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AQUEOUS EXTRACT OF POMEGRANATE PEELS (*PUNICA GRANATUM*) ENCAPSULATED BY SPRAY DRYING

R. I. Nogueira[1]*, M. C. A. Santiago[1], D. R. S. F. Paim[2], L. F. Santos[2], A. L. M. Carlos[2], V. C. Wilberg[1], R. L. O. Godoy[1], S. P. Freitas[3]

[1]*Embrapa Food Tecnology, Av. Das Américas, 29501, Rio de Janeiro, 23020-470, Brazil*

[2]*UEZO- Fundação Centro Universitário Estadual da Zona Oeste*
Av. Manuel Caldeira de Alvarenga, 1203, Rio de Janeiro, 23070-200, Brazil

[3]*UFRJ/ Escola de Química, Av. Athos da Silveira Ramos, 149 Bloco E - Sala E-209, Ilha do Fundão, 21941-909, Rio de Janeiro, Brazil*

**Corresponding author: Tel.:+55 21 3622 9611, e-mail: regina.nogueira@embrapa.br*

Abstract: Tannins, as punicalagin, represent the predominant class of bioactive substances from pomegranate, concentrating mostly in the peels and fruit mesocarp. The aim of this study was to select the wall materials (gum Arabic, Capsul™ and maltodextrin) for microencapsulating by spray drying an aqueous extract with 14% in soluble solids obtained from pomegranate peels. It was observed no statistical differences on punicalagin concentration in all powdered products. However the agents Capsul™ and maltodextrin were responsible for the better retention of punicalagin in the powdered product. So the stability of dried extracts makes it suitable for industrial or in agricultural applications.

Keywords: phenolic compound, punicalagin, green chemistry, by-product

INTRODUCTION

The pomegranate is a shrub native to western Asia and the Mediterranean countries, whose cultivation occurs in countries like Spain, United States, Iran, Turkey, India, Israel, China and countries of the northern coast of Africa, among others. Its cultivation in Brazil occurs mainly in irrigated areas of the semiarid region due its economic potential and health benefits associated with the bioactive compounds of this fruit.

Pomegranate juice is the main product, but the processing waste such as peels and seeds can be used as animal feed or for developing other products with high added value.

The peels are 42% of the residue of pomegranate and analysis of different parts of the fruit results that they stand out as the amount of antioxidant activity and phenolic compounds demonstrating its potential as a functional ingredient. But the extract of pomegranate peels have an astringent taste that is unpleasant sensory[1].

The main phenolic compound present in the pomegranate peels is punicalagin, a polyphenol, classified as hydrolysable tannin (ellagitannin) of high molecular weight, with recognized antioxidant and anti-inflammatory activities, emerging as a promising molecule with multiple functions beneficial to health[2,3]. The complexation between punicalagin and proteins is the basis for its properties as insect control, fungal and bacterial factors and their pharmacological activities[4].

Solid-liquid extraction in ethanol process, such as maceration or soxhlet extraction followed by evaporation of the alcohol under vacuum, was used to obtain punicalagin from pomegranate peels, resulting in a product in liquid form. This extract can be stabilized by microencapsulation process, but special care in handling for the maintenance the bioactivity of its component must be observed.

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 [5]. For microencapsulation by spray drying a step of formulating with carriers that do not interact with the molecule to be encapsulated, for protection from heat and facilitate its flow into the tower to prevent adherence of product to the walls of the equipment is required.

Encapsulating agents

The encapsulating agents most commonly used are modified or hydrolyzed starch and gum Arabic.

Maltodextrin is a hydrolyzed starch widely used in the food industry because of its property of aiding the dispersion of a product and preventing its agglomeration pipelines. Additionally facilitates the production of a powder product with a homogeneous granulation [6]. The properties of the emulsion as total solids content, viscosity, stability and droplet size directly affects the efficiency of encapsulation. Maltodextrin is widely used in microencapsulation and offers advantages such as low cost, aroma and mild flavor and low viscosity at high solids concentrations, but has lower emulsifying property[7]. Therefore, it is common to use combinations of maltodextrin with other wall materials which have good emulsifying capacity and can thus compensate for the lack of this property[8].

G*um arabic* is a well-known encapsulating agent for its property of producing stable emulsions with good retention of volatiles [9]. But there are limitations to its use in Brazil due to the high cost and limited supply.

Gum Arabic and maltodextrin were tested as carrier agents for camu camu microencapsulation by spray-drying. According the reported data, the gum arabic was more effective than maltodextrin to preserve the bioactive compounds[10].

Modified starch[11] is a food additive which is prepared by treating starch or starch granules, by the incorporation of lipophilic groups making it easier to the solubility of the powder products. The purposes of this modification are to enhance its properties particularly in specific applications such as to improve the increase in water holding capacity, heat resistant behavior, reinforce its binding, minimized syneresis of starch and improved thickening. In this study it was select a very used modified starch named Capsul^{TM} that is especially developed for the encapsulation due its excellent film-forming properties and low viscosity in high solid soluble concentration. In some cases it is used in formulations to completely replace the gums.

The utilization of encapsulated phenolics instead of free compounds can overcome the drawbacks of their instability and improve the bioavailability and half-life of the compound *in vivo* and *in vitro*.

The aim of this study was select the wall materials for encapsulating an aqueous extract obtained from pomegranate peels in order to obtain a stable form to be used in agriculture as fungicide agent or others applications in food industry.

MATERIALS AND METHODS

Fresh pomegranates (*Punica granatum* L.) fruits were supplied by Boa Fruta farm, located in Petrolina, brazilian semiarid region. The peels were manually separated, longitudinally sliced on dimensions of 1 cm, dried in a cabinet dryer at 40ºC during 24 hours and crushed in a hammer mill. Then it was packed in a sandwich packing "stand up pouch", sealed and maintained at 7° C until the processing.

Processing

The aqueous extract was prepared on a soxhlet system consisting of a 5-liter flask attached to a heater blanket containing a cartridge which was placed about 1 kg of crushed pomegranate peel, Figure 1.

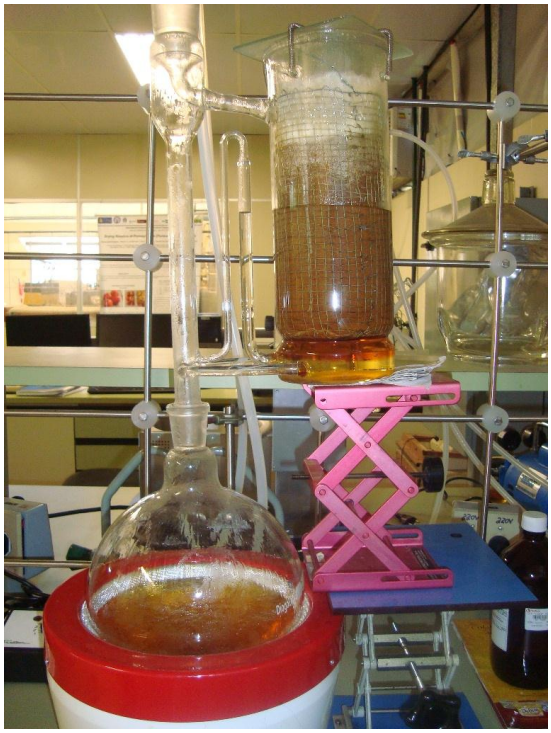

Fig. 1. Extraction process in a soxhlet equipment

Subsequently three liters of an extraction solution of ethanol (80%) were transferred, resulting in a ratio of extraction 1:3. The process was conducted at 78 °C and was interrupted when 8 siphoned been completed. The alcohol present in the extract was removed in a rotary evaporator and this aqueous extract was placed in hermetic container and kept under refrigeration at 7°C until the moment of analytical determinations and formulated to feed a spray dyer.

Formulation

Three different encapsulating agents were used in the formulations according to an experimental planning (Table 1): (GA) instantaneous gum Arabic (Vetec), (MS) modified starch (Capsul^{TM} AKY-0800,

National Starch) and (MD) maltodextrin DE5 (Globe® 1805, Corn Products Brazil). The aqueous extract was mixed with the encapsulating agents using an ultra turrex forming a emulsion to be atomized in a spray dryer. The percentage of encapsulating agent was established according to the solids content of pomegranate extract, 14 °Brix.

Table 1. Experimental design used to prepare the suspension for the spray dryer feed

Treatment	suspensions
1	MD
2	GA
3	MS
4	MD + GA
5	GA+ MS
6	MD + MS
7*	MD + GA+ MS

* Central point: assay in triplicate.

Spray drying

A laboratory scale spray dryer Buchii model B190, Figure 2, atomized with a nozzle was operated with a flow rate of 1 $kg.h^{-1}$. The inlet temperatures of the drying air was controlled from 162°C to 170°C and outlet temperatures from 89°C to 93°C. The formulated suspension was fed into a spray drying and the powder product was packaged in vacuum sealed packages, which remained stored at 25°C until analysis of punicalagin.

Figure 2. A laboratory spray dryer used to obtain punicalagin in dry form

Chemicals. HPLC grade acetonitrile, formic acid 96% and methanol were purchased from Tedia (USA). Ultrapure water was obtained from Milli-QTM Gradient 10A System (Merck Millipore, USA). Punicalagin analytical standard (Sigma-Aldrich, USA).

Punicalagin analysis

The punicalagin analysis was conducted using about 1 g of sample for the extraction with methanol: water (80:20, v/v) in the ultrasonic bath with subsequent centrifugation, being this procedure repeated three times [12]. Then, an aliquot of the extract was filtered for chromatographic analysis. All assays were performed in triplicate. Chromatography[13] was performed, with adaptations, on a Waters™ Alliance 2695 system, with a Waters™ 2996 photodiode array detector, with a Thermo™ Scientific C_{18} BDS (100 mm x 4.6 mm; 2.4 µm) column, flow 1.0 mL min^{-1}, column temperature of 40°C, injection volume of 1 µL and gradient elution method with acetonitrile and formic acid. The quantification of punicalagin was performed by external standardization, based on calibration curves made with commercial analytical standard.

Statistical analysis

The experimental planning simplex centroid was used to analyze the punicalagin retention. According to this methodology, experiments were conducted with 7 mixtures of different compositions: three experiments with pure components corresponding to experiments located at the vertices of the diagram, three experiments of binary mixtures corresponding to the midpoint of the edges and a center point, the centroid of the diagram, with the three components. Triplicates were made at the center point, being possible to evaluate the error associated with the model. Data were analyzed using the software Statistica version 9.0 and XLSTAT version 2013. Each assay was conducted in duplicate.

RESULTS AND DISCUSSION

An important operating parameter in spray drying is that the product has not adhering to the walls allowing its perfect flow in the atomization chamber.

The Figure 3 shows the punicalagin powder collected in the output of spray dryer. It was observed that all formulations resulting in an adequate performance of the drying.

Fig. 3. Punicalagin in powder form

With chromatographic analysis it was possible to observe an average of 49% on punicalagin retention in the powder product regarding the initial concentration of the extract, Table 2. All extracts showed the same punicalagin chromatographic profile, Figure 4.

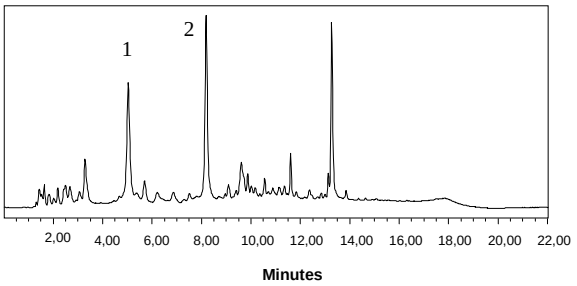

Fig. 4. Chromatogram: peaks 1 and 2 are punicalagin isomers

According Table 2 it was observed no statistical differences on punicalagin concentration in all powdered products. This result allows for a greater choice of carriers drying and can be used three encapsulating agents alone or in binary or ternary mixtures according to the interest of application or processing costs.

By the other side, the effects of encapsulating agents on the punicalagin retention are illustrated in the Figure 5. This parameter is dependent upon carrier type and its concentration.

Table 2. Concentration of punicalagin in the peel extract and in the microcapsules

Treatment	concentration of punicalagin * ($mg.100g^{-1}DB$)	Punicalagin retention*** (%)
Peel extract	5535.57 ± 15.56^{a}	-
1	2985.54 ± 144.85^{b}	54
2	2591.38 ± 81.87^{b}	47
3	3017.83 ± 58.28^{b}	55
4	2548.02 ± 70.63^{b}	46
5	2658.23 ± 45.69^{b}	48
6	2985.32 ± 131.36^{b}	54
7**	2790.41 ± 228.99^{b}	50

* values expressed as a mean of two determinations ± standard error; ** values corresponding to the three treatments of the central point, with average concentration of punicalagin expressed as mean of two determinations ±standard error; *** punicalagin retention relative to the initial concentration in the feed solution.
^{a,b} different letters at the same column represent statistical differences (Tukey, $p<0,05$).

Simultaneously analysing the three encapsulating agents it was possible to observe, Figure 5, that the Capsul™ agent exerts the greatest influence on punicalagin retention. Maltodextrin has a behaviour very similar to Capsul™, and gum Arabic was the agent that less contributed in retaining punicalagin.

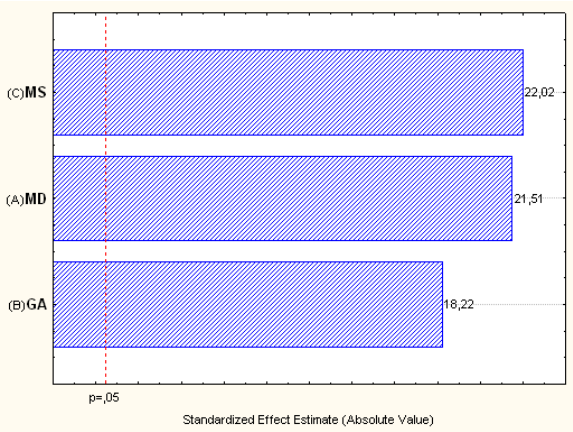

Fig. 5. Influence of encapsulating agents on punicalagin retention

From Figure 6 it was possible to establish the best conditions for the formulation of suspensions of pomegranate extract to be atomized in order to obtain a powdered product with retention of up to 55% of punicalagin.

A microcapsule can be elaborated through matrix containing the material to be protected and mixtures of polymers that act as a protective film avoiding the effect of their inadequate exposure. So the losses on punicalagin retention may be occurred due the molecule size, Figure 7, which probably causes difficulties at its full recovery and consequent protection.

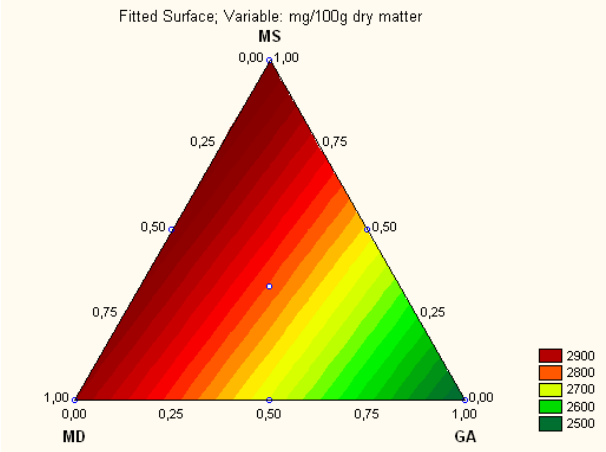

Fig.6 - Diagram showing the influence of ternary mixture of encapsulating agents on punicalagin retention.

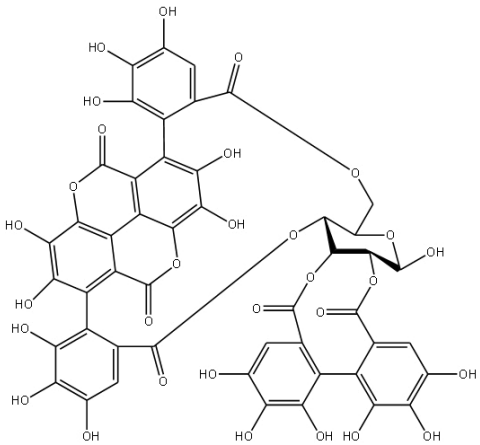

Fig.7 – Punicalagin structure

By the diagram of the Figure 6 it is possible to choose different formulations depending on the interest and availability of encapsulating agents. Within the region of optimum retention of punicalagin it is possible to use up to 20% gum Arabic and 80% Capsul™ or mixtures of maltodextrin and Capsul™ in any desired combinations.

Bioactive compounds from the ethanol extract of pomegranate peels were encapsulated with maltodextrin and soy protein concentrate being observed that the maltodextrin exerted a greater protective effect in polyphenols present in the powder, with over 90% recovery [14].

Similar process of spray drying were conducted to obtain encapsulated pomegranate juice[15] and the best anthocyanin protection was reached with a binary mixture of gum Arabic and Capsul™ .

The advantage of using more than one carrier is the obtaining of microcapsules with more favorable conditions for their use in new food formulations in hydrophilic or lipophilic bases.

CONCLUSIONS

This work confirms the feasibility of ternary mixture of gum Arabic, Capsul™ and maltodextrin as encapsulant agents for spray drying of pomegranate peels aqueous extract. The highest concentration of punicalagin retentions were observed in the formulations containing only Capsul™ or maltodextrin and in its binary mixtures.

NOMENCLATURE

DB	Dry Basis	$mg.100g^{-1}DB$
MD	Maltodextrin	
GA	Gum Arabic	
MS	Modified Starch	

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