EXTRACTION OF PHENOLIC COMPOUNDS FROM MANGO PEELS

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***Abstract*— Scientific studies have shown that mango peel is a rich source of phenolic compounds, which present antioxidant activity. In order to contribute to the future applications of this material, this paper aims the optimization of the extraction process of phenolic compounds from mango peels. The following process parameters were evaluated in this study: mango variety, peel drying time, ethanol concentration and solvent temperature. A two-level, four-factor full- factorial design (2^4) was used in the design of the experiments and in the analysis of the results. With the preliminary results a star experimental design around the central point was performed to obtain a response surface. A maximum extraction yield of 33.7% of total phenolics was obtained for the "Espada" variety, with ethanol concentration of 70% and 10 hours of mango peel drying time.**

***Keywords*— mango, phenolic compound, extraction.**

I. INTRODUCTION

Considering the importance of bioactive compounds on human health, studies have been undertaken for their characterization and quantification in different kinds of foods. A recent research performed by Ajila *et al.* (2007) and Berardini *et al.* (2005) revealed that mango peels (*Mangifera indica L.*) are composed of various phenolic compounds. The authors characterized approximately fifteen phenolic compounds in the mango peels, showing the feasibility of obtaining flavonoids and also xanthones in the peel of the fruit.

Clinical studies have found that the phenolic compounds which in addition to antioxidant activity, they also have antiviral, antibacterial and anti-inflammatory activity (Zgórka and Kawka, 2001; cited in Santos and Hasman, 2002). Some epidemiological studies have shown that phenolic compounds present in fruits, grains and vegetables have contributed significantly for the reduction of chronic and degenerative diseases in populations with high doses of these foods in their daily diet. There is evidence that the intake of polyphenols is related with the reduction of cancer incidents.

Mango is a fruit that grows abundantly in 85 different countries and is considered one of the most important tropical fruit in the world since its consumption corresponds to 50% of the entire consumption of all tropical frutis. According to FAO (Food and Agriculture Organization of United Nations) mangos world's supply in 2004 was approximately 26.3 million tons. Brazil's annual production is about 0.84 million tons, corresponding to 3% of the total volume offered (Manica and Oliveira, 2005).

The Northeast region of Brazil accounts for more than 50% of the national production and the production at São Francisco Valley is about 250,000 tons / year. Part of this production is consumed as fresh fruit in the domestic market, another part is exported and the final portion is absorbed by juice processing industries. Around 35 to 60% of the total mass of processed fruits is considered by-products, which are discarded in landfills. The reuse of this great mass of natural waste product represents an important opportunity from both environmental and socioeconomic points of view.

So seeking forward a future use of this material, this work aims to study the extraction process of phenolic compounds from mango peels, trying to determinate optimized operational conditions of this process that may lead to a maximum extraction yield.

Full four factors (24) factorial design with a response surface methodology were used in the design of the experiments and in the analysis of the results. Process parameters analyzed in this work in order to evaluate their effect over extraction yield are: mango variety, peel drying time, ethanol concentration and solvent temperature. The Folin-Ciocalteau method and UV-vis spectrophotometry were used to measure the content of total phenolic compounds.

II. METHODS

A. Reagents

The following reagents were used in this study: gallic acid P.A., sodium carbonate anhydrous, Folin-Ciocalteu reagent, ethanol, and distillated water Folin-Ciocalteu reagent is a mixture of phosphomolybdate, phosphotungstate and phosphoric acid used for the colorimetric assay of phenolic and polyphenolic antioxidants which causes the oxidation of the phenolate, reducing them to blue oxides of molybdenum and tungsten (W_8O_{23} and Mo_8O_{23}).

B. Samples

Mango peels used were from two different kinds of mango: "Espada" and "Tommy Atkins", both acquired in open markets in the region of Feira de Santana/Bahia/ Brazil. Fruits of the variety "Espada" were purchased in October/2006 and of the variety "Tomy Atkins" in March/2007. These fruits were promptly peeled with a stainless steel knife and the peels were frozen and stored

inside a freezer in polyethylene plastic bags at a temperature under -5 ºC. Samples of the "Spada" were kept stored for five months and of the "Tommy" variety for only a week, before the extraction process has begun.

C. Experimental Procedure

The general procedures for sample preparation, extraction and determination of the concentration of phenolic compounds are represented in the flowchart shown in Fig. 1. According to the flow diagram, mango peels were firstly dried and grinded. Later, the material obtained was mixed with the solvent specified for the extraction process. The suspension was kept in a process of dynamic steeping for 30 minutes and then filtered under vacuum. Finally, extract phenolic content was determined by the method of color reaction of Folin-Ciocalteu (Georgé, 2005) and a Cary Varian optical fiber UV-vis spectrophotometer gave a direct measurement of the phenolic compounds concentration at 760 nm.

D. Design of Experiments

In this work the effect of the following parameters: mango variety; peel drying time; ethanol concentration in an aqueous solution and solvent temperature; were selected in order to evaluate the optimization of total phenolic compounds extraction process from mango peels. A two-level, full four factorial design (2^4) was performed and Table 1 shows the levels of these parameters used in the experiments.

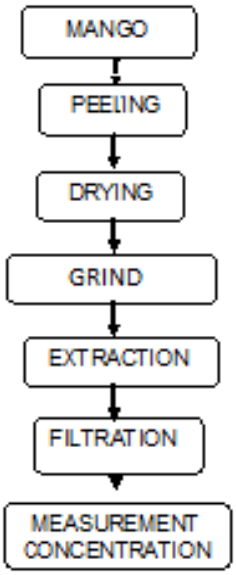

Fig. 1. Flow chart concerning the experimental procedure

Table 1: Parameters analyzed and their corresponding levels.

Parameter	Levels	
	(-)	(+)
Mango Variety	Espada	Tommy
Peel drying time (h)	15.0	24.0
Ethanol Concentration (% v/v)	50.0	0.0
Solvent Temperature (°C)	70.0	90.0

Table 2: List of tests of the 2^4 factorial experimental design.

Test	Variety	Drying time (h)	Solvent Temperature (°C)	Solvent Concentration (% v/v)
1	Espada (-)	15 (-)	70 (-)	50(-)
2	*Tommy* (+)	15 (-)	70 (-)	50(-)
3	Espada (-)	24 (+)	70 (-)	50 (-)
4	*Tommy* (+)	24 (+)	70 (-)	50 (-)
5	Espada (-)	15 (-)	90 (+)	50 (-)
6	*Tommy* (+)	15 (-)	90 (+)	50 (-)
7	Espada (-)	24 (+)	90 (+)	50(-)
8	*Tommy* (+)	24 (+)	90 (+)	50(-)
9	Espada (-)	15 (-)	70 (-)	0 (+)
10	*Tommy* (+)	15 (-)	70 (-)	0 (+)
11	Espada (-)	24 (+)	70 (-)	0 (+)
12	*Tommy* (+)	24 (+)	70 (-)	0 (+)
13	Espada (-)	15 (-)	90 (+)	0 (+)
14	*Tommy* (+)	15 (-)	90 (+)	0 (+)
15	Espada (-)	24 (+)	90 (+)	0 (+)
16	*Tommy* (+)	24 (+)	90 (+)	0 (+)

Table 3: Concentration of total phenolic compounds (FET's) obtained in the extraction process.

Test	Total Phenolics concentration (ppm)		
	Experiment 1	Experiment 2	Average
1	393.0	370.0	381.5
2	315.0	203.0	259.0
3	340.0	363.0	351.5
4	198.0	200.0	199.0
5	365.0	340.0	352.5
6	178.0	203.0	190.5
7	370.0	340.0	355.0
8	165.0	178.0	171.5
9	150.0	285.0	217.5
10	65.0	68.0	66.5
11	113.0	113.0	113.0
12	60.0	55.0	57.5
13	158.0	113.0	135.5
14	68.0	133.0	100.5
15	140.0	103.0	121.5
16	83.0	70.0	76.5

Considering the 2^4 factorial design described later, excluding replications, there are sixteen different ways of combined settings, which imply in 16 experimental runs, shown in Table 2.

III. RESULTS

The results presented in Table 3 were subjected to statistical analysis using Statistica© software version 6.0 to determine the effect of each extraction parameter on total phenolic compound content in the extracts.

A. Calculation and evaluation of parameter effects

Considering the results presented in Table 3, the effects of each parameter over the extraction process of phenolic compounds from mango peels were calculated, at the significance level of 5% ($p \leq 0.05$). So, ten effects were calculated, classified as follow: four primary (variety, drying time, temperature and concentration of solvent) and six secondary effects, resulting from interact

Table 4: Analysis of variance showing significance of the primary effects.

Parameter	Effect	*p*
Variety	-113.375	0.000000
Drying time (h)	-32.250	0.022893
Solvent Temperature (^oC)	-17.750	0.186493
Solvent Concentration	-171.500	0.000000

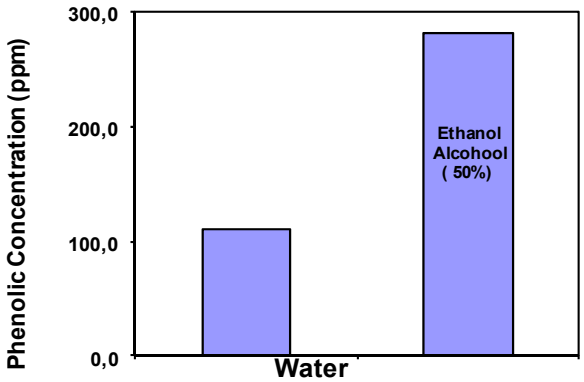

Fig. 2: Effect of concentration of solvent in the concentration of phenolic compounds

tions between two factors. A discussion about these effects is presented as follows.

Primary effects

Table 4 illustrates the values of the main effects found in this study. It can be observed firstly that the largest effect is due the concentration of the solvent (-171.50), followed by mango variety (-113.37) and peel drying time (-32.25). On the other hand, the effect of temperature of the solvent was non-significant ($p > 0.05$).

Figures 2, 3 and 4 clearly identify the influences of these three parameters on the extraction through the values of average concentrations of phenolic compounds. Figure 2 compares the values of global average of total phenolic concentrations obtained for two different solvents used (pure water and ethanol aqueous solution 50% v/v). In this case the averages of total phenolic concentrations obtained with pure water (1 to 8 tests) was 111.1 ppm and with ethanol 50% (9 to 16 tests) was 282.6 ppm.

The results show that ethanol-water solution is a better solvent for the extraction of phenolics compounds from mango peel when compared with pure water, leading to a higher extraction yield.

Rangkadilok *et al.* (2005) attributed this behavior to the variation of solubility of different compounds in water. According to these authors, the use of alcohol as a solvent can increase the solubility of poorly soluble compounds in water. This reference can also be related to the work of Prabha and Patwardhan (1986) (cited by Berardini *et al*., 2005) who claimed that the ellagic acid compound, which is slightly soluble in water, is present in a greater proportion in mango peels.

Figure 3 compares the values of global average of total phenolic compounds concentrations in the extracts obtained from two mango varieties. An arithmetic mean of phenolic compounds concentrations was obtained in the tests where only the "Espada" variety was used (1,3, 5, 7,9,11,13 and 15), and the same was done for the tests where only the "Tommy" variety was used (2,4,6, 8, 10, 12,14 and 16). The overall average for the 1st test group was 253.5 ppm, while for the 2nd group of tests the average was 140.1 ppm.

Analyzing data shown in Fig. 3, it is possible to observe that the extracts obtained from "Espada" variety have presented phenolics concentration higher than the ones from Tommy variety. According to Sharma *et al.* (2001) this variation in total phenolic content is mainly due to genetic differences that exist between these mango varieties.

Figure 4 compares the concentrations of the extracts obtained for two different peel drying times used. The arithmetic mean of phenolic concentrations obtained in the tests where only drying time of 15 hours was used (1,2,5,6,9,10,13 and 14), and the average of the tests where only drying time of 24 hours (3,4,7,8,11,12, 15 and 16) was used are compared. Thus, the effect of drying time over phenolic compounds extraction was also evaluated. An average of 212.9 ppm phenolic concentration was found in extracts from mango peels after 15 h of drying in comparison to 180.9 ppm phenolic concentration after 24 h.

It is possible to conclude that increasing peels drying time reduces phenolic concentration in the extracts and therefore the yield of extraction. The explanation of this effect may be that the extended thermal treatment caused the decomposition of some compounds, however there no references in the literature to justify this fact were found.

Effects of interactions between the parameters

The effects of interactions between the parameters were obtained from six different combinations between them:

- Mango variety versus drying time;
- Mango variety versus solvent temperature;
- Mango variety versus solvent concentration;
- Rinds drying time versus solvent temperature;
- Shell drying time versus solvent concentration;
- Solvent temperature versus solvent concentration.

The calculated values of the interaction effects are listed in Table 4.

According to Table 5 it is clear that only the interaction between mango variety and solvent (ethanol) concentration has presented a significant effect over the extraction process, while the others had no significant effect ($p \leq 0.05$).

B. Optimization

Considering the results discussed later, that the "Espada" variety and the use of an ethanol-water mixture as a solvent have resulted in higher yield of extraction and that an increase in peel drying time reduced the yield of the extraction, new experiments were performed. A second design experiment was fitted using only "Espada" variety peels. A star planning around the center

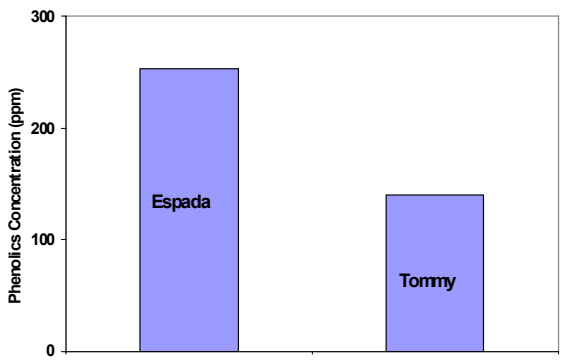

Fig. 3: Effect of mango variety over phenolics concentration in the extracts.

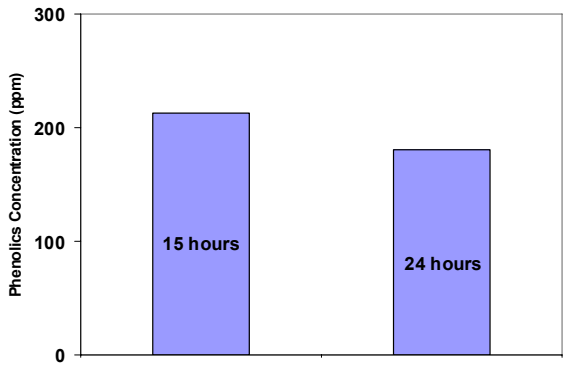

Fig. 4: Effect of drying time on the phenolic concentration in the extracts.

point was taken, using a 10 h drying time and a 70% concentration of the ethanol solution. To obtain this star planning the experimental design showed in Table 6 was used.

With the results of star planning showed in the Table 6 and using the software Statistica© a response surface was obtained and their respective contour plots are showed in Fig. 5.

Looking at Fig. 5 it is possible to see that the maximum yield reached was 33,7% and this can be obtained using an ethanol concentration between 65% and 75% and drying time between 9 and 11 hours.

Concerning to the solvent concentration the results are in agreement with the ones obtained in the literature, which claim that the best point was in a range of solvent concentration between 60% to 80%. A study published by Georgé *et al.* (2005), where an optimization of Folin Ciocalteu method was performed to extract phenolic compounds from fruit juice, relates that the best solvent concentration was 70% ethanol-water solution. Luthria and Mukhopadhya (2006, cited by Luthria, 2006) found that a 60% methanol-water solution was more effective in extracting phenolic compounds than pure solvents like acetone, methanol and ethanol when used in *Cimicifuga racemosa*. Keinänen (1993; cited by Lima, 2004) also noted that both 80% methanol-water and ethanol-water solutions are also more efficient in the extraction of phenolic compounds in leaves of *Betula pendula*.

Table 5: Variance analysis showing the significance of interaction effects between the parameters.

Interaction	Effect	*p*
Variety - Drying time	4.250	0.757407
Variety – Solvent Temperature	7.000	0.611627
Variety – Solvent Concentration	41.750	0.005754
Drying time – Solvent Temperature	18.625	0.184713
Drying time– Solvent Concentration	-5.625	0.682931
Solvent Temperature – Solvent Concentration	12.625	0.363122

Table 6: Star planning structure and results.

Test	Ethanol Concentration (%)	Peel drying time (h)	Yield (%)
1	60	8	29,7
2	80	8	29,2
3	80	12	29,9
4	60	12	29,7
5	70	10	34,4
6	70	10	33,0
7	70	10	33,7
8	56	10	30,2
9	70	12,8	29,8
10	84	10	30,9
11	70	7,2	29,7

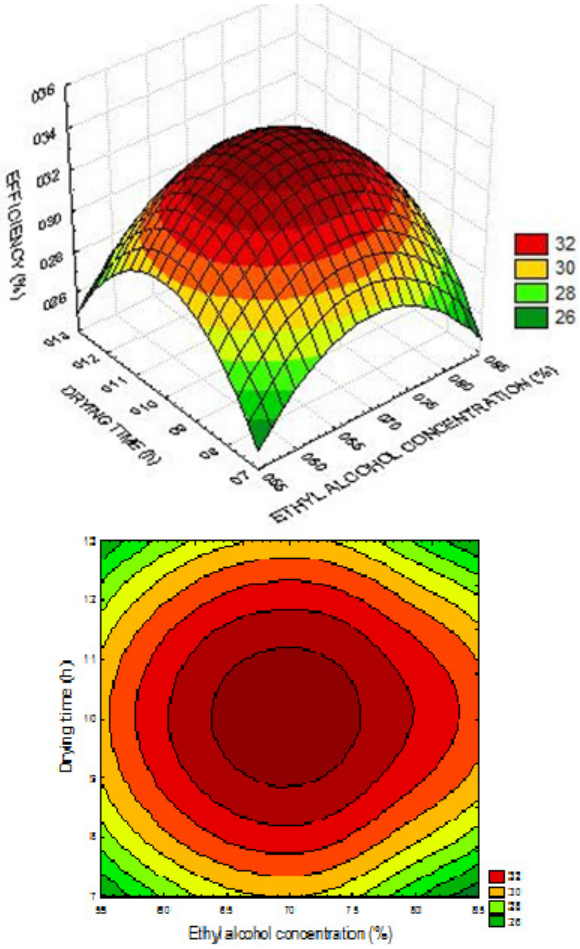

Fig. 5: Response surface and contour plots showing the effect of ethanol concentration and drying time on the extraction yield of phenolic compounds.

IV. CONCLUSIONS

The results obtained in this study suggest that the parameters ethanol concentration and peel drying time directly influence extraction yield of phenolic compounds in mango peels. Furthermore, mango peels of the "Espada" variety have presented higher total phenolic content than the "Tommy Atkins" variety.With respect to the solvent used in extraction, an ethanol-water solution proved to be much more effective than pure water. Also it was found that increasing peel drying time decreases the extraction yield.

Finally the highest empirical extraction yield obtained was 33.7% based on dry matter from the "Espada" variety, using the following process parameters: ethanol-water solution concentration between 65% and 75% and peel drying time between 9 and 11 hours.

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