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MICROENCAPSULATION OF BIOACTIVE COMPOUNDS FROM POMEGRANATE (PUNICA GRANATUM L) JUICE USING SPRAY DRYING

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Abstract: The aim of this work was to evaluate the effect of operational conditions on anthocyanins concentration and antioxidant capacity of the microcapsules from pomegranate juice obtained by spray drying. The effect of air conditions (temperature and air flow rate) on the anthocyanins concentration of pomegranate microcapsules was investigated. The experimental results showed that the identified bioactive compounds were preserved for all conditions evaluated.

Keywords: *anthocyanins, functional compounds, spray drying*

The pomegranate (*Punica granatum* L.) is a fruit from the Middle East and grows in arid regions. It is a fruit with a long medical history, widely used by many people, especially Asians. The fruit has seeds covered with a reddish pulp attended by phenolic compounds, especially anthocyanins. This is an important commercial fruit widely cultivated in Asia, North Africa, the Mediterranean and Middle East (Sarkhosh et al. 2006), and it has a good consumer preference for its attractive juice, sweet acidic and refreshing arils. There are great interests of scientists who engage themselves in pharmaceutical, nutriological and pharmacological research, and new drug development, due to its distinctive multiple officinal parts and multiple bioactive such hypolipidemic, antioxidant, antiviral, anti-neoplastic, antibacterial, anti-diabetic, antidiarrheal and helminthic effects (Wang et al, 2010). Human consumption of anthocyanins is increasing because of the rising awareness and interests in their potential health benefits and pomegranate is one of the major sources of polyphenolic such as the anthocyanins.

There are a lot of products made from pomegranate, like jellies juices and dry fruit. However, during the processing, may be occur the degradation of their constituents. The transformation process should preserve the nutritional and functional characteristics of the raw material from which it originates. Jaiswal et al (2010) evaluated the effect of drying pomegranate arils anthocyanins and observed anthocyanins losses of 60 to 80% by vacuum and sun drying, respectively. They concluded that anthocyanins are stable at high temperatures, but the enzymatic actions contributed to color changes in the product.

In conventional drying processes, high anthocyanins losses are observed, but the use of microencapsulation by spray drying may allow the preservation of bioactive compounds present in pomegranate juice. Microencapsulation is a process of packaging of solid, liquid or gas in extremely small capsules which can release content in a controlled manner and under specific conditions, increasing the product stability (Favaro-Trindade et al, 2008).

The aim of this work was to evaluate the effect of operational conditions (inlet air temperature and air flow rate) on anthocyanins concentration and antioxidant capacity of the microcapsules from pomegranate juice obtained by spray drying.

MATERIALS AND METHODS

Fresh pomegranates (*Punica granatum* L.) were supplied by Special Fruit farm, located in Brazilian semiarid. They were selected, washed and manually prepared to separate the arils from the shell.

Processing

The arils were processed in horizontal device marc Itametal, model Bonina 0,25df, in order to separate the juice from the seeds. The juice was frozen and maintained at ˉ20° C until the processing.

Formulation

For encapsulation purposes, a mixture of 50% modified starch (Capsul®,National Starch) and 50% maltodextrin (MD14P, National Starch), was hydrated with pomegranate juice (15°Brix), to be used as the wall material. The ratio between the juice solid content and the wall material was 1:1.

Spray drying

The formulated material was fed into a spray drying Buchii model B190, and atomized with a nozzle. The water was evaporated by the hot air contacting the atomized material. The microcapsules were collected after they fall to the bottom of the dryer and kind packed in stand up pouch, vacuum sealed and stored at 25° C until the analytical determinations. The yield was calculated as the ratio of the mass of product (dry basis) and mass of solids in the feed.

Analytical determinations

Anthocyanins analysis: about 1 g of sample was weighed and the extraction was made with a solution of methanol and formic acid in the ultrasonic bath with subsequent centrifugation until discoloration of the solution. Then, an aliquot of the extract was dried under compressed air being re-suspended in methanol and formic acid for chromatographic analysis. Chromatography was performed on a Waters® Alliance 2695 system, with a Waters® 2996 photodiode array detector, with a Thermo® Scientific C18 BDS (100mm x 4.6mm; 2.4µm) column, flow 1.4 mL / min., column temperature of 40° C, injection volume of 20µL and gradient elution method with acetonitrile and formic acid. Characterization of anthocyanins present in pomegranate was performed by comparing the chromatographic profile with the literature and by comparing the retention times obtained with chromatograms of other fruits previously characterized by the laboratory in similar conditions analysis (Santiago et al, 2010). The quantification of anthocyanins was performed by external standard curve and the results have been reported by the equivalence cyanidin-3-O-glycoside, which was the external standard used to plot the calibration curve. Anthocyanins determination was conducted in duplicate analysis.

Antioxidant Activity: measured by the Trolox Equivalent Antioxidant Capacity (TEAC), also known as ABTS cationic radical scavenging activity, according to the method proposed by Re *et a*l. (1999) and Rufino *et al.* (2010). Antioxidant activity analysis was performed in triplicate.

Experimental design

The response surface method was applied to optimize the process parameters: inlet air temperature (X_1) and air flow rate (X_2) were the independent variables studied to optimize the process yield, anthocyanins concentration and antioxidant activity. The levels of the independent parameters were based on preliminary experimental results.

Table1. Uncoded and coded levels of independent variables used in the experimental design.

Uncoded-variables	Air inlet Temperature (°C)	Air flow (m^3/h)
-1	180	500
0	190	600
1	200	700

RESULTS

The overall yield (product mass in dry basis/solids in the feed) reached was about 57%. The yield of spray drying on a laboratory scale observed by Li *et al.* (2010) was between 50 to 70%.

Characterization of phenolic compounds

The anthocyanins profiles and content of the formulated pomegranate juices and microcapsules were similar in a dry basis. So, the identified bioactive compounds were preserved during spray drying processing for all operational conditions (Table 1). The results showed that the average antioxidant activity of microcapsules, expressed as µmol Trolox eq/g in dry basis, was 121.77 ± 9.93 representing about 21% losses as compared with formulated juice, 154.33 ± 5.75

Table1. Anthocyanins concentration (mg/100 g db) in the formulated juice and microcapsules

Anthocyanin	Average concentration	
	Feed*	Powder**
Delphinidin 3,5 - diglucoside	14.99 ±1.52	14.38 ± 2.36
Cyanidin 3,5 - diglucoside	33.00 ±3.84	28.91 ±4.64
Delphinidin 3 - glucoside	2.63 ±0.09	2.06 ±0.33
Pelargonidin 3,5 - diglucoside	2.07 ±0.13	1.39 ±0.19
Cyanidin 3 - glucoside	5.87 ±0.34	4.66±0.67
Pelargonidin 3 - glucoside	1.29 ±0.06	0.66±0.05

* Values expressed as means of two determinations ± standard error; ** values expressed as means of seven experiments ± standard error.

Figures 1, 2 and 3 are the Pareto chart showing the effect of air inlet temperature and air flow on the process yield, antioxidant activity and cianidin-3-glucoside content, respectively. No significant effect of the studied independent variables was observed on process yield and anthocyanins content, at 95% of confidence. However, a negative effect on antioxidant activities occurred as air temperature and air flow increased simultaneously (Figure 2).

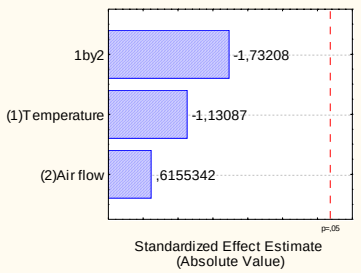

Figure1. Pareto chart: Effect of air temperature and air flow on total mass product.

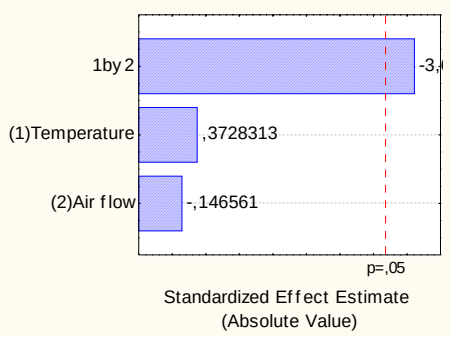

Figure 2: Pareto chart: Effect of air temperature and air flow on Antioxidant activity.

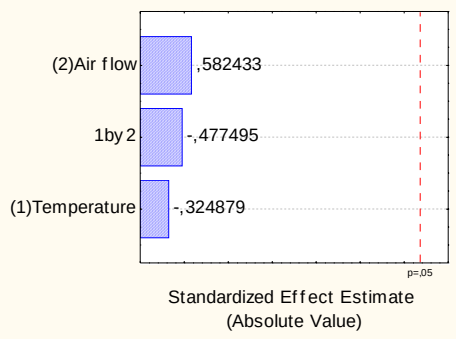

Figure 3: Pareto chart: Effect of air temperature and air flow on Cianidina-3-5-diglicosídeo

CONCLUSIONS

This study investigated the effects of operational air conditions on anthocyanin content of pomegranate microcapsules prepared by spray drying from formulated juice, using modified starch and maltodextrin as wall materials. No statistical difference, at 95% of confidence, was observed for process yield and anthocyanins content, at the selected operational conditions. Very satisfactory results were observed for all anthocyaninsns retention in pomegranate microcapsules (about 100%). So, the experimental results showed that the identified bioactive compounds were preserved for all conditions evaluated.

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