODs) are enzymes that are able to oxide different compounds using peroxides and creating free radicals. Their main substrate is hydrogen peroxide (H_2O_2), though they may also act upon phenols and aromatic amines [7]. Upon the absence of peroxides, PODs may also catalyze the oxidation of certain substrates with the aid of molecular oxygen.

As a result of these catalyzed reactions, it have quinones, which are highly reactive substances that combine in order to generate condensation products with high molecular mass and dark color [7]. This process is called enzymatic darkening that, in many cases, is undesirable, because it also alters flavor, odor and nutritional value [8]. When most fruits are mashed, cut or triturated, they darken due to reactions catalyzed by polyphenoloxidase (PPO). This action upon many fresh fruits and vegetables causes considerable economic losses, in addition to decreasing nutritional quality and altering flavor. Therefore, pulp powders aim at maintaining their quality and increasing their useful life. These factors are strongly dependent on water content, which influences palatability, digestibility, physical structure and handling. Practically all deteriorative processes that happen to food are also influence by water concentration and mobility within it [9].

In order to obtain fruit pulp powders, food processing industry has been using drying processes, such as spray drying atomization and lyophilization. Drying by atomization consists of transforming fluid products into dry particles (powder) and this material should present as many characteristics as in the beginning, in order to protect it from external adverse conditions. Thus wall materials or microencapsulating substances are added to the product [10,11]. Lyophilization is a drying process, in which water is eliminated by sublimation. For that, the fruit pulp is frozen and it is not submitted to high temperatures, as in other drying processes. Freezing must be quick, so that ice microcrystals are formed, otherwise, the cell membranes may be torn and, consequently, lose their cytoplasmic content, causing the food to wilt [12]. Afterwards, the frozen pulp is placed in the vacuum lyophilizer, where dehydration happens. With this vacuum system, pressure drops to 1 mmHg and it should be maintained like this up to the end of the drying process [13].

As for fruit juice, the high sugar content may cause the obtention of highly viscous and hygroscopic products, hampering the process yield. Therefore, it is fundamental to use carriers with high molecular weight - such as polymers and gums - before atomization, aiming at easing drying process and transportation and storage operations. Maltodextrin is a carrier commonly employed at atomization, due to its low hygroscopicity, high solubility in cold water and low cost [14].

Given the above, this work aimed at assessing the characteristics of avocado pulp, lyophilizing them and evaluating their physicochemical and biochemical features with and without maltodextrin.

2. Material and Methods

In this research were evaluated fresh and powdered pulps of different avocado (*Persea americana* Mill.) varieties, cultivated in the Farm-School in the Northwestern Regional Campus from Maringá State University (UEM), in Diamante do Norte, State of Paraná. Fruits of cultivars “Beatriz”, “Breda” and “Ouro Verde” were harvested in a given state of maturation. We separated skin and pit manually. The pulp powders were obtained by lyophilization and characterized as for physicochemical and biochemical features. The pulp was also assessed the cultivar “Ouro Verde” added with 10% and 30% of maltodextrin. For lyophilization, the pulps were frozen in trays at -40ºC for 24 hours, and the layers of pulp had approximately 1.5 cm in height. Then, they were lyophilized for 48 hours, protected from light.

2.1. Physicochemical Analyses

2.1.1. Pulp color

The color pulp was analyzed by using a reflectance colorimeter MiniScan EZ, brand HunterLab, model MSEZ-4000S, and obtained parameters for luminosity (L), varying from 0% (black) to 100% (white) and tendencies to green (a-), red (a+), blue (b-) and yellow (b+), chromaticity, or Chroma (C*) and color angle (Hue). We calibrated the device using a standard plain white plaque of magnesium oxide. We carried out readings at five spots in the sample. Values for L, a, C and Hue were read directly at the colorimeter display.

2.1.2. Moisture determination

The moisture content was determined by drying the sample in a hothouse at 105ºC, until it reached constant weight [15].

2.1.3.Determination of titratable acidity (TA)

To determinations of titratable acidity was transferred 10 g of sample to a 100mL-volumetric flask and completed the volume with distilled water. This solution was titrated with a solution of 100mM of sodium hydroxide until it became rosaceous. We used phenolphthalein as indicator [15].

2.1.4. Determination of reducing and total sugars
The determination of total sugars was carried out using Lane-Eynon method (IAL, 2008). We used 5 g of sample, transferring it and completing its volume with distilled water (200 mL). We added 3.0 mL of concentrated hydrochloric acid (HCl) and homogenized by heating up the mixture and boiling it for 3 hours. After cooling and neutralization with NaOH at 10%, we transferred it to a 250 mL-Erlenmeyer flask and completed the volume with distilled water. Titration was made with 5.0 mL of each Fehling solution - A and B - adding 20 mL of water and boiling it for two minutes. The solution was titrated until the Fehling solutions changed from blue to clear, with a residue of Cu_2O in the bottom of the flask. As for reducing sugars, they were quantified by using 5 g of sample and completing the volume to 100mL with distilled water. The solution was homogenized and titrated with Fehling solutions A and B, as described for total sugars [15].

2.1.5. Lipid determination
The lipid content was carried out the extraction in accordance with Bligh and Dyer method, using a mixture of chloroform, methanol and water and determined the content of total lipids gravimetrically [16].

It was weighed 15 g of sample of avocado pulp in a 250 mL-beaker and added 10 mL of distilled water and 30 mL of methanol. The mixture was homogenized for 1 minute in a magnetic stirrer and then, we added 15 mL of chloroform and homogenized it for more 5 minutes. Afterwards, we added 15 mL of chloroform, stirred it for 2 minutes, added 15 mL of distilled water and stirred it for more 5 minutes. This mixture was filtrated under vacuum in a Buchner funnel and the residue was places into the beaker again and stirred for more 5 minutes, with 10 mL of chloroform. Then, the sample was filtered it again and rinsed it with 10 mL of chloroform and the result was transferred to a 250 mL-separation funnel. After separating phases, the bottom one (with chloroform and total lipids) was drained into a 250 mL flat bottom flask, previously weighed, and the solvent was evaporated in a rotational evaporator, with water bath at 34-36ºC. Lipid content was determined gravimetrically.

2.1.6. Protein determination
Total protein content was quantified by Kjeldahl method for nitrogen determination, using factor 6.5 for protein conversion, according to the methodology [15].

2.1.7. Determination of total ash content
The ashes were obtained by incinerating samples in muffle furnace, at 550ºC, for six hours and then, were determined by weight difference between tared crucibles taken from the muffle [15].

2.1.8. Quantification of phenolic compound content
Determination of phenolic compounds was realized in accordance with Bucic-Kojic et al. In order to obtain the extract, it use ethanol 50% and the determination made using Folin-Ciocateau reagent. It was use 0.2 mL of extract, 1.8 mL of water and 10 mL of the reagent. Between 30 seconds and 8 minutes, we shall add 8.0 mL of a solution of sodium carbonate (7.5%) and stir it in a vortex tube stirrer, followed by a water bath at 45ºC for 15 minutes. Reading was made in spectrophotometer UV/VIS (PG Instruments T80+) at 756 nm. The white was prepared, replacing the ethanolic extract with distilled water and the curve calibration will be made with gallic acid [17].

2.1.9. Antioxidant activity
Total antioxidant activity (AA) was determined by using radical DPPH (1,1-diphenil-2-picrylhydrazyl), according to the method described by Mensor et al. (with modifications), where the reactive medium (extract + DPPH solution and absolute ethanol) had a volume of 3.5 mL. Into 5.0 mL flasks we added: 2.4 mL of absolute ethanol (solvent), 1.0 mL of DPPH solution (6 mg/50 mL) and 0.1 mL of extract.

For each sample, we carried out, concomitantly, in order to correct a likely contribution of extract color, a white test, consisting of the extract volume (0.1 mL) and 3.4 mL of absolute ethanol. The control was prepared by mixing 1.0 mL of DPPH solution (6 mg/50 mL) with 2.5 mL of absolute ethanol.

After 45 minutes of incubation in dark, at room temperature, the sample absorbance values were registered against a white at 517 nm [18]. The tests were carried out in triplicates and DPPH inhibition (in %) was calculated by equation 1:
$AA\% = 100 - [(Aa - Ab) \times 100] / Ac$ (1)
Where:
Aa = sample absorbance;
Ab = white absorbance;
Ac = control absorbance.

2.2. Biochemical Assessments
The avocado pulp were measured before and after the drying process, such as for activity of polyphenol oxidase oxidative enzymes (PPO) and peroxidase (POD). The extract was obtained for the analysis of enzymatic activity by homogenizing the pulp with a buffer solution of sodium phosphate 100 mM and pH (pH 6.0 for POD and 7.4 for PPO). Afterwards, the sample was filtered the homogenized extract with a cotton cloth and centrifuged it (7,000 rpm at 4ºC), separating the supernatant. In order to extract both enzymes from the fruit, it was added

polyvinylpyrrolidone (PVPP) 5% to the homogenized solution in order to prevent the action of phenolic compounds. The avocado pulp extracts were stored at -18ºC and defrosted as needed, to proceed with the analysis. The activity of the enzyme will be monitored by spectrophotometry.

2.2.1. Assessment of the enzymatic activity of polyphenol oxidase (PPO) EC 1.10.3.1

PPO activity was determined in accordance with Fujita et al. In the mix was addition 0.5 mL of PPO extract with 0.8 mL of a buffer solution of sodium phosphate 100 mM and 0.05 mL of a catechol solution 0.01 M, and incubated it at 30ºC for 30 minutes. Afterwards, we added 0.8 mL of perchloric acid 2M and put it in an ice bath. PPO activity was determined by measuring absorbance with spectrophotometer (λ = 395 nm). The unit of PPO activity was defined with the increasing of a unit of absorbance per minute mL^{-1} of sample [19].

2.2.2. Assessment of the enzymatic activity of peroxidase (POD) EC. 1.11.1.7

POD activity was determined in accordance with the methodology described by Clemente. To prepare this solution was added 0.2 mL of the sample, 2.7 mL of a solution of H_2O_2 0.1% in buffer (Sodium phosphate 100 mM, pH 6.0) and 0.1 mL of an alcoholic solution of ortho-dianisidine 1%. Readings were made by spectrophotometer (λ = 460nm). The unit of POD activity was defined with the increasing of a unit of absorbance per minute mL^{-1} of sample [20].

2.3. Statistical Analyses

The results obtained were assessed statistically by Analysis of Variance (ANOVA) and the averages were compared by Tukey test ($p<0.05$), using SAS [21].

3. Results and Discussion

The results obtained for parameters of color characterization of avocado pulp are displayed on Table 1. Values for 'a' and 'b' highlighted the green for both fresh and powder pulps. According to the sequence CIELAB Hue angle [22], which defines red as 0º*h*, yellow as 90º*h*, green as 180º*h* and blue as 270º*h*, the cultivars in this study were greenish yellow. Chroma (C*) defines color intensity, i.e., values close to zero indicate neutral colors (white and/or gray) and values around 60 represent vivid and/or intense colors. Cultivar "Ouro Verde" had the most intense color (highest C* values), after being processed into powder, without maltodextrin. It can be observed that the pulp powders had higher values for luminosity (L) than the fresh pulps.

Table 1. Parameters for color assessment of avocado pulp

Cultivars	Fresh pulp				
	a	b	C	L	H
Beatriz	- 3.78 a*	45.97 a	41.66 a	68.97 a	132.44 a
Breda	- 3.96 a	48.88 a	39.97 a	64.12 a	139.23 a
Ouro Verde	- 0.88 b	35.12 b	40.08 a	69.78 a	128.38 a
Cultivars	Pulp powder				
Beatriz	- 5.74 a	47.71 a	39.46 b	83.94 a	133.12 a
Breda	- 3.96 b	50.41 a	32.00 c	79.11 ab	134.52 a
Ouro Verde	- 2.88 c	38.75 b	52.68 a	76.84 b	118.50 b
Ouro Verde + 10% maltodextrin	- 3.78 b	31.99 c	39.45 b	77.43 b	112.78 b
Ouro Verde+ 30% maltodextrin	- 3.90 b	47.19 a	37.99 b	79.10 a	109.00 b

* Average values followed by the same letter in the columns, for each type of pulp, do not differ significantly by Tukey test ($p>0.05$).

The physicochemical characteristics of the avocado pulps, both fresh andpowdered, can be seen in Table 2. The cultivars had similar values for acidity and did not differ statistically ($p>0.05$). Such parameter indicates the total amount of organic acids in the pulp. Cultivar "Ouro Verde" had the lowest amount of acids, when compared to the other two varieties. The drying process did not change this chemical characteristic in the pulps.

Our values for moisture content in fresh avocados were similar to the ones found in literature.

There were some significant differences among the samples as for ash content; cultivar "Breda" had the lowest content. Ash content represents the amount of minerals in the pulp.

Table 2. Chemical characteristics of avocado pulp.

Cultivars	Fresh Pulp				
	Acidity (g of acid 100 g^{-1})	Moisture (%)	Ashes (%)	AR[1] (%)	AT[2] (%)
Beatriz	0.180 a*	77.03 a	1.10 b	1.70 a	1.77 a
Breda	0.157 b	76.39 a	0.80 c	1.60 a	1.67 a
Ouro Verde	0.083 c	75.44 a	1.29 a	1.83 a	1.95 a
Cultivars	Pulp powder				
Beatriz	0.192 a	2.97 c	1.12 b	1.88 b	2.00 ab
Breda	0.166 b	3.59 b	0.89 c	1.80 b	1.84 b
Ouro Verde	0.085 c	4.24 a	1.34 a	2.02 ab	2.03 ab
Ouro Verde + 10% maltodextrin	0.080 c	2.59 c	1.29 a	2.19 a	2.19 a
Ouro Verde + 30% maltodextrin	0.079 c	4.00 a	1.23 ab	2.30 a	2.32 a

* Average values followed by the same letter in the columns, for each type of pulp, did not differ significantly by Tukey test ($p>0.05$).
AR[1]: Reducing sugars;
AT[2]: Total sugars.

Reducing sugars (glucose and fructose) are usually present in fruit pulps. Total sugars represent the sum of the amount of different sugars in the pulp. Both fresh and processed, cultivar "Ouro Verde" had the highest amount of sugars. These compounds are responsible for their sweet flavor. The values obtained are in accordance with the ones in literature.

In Table 3, the average results for the other chemical compounds in avocado pulps. As for lipid content, it varied among the cultivars, being that "Breda" and "Ouro Verde" had the highest values. The drying process (lyophilization) does not alter the amount of lipids in the pulps, though there are higher amounts in powders, since the quantity of compounds that do not evaporate with water increases in the process.

Protein content varied from 0.96% to 1.22% in fresh pulps and from 1.08% to 1.30% in powdered pulps. Proteins are complex macromolecules, composed by amino acids, fundamental for the chemical processes that occur in living organisms. It can be observe that cultivar "Breda" had the highest rate of proteins in its pulp.

The outcome of the determination of phenolic compounds and their antioxidant activity can also be observed in Table 3.

Table 3. Average values for chemical compounds found in avocado pulp.

Cultivars	Fresh pulp			
	Lipids (%)	Proteins (%)	Phenolic compounds (mg GAE g^{-1})	Antioxidant Activity (%)
Beatriz	11.00 b*	1.10 ab	2.03 a	39.15 a
Breda	14.45 a	1.22 a	0.77 b	31.27 b
Ouro Verde	16.69 a	0.96 b	0.80 b	44.34 a
Cultivars	Pulp powder			
Beatriz	13.74 b	1.23 ab	2.55 a	37.72 a
Breda	19.15 a	1.30 a	0.90 b	26.12 b
Ouro Verde	19.13 a	1.14 b	0.88 b	40.98 a
Ouro Verde + 10% maltodextrin	18.13 ab	1.11 b	0.89 b	33.00 ab
Ouro Verde + 30% maltodextrin	17.30 ab	1.08 b	0.82 b	30.23 ab

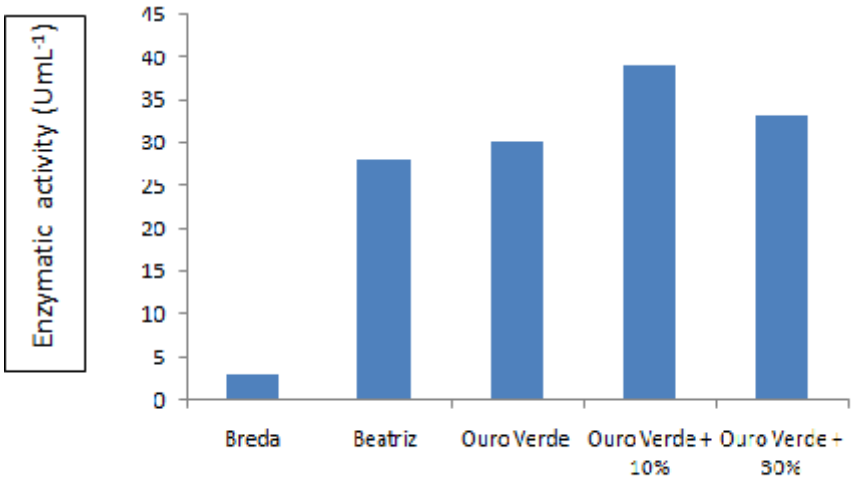
* Average values followed by the same letter in the columns, for each type of pulp do not differ significantly by Tukey test ($p>0.05$).

The numbers for total phenolic compounds varied from 0.77 to 2.55 mg GAE g^{-1} of avocado pulp, considering that cultivar "Beatriz" had the highest rate. Wang et al. [23], while assessing different cultivars, had rates of total phenolic compounds ranging from 0.6 to 4.9 mg GAE g^{-1} of avocado pulp, being that the lowest values were found in cultivars "Simmonds" and "Choquete" and the highest ones in cultivar "Hass".

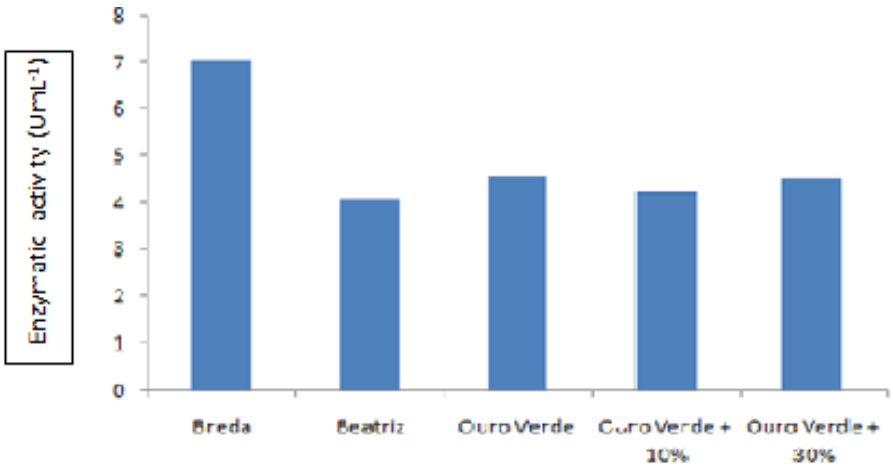
The outcome of the evaluation of antioxidant activity varied from 31.27 to 44.34% for fresh pulps and from 26.12 to 40.98% for avocado pulp powders. Antioxidants compounds are substances that retard oxidation by one or more mechanisms, such as inhibition of free radicals and complexation with metals [24], and they can be defined as compounds that protect cells against the damages caused by oxygenated and nitrogenous free radicals, formed in oxidative processes [25]. Vieites et al. determined the antioxidant activity in avocado pulp, cultivar "Fuertes" and obtained values that ranged from 17.6% to 68.7% [26].

Phenolic compounds are greatly responsible for the antioxidant activity in fruit, thus being a natural source of antioxidants [27]. However, the content of phenolic compounds in vegetable food depend on a series of intrinsic factors, such as genus, species and cultivar, and extrinsic factors, like agronomy, environment, handling and storage [28].

The enzymatic activity of polyphenol oxidase (PPO) in avocado pulp can be observed in Figure 2. Several researches related to reduction or inactivation of such activity in fruit may provide good information to the industrial sector, allowing the commercialization of fruit pulp or byproducts with little or no chemical additives and without altering their original organoleptic quality [29]. The action of these enzymes indicates that drying by lyophilization increased PPO activity, but remained among the other cultivars, as for POD activity.



(A)



(B)

Figure 2. PPO (A) and POD (B) enzymatic activity

Darkening in avocado pulp is directly related to the degradation of phenolic compounds by PPO, which, together with POD and in contact with oxygen, affects the appearance of avocado pulp. Therefore, the drying process decreases water content and alters, as a consequence, the reactive medium of these enzymes, reducing their influence upon flavor and this dark appearance.

4. Conclusion

Obtaining pulp powders by lyophilization is a means of processing avocado pulp that maintains its physicochemical quality. The phenolic compounds found in this fruit were determined with the highest rate in cultivar "Ouro Verde". Contrary to the process, the avocado pulp had high rates of enzymatic reactions.

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