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AN OPTIMIZED ALTERNATIVE FOR PHENOLIC COMPOUND-EXTRACTION OF STRAWBERRY BAGASSE AGRO-INDUSTRIAL RESIDUES

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ABSTRACT

The optimum extraction conditions for highest recovery of total phenolics content and antioxidant capacities were analyzed for strawberry (*Fragaria ananassa*) using response surface methodology. Phenolic compounds have been used in food technological processes, and thus it can contribute to the prevention of some degenerative diseases and used as antioxidant or antimicrobial. The assessment of the concentration of total phenolics, as well as their capacity to scavenge ABTS and the antioxidant capacity, determined by the modified DPPH method, were investigated based on distinct combinations of time, temperature, and solvents concentration. It was investigated that the optimum condition for getting the highest antioxidant yield was obtained under water-acetone (80:20, v/v) at 36 ºC and 36 min. We have found that the maximum yield of total phenolic was 1707.66 ± 38.43 (mg GAE/100 g), preserving the antioxidant capacity which was measured by using DPPH and ABTS assays, showing an EC50 of 1085.20 ± 32 (g fruit/g of DPPH) and 66.67 ± 2.4 (μM trolox/g fruit) respectively. This method is also easier and cheaper than other methods to perform polyphenols extractions since does not require expensive reagents or high quantities of organic solvents.

Keywords: antioxidants, strawberry, optimization, phenolic compounds

INTRODUCTION

The strawberry (*Fragaria ananassa*) belongs to the Rosaceae family and is one of the most consumed and currently investigated non-climacteric fruit (**Brito de Figueirêdo *et al*., 2015**). The ripe fruit, which has a pleasant flavor, is consumed fresh as natural drinks, eat with or without the peel and seed (**Tomadoni *et al.* 2016**).

The health benefits of strawberries have been linked mostly to their biological activities in the prevention of cardiovascular disease, inflammation, oxidative stress, obesity, and diabetes. Thanks to its high amount of folate, vitamin C, as well as phytochemicals, attention has been given to strawberry consumption effects (**Scalzo *et al.* 2005**). Consumption of antioxidant-rich strawberries in healthy volunteers diminished mortality rate of mononuclear cells and increased erythrocytes resistance to hemolysis (**Tulipani *et al.* 2014**). Cranberry and strawberry polyphenols compounds improve insulin sensitivity in insulin-resistant, non-diabetic adults (**Paquette *et al*., 2017**). Anthocyanin-rich strawberry supplementation positively improved lipid profiles by significantly decreasing of total cholesterol concentrations, triglycerides and low-density lipoprotein cholesterol (LDL) (**Alvarez-Suarez *et al*., 2014**). Numerous studies and health benefits related to the strawberries were described in previous review. However, clinical trials on cancer chemoprevention are still limited (**Afrin *et al.* 2016**).

Residues of the industry juices can trigger serious environmental problems, these residues, consisting of seeds, peels, husks, whole pomace, among others, are generated every year in the form of wastes, and are poorly valorized (**S. Martins *et al*., 2011**). Recently, increased attention has been given to these materials as abundantly available and cheap renewable feedstock for the production of compounds like the polyphenols (**Sójka *et al.* 2013; Tumbas Šaponjac *et al.* 2015; Zhu *et al.* 2015**). Phenolic compounds are plant secondary metabolites commonly found in plants and derived products such as berries, apples, citrus fruit, cocoa, grapes, onions, olives, tomatoes, broccoli, lettuce, soybeans, grains and cereals, green and black teas, coffee beans and red and white wines (**Alu'datt *et al*., 2017; Magalhães *et al*., 2017**). Recently, phenolic compounds have been used in food technological processes, and thus it can contribute to the prevention of some degenerative diseases and used as antioxidant or antimicrobial agents (**Dzoyem *et al.* 2017**). Polyphenols may exert an indirect antioxidant effect, by protecting endogenous antioxidant enzymes in the human body (**Pradeep & Sreerama, 2018; Zhang *et al*., 2015**), moreover these compounds prevent amyloid β-protein oligomerization and synaptic dysfunction by site-specific binding (**Ono *et al*., 2012**). Related to this, the interest in food phenolics has increased due to their antioxidant and free radical-scavenging abilities (**Santos Felix *et al*., 2018**), anti-inflammation, modulation of signal transduction and anti-proliferation activities (**Banothu *et al.* 2017**).

It should be pointed out that the effectiveness of nutraceutical compounds in preventing diseases depends on the bioactivity, bioavailability and stability of the active substances. Thereby, the "medical use" of these agents need food formulations and processes able to maintain the active molecular structures until their consumption and release in the physiological target (**Vieira da Silva, Barreira, & Oliveira, 2016**). The bioactive phenolic compounds feature different polarities and chemical properties that are related to their structures. The extraction process is affected by the polarity of the bioactive compounds; therefore, the matrix type, time, and temperature used in the process can influence the efficiency of extraction of phytochemicals (**Andrade *et al*. 2015; Rufino *et al.* 2010**).

The extraction process is an important step for isolating and identifying polyphenols. Phenolic compounds are the major phytochemicals in strawberry fruits, mainly represented by anthocyanins (41%), flavan-3-ols (28%), cinnamic acid conjugates (13%), ellagitannins (14%), flavonols (3%) and ellagic acid conjugates (1%), which have been considered to be the most important contributors to the various biological potentialities of strawberries (**Zhu *et al*., 2015**).

Response surface methodology (RSM) are techniques used for optimization of

processes and explores the relationships between explanatory variables and response variables (**Bezerra *et al.* 2008**). It is widely used to optimize conditions for extracting active compounds from herbs (**Lai *et al*. 2013; Ji *et al*. 2012**). The use of RSM is useful to determine optimum values of the independent variables to achieve better response, and enables the user to investigate the interaction among variables, being more efficient than the traditional process using the one-variable-at-a-time method (OVATM). Also, the application of RSM provides a faster and less expensive procedure to obtain optimal values of chemical and instrumental variables affecting the extraction, in accordance with the principles of the green chemistry (**Altemimi *et al*. 2015; De Souza *et al.* 2014**). Hence, in this study two factorial designs (mixture and three level factorial) were applied to optimize the best proportion of solvents, time and temperature for the extraction of phenolic compounds from strawberry (*Fragaria ananassa*) bagasse agroindustrial residues. Free radical scavenging capacities were also measured using DPPH and ABTS radical cations.

MATERIALS AND METHODS

Chemicals and reagents

Gallic acid monohydrate (98 %), sodium carbonate P.A, Folin & Ciocalteu's phenol reagent and Trolox (2,5,7,8-tetramethylchroman- 2-carboxylic acid) were purchased from Sigma-Aldrich (São Paulo, SP, Brasil). Acetone P.A., distilled water, and ethanol 95 % P.A. were obtained from Vetec (Rio de Janeiro, RJ, Brasil). Methanol 99,8 % P.A. was purchased from Chemis (São Paulo, SP, Brasil). DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS, 2,2 azino bis (3-ethylbenzo thiazoline 6 sulfonic acid), diammonium salt were purchased from Sigma-Aldrich (São Paulo, SP, Brasil).

Sample

The strawberry bagasse agro-industrial residues sample employed for the development of the extraction method were obtained from local industry (Frutisol, Jequié, Bahia, Brazil). Residues was dried in an air-circulating oven at 50°C for 72 h and then powdered in a grinder and stored at 20°C until extraction and analysis.

Extraction total phenolic contents

The polyphenols of strawberry bagasse agro-industrial, 10-fold volume (w/v) were extracted with 50 mL of a mixture (ethanol, acetone, and water) for 2 h at 35°C in a shaking incubator (Shaker SL 222, Solab) at 200 rpm. After completion of the extraction time, the crude extract was centrifuged at 5000 rpm (Fanem-Tecnal, São Paulo, Brasil) for 10 min. The extract was filtered for removal of solids particles, and the supernatant was collected and analyzed for apparent phenolic content, ABTS and DPPH radical scavenging activity.

Initially, a mixture design was performed to optimize the best proportion of the three solvents used. After this preliminary evaluation a three level factorial design (TLFD) was performed involving the variables time and temperature. The factors optimized by mixture design remained constant during execution of the 3k factorial designs. All the experiments were also performed randomly. The experimental dominions as coded and real values for the factors and the response (Total Phenolic) obtained are shown in Table 1. Experimental designs and the statistical analysis was conducted using the statistic software 10® (Statsoft) with 95% of confidence level. All determinations were carried out in triplicate (n=3) and the data recorded as mean and standard deviation.

Determination of total phenolic contents

Total phenolics content was determined according to the adapted Folin–Ciocalteu method (**Rebaya *et al*., 2015**). Briefly, the extracts (0.5 mL) were mixed with 2.5 mL of Folin–Ciocalteu reagent (1:10) and 2 mL of sodium carbonate solution (4%). The mixture was stirred and kept at room temperature for 2 h in the dark. Then, the sample absorbance was measured by spectrophotometer (MARTE SPECTRO 560**)** at 750 nm against a blank. Aqueous solutions of gallic acid were used for calibration. The results were expressed as milligrams (mg) of gallic acid equivalents per 100 g of residue (mg GAE/100 g). All measurements were performed in triplicate.

Determination of antioxidant activity

DPPH (free radical-scavenging) assay

The antioxidant capacity was determined by the modified DPPH method (**Brand-Williams et al. 1995**), which is based on the quantification of free radical-scavenging. A methanol solution containing 0.06 mM DPPH was prepared. After adjusting the blank with methanol, an aliquot of 100 µL of fruit extract was added to 3.9 mL of this solution. The reduction in absorbance at 515 nm was measured at 1 min intervals for the first 10 min, and then at 5 min intervals until stabilization. The antioxidant capacity was expressed as the concentration of antioxidant required to reduce the original amount of free radicals by 50% (EC_{50}) and the values expressed as g fruit/g DPPH.

$ABTS^+$ assay

The $ABTS^+$ assay was based on a method developed by Evans Re et al with modifications (**Re *et al*., 1999**). $ABTS^+$ radical were produced by reacting 7 mM ABTS stock solution with 145 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 14 h before use. The ABTS+ solution was diluted with ethanol until an absorbance of 0.7 ± 0.02 at 734 nm was reached. 30 µL of the extract were added in 3.0 mL of diluted $ABTS^+$ solution. After the addition of 30µl of the extract, samples absorbances were recorded at 6 min after mixing. The results were expressed as µM trolox/g fruit.

RESULTS AND DISCUSSION

Application of the experimental designs in the extraction process

In this study, we have determined the optimum values of the independent variables to achieve the maximum response for the extraction of total phenolics compounds, preserving their antioxidant activity in strawberry (*Fragaria ananassa*) bagasse agroindustrial residues. The assessment of the concentration of total phenolics, as well as their capacity to scavenge ABTS and the antioxidant capacity, determined by the modified DPPH method, were investigated based on distinct combinations of time, temperature, and solvents concentration.

The maximum yield of total phenolic were 1707.66 ± 38.43 (mg GAE/100 g). The antioxidant capacity which was measured by using DPPH and ABTS assays, showing an EC50 of 1085.20 ± 32 (g fruit/g of DPPH) and 66.67 ± 2. 4 (µM trolox/g fruit) respectively.

Actually, relevant attention has been given to the health benefits provided by polyphenols derived from plants. Berries are rich in nutrients, vitamins, dietary fibers, and minerals, as well as polyphenols (**Nile and Park 2014**). The content of flavonoids, micro-nutrients such as folate, vitamin C, and minerals determine the potential health effects of strawberries (**Giampieri *et al*., 2012**). In spite of that, it is not always possible to extract all the target compounds with a unique solvent. Therefore, different solvents are often required to extract species of varying polarities in compound mixtures. The mixture consisting of water and organic solvent may facilitate the extraction of species that are soluble in water and/or organic solvent (**Boeing *et al*. 2014**). Indeed, in this study, a mixture design was performed to optimize a better combination involving three solvents of different polarities (acetone, ethanol and water) in the extraction of phenolic compounds from strawberry.

The evaluated response was the total phenolic content extracted. In this study, we applied two response surface methodology techniques to determine the optimum values of the independent variables to achieve the maximum response for the extraction of total phenolics compounds: (1) design matrix and (2) 3^K factorial design. The response expressed as total phenolic content were calculated by the adapted Folin–Ciocalteu method and the results achieved are shown in Table 1.

Table 1 Design matrix and results for optimization of the extraction of compounds phenolic.

Mixture design

Run	Variables			Total Phenolic* (mg GAE/100 g)
	Acetone (%)	Water (%)	Ethanol (%)	
1	100.0	0.0	0.0	125.43 ± 5.55
2	0.0	100.0	0.0	1067.45 ± 10.7
3	0.0	0.0	100.0	218.68 ± 9.85
4	50.0	50.0	0.0	935.20 ± 8.54
5	50.0	0.0	50.0	334.66 ± 13.44
6	0.0	50.0	50.0	595.93 ± 7.75
7	66.7	16.66	16.7	504.72 ± 6.65
8	16.7	66.7	16.7	695.12 ± 12.54
9	16.7	16.7	66.7	643.06 ± 12.05
10 (CP)	33.3	33.3	33.3	707.83 ± 10.40
11 (CP)	33.3	33.3	33.3	691.04 ± 9.15
12 (CP)	33.3	33.3	33.3	693.76 ± 7.25

3^k factorial design

Run	Variables		Total phenolic* (mg GAE/100 g)
	Time (min)	Temperature (°C)	
1	(-1) 30	(-1) 25	751.85 ± 77.46
2	(-1) 30	(0) 35	1110.26 ± 51.05
3	(-1) 30	(+1) 45	996.26 ± 94.09
4	(0) 60	(-1) 25	996.04 ± 104.81
5 (CP)	(0) 60	(0) 35	1122.08 ± 39.50
6 (CP)	(0) 60	(0) 35	1053.2 ± 13.01
7 (CP)	(0) 60	(0) 35	826.42 ± 5.57
8	(+1) 90	(-1) 25	1052.83 ± 35.00
9	(+1) 90	(0) 35	1004.45 ± 8.50
10	(+1) 90	(+1) 45	910.26 ± 2.50
11	(0) 60	(+) 45	1061.18 ± 36.50

CP – Central Point; *Mean ± Standard Deviation,

The extraction of phenolic compounds is dependent on the solvent used and their polarity. In this work, were used ethanol, water, and acetone, which present a dipolar moment (μr) of 1.69 Debye (D), 1.85 D and 2.88 D, respectively (**C. R. Martins *et al*. 2013**). The highest value of phenolic contents was obtained with water and the lowest with acetone or ethanol (Table 1: Run 1 and 3). According to Fig. 1a and 1b, an increase of water concentration resulted in enhancement of the polyphenol content. Figure 1 shows the effect of the interaction of different solvents concentration and extraction power. **Boeing *et al.* (2014)** found that acetone is the least efficient solvent when used pure, but showed good results when combined with water due to increased solvation provided by the presence of water (**Boeing *et al.*, 2014**). Moreover, a constrained mixture design was used to investigate the best proportion of the three solvents used for the extraction. When working with mixtures variables, this freedom of combination between levels does not exist, because they cannot vary without taking into consideration the levels of other variables. In this case, the variables are the components of a mixture, and the sum must be constant and equal to 1 (**Novaes *et al*., 2016**).

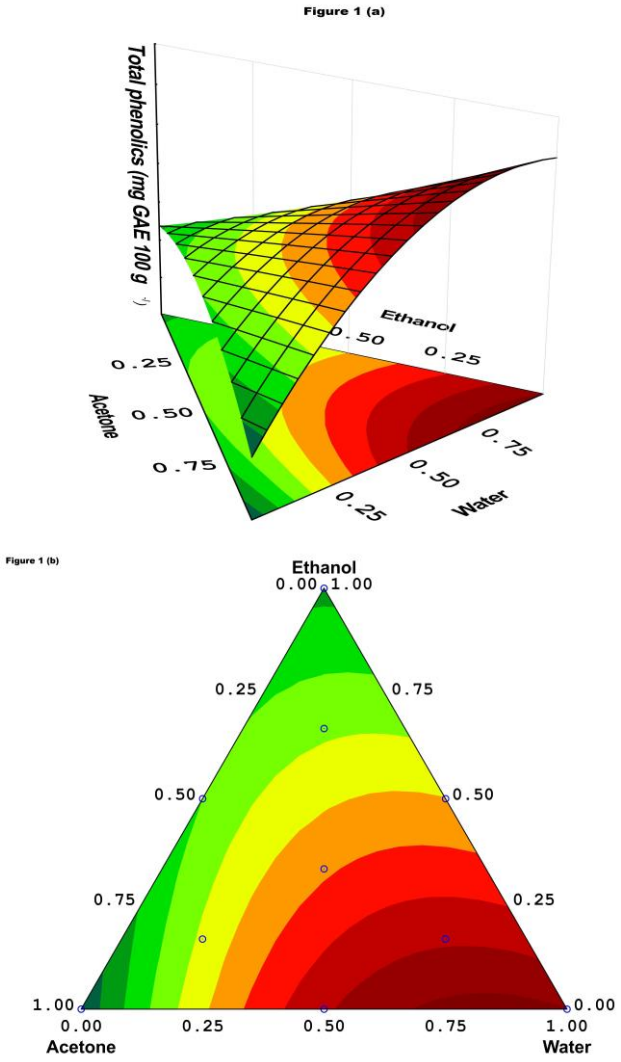

Figure 1 (a) Response surface and (b) Contour plot for optimization of the total phenolic contents of strawberry extracts as a function of acetone, ethanol and water concentration.

By applying the Lagrange criteria, the critical point of the mixture design is characterized as a saddle point. However, the coordinates of the saddle point will not show the optimum conditions of extraction (**Ferreira *et al*., 2007**). Therefore, in order to find the optimum conditions for the extraction, a visual inspection of the surfaces was performed, by analyzing the response surfaces presented in the Fig. 1 is possible to observe that polyphenols content increased parallel to water concentration up to almost 80%, which was further confirmed by the contribution of each factor displayed by the polynomial equation obtained. Furthermore, we were able to find that a high ratio of water (80%) in the solvent mixture with acetone (20%) enhanced the extraction yield. According to **Alothman *et al* (2009)**, acetone-water mixtures are good solvent combinations for the extraction of polar antioxidants (**Alothman *et al*. 2009**). These results are interesting, since it is the first attempt to identify the best solvents used for the extraction of antioxidant compounds from three different berries cultivated in southern Brazil. Previously, **Boeing (2014)** found that the highest total phenolic contents for strawberry extracts were obtained for acetone/water (50/50, v/v) and (70/30, v/v) extraction solution (**Boeing *et al*., 2014**). In addition, with a high ratio of water (80%) in the solvent mixture, we can infer that our extraction procedure can be a clean alternative to procedures involving only organic solvents or mixtures containing a high proportion of organic solvents. This behavior was supported by the response surface plots and polynomial equations (Fig. 1). The surface presented in the Fig. 1 (a) has a maximum as critical point. The proportions of the components that generate the better response in the extraction are the coordinates of this point. Therefore, the optimum conditions for the total phenolics extraction were water/acetone mixture (80:20, v/v) without the presence of ethanol. The surface presented in the Fig. 2 (b) is the contour graphic that is generated by the projection of the surface shown in Fig. 2 (a) in the two-dimensional plane.

The matrix of the experimental design and the response (Total Phenolic - TP) obtained are shown in Table 1 (n = 3). The surfaces response can be described for Eq. (1), resulting from an adjustment of the quadratic model:

TP = 119.46(A) + *1003.98(W) + *272.83(E) + *1282.51(AW) + 812.95(AE) - 140.92(WE) Eq. (1)

The values marked with * are considered significant; A – Acetone, W – Water and E – Ethanol. The coordinates of the maximum point are found by means of the first derivative of the mathematical function (**Lemos *et al*. 2009**). Analysis of variance (ANOVA), P-value significant levels and the analysis of the residues generated between the predicted values and the observed values were used to check the significance of the effects, at the 95% confidence level ($p < 0.05$).

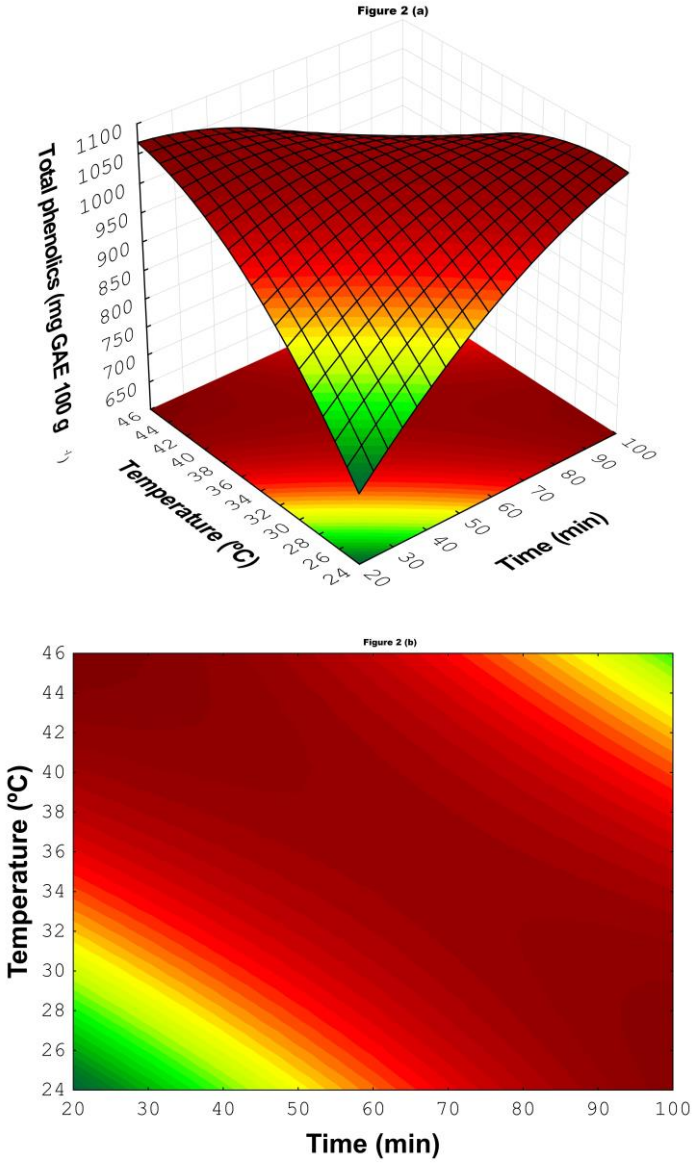

Figure 2 (a) Response surfaces and (b) Contour plot of the total phenolic contents of strawberry extracts as a function of time and temperature

Effects of time and temperature on the extraction yield

It is known that high temperatures improve the efficiency of the extraction; however, the excessive temperature may degrade phenolic compounds. Therefore, extraction temperature and time were another parameters studied in the extraction procedure. After the preliminary evaluation by mixture design, a

three level factorial design (TLFD) was used to screening new variables that significantly affect polyphenol extraction. TLFD presents three levels (−1, 0, and +1) and is generated by the expression 3k. The experimental matrix is composed of all three level combinations of these levels (**Novaes *et al*., 2016**). Table 1 showed the matrix of the experimental design of the two factors expressed as coded and real values and the response (Total Phenolic - TP) obtained (n=3). The ratio of water (80%) and acetone (20%) was kept according to the results obtained in design 1. The function that represents the relationship between the time (Ti), temperature (Te) and Total Phenolic (TP) for polyphenol extraction from strawberry is presented by Eq. (2):

$$TP = 1033.82 + 36.43(Ti) - 52.72(Ti)^2 + 55.85(Te) - 109.52(Te)^2 - 193.42(Ti)(Te) \quad \text{Eq. (2)}$$

This model fits the experimental data. The quadratic model allowed one to locate the optimal conditions and generated the corresponding response surfaces (Fig. 2 (a)) and contour plot (Fig. 2 (b)). The surface presented in the Fig. 2 (b) is the contour graphic that is generated by the projection of the surface shown in Fig. 2 (a) in the two-dimensional plane. The Fig. 2 a-b shows the effect of the interaction of the extraction time and temperature variables in the extraction of the polyphenols. Response surface plots showed that polyphenols extraction increased under experimental conditions optimized. The contribution of each factor is displayed by the polynomial equation obtained. The critical points were calculated by solving the equation system formed by the partial derivatives of the function (**Martendal *et al*. 2007**):

$$\partial TP/\partial(Ti) = 0 = 36.43 - 105.44(Ti) - 193.42(Te) \quad \text{Eq. (3)}$$

$$\partial TP/\partial(Te) = 0 = 55.85 - 219.04(Te) - 193.42(Ti) \quad \text{Eq. (4)}$$

(Ti) = 66 min and (Te) = 36 °C

The critical points also can be observed by visual inspection of the charts depicted in Fig. 2. In this way, for the evaluated experimental domain, an ideal experimental condition is obtained when using intermediate values of time and temperature. Under these experimental conditions optimized (water/acetone mixture (80:20, v/v); time = 36 min and temperature = 36°C), the extraction of polyphenolic compounds from strawberry was 1707.66 ± 38.43 (mg GAE/100 g). The model generated by mixture design was able to establish better conditions for the extraction of compounds from strawberry. The phenolic content of the strawberry was higher than that reported by De Souza (**De Souza *et al*., 2014)** who found 621.92 ± 15.51 (mg GAEs/100 g) by extracting sequentially with methanol/water mixture (50:50, v/v) at room temperature for 1 h. Moreover, **Patras *et al*. (2009)** reported 855.02 ± 6.52 (mg GAEs/100 g) when using with unprocessed purées (**Patras *et al*. 2009**). To evaluate the significance of the model and the effect of the main parameters, analysis of variance (ANOVA) was assessed (Table 2). The p-value for lack of fit of the model was 0.7535 ($p > 0.05$, not significant). The results demonstrated that the mathematical model is well suited to the obtained values.

Table 2 Analysis of variance (ANOVA) for response surface quadratic model.

Factor	SS	d.f.	M.S.	*F*-value	*p*-value
Ti(L)	1990.7	1	1990.7	0.083184	0.8002
Ti(Q)	1760.0	1	1760.0	0.073545	0.8117
Te(L)	4678.8	1	4678.8	0.195510	0.7016
Te(Q)	7596.1	1	7596.1	0.317411	0.6299
Ti by Te	37411.3	1	37411.3	1.563272	0.3376
Lack of Fit	31011.3	3	10337.1	0.431947	0.7535
Pure Error	47862.8	2	23931.4		
Total SS	135126.7	10			

Ti - Time; Te - Temperature; L - Linear; Q - Quadratic; SS - sum of squares; df - degree of freedom; MS - mean square.

Effects of conditions on antioxidant activity

The antioxidant capacity of extracts obtained in the optimal conditions producing the maximum extraction yield of polyphenolic compounds, water/acetone mixture (80:20, v/v); time = 36 min and temperature = 36°C, was measured by using DPPH and ABTS assays, showing an EC_{50} of 1085.20 ± 32 (g fruit/g of DPPH) and 66.67 ± 2.4 TEAC (µM/g fruit), respectively.

The DPPH assay is employed to test the ability of compounds to act as free radical scavengers, and frequently used to evaluate the antioxidant capacity of foods. When a solution of DPPH• radical is mixed with an antioxidant substance, its color turns from purple to yellow. ABTS assay estimates more accurately the antioxidant capacity of foods, particularly those containing hydrophilic, lipophilic and highly pigmented compounds (**Floegel *et al*., 2011**). It is worthwhile noting that the solvent extraction of antioxidants can be improved increasing, for example, the solvent temperature; however, one of the major problems of the antioxidant capacity of the extracts is their preservation. In spite of that, the results from this study demonstrated that strawberry bagasse agroindustrial residues could be considered a potential source of phenolic bio-active compounds with strong antioxidant capacity. With our optimal conditions of extraction, we were able to obtain a high antioxidant capacity comparing with others works. For example, **De Souza *et al* (2014)** found an EC_{50} of 3778.94 ± 333.88 (g fruit/g of DPPH) and 7.87 ± 0.87 TEAC (µM/g fruit) when the extracts were obtained with methanol/water (50:50, v/v) at room temperature for 1 h (**De Souza *et al*., 2014**). Although there exist some studies with extraction times ranging from a few minutes to several hours (**Boeing *et al*. 2014**), in this work we have found parameters that can obtain not only the maximum extraction of total phenolic, but also an effective antioxidant capacity by using a short period of time (36 min). Although we worked with bagasse agroindustrial residues, it should be remarked that antioxidants can perform protective roles against free radicals through a variety of different mechanism like catalytic systems to neutralize or divert reactive oxygen species (ROS) (**Hwang et al., 2015**). It should be pointed out that in biological conditions the antioxidant capacity could change, however, many reports support the potential protective effects of various polyphenol-rich foods against chronic disease, including cancer, cardiovascular disease and neurodegeneration. All of these biological activities can be ascribed to the wide range of bioactive compounds present in the strawberry (**Giampieri *et al*., 2017**). According to Del Rio, to evaluate if polyphenols cause these effects, well-powered and well-controlled human intervention trials are necessary (**Del Rio *et al*., 2013)**. Related to this, positive results were obtained and confirmed in *in vivo* studies on animals and humans, showing the effects of strawberry phenolics on oxidative stress in both physiological and pathological situations. Tulipani et al. reported that strawberry intake increases blood fluid, erythrocyte and mononuclear cell defenses against oxidative challenge, thus suggest that regular consumption of strawberries may improve human defenses against oxidative challenges (**Tulipani *et al*., 2014)**. Strawberry consumption alleviates doxorubicin-induced toxicity by suppressing oxidative stress (**Giampieri *et al*., 2016**).

The recovery of antioxidants reflects not only the need for biofunctional compounds, which may also be interesting from a technological point of view as valuable components of nutraceuticals in food and pharmaceutical preparations or cosmetics industry, but can also exploit the food production chain (**Van Der Goot *et al*., 2016**).

CONCLUSION

To sum up, the importance of this work is that we were able to find optimal conditions producing the maximum extraction yield of polyphenolic compounds with strong antioxidant capacity. It was investigated that the optimum condition for getting the highest antioxidant yield was obtained under water/acetone mixture (80:20, v/v) at 36ºC and at 36 min of time. This method is also easier and cheaper than other methods to perform polyphenols extractions since does not require expensive reagents or high quantities of organic solvents. Fruit sources like waste strawberry may bring new natural products into the food industry with safer and better antioxidants qualities against oxidative damage thus suggesting a new interesting target to search for a novel technological extraction method not yet patented.

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