Research, Society and Development, v. 9, n. 10, e9939109273, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.9273

Optimization of the extraction process of compounds with antioxidant activity of tommy atkins mango peel

Otimização do processo de extração de compostos com atividade antioxidante da casca de manga tommy atkins

Optimización del proceso de extracción de compuestos con actividad antioxidante de la cáscara de mango tommy atkins

Received: 10/18/2020 | reviewed: 10/22/2020 | accept: 10/27/2020 | published: 10/30/2020

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Abstract

It is well known that vegetable residues from the food industry can be used as a possible source for the extraction of compounds with antioxidant activity; in the case of mango, approximately 40 to 60% of the total mass of the fruit is considered as residue after processing. This work focused on the optimization of the extraction process of compounds with antioxidant activity from the rind of Tommy Atkins mango. Initially a Fractional Factorial Design 2^{5-1} (FFD) was applied to study the effect of five variables on the extraction process, namely: extraction time (min), percentage of ethanol in aqueous solution (%), pH, dry/solvent mango peel ratio (g/mL) and ultrasound power range (%). The variables extraction time and dry/solvent mango peel ratio were selected, which were evaluated sequentially by the application of a Central Composite Rotatable Design (CCRD) to

determine the conditions of maximum extraction of antioxidant compounds by the response surface analysis. The $ABTS^{\bullet+}$ and Folin-Ciocalteu methods were used for the quantification of the antioxidant activity. The maximum extraction occurred with the use of water, extraction time of 30 min, dry mango peel/solvent ratio of g/mL (1:100), at natural pH of the mixture (pH 4.6 ± 0.20) and sonication amplitude at 50%. It was possible to develop an extraction process of compounds with antioxidant activity from the mango peel, in order to maximize yield through the use of non-toxic solvents and using an agro-industrial residue as raw material.

Keywords: Tommy Atkins; Antioxidant potential; Sequential experimental planning strategy.

Resumo

É notório que os resíduos vegetais oriundos da indústria de alimentos podem ser utilizados como possível fonte para a extração de compostos com atividade antioxidante; no caso da manga, aproximadamente 40 a 60% da massa total da fruta é considerada como resíduo após seu processamento. Este trabalho teve como foco a otimização do processo de extração de compostos com atividade antioxidante da casca da manga *Tommy Atkins*. Inicialmente foi aplicado um Planejamento Fatorial Fracionário 2^{5-1} (PFF) para o estudo do efeito de cinco variáveis sobre o processo de extração, a citar: tempo de extração (min), porcentagem de etanol em solução aquosa (%), pH, razão casca de manga seca/solvente (g/mL) e amplitude de potência do ultrassom (%). Foram selecionadas as variáveis tempo de extração e razão casca de manga seca/solvente, as quais foram avaliadas sequencialmente pela aplicação de um Delineamento Composto Central Rotacional (DCCR) para determinação das condições de máxima extração de compostos antioxidantes pela análise de superfície de resposta. Para a quantificação da atividade antioxidante utilizou-se o método $ABTS^{\bullet+}$ e Folin-Ciocalteu. A máxima extração ocorreu com utilização de água, tempo de extração de 30 min, razão casca de manga seca/solvente de g/mL, no pH natural da mistura (pH 4,6 ± 0,20) amplitude de potência do ultrassom 50%. Foi possível desenvolver um processo de extração de compostos com atividade antioxidante da casca de manga, de forma a se maximizar o rendimento por meio da utilização de solventes não-tóxicos e utilizando um resíduo agroindustrial como matéria-prima.

Palavra-chave: Tommy Atkins; Potencial antioxidante; Estratégia sequencial de planejamento experimental.

Resumem

Es bien sabido que los residuos vegetales de la industria alimentaria pueden utilizarse como una posible fuente para la extracción de compuestos con actividad antioxidante; en el caso del mango, aproximadamente del 40 al 60% de la masa total de la fruta se considera como residuo después de su procesamiento. Este trabajo se centró en la optimización del proceso de extracción de compuestos con actividad antioxidante de la corteza del mango de Tommy Atkins. Inicialmente, se aplicó un Plan de Factores Fraccionales 2^{5-1} (PFF) para estudiar el efecto de cinco variables en el proceso de extracción, a saber: tiempo de extracción (min), porcentaje de etanol en solución acuosa (%), pH, relación cáscara de mango seca/solvente (g/mL) y rango de potencia de ultrasonido (%). Se seleccionaron las variables tiempo de extracción y relación cáscara de mango seca/solvente, que se evaluaron secuencialmente mediante la aplicación de uno Diseño Compuesto Central Rotacional (DCCR) para determinar las condiciones de máxima extracción de compuestos antioxidantes mediante el análisis de la superficie de respuesta. Se utilizaron los métodos $ABTS^{\bullet+}$ y Folin-Ciocalteu para la cuantificación de la actividad antioxidante. La máxima extracción se produjo con el uso de agua, tiempo de extracción de 30 min, relación cáscara de mango seco/solvente de g/mL, a pH natural de la mezcla (pH 4,6 ± 0,20) 50% de rango de potencia del ultrasonido. Fue posible desarrollar un proceso de extracción de compuestos con actividad antioxidante de la cáscara del mango a fin de maximizar el rendimiento mediante el uso de solventes no tóxicos y la utilización de un residuo agroindustrial como materia prima.

Palabras clave: Tommy Atkins; Potencial antioxidante; Estrategia secuencial de diseño experimental.

1. Introduction

During the industrial processing of fruit there is the generation of large quantities of waste. It is estimated that for the mango, in relation to the total volume, about 40 to 60% of the fruit mass becomes residue (peel and seed) (Santos et al., 2018). These residues have considerable concentrations of bioactive compounds, and it has been mentioned that their use provides improvements in health and can be applied for technological purposes (Barbulova et al., 2015).

Bioactive compounds consist of chemical compounds found in different tissues (peel, root, stem, seed, pulp, etc.) of food or non-food plants, which present some kind of interaction on living organisms, tissues or cells; they are found in the form of lipids, vitamins, peptides

and antioxidants (Moreira-Araújo et al., 2019) Their relevance is due to the fact that they have a broad spectrum of action, in the specific case of mango, there are studies in the literature that indicate that the bioactive compounds extracted from the peel have antioxidant activity (Agatonovic-Kustrin et al., 2018; Huang et al., 2018; Mugwagwa & Chimphango, 2019) and antimicrobial (Poomanee et al., 2018) and antifungal (Gómez-Maldonado et al., 2020).

The use of antioxidants as additives in the food industry is of relevant importance, since these compounds give foods longer periods of conservation due to their neutralizing action of free radicals that would otherwise alter the properties of the food (Adegbola et al., 2020). There are studies in the literature that demonstrate the feasibility of using vegetal extract to replace the synthetic antioxidant BHA in the formulation of cookies without significant loss in sensory characteristics and generating the increase in antioxidant activity of the food (Caleja et al., 2017).

In view of the relevance of the use of bioactive compounds in the food industry and the importance of minimizing the generation of plant residues, it becomes relevant to study methods that allow the use of these residues, such as the processes of extraction of bioactive compounds.

The methods commonly used for the extraction of compounds with vegetable bioactivity usually involve the use of toxic solvents, generating high environmental impact, several steps and the use of fixed variables (Vardanega et al., 2014). Such factors decrease the method's usability and the understanding of which variables significantly affect the process (Silva et al., 2019).

During the extraction process of bioactive compounds from a given matrix, several factors can influence the yield and quality of the extracted products, such as temperature, pH, extraction methodology used, solvent and its concentration, matrix characteristics, etc. The investigation of how these variables act and how they interact with each other is fundamental for a better understanding of the extraction process, thus allowing adaptations and optimizations to obtain a better efficiency in the process (Both et al., 2014).

To enhance the traditional methods of extracting antioxidant compounds, such as solvent extraction, and also as an innovative method, sonication has been used for several matrices (Albuquerque et al., 2016; Guandalini et al., 2019; S. Rodrigues et al., 2015). In general, ultrasonic waves assist in cell disruption of the tissues and particle size minimization, resulting in to enhance release of cellular contents (Luo et al., 2014), due to the acoustic cavitation effect produced in the solvent, providing benefits during extraction, such as the use

of ambient temperature, increased process safety and shortened process time (Chemat et al., 2011; Gogate & Kabadi, 2009).

The use of fractional factorial design (FFD), a statistical method for prospective data analysis, allows the conclusion of which factors have an influence on the process, but does not allow the determination of the optimal point of the process under analysis or how the factors interact with each other (Rodrigues & Iemma, 2014). To find the optimal point values of an extraction process, for example, it is necessary to use a mathematical tool called Response Surface Methodology (MRS).

MRS was first described by Myers and Montgomery (1995), according to the authors MRS consists of a set of statistical techniques used for problem modeling and analysis, where the responses of interest can be influenced by many variables and in which the aim is to reach the optimal point. The adjustment of the data obtained in the response surface methodology for generating a mathematical model was carried out by calculating the least squares from the second order polynomial equation (Equation (1) below.

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1;j>i}^{k-1} \sum_{j=2}^{k} \beta_{ij} x_i x_i \quad (1)$$

Y corresponds to the dependent variable (response) to be modeled, xi and xj define the independent variables, β_0 is a constant coefficient (mean), βi the linear effect coefficient, βij the interaction effect coefficient, βii the quadratic effect coefficient and n the number of variables.

The main objective of this work was to determine the optimal conditions for the extraction of compounds with antioxidant potential from the mango peel, applying an ultrasound-assisted maceration process by a sequential strategy of experimental design.

2. Methodology

2.1 Reagents

The reagents used were ABTS$^{\bullet+}$ (2,2'-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid, acquired from Sigma-Aldrich, and Folin-Ciocalteu reagent, anhydrous sodium carbonate (Na2CO3), absolute ethyl alcohol and potassium persulphate, acquired from Dinamic.

2.2 Sample preparation

The mango, purchased in the local commerce of the city of Medianeira (PR), was washed, sanitized, peeled and the peel was dried in a greenhouse with forced air circulation at 40°C ± 1°C (Novainstruments, NI-1705, Brazil) until the mass stability (approximately 16 h). The dried peel was crushed in a knife mill (SL31, Solab, Piracicaba, Brazil) with a 30 mesh screen. Finally, the samples were packaged in plastic containers and frozen in a commercial freezer at -18°C ± 1°C (-4°F ± 1°C) until use.

2.3 Extraction of antioxidant compounds

The methodology used for the extraction of antioxidant compounds from the mango peel was maceration assisted with ultrasound (MAU). The sample was weighed in all falcon and then transferred to a 100 mL beaker in which the solvent was added (distilled water, 50% ethanol and 100% ethanol). The pH was adjusted with 2 M citric acid and the mixture was taken to the ultrasonic bath (Elma P 120, Elma, Germany). The bath temperature was 35°C ± 5°C. The extracts were filtered on filter paper (Whatman n°4) and then the total phenolic compound content was evaluated (Folin-Ciocalteu method) and the antioxidant activity measured ($ABTS^{\bullet+}$ method).

2.4 Sequential strategy of experimental design

To optimize the MAU process, a Fractional Factorial Design (FFD) (2^{5-1}) (3 central points, 19 runs) was first used, as describe by Rodrigues and Iemma (2014), to evaluate the effect of 5 selected variables on the extraction of antioxidant compounds from the dry mass of the Tommy Atkins type mango peel, to mention: time (x1), percentage of ethanol in aqueous solution (x2), pH (x3), dry mango peel/solvent ratio (x4), and sonication amplitude (x5). The real and coded levels of the variables studied in the FFD are presented in Table 1.

Table 1 - Real and coded levels of the variables studied in FFD 2^{5-1}.

Variables / Levels	x_1[a]	x_2[b]	x_3[c]	x_4[d]	x_5[e]
-1	5	0	2	(1/40)	30
0	15	50	3	(1/20)	50
1	25	100	5	(1/10)	70

[a]Time (min); [b]Percentage of ethanol in aqueous solution (%); [c]pH; [d]Dry mango peel /solvent ratio (g/mL); [e] sonication amplitude (%). Source: Authors.

From the FFD shows in Table 1, the effects of the variables on the extraction process were evaluated, and three of them were selected for the next step, which consisted of a CCRD (Central Composite Rotatable Design) with 6 axial points and 3 repetitions at the central point (17 runs), with the objective of optimizing the process of extraction of antioxidants from the peel of the mango using response surface methodology. The adjustment of the data obtained in the response surface methodology (MSR) for the generation of a mathematical model was carried out by the method of calculating the least squares, from the second order polynomial equation (Equation 1).

All the FFD and CCRD runs were randomly performed and the results were treated with the website *Protimiza Experimental Design* (https://experimental-design.protimiza.com.br/, accessed February 15, 2020). This program was also used to generate the response surfaces obtained from the CCRD. The adequacy of the models was evaluated through the analysis of variance (ANOVA).

2.5 $ABTS^{\bullet+}$ method

The evaluation of the antioxidant activity of extracts by the $ABTS^{\bullet+}$ method was carried out according to the methodology described by Re and collaborators (1999). An aqueous solution of the radical $ABTS^{\bullet+}$ 7 mM was prepared from $ABTS^{\bullet+}$ stock solution with potassium persulphate (2.45 mM). In the absence of light and at room temperature, the solution remained at rest for 12 to 16 hours. After that, ethanol was added to the solution up to absorbance of 0.700 ± 0.020 at 734 nm. In a test tube, 50 μL of the extract and 5 mL of

$ABTS^{\bullet+}$ solution was added, and the absorbance was read at 734 nm in a spectrophotometer (Biospectro, Sp-22, Brazil) after 6 minutes of reaction. For the white one 50 µL of ethanol were used instead of the extract. The antioxidant activity was determined from a standard curve prepared for Trolox and the results were expressed in terms of Trolox equivalent (Trolox-Eq) (µmol Trolox-Eq/g sample). The percentage of inhibition consists of the amount of $ABTS^{\bullet+}$ neutralized by the sample based on the Trolox equivalent. The percentage of inhibition was determined by Equation (2). Where Abs_{SAMPLE} correspond to the absorbance of the sample in the specific wavelength and the $Abs_{WHITENESS}$ was the analysis without the analyte.

$$\% \text{ of inhibition} = 100 - \left(1 - \frac{Abs(734nm)_{ASAMPLE}}{Abs(734nm)_{WHITENESS}}\right) x\ 100 \quad (2)$$

2.6 Folin-Ciocalteu method

The Folin-Ciocalteu method was used to determine the total phenolic content of extracts according to the procedure described by Ainsworth and collaborators (Ainsworth & Gillespie, 2007) In the absence of light and at room temperature, in tubes with 500 µL of extract, 1 mL of the Folin-Ciocalteu 20% reagent and 4 mL of sodium carbonate 700 mM were added. Ethyl alcohol was used to replace the extract in the preparation of white. After 2 hours of reaction at room temperature and under light, the absorbance was measured at 765 nm in spectrophotometer (Biospectro, Sp-22, Brazil). From a standard curve prepared with gallic acid (5 to 100 µg/mL) the results were obtained and expressed in terms of gallic acid equivalent (GA-Eq), mg GA-Eq/g sample.

3. Results and Discussions

3.1 Fractional Factorial Design 2^{5-1} (FFD)

For the study of the extraction process of antioxidant compounds with FFD 2^{5-1}, the ranges of the five independent variables (time, percentage of ethanol in aqueous solution, pH, dry/solvent mango peel ratio, and ultrasound power range) were defined based on the results of previous studies (Albuquerque et al., 2016; S. Rodrigues et al., 2015).

The standard curve used for the $ABTS^{\bullet+}$ method presented a straight-line equation (Equation 3), with an $R^2 = 0.9989$; the straight-line equation (Equation 4) for Folin-Ciolcateu presented an $R^2 = 0.9992$.

$$\text{Trolox} - \text{Eq}\ (\mu\text{mol/L}) = 0{,}0447(\%\ \text{inhibition}) - 1{,}2335 \quad (3)$$

$$\text{Absorbance} = 0{,}0081\ (\text{Gallic Acid}(\mu\text{gAG} - \text{Eq/mL})) + 0{,}0067 \quad (4)$$

The design matrix of the FFD with the real and coded values of the studied variables, as well as the results obtained for the $ABTS^{\bullet+}$ and Folin-Ciocalteu methods, are presented in Table 2. It was observed that the extracts with higher antioxidant activity, for both analyses, corresponded to the 17, 18 and 19 tests (central points). The evaluation of the central point's responses also indicates that there was a good repeatability of the process, since they showed low variation (242.78 ± 16.83 µmol Trolox-Eq/g).

Table 2 - FFD 2^{5-1} matrix (real and coded values) with the responses of the $ABTS^{\bullet+}$ and Folin-Ciocalteu methods.

Runs	x_1[a]	x_2[b]	x_3[c]	x_4[d]	x_5[e]	$ABTS^{\bullet+}$ (µmol Trolox-Eq/g) *	Folin-Ciocalteu (mg GA-Eq/g) *
1	-1 (5)	-1 (0)	-1 (2)	-1 (1/40)	1 (70)	128,85 ± 24,64	9,96 ± 0,05
2	1 (25)	-1 (0)	-1 (2)	-1 (1/40)	-1 (30)	129,95 ± 14,77	7,85 ± 0,09
3	-1 (5)	1 (100)	-1 (2)	-1 (1/40)	-1 (30)	98,48± 8,93	8,40± 0,22
4	1 (25)	1 (100)	-1 (2)	-1 (1/40)	1 (70)	147,81 ± 5,94	11,59 0,55
5	-1 (5)	-1 (0)	1 (5)	-1 (1/40)	-1 (30)	143,31 ±16,46	7,57± 0,09
6	1 (25)	-1 (0)	1 (5)	-1 (1/40)	1 (70)	149,47 ± 7,41	7,66 ± 0,20
7	-1 (5)	1 (100)	1 (5)	-1 (1/40)	1 (70)	128,77 ± 12,03	8,61 ± 0,15
8	1 (25)	1 (100)	1 (5)	-1 (1/40)	-1 (30)	147,68 ± 6,26	8,59 ± 0,27
9	-1 (5)	-1 (0)	-1 (2)	1 (1/10)	-1 (30)	78,75 ± 8,06	5,67 ± 0,06
10	1 (25)	-1 (0)	-1 (2)	1 (1/10)	1 (70)	101,33 ± 7,44	7,21 ± 0,08
11	-1 (5)	1 (100)	-1 (2)	1 (1/10)	1 (70)	43,11 ± 2,83	2,50 ± 0,02
12	1 (25)	1 (100)	-1 (2)	1 (1/10)	-1 (30)	38,06 ± 0,47	3,11 ± 0,05
13	-1 (5)	-1 (0)	1 (5)	1 (1/10)	1 (70)	24,85 ± 9,29	5,86 ± 0,43
14	1 (25)	-1 (0)	1 (5)	1 (1/10)	-1 (30)	49,55 ± 3,60	2,91 ± 0,17
15	-1 (5)	1 (100)	1 (5)	1 (1/10)	-1 (30)	88,83 ± 5,37	6,71 ± 0,39
16	1 (25)	1 (100)	1 (5)	1 (1/10)	1 (70)	86,42 ± 9,13	4,60 ± 0,22
17	0 (15)	0 (50)	0 (3)	0 (1/20)	0 (50)	217,54 ± 16,44	15,17 ± 0,37

18	0 (15)	0 (50)	0 (3)	0 (1/20)	0 (50)	252,72 ± 11,53	17,40 ± 0,68
19	0 (15)	0 (50)	0 (3)	0 (1/20)	0 (50)	258,08 ± 14,98	17,73 ± 0,34

*Average of triplicate measurements ± standard error; [a]Time (min); [b]Ethanol percentage in aqueous solution (%); [c]pH; [d]Dry mango peel /solvent ratio (g/mL); [e] sonication amplitude (%). Source: Authors.

In Table 2 it is possible to notice that in the central points for $ABTS^{\bullet+}$ (runs 16, 17, 18) the values are close, presenting a variation coefficient of 6.93%, that is, the difference between the answers was approximately 6.93%. For the central points of Folin-Ciocalteu, the same occurred, the results showed a coefficient of variation of 6.35%, close values in the results at the central point's shows that the process showed good reproducibility. According to the Fonseca and Martins (2011, p.148), values of variation coefficients less than 10% say that the data distribution has a low dispersion.

Based on the results of Table 2 it was possible to determine the effects of the five variables studied on the responses of each of the investigated methods, which are presented in Table 3.

Table 3 - Effect of the factors studied in FFD 2^{5-1} for the $ABTS^{\bullet+}$ and Folin-Ciocalteu methods.

Factors	$ABTS^{\bullet+}$ (µmol Trolox-Eq/g)				Folin-Ciocalteu (mg GA-Eq/g)			
	Effect	Standard error	t calculated	p-valor	Effect	Standard error	t calculated	p-valor
Averege	99,08	6,23	15,9	< 0,0001*	68,01	4,32	15,76	< 0,0001*
x_1[a]	14,41	12,46	1,16	0,27	-2,19	8,63	-0,25	0,8042
x_2[b]	-3,36	12,46	-0,27	0,792	-0,71	8,63	-0,08	0,9358
x_3[c]	6,569	12,46	0,53	0,6078	-4,73	8,63	-0,55	0,5937
x_4[d]	-70,43	12,46	-5,65	< 0,0001*	-39,58	8,63	-4,59	< 0,0001*
x_5[e]	4,5	12,46	0,36	0,7243	9,01	8,63	1,04	0,317

The effects are presented in µmol Trolox-Eq/g for the test with $ABTS^{\bullet+}$ and mg GA-Eq/g for Folin-Ciocalteu; * $p \leq 0,05$; [a]x_1: time (min); [b]x_2: percentage of ethanol in aqueous solution v/v (%); [c]x_3: ph; [d]x_4: ratio of dry/solvent mango (g/ml); [e]x_5: sonication amplitude. Source: Authors.

The dry mango/solvent ratio (x4) was the only variable investigated that showed statistically significant effects ($p \leq 0,05$) on the studied response, and for this reason it was selected as a variable for the CCRD.

The time variable (x1) presented a positive effect for the $ABTS^{\bullet+}$ method (Table 3), indicating that the highest concentrations of compounds with antioxidant activity were obtained at the highest extraction times, however, this effect was not significant. For the Folin-Ciocalteu response, time presented a negative effect, indicating that the best results

were found in short extraction times. Due to the difference in the effects for both methods evaluated and also because the literature reports time as a variable of direct relevance in the efficiency of the extraction process (Ye & Jiang, 2011), it was decided to keep it as a variable in the sequence of the study (CCRD), to evaluate more specifically its influence on this extraction process.

Regarding the variable percentage of ethanol in aqueous solution (x2), it was not significant and it was also observed that, for both methods evaluated, the effect was negative, i.e., the highest responses were observed in the lower levels of the studied range. As the assessed levels were 0% ethanol (use of pure water as a solvent) to 100% ethanol, based on the above results, it was decided to maintain pure water as an extraction solvent in the CCRD tests.

The pH (x3) also had no statistically significant effect on the evaluated responses. For the CCRD, the pH was fixed at 4.60 which is the natural pH value of the Tommy Atkins mango peel. According to the study conducted by on the effect of pH on the extraction of phenolic compounds from the fruit peel, the authors identified that the maximum extraction occurred at pH values close to 4.0.

The ultrasound power range (x5) was also not significant in the responses, but showed positive effects in both investigated methods, which corresponds to the highest responses when the highest powers were used. The ultrasound helps in the extraction process because the ultrasound waves generate cavitation bubbles that help in the penetration of the solvent facilitating the extraction of compounds (Lavilla & Bendicho, 2017) Thus, although this variable did not present significant effects, considering the positive effect presented, the sonication amplitude was fixed at 50 %. for the CCRD runs, intermediate value of operation within the range investigated in the FFD (30 - 70 %).

In the second stage, the optimization of the extraction process of compounds with antioxidant potential from the mango peel by the application of a CCRD was carried out. Based on the discussions presented above, regarding the results obtained in FFD 2^{5-1}, the dry/solvent mango peel and extraction time ratios were selected as independent variables, keeping the parameters pH (4.60), sonication amplitude (50%) and using water as solvent.

3.2 Central Composite Rotatable Design (CCRD)

The matrix of the tests with the real values (in brackets) and coded of the independent variables, as well as the responses obtained for the $ABTS^{\bullet+}$ and Folin-Ciocalteu methods are

presented in Table 4. Both independent variables considered in the CCRD had their investigation range expanded compared to the results indicated by the FFD. For the dry/solvent mango peel ratio the investigation range was 1/20 to 1/100 g/mL and for the extraction time variable it was 10 to 40 min.

Table 4 - CCRD matrix with real and coded levels of the studied variables and responses of the $ABTS^{\bullet+}$ and Folin-Ciocalteu methods.

Test	x_1^a	x_2^b	$ABTS^{\bullet+}$ (µmol Trolox-Eq/g)*	Folin-Ciocalteu (mg GA-Eq/g)*
1	-1 (14)	-1 (1:62,5)	85,58 ± 1,35	3,94 ± 0,07
2	1 (36)	-1 (1:62,5)	203,67 ± 79,18	4,67 ± 0,22
3	-1 (14)	1 (1:22,5)	132,30 ± 19,76	4,79 ± 0,17
4	1 (36)	1 (1:22,5)	138,49 ± 32,30	4,90 ± 0,10
5	-1,41 (10)	0 (1:33,35)	120,26 ±21,41	4,91 ± 0,85
6	1,41 (40)	0 (1:33,35)	134,08 ±17,61	5,10 ± 0,03
7	0 (25)	-1,41 (1:100)	183,32 ± 41,40	3,31 ± 0,33
8	0 (25)	1,41 (1:20)	137,77 ± 55,30	5,23 ±0,17
9	0 (25)	0 (1:33,35)	172,39 ± 8,61	6,04 ± 0,14
10	0 (25)	0 (1:33,35)	174,66 ± 10,64	4,83 ± 0,18
11	0 (25)	0 (1:33,35)	154,12 ± 4,97	4,32 ± 0,23

[a]x_1: time (min); [b]x_2: dry/solvent sleeve mass ratio (g/mL); * Average of triplicate measurements ± standard error. Source: Authors.

In Table 4 it is possible to notice that in the central points, for $ABTS^{\bullet+}$, the values are close, presenting a variation coefficient of 5,16 %, that's represent low dispersion. For the central points of Folin-Ciocalteu, the same occurred, the results showed a coefficient of variation of 12,59 % , that's represent medium dispersion, close values in the results at the central point's shows that the process showed good reproducibility (Fonseca & Martins 2011, p.148).

The standard curve used for the $ABTS^{\bullet+}$ method presented a straight-line equation (Equation 5), with an $R^2 = 0.9977$; the straight-line equation (Equation 6) for Folin-Ciolcateu presented an $R^2 = 0.9892$.

$$\text{Trolox} - \text{Eq}\ (\mu\text{mol/L}) = 0{,}0469(\%\ inhibition) - 2{,}3513 \quad (5)$$

$$\text{Absorbance} = 0{,}0098(\text{Gallic Acid}\ (\mu\text{gAG} - \text{Eq/mL})) + 0{,}0464 \quad (6)$$

Comparing the results of Tables 2 (FFD) and 4 (CCRD), one notices that the results observed in the CCRD (Table 4), in general are similar to those presented in Table 2 (FFD), not having been observed an increase of the results in the analysis of the CCRD assays, probably due to the low variation in the study ranges of the variables, between one planning and another or also due to saturation of the extraction process of phenolic compounds.

For the $ABTS^{\bullet+}$ method (Table 4) the highest and lowest concentration of antioxidant compounds, respectively, occurred in tests 2 (203.67 ± 79.18 µmol Trolox-Eq/g sample) and 1 (85.58 ± 1.35 µmol Trolox-Eq/g sample), values that are higher than those found in the work performed by Sogi and collaborators (2015), in which the authors used 80% methanol as a solvent and the mango peel was dried by lyophilization, demonstrating that the use of solvents with low toxicity and ecofriendly, such as ethanol and water, as a substitute for more aggressive solvents to the environment, such as methanol, is a viable alternative from the point of view of the extraction process of compounds with antioxidant activity.

Also in Table 4 it can be observed that the greatest results for the Folin-Ciocalteu method were obtained in tests 6 (6.04 ± 0.14 µg GA-Eq/g) and 8 (5.23 ± 0.17 µg GA-Eq/g), values close to those found by other authors (Gentile et al., 2019; Guandalini et al., 2019; Nyangena et al., 2019), who concluded that the maximum extraction of phenolic compounds occurred with 50% ethanol solution. The concentration of phenolic compounds observed in some mango cultivars varies from 2.66 to 55.36 mg GA-Eq/g (Lim et al., 2019; Pal & Jadeja, 2019; Sogi et al., 2015), so that the values found in this work are in accordance with those described in the available literature.

Based on the evaluation of the CCRD test matrix the regression coefficients were determined, which are presented in Table 5 for the $ABTS^{\bullet+}$ and Folin-Ciocalteu methods. The behavior regarding the effects previously obtained in FFD were confirmed, that is, for the $ABTS^{\bullet+}$ method the time variable presented a value of positive regression coefficient and a negative coefficient for the dry/solvent mango mass ratio, and for the Folin-Ciocalteu method both variables presented values of positive regression coefficients.

Table 5 - Regression coefficients for the $ABTS^{\bullet+}$ and Folin-Ciocalteu methods response from CCRD.

$ABTS^{\bullet+}$				
	Coefficient of Regression	Standard Error	t-estimated	p-valor
Média	167,09	11,31	14,77	<0,0000*
x_1	17,98	6,92	2,6	0,0483
x_1^2	-20,91	8,24	-2,54	0,0522
x_2	-10,36	6,92	-1,5	0,1954
x_2^2	-4,22	8,24	-0,51	0,6303
$x_1 \cdot x_2$	-27,974	9,79	-2,86	0,0349
Folin-Ciocalteu				
	Coefficient of Regression	Standard Error	t-estimated	p-valor
Média	5,06	0,36	14,12	<0,0000*
x_1	0,14	0,22	0,63	0,5576
x_1^2	-0,04	0,26	-0,17	0,8703
x_2	0,47	0,22	2,16	0,0829
x_2^2	-0,41	0,26	-1,58	0,1748
$x_1 \cdot x_2$	-0,15	0,31	-0,5	0,6415

* $p \leq 0,05$; L- linear terms; [a]Time (min); [b]Dry mango peel/solvent ratio (g/mL). Source: Authors.

Based on the results presented in Table 5 it can be observed that in the case of the $ABTS^{\bullet+}$ method the variable that presented statistical significance was x1 (time), thus this variable was considered to obtain the model presented in Equation 7, which represents the quadratic coded model of the response to the $ABTS^{\bullet+}$ method as a function of the variables studied. The x_2 variable did not present statistical significance, but it was used for the development of the mathematical model, because its inclusion in the model allowed obtaining a mathematical model of better adjustment to experimental data. For the answer obtained by the Folin-Ciocalteu method no variable presented statistical significance within the studied range. Thus, no model was generated for this method.

$$ABST(\mu molTrolox/g) = 163,09 + 17,98X_1 - 19,67X_1^2 - 10,36X_2 - 27,97X_1X_2 \quad (7)$$

The non-significant parameters were incorporated to the residues for the calculation of the analysis of variance (ANOVA), presented in Table 6. According to the F test, the model is predictive, as the F test value (6,66) was higher than the critical value (4,53) (p=0.021). The percent of variation explained by the model (R2 =82%) were good enough (Haaland, 1989), confirming the fit of the statistical model to the experimental data. Based on these observations, the model was used to generate the contour plot and response surface (Figure 1).

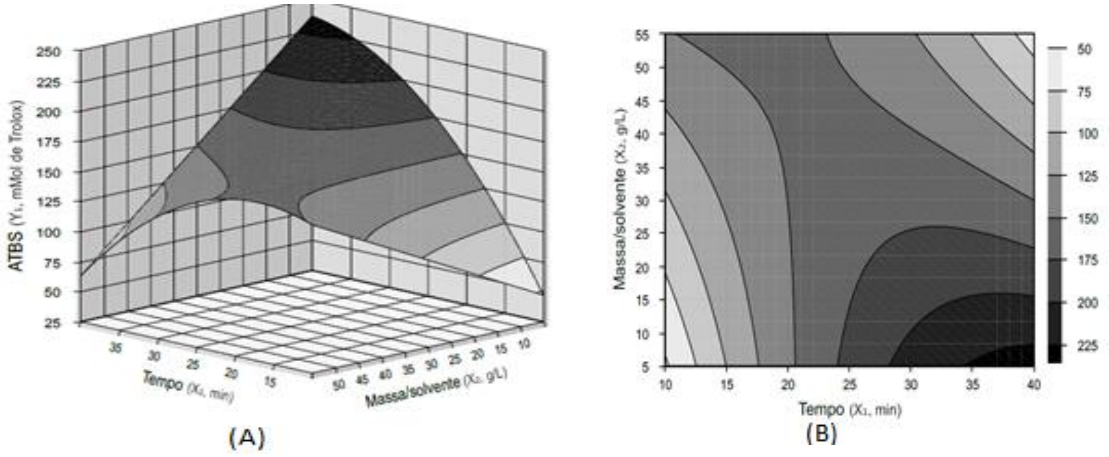
Table 6 - ANOVA of the quadratic models generated for the $ABTS^{\bullet+}$ responses.

ANOVA	$ABTS^{\bullet+}$				
Source of Variation	SQ[a]	GL[b]	QM[c]	$F_{estimated}$	p-valor
Regression	8965,24	4	2241,31	6,66	0,0210
Waste	2018,71	6	336,45		
Total	10983,95	10			

[a]sum of squares; [b]degrees of freedom; [c]Mean squares. Source: Authors.

From the analysis of the response surface and contour plot (Figure 1) it was observed that there is an optimum region for the extraction of antioxidant compounds for the $ABTS^{\bullet+}$ method tests, in the extraction time range between 30 to 40 min and, dry/solvent mango peel ratio (g/mL) from 1:65 to 1:100.

Figure 1 - Response surface (A) and contour plot (B) for the ABTS$^{\bullet+}$ (µmol Trolox/g) as a function of time (min) and dry/solvent mango mass ratio (g/mL) for the Tommy Atkins mango peel antioxidant compounds extraction process.



Source: Authors.

3.3 Validation of the descriptive model - abts$^{\bullet+}$ method

Analyzing the above results for the ABTS$^{\bullet+}$ method it is noted that the conditions under which the extraction process was maximized are: pH 4.6 ± 0.2; sonication amplitude 50%; solvent distilled water; extraction time of 30 min, dry mango peel/solvent ratio (g/mL) of 1:100, using the ABTS $^{\bullet+}$ method.

Based on the discussion of the results obtained from the CCRD for the ABTS$^{\bullet+}$ method, the parameters were considered for the process validation: extraction time of 30 and 40 min, and dry peel/solvent ratio (g/mL) of 1:100. The validation with these conditions is important because they are in the maximization range, thus, to be possible to determine if was statistical variations within this range, we used the two values mentioned above.

The validation process began with the extraction of the antioxidant compounds from the mango peel under the conditions described above, with the extraction tests performed in triplicate. Table 7 shows the experimental values observed and the values predicted by the model (Equation 7).

Table 7 - Experimental values and values predicted by the model - $ABTS^{\bullet+}$ method.

	Experimental response Eq Trolox (µmol/g sample)	Answers predicted by the model* Eq Trolox (µmol/g sample)
Test 1	254,68 ± 1,82[A]	220,52 ± 13,41
Test 2	251,11 ± 1,73[A]	201,02 ± 19,95

* relative deviation= ((y - ŷ)/y)*100; where y= experimental response and ŷ= predicted response by the model; [a] without significant difference between means for p≤0.05 by tukey's test. assay 1: extraction time 40 min and dry mango peel/solvent ratio (g/ml) of 1:100; assay 2: extraction time 30 min and dry/solvent mango peel ratio (g/ml) of 1:100. source: authors.

Based on the information provided in Table 7 it can be concluded that Equation (2) described in a statistically satisfactory way the extraction process based on the $ABTS^{\bullet+}$ method, presenting a relative deviation of less than 20% in relation to the experimental data, confirming the validity of the model demonstrated by ANOVA (Table 6). The results obtained in the experimental analyses do not show significant differences by Tukey's test and when analyzing Table 7 it is noted that the difference between the two time values used (30 and 40 min) does not generate a statistically significant difference (p≤0,05) between them, and the values obtained are similar to those found experimentally by means of Equation 2, demonstrating that the predictive model proposed is valid and that the use of an extraction time of 30 min is sufficient to reach the optimal region of the process.

Thus, the optimal conditions for the extraction process of compounds with antioxidant activity of the Tommy Atkins mango peel are: pH of approximately 4.6; sonication amplitude 50%, distilled water as solvent; ratio of dry/solvent mango peel (g/mL) of 1:100; extraction time of 30 min

4. Conclusions

For the Tommy Atkins type mango peel, it was inquired that the best parameters for the maximum extraction of antioxidant compounds occurred with the use of water as solvent, extraction time of 30 min, dry/solvent mango peel ratio (g/mL) of 1:100, in the natural pH of the mixture (pH of 4.6 0.20) and sonication amplitude of 50%. With these parameters it was possible to obtain the best results for the extraction process.

Based on the information presented in this work it was possible to develop an extraction process of compounds with antioxidant activity from mango peel, in order to maximize yield and aiming the application of such compounds in products for use in humans and animals, through the use of non-toxic solvents and using an agro-industrial residue as raw material, highlighting also the economic feasibility of the process, because the necessary solvents and extraction technique are low cost.

Suggestions for Future Works

For a better understanding of the extraction process and its ability to remove compounds from the plant matrix, it is recommended for future work to characterize the extracted compounds, analysis of bioactivity, aiming at their application in foods or drugs.

Project Performance

To describe the performance of each author on this article: Felipe da Silva Veloso did 40% of the article, Eliane Colla and Aziza Kamal Genena 30% each.

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