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Application of Response Surface Methodology and Simplex-Centroid Design to Extraction of Phenolic Compounds from Avocado (*Persea americana*) Using UV-Vis Spectrophotometry

Aplicação da Metodologia de Superfície de Resposta e Design Simplex-Centróide para Extração de Compostos Fenólicos de Abacate (Persea americana) Usando Espectrofotometria UV-Vis

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Avocado (*Persea americana*) is a fruit rich in bioactive compounds, such as phenolic acids substances involved in combating oxidative stress. Considering that the single solvent extraction method can show low-efficiency, this work investigated the effect of different solvents (acetone, ethanol, methanol, and water) in the extraction of phenolic compounds in the avocado pulp and peel through simplex-centroid design, using the response surface methodology. The results, evaluated in mg of Gallic Acid Equivalent (GAE), show that most of the phenolic compounds are present in the peel. Optimized conditions were acetone:ethanol mixture (57.61 ± 0.92 mg GAE g^{-1} dry weight) for peel and the ethanol:water mixture (10.71 mg GAE g^{-1} dry weight) for pulp. Furthermore, the quadratic model from ANOVA proved to be significant, describing 99.30% of the experimental results for the peel and 99.92% for the pulp, evidencing the importance of mixture designs to increase the extraction yield of these compounds. The accuracy has been confirmed by spike with a gallic acid standard the avocado samples (peel and pulp), showed recovery >94% (RSD < 3.5%). The significant phenolic compounds concentration in avocado peel makes this fraction of fruit an interesting source of natural antioxidants, mainly because it is an agro-industrial residue.

Keywords: Avocado; phenolic compounds; simplex-centroid; response surface methodology; UV-Vis Spectrophotometry.

1. Introduction

Phenolic compounds are naturally occurring secondary metabolites in fruits and vegetables.[1] The main types of phenolic compounds found in plant matrices are flavonoids, anthocyanins, tannins, and phenolic acids.[2] The structure of phenolic compounds constitutes aromatic rings associated with hydroxyls.[3] This structural profile confers antioxidant properties on phenolics, which inhibit oxidative damage associated with various health problems, such as cancer and cardiovascular and neurodegenerative diseases.[4]

Natural antioxidants, especially phenolic compounds, have attracted scientific interest due to their functional properties and beneficial health effects and their potential to be used in the industrial activities, particularly as alternatives to synthetic antioxidants, such as BHA (hydroxyanisole butylated) and BHT (butylated hydroxytoluene).[5,6] Among the limitations of synthetic antioxidants is the carcinogenic potential, which has stimulated the study of phenolic compounds from natural sources.[7,8]

In the literature, several sources of phenolic compounds are reported, such as in different grape cultivars,[9] rice (*Oryza sativa*),[10] orange (*Citrus xsinensis*)[11] and avocado (*Persea americana*).[12] Due to its high content of fatty acids, proteins, fiber, vitamins, minerals, and phenolic compounds, avocados are well credited for their excellent nutritional and medical properties and applications.[13] According to the American Dietetic Association (ADA), the rich dietary composition of the avocado and its positive effects on human health classify it as a functional fruit.[14]

The avocado belongs to the Lauraceae family, and it is a climacteric fruit, so its maturation is completed after harvest. Among numerous varieties of avocado around the world, the 'Hass' variety is the most cultivated and imported type.[15,16] As for the national production of the fruit,

Brazil is the eighth world producer, having produced 266 thousand tons in 2020 (3.2% of the world production), and the seventeenth in terms of cultivated area, presenting, therefore, effective productivity.[17] The avocado is divided into three basic anatomical portions: the peel, the seed, and the pulp, which, in particular, correspond to 65% of the weight of the fruit.[18]

The avocado pulp is the edible part of the fruit, the ingestion of which can act in the fight against free radicals.[19] In addition, the avocado peel, one of the by-products of the fruit, is also an excellent source of phenolic compounds. The extraction of phenolics from the avocado epicarp presents itself as an alternative to reduce the economic and environmental problems caused by the industrial processing of the fruit since the rind is considered a residue, being often discarded inappropriately.[15,20]

In the literature, there are different economic and ecological extraction methods that have been proposed for sample preparation and analysis of plant and fruit phenolic extracts. Among these, conventional solid-liquid extraction, ultrasound and microwave assisted extraction, techniques employing supercritical fluids and subcritical water extraction stand out.[21] Conventional solid-liquid extraction makes use of the simple maceration of the sample in a solvent, at atmospheric pressure and at room temperature, with agitation. The absence of high temperatures in the extraction process is due to the thermal degradation of the phenolic compounds, especially anthocyanins.[22] That extraction method has the main advantage of not using sophisticated analytical instrumentation.[23]

As the solvents, the extraction of phenolic compounds from plant matrices employs individual solvents,[24] which can produce inefficient results, being necessary to opt for extracting solvent mixtures. The polarity of the solvent and the different phenolic compounds is the main factor influencing the extraction efficiency and the composition of the extracts obtained.[25] Among the extractive systems most used in the phenolic recovery of plant matrices, water, methanol, ethanol, acetone, and ethyl acetate are the main ones.[26,27]

Thus, simplex-centroid designs become a convenient tool to improve the phenolic extraction yield.[24] Simplex-centroid designs clarify the relationship between the solvent system composition and the extraction yield of the target compounds by evaluating the synergistic and antagonistic effects of solvent composition on the response variable.[28] Simplex-centroid designs describes a mixture system in which the points corresponding to the mixture-related experiments present the components in equal proportions. In total, the number of experiments is defined by the expression 2^q - 1, where q consists of the number of components. As an example, in a mixture of three components, all sets of components have the same proportion, that is, zero (0), one half (1/2), one third (1/3) and one (1), totaling 7 experiments. In this case, the experiments are three corresponding to the pure ones, three references to the binary mixtures involving all components and one assay related to the centroid, the ternary mixture.[29]

The simplex-centroid design has been widely applied to evaluate the best extraction conditions of phenolic compounds from plant matrices. Simplex-centroid design was applied to evaluate the effect of ethanol, dichloromethane and hexane on pigment extraction and antioxidant activity from *Coffea arabica* L. Leaves.[30,31] In another study, it was used to increase the efficiency of carotenoid extraction from cashew apple by Spectrophotometry UV-Vis.[32]

In this context, this article investigates the effect of different extracting solvents (acetone, ethanol, methanol, and water) and their mixtures on extracting phenolic compounds from the pulp and peel of the avocado (*Persea americana*) by the simplex-centroid design, developing a response surface methodology to obtain the best extraction conditions.

2. Experimental

2.1. Reagents, standards, and materials

All reagents used in this work were of analytical grade. Solutions were prepared with ultrapure (18.2 MΩ cm) water obtained from Millipore RiOs-DI™ purchased from Milli-Q (Billerica, MA, USA). The solvents used in the extraction procedure: acetone, ethanol, and methanol, were from J. T. Baker® (Geel, Belgium). Phenolic determination was performed using the Folin-Ciocalteu reagent purchased from Sigma-Alderich® (St. Louis, MO, USA).

2.2. Avocado samples

The avocado samples used to optimize the phenolic extraction were acquired from commercial locations in Teresina, Piauí, Brazil. The fruits were selected based on the visual integrity criterion, without the presence of bruises, fungi, and parasites or in an advanced state of maturation.

2.3. Sample preparation

The avocado fruit was divided into peel (epicarp), pulp (mesocarp), and seed (endocarp). The avocado peels were separated and homogenized with a mortar and pestle in the presence of liquid nitrogen. The fruit pulp was frozen, and the moisture was removed in a lyophilizer consisting of a Micro Modulyo Edwards coupled to a high-performance reduced pressure pump valPump, VLP80 Salvant. Then, the dehydrated pulp sample (*ca.* 20 g) was compressed to reduce the sample and provide greater homogeneity. Subsequently, the freeze-dried pulp was degreased, following the methodology proposed by Chen *et al.* (2018),[33] with slight modifications. For this purpose, *ca.* 30 mL of hexane was added to the dehydrated pulp. The sample was stirred with a magnetic stirrer for 15 minutes. Next, the supernatant was

collected, and the precipitate (pulp) was defatted twice more. Finally, the residual hexane in the sample was evaporated at room temperature, and the dry pulp residue was separated for phenolic extraction.

2.4. Procedure for the extraction of total phenolic compounds

The extraction of phenolic compounds was conducted following the methodology described by Mokrani and Madani (2016)[34] with minor adjustments. First, the extracts were prepared with *ca*. 0.1 g of homogenized avocado sample, pulp, and peel, then 10 mL of extracting solvent (1:100 w v^{-1}) was added. Next, the mixtures were subjected to extraction on a shaker table for 30 minutes at 290 rpm. Then, the samples were centrifuged for 10 minutes at 3520 rpm to decant the solid portions. Finally, the supernatants were collected and organized for the quantification procedure in the final step.

2.5. Mixture design

The study of the mixture design was carried out to obtain the best extracting solution for the extraction of phenolic compounds from the avocado pulp and peel. The design of mixtures of the simplex-centroid type was used to evaluate the best extraction yeld. The choice of solvent types was performed according to the phenolic composition of the samples for the pulp; ethanol, methanol, and water were used, according to the methodology proposed by SEKE *et al.* (2021);[35] ethanol, methanol and acetone, were used according to BOUAFIA *et al.* (2021)[36] for the extraction from the avocado peel.

In the simplex-centroid design for three components, the experimental space is represented by a triangle, as shown in Figure 1. The points located at the vertices correspond to the tests carried out with only one extracting solvent. The ones arranged on the edges represent the binary mixtures between the solvents, and in the center, is the ternary mixture, composed of the three extracting solvents in equal proportions.

The mixing design modeling coupled to a regression

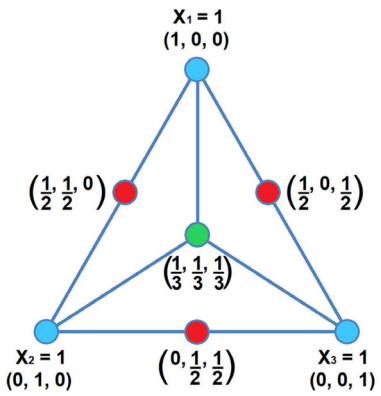

Figure 1. Experimental triangle for design ternary mixtures

model can be adjusted by expressing the response as a function of the variables through a polynomial equation. In general, a polynomial for a 3-component system quadratic model is defined by Eq. 1:[37]

$$y = b_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i<j}^{3} \sum_{j}^{3} \beta_{ij} x_i x_j \quad (1)$$

The parameter βi, called the linear coefficient, represents the response expected by the model for the pure component i; and βij corresponds to the interaction coefficient between components i and j. From these parameters, the model provides information on synergistic or antagonistic interaction effects, according to the coefficients, for experiments involving combinations between the constituents of the mix design.[37]

2.6. Determination of Total Phenolic Content (TPC)

The content of total phenolic compounds (TPC) present in the avocado pulp and peel was determined spectrophotometrically according to the Folin-Ciocalteu method adapted from Páramos *et al.* (2020)[38] with some modifications. For the determination procedure, 250 µL of the Folin-Ciocalteu reagent was added to a 1.0 mL aliquot of the phenolic extracts and then 2.0 mL of a 15% sodium carbonate solution (w v^{-1}). The final volume was adjusted to 10 mL with ultrapure water. After incubation for one hour at room temperature and protection from light, the absorbances of the samples were read at a wavelength of 745 nm using a UV-1800 ultraviolet-visible spectrophotometer (Shimadzu, Kyoto, Japan). From a analytical curve (standard curve equation: A = 0.0123C – 0.0367; r = 0.9915) prepared with a standard solution of gallic acid, with concentrations ranging from 7.0 to 84.0 µg mL^{-1}, the contents of the total phenolic compounds of the avocado pulp and peel extracts were quantified and expressed as mg of gallic acid equivalent per g of dry weight sample (mg GAE g^{-1} dry weight). Gallic acid is often used as reference standard because, as well as other phenolic compounds, react to the Folin-Ciocalteu reactive (phosphomolybdate and phosphotungstate) resulting in a blue coloration that can be determined by UV-Vis spectroscopy between 730 and 760 nm.[30]

2.7. Validation of the optimal conditions

The verification of the accuracy of the analytical procedure was carried out by the standard addition experiment. In this test, three concentration levels of the gallic acid standard (10, 15 and 20 ug mL^{-1}) were added to the samples (peel and pulp). Then the percentage of recovery was evaluated in terms of the ratio between the average concentration of the sample without spiking and the concentration after spiking. Precision was determined regarding method repeatability, considering the relative standard deviation (RSD).

2.8. Statistical analysis

The analysis of variance (ANOVA), response surfaces, and statistical data treatment was obtained using Statistica 12.5 and Excel software. ANOVA and Tukey's multiple comparison test were used to determine significant differences between assay responses at the 95% confidence level. The analyzes were performed in duplicate to attend to the minimum degrees of freedom of the experimental design. Results were expressed as mean ± standard deviation.

3. Results and Discussion

3.1. Selection of solvent sets

A set of solvents of higher polarity (ethanol, methanol, and water) was used to optimize the extraction of phenolics from the pulp. However, for the procedure done with the peel, the water was replaced by acetone, a solvent of more intermediate polarity. The selection of different sets of solvents to optimize the process of extracting phenolic compounds from the pulp and peel of the avocado was based on the phenolic composition of the avocado matrices. These studies indicate the majority presence of flavonoids in the peel, that are more soluble in solvents of intermediate polarity; and of phenolic acids in the pulp, which are better extracted with more polar solvents.[34,39]

3.2. Optimization of phenolic extraction from avocado peel

Table 1 summarizes the contents of total phenolic compounds in avocado peel obtained using acetone, ethanol, and methanol solvents. According to Table 1, for the design of mixtures with the three components, the TPC of avocado peel ranged from 27.69 ± 0.21 to 57.61 ± 0.92 mg GAE g^{-1} dry weight.

The highest total phenolic content was found in the extract using the binary mixture acetone:ethanol (57.61 ± 0.92 mg GAE g^{-1} dry weight). Among the pure solvents, the acetone extract had the highest content of total phenolic compounds (44.33 ± 1.00 mg GAE g^{-1} dry weight), followed by the methanolic extract (32.07 ± 1.05 mg GAE g^{-1} dry weight). Pure ethanol was the least efficient solvent in the phenolic extraction of avocado peel, with a TPC of 27.69 ± 0.21 mg GAE g^{-1} dry weight. According to the paired *t* test, the results for the extracts of pure acetone and the ternary mixture acetone:ethanol:methanol can not be considered different at a 95% confidence.

As seen in Table 1, the tests involving mixtures (binary and ternary mixtures) have shown average levels of phenolic compounds higher than the average of the tests using pure solvents. In raw values, concentrations increased in the following order: pure solvents (34.70 ± 1.46 mg GAE g^{-1} dry weight), binary mixtures (41.63 ± 0.92 mg GAE g^{-1} dry weight), and ternary mixture (41.88 ± 0.56 mg GAE g^{-1} dry weight). These results suggest that the application of mixtures has greater efficiency in extracting polyphenols for avocado and possibly other plant matrices, compared to the use of individual solvents, as reported in studies by Fernández-Agulló *et al.* (2013)[40] and Martínez-Ramos *et al.* (2020).[41]

The quadratic mathematical model was adjusted to optimize the phenolic extraction of avocado peel involving the three solvents. The quadratic model consists of expanding the linear model, with an additional term that allows evaluating the influence of binary mixtures on the response.[42] According to Eq. 2. Table 2 presents the ANOVA values for the model.

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad (2)$$

The quadratic model described 99.30% (R^2 = 0.9930) of the response data variance, indicating the model's high efficiency in predicting, with significant precision, the experimental values for the TPC of the avocado peel. The significance of the quadratic model is also justified by the calculated F value, which, according to the ANOVA test, presented *ca.* 62 times higher than the tabulated F (229.58>3.69) at a confidence level of 95%. On the other hand, the model showed a lack of fit, as the calculated value of F for the lack of fit, 10.92, was greater than the value of the F tabulated. However, the Fcalc/Ftab ratio for lack of

Table 1. Effects of solvent proportions on the total phenolic content of avocado peel extracts. Results as mean ± standard deviation

Assay	Acetone (a)	Ethanol (e)	Methanol (m)	TPC (mg GAE g^{-1} dry weight)
E1	1	0	0	44.33 ± 1.00
E2	0	1	0	27.69 ± 0.21
E3	0	0	1	32.07 ± 1.05
E4	0.5	0.5	0	57.61 ± 0.92
E5	0.5	0	0.5	33.76 ± 0.07
E6	0	0.5	0.5	33.53 ± 0.07
E7	0.33	0.33	0.33	41.88 ± 0.56

Table 2. ANOVA table for the quadratic model

Source of variation	Quadratic sum	Degrees of freedom	Mean square	Fcalc	Ftab
Regression	1226.300	5	245.259	229.58	$F_{5,8} = 3.69$
Residue	8.546	8	1.068		
Lack of fit	5.208	1	5.208	10.92	$F_{1,7} = 5.59$
Pure error	3.338	7	0.4769		
Total adjustment	1234.846	13	94.988		
R^2	0.9930				
R^2_{ajus}	0.9887				

fit did not deviate much from the unit value, which did not compromise the practical purpose of the optimization.[31]

The Pareto chart, shown in Figure 2, evaluated the effects of the factors studied (independent variables), adjusted to the quadratic model, as can be seen among the pure solvents, acetone stands out with greater significance. The mixture composed of acetone:ethanol is the most significant, on the other hand, the least significant is the one composed of ethanol:methanol. The red line corresponds to the region where the factors must reach to exhibit significance, at 95% confidence. In this case, all independent variables (pure solvents and binary mixtures) were significant for the TPC of avocado peel. Therefore, all factors are involved in the model's equation (Eq. 2).

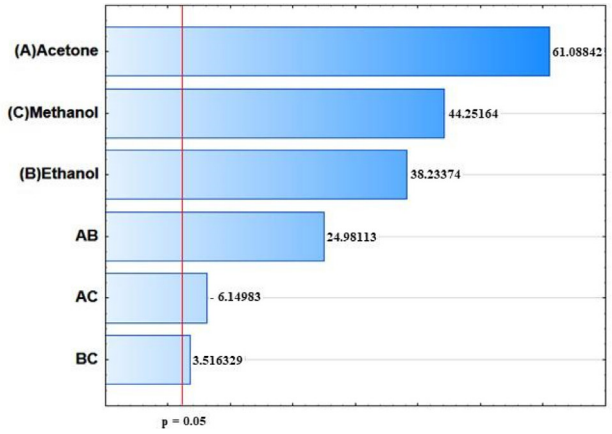

Figure 2. Pareto graph for the effects of solvents on the TPC concentration of avocado peel

$$TPC = +44.47a + 27.83e + 32.21m + 83.59ae - 20.58am + 11.77em \quad (3)$$

The mathematical model equation for the TPC of avocado peel, Eq. 3, shows the efficiency of extracting solvents, arranged in the following decreasing order: acetone:ethanol, acetone, methanol, ethanol, and ethanol:methanol. The acetone:ethanol binary mixture showed a more efficient interaction. The mixture coefficient (83.59) was higher than the average of the isolated solvents (36.15), which indicates that the two components act in synergy. The negative quadratic term of the equation for the acetone:methanol mixture, on the other hand, shows that the interaction between the solvents is antagonistic; that is, the response in the phenolic recovery with the combination of acetone and methanol is smaller than that with the sum of the pure solvents. Figure 3 represents the two-dimensional contour plot relating solvent type and TPC.

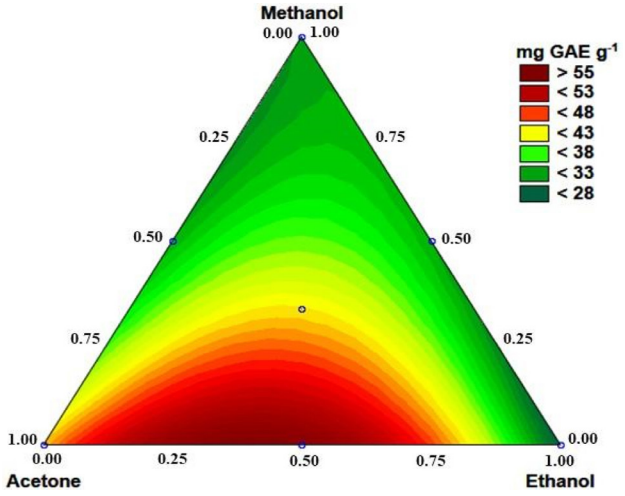

Figure 3. Contour graph of the influence of solvent interactions (acetone, ethanol, and methanol) on the TPC of avocado peel

The contour plot shows that the maximization of the TPC, represented by the red color, occurs with the treatment of the combination between the solvents acetone and ethanol, indicating higher levels than 55 mg GAE g^{-1} dry weight. The acetone:ethanol binary mixture showed the highest extraction yield (57.61 ± 0.92 mg GAE g^{-1} dry weight) in the avocado peel design, mainly due to its intermediate polarity. In addition to the polarity factor, the presence of ethanol in the composition leads to a better permeation of the mixture in the plant tissues, causing the cytoplasm layer to be directly exposed to the solvent.[43,44]

Among the pure solvents, acetone had the highest content of total phenolic compounds, whose extraction yield was 44.33 ± 1.00 mg GAE g^{-1} dry weight. The higher TPC found in the acetone extract can also be attributed to the intermediate polarity of the solvent, classified as a polar aprotic solvent, due to the absence of hydrogen atoms attached to an electronegative atom.[45] In comparison with the other pure solvents in the study, acetone has the ET(30) polarity parameter of 42.2, lower than ethanol and methanol

(51.9 and 55.4, respectively).[46] Acetone has the lowest polarity yet has the highest phenolic compounds content compared to the other pure solvents.

The greater capacity of the extractor systems of intermediate polarity (the binary mixture acetone:ethanol and pure acetone) in the phenolic extraction of avocado peel is also related to the molecular characteristics of the phenolic compounds contained in the matrix, such as the number of hydroxyls, the dimerization of these structures, the presence of glycosyl groups, the interactions between phenolics and the structure of insoluble complexes, which, as well as the aforementioned physicochemical properties of solvents, also influence the solubility of these phenolic compounds.[45,47] Studies of chemical characterization of the avocado peel affirm that the matrix presents, in a more significant proportion, the polyphenol catechin and quercetin, and procyanidins dimers, all belonging to the class of flavonoids.[16,48]

Acetone is generally the best solvent for extracting oligomeric flavonoids, condensed flavones, non-glycosylated polyphenols, and other high molecular weight phenolic compounds similar to those found in avocado and other fruits.[27,34] This fact is based on the principle that the higher the molecular weight of the solvent, the lower the polarity, so that substances with approximately the same molecular weight are preferentially extracted.[34,49]

The significant content of phenolic compounds present in the avocado peel, revealed in this work, indicates a potential use of the peel to reduce the use of synthetic antioxidants used on a large scale in the food, fuel, and cosmetics industries. In addition, it is possible to minimize the disposal of this by-product in open environments, minimizing environmental disturbances, such as soil and water pollution and the proliferation of diseases.[50]

3.3. Optimization of phenolic extraction from avocado pulp

Table 3 presents the TPC for the pulp extracts of avocado samples by applying a simplex-centroid mixture design for ethanol, methanol, and water solvents. The results identify the effect of the type of solvent and the nature of the mixtures on the recovery of phenolics present in the fruit pulp.

The content of phenolic compounds extracted from the avocado pulp by different solvents and extractor mixtures ranged from 3.06 to 10.71 mg GAE g^{-1} dry weight. Among the unitary solvents, pure water showed the highest efficiency in extracting phenolics from the pulp (8.83 ± 0.02 mg GAE g^{-1} dry weight). The content obtained using only water as extracting solvent was *ca*. 49% higher than the TPC obtained using pure methanol (5.92 ± 0.03 mg GAE g^{-1} dry weight). The ethanol extract showed the lowest value for TPC (3.06 ± 0.01 mg GAE g^{-1} dry weight). This order suggests that increasing polarity leads to increased phenolic recovery, reinforcing the influence of solvent polarities.

The highest TPC for avocado pulp was found in the binary mixture ethanol:water (10.71 mg GAE g^{-1} dry weight). The phenolic contents of the extracts of the binary mixture water: methanol (8.72 ± 0.04 mg GAE g^{-1} dry weight) and the ternary mixture ethanol: methanol: water (8.73 ± 0.24 mg GAE g^{-1} dry weight) are statistically equal to a 95% confidence level, according to the *t* test. In the same way, for the design of avocado pulp, the tests involving mixtures (binary and ternary mixtures) in the avocado peel showed average levels of phenolic compounds higher than the average of the tests using pure solvents.

Considering the response values (TPC) and the three variables (ethanol, methanol, and water), the ANOVA results were obtained for the quadratic mathematical model elaboration, shown in Table 4.

According to ANOVA, coefficient R^2 presented the value of 0.9992; the quadratic model described 99.92% of the data variance. In addition to being satisfactory, the model showed high significance, expressed by the F value calculated for (1951.72), *ca*. 528 times higher than the tabulated F value (3.69); this reveals that the modeling used was highly significant. Furthermore, there was no lack of fit since Fcalc = 0.14 < Ftab = 5.59, reinforcing the model's adequacy with the experimental results. According to the Pareto chart (Figure 4), all variables, including pure solvents and binary mixtures, were significant for the response. Therefore, the polynomial equation (Eq. 4) that describes the model considers all experimental variables.

Table 3. Effects of solvent proportions on the total phenolic content of avocado pulp extracts. Results as mean ± standard deviation

Assay	Ethanol (e)	Methanol (m)	Water (a)	TPC (mg GAE g^{-1} dry weight)
E1	1	0	0	3.06 ± 0.01
E2	0	1	0	5.92 ± 0.03
E3	0	0	1	8.83 ± 0.02
E4	0.5	0.5	0	4.73 ± 0.04
E5	0.5	0	0.5	10.71 ± 0.08
E6	0	0.5	0.5	8.72 ± 0.04
E7	0.33	0.33	0.33	8.73 ± 0.24

Table 4. ANOVA table for the quadratic model

Source of variation	Quadratic sum	Degrees of freedom	Mean square	Fcalc	Ftab
Regression	89.007	5	17.801	1951.7	$F_{5,8} = 3.69$
Residue	0.073	8	0.009		
Lack of fit	0.001	1	0.001	0.14	$F_{1,7} = 5.59$
Pure error	0.072	7	0.010		
Total adjustment	89.080	13	6.852		
R^2	0.9992				
R^2_{ajus}	0.9987				

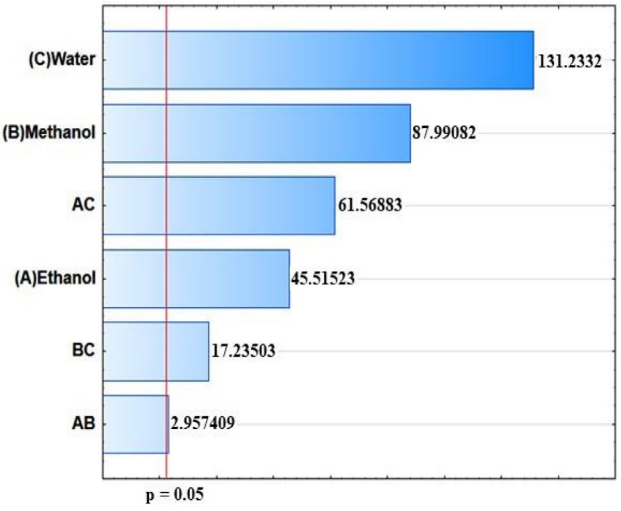

Figure 4. Pareto chart for the effects of solvents on the TPC of avocado pulp

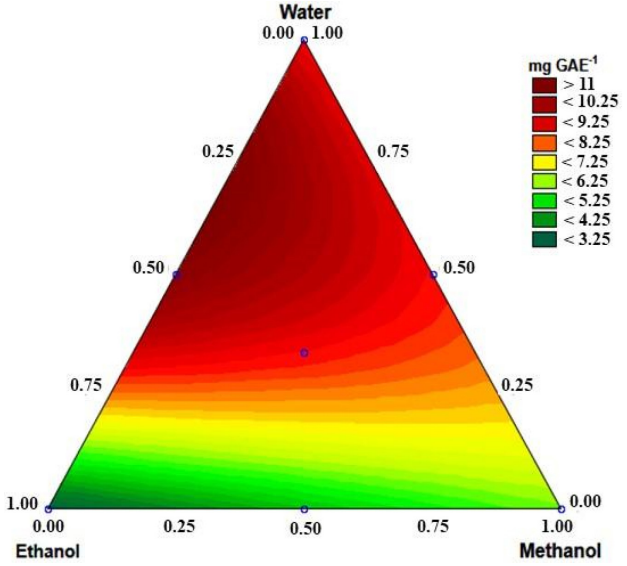

Figure 5. Contour graph of the influence of solvent interactions (ethanol, methanol, and water) on the total phenolic compounds content of the avocado pulp

$$TPC = +5.92m + 3.06e + 8.83a + 091em + 5.33am + 19.04ea \quad (4)$$

For the three binary mixtures (ethanol:water, ethanol:methanol, and water:methanol), the two components in each mixture act synergistically and, consequently, the response in phenolic recovery with the combination of each solvent pair is greater than the sum of the income provided by each component. The contour plot of the mixture for the quadratic model (Figure 5) illustrates how the interactions of different proportions of solvents affect the total phenolic content.

The contour plot shows that the maximum extraction of phenolic compounds from avocado pulp is located between the vertices of ethanol and water, being closer to the latter. The extraction efficiency is reduced when approaching the ethanol apex and the region between the methanol and ethanol apexes. The composition of the mixture that provides the optimal value predicted by the model for the TPC of avocado pulp corresponds to 69.2% water and 30.8% ethanol.

Adding water to the ethanol and methanol organic solvents improved the extraction efficiency, which can be attributed to the high polarity of the phenolic compounds in the matrix and other compounds that may have been extracted, such as sugars and proteins.[24] Chemical characterization studies of avocados report that the phenolic profile of the fruit pulp is mainly composed of hydroxybenzoic and hydroxycinnamic acids from the phenolic acid class, such as sinapic acid, ferulic acid, coumaric acid, and their glycoside derivatives;[15,39] in addition to protocatechuic and feruloylquinic acids.[15] The greater polarity of phenolic acids, compared to the other classes of polyphenols, added to the presence of phenolic glycoside compounds, justifies the preferential extraction of the aqueous solvent due to the establishment of hydrogen bonds.[41]

The aqueous ethanol extract provided the highest total phenolic content, despite the fact that the methanol:water mixture presented the highest ET(30) polarity parameter (57.4 and 57.7, respectively).[51] In this case, the affinity of the phenolic compounds for the solvent does not satisfactorily explain the dependence of the composition of the binary mixture on the TPC. In fact, before solubilizing the solutes, the solvent must break the cell walls of the plant tissue, composed of cellulose, hemicellulose, and pectin.[52] The plant tissue, although poorly permeable, suffers structural disarray through swelling, resulting from the interaction

Table 5. Addition and recovery (%) applied in the method of extracting phenolic compounds in μg mL^{-1} of avocado peel and pulp

Sample	No addition	low level (10)	Rec. (%)	Middle level (15)	Rec. (%)	High level (20)	Rec. (%)
Peel	25.87 ± 0.63	34.29 ± 0.98	95.6	41.00 ± 0.57	100.2	46.79 ± 0.27	102.0
Pulp	17.97 ± 0.27	29.03 ± 0.25	103.8	31.12 ± 0.21	94.4	36.0 ± 0.50	94.8

between the solvent molecules with the hydroxyl and carboxyl groups of the plant membranes, causing the expansion of the plant tissue accompanied by the penetration of the solvent.[53] The presence of ethanol, as already mentioned, contributes to more excellent permeability of the extractor system in plant tissues, resulting in greater diffusion of the solvent in the matrix.[43]

3.4. Figures of merit

After the optimization procedures of the extractor solution, the optimal composition obtained by mathematical modeling was applied to the samples to verify the reliability of the results. The accuracy was measured regarding recovery percentage, as observed in Table 5. For the peel, the three levels of addition showed recovery values greater than 95.6%, while for the pulp, the levels were greater than 94.8%, indicating adequate accuracy for the developed method. The precision of the proposed was evaluated considering the relative standard deviation (rsd), that converts the error or uncertainty of the result to a percentage. It was calculated by dividing the standard deviation by the mean and multiplying the result by 100%. The rsd values were in the range of 1.38 to 2.88% for the peel and 0.67 to 1.38% for the pulp. These findings show that the developed method is accurate and precise.

The limit of detection (LOD) is the lowest concentration that can be detected, but it is not necessarily quantified by the method. On the other hand, the limit of quantification (LOQ) is the concentration with a different signal than the analytical blank that can be measured, the LOD and LOQ are presented in equations 5 and 6, where σ represents standard deviation of the blank and *S*, the slope of the analytical curve. The values of LOD and LOQ were 0.018 and 0.060 mg GAE g^{-1}, respectively.

$$LOD = 3\frac{\sigma}{S} \quad (5)$$

$$LOQ = 10\frac{\sigma}{S} \quad (6)$$

The results found in this study, which show higher amounts of phenolic compounds in the avocado peel than in the fruit pulp, agree with the results reported in the literature.[54,55] Compared with the other fruit fractions, the avocado peel has a higher TPC since these antioxidant compounds protect the fruit from oxidative damage induced by ultraviolet radiation and high temperatures.[56] In this sense, the avocado peel, which is usually discarded after the industrial processing of the fruit, presents itself as a potential source of natural antioxidants due to its high TPC.[15] In the nutritional view, due to their high occurrence in vegetables and fruits, such as avocado, the daily intake of phenolic compounds is higher than that of other natural antioxidants, such as carotenoids and vitamins. According to Corrêa *et al.* (2015),[57] the daily dietary intake of phenolic compounds is approximately 1 g, while for vitamins and carotenoids, they are 110 and 9.4 mg, respectively. Therefore, from the quantification results for the avocado pulp obtained in the present work, the consumption of the edible part of the fruit may represent a good source of antioxidants that inhibit several health problems through routine eating habits.

4. Conclusions

The study satisfactorily applied of the simplex-centroid design to extract phenolic compounds from the avocado peel and pulp. Using the set of acetone, ethanol and methanol solvents for the shell, the design adjusted to the quadratic model proved to be significant, reproducing 99.30% of the experimental data. The design showed that the binary mixture of acetone:ethanol showed synergistic interaction; on the other hand, the solvents acetone and methanol interacted antagonistically. The highest TPC for the peel was obtained by the extract of the acetone:ethanol mixture of intermediate polarity. The quadratic model described 99.92% of the experimental results and showed no lack of fit for the pulp. It was found that water, compared to the other pure solvents, showed greater efficiency in extracting phenolics from the pulp. In contrast, the binary mixture ethanol:water was the extractor system that provided the highest value of TPC for the pulp. Moreover, the results obtained in this work indicate a higher phenolic concentration in the peel than in the pulp to the analyzed avocado sample. The accuracy has been confirmed by spike with a Gallic Acid standard the avocado samples (peel and pulp), showed recovery >94% and precision <3.5%. After the optimization procedures, it was verified that the mixtures were more efficient in extraction. The significant concentration of phenolic compounds in the avocado peel makes this fraction of the fruit an interesting source of natural antioxidants, mainly because it is an agro-industrial residue.

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