PRODUCTO NATURAL

Ultrasound assisted extraction of polyphenols from *Punica granatum* (Grenada) fruit

Extracción por ultrasonido de los polifenoles de la fruta del *Punica granatum* (Granada)

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ABSTRACT

Introduction: extraction of bioactive compounds from vegetable materials is a classical operation applied in many industrial processes. Few studies have made reference to processes to obtain extracts from Punica granatum. This fruit is generally consumed as such or as fermented juices. In Cuba, it is known as grenade and numerous studies have shown antiviral, antioxidant and antimicrobial properties. Maceration process is the main used technology in the preparation of extracts from Punica granatum fruit, being this a long and expensive process. Ultrasound-assisted extractions have been proven to significantly decreased extraction time and increased extraction yields in many vegetable materials. However, few papers report the use of this methodology in the extraction processes of Punica granatum.
Objective: to evaluate an ultrasound-assisted extraction process to extract polyphenols from Punica granatum fruit.
Methods: an experimental surface, response 3^2, randomized and replicate design was made to identify the effect of the extraction time and the alcoholic concentration of the menstruum as well as an extraction study in the course of time, keeping the best extraction conditions set in the design.
Results: the studied parameters did not show significant influence over the process ($p= 0.0981$ and $p= 0.8504$ for time of extraction and alcoholic content, respectively). The behaviour curve of the polyphenol extraction in time showed that top concentration values were reached at 60 minutes.
Conclusions: according to results, the optimal conditions of polyphenol extraction were as follows: extraction time of 60 min, and 50 % alcoholic concentration (v/v) as menstruum.

Keywords: Punicam granatum, pomegranate, ultrasound-assisted extraction, extraction time, polyphenols, grenade.

RESUMEN

Introducción: la extracción de compuestos bioactivos a partir de material vegetal es una operación clásica aplicada en muchos procesos industriales. Pocos estudios han hecho referencia a la obtención de extractos a partir de la fruta del *Punicam granatum*. Esta fruta es generalmente consumida en forma natural o en jugos fermentados. En Cuba es conocida como granada y numerosos estudios han demostrado sus propiedades antivirales, antioxidantes y antimicrobianas. La maceración es el principal proceso empleado en la elaboración de extractos a partir de esta fruta, siendo un proceso largo y costoso. La extracción asistida por ultrasonido es una metodología que garantiza una disminución del tiempo de extracción e incrementa los rendimientos extractivos en muchos materiales. Sin embargo, pocos trabajos informan el empleo de esta metodología en procesos extractivos a partir de *P. granatum*.
Objetivo: evaluar un proceso de extracción asistida por ultrasonido para extraer los polifenoles de la fruta *P. granatum*.
Métodos: se realizó un diseño experimental de superficie respuesta 3^2 aleatorizado replicado en el punto central, para estudiar la influencia del tiempo de extracción y la concentración alcohólica del menstruo. Así como un estudio de extracción en el tiempo manteniendo las mejores condiciones establecidas en el diseño.
Resultados: los parámetros estudiados no presentaron una influencia significativa para el proceso (p= 0,0981 y p= 0,8504 para el tiempo de extracción y la concentración alcohólica del menstruo, respectivamente). La curva del comportamiento de la extracción de polifenoles en el tiempo demostró que a los 60 min se alcanzaban valores máximos de concentración.
Conclusiones: de acuerdo con los resultados, las condiciones óptimas de extracción fueron: tiempo de extracción 60 min y solución hidroalcohólica al 50 % (v/v) como menstruo.

Palabras clave: *Punicam granatum* , pomegranate, extracción ultrasónica, tiempo de extracción, polifenoles, granada.

INTRODUCTION

Punica granatum (pomegranate) plant is an erect shrub and its fruit is known to be a rich source of bioactive compounds. It has been used for many peoples for medicinal purpose. The fruit is a globose berry, crowded by persistent calyx lobes, having a leathery pericarp filled with numerous seeds, which are surrounded by a pink to red, transparent, juicy, acidic, pleasantly tasting pulp.[1] In Cuba is known as grenade and numerous studies have shown antiviral, antioxidant and antimicrobial property.[2-7]

Punicalagin is the main ingredient of pomegranate husk, is a high molecular weight polyphenolic compounds. It has shown remarkable pharmacological activities attributed in the presence of dissociable OH groups.[8-10]

Maceration process is the main used technology in the elaboration of extracts from *P. granatum* fruit, being this a long and expensive process.[11] Ultrasonic assisted extractions have been proven to significantly decreased extraction time and

increased extraction yields in many vegetable material.[12] However, few works report the use of this methodology in the extraction processes from *P. granatum*.[13,14] The aim of this study was to evaluate a process of ultrasound assisted extraction of polyphenols from *P. granatum* fruit.

METHODS

PLANT MATERIAL

P. granatum ripe fruit were collected in the Experimental Station of Medicinal Plants "Dr. Juan Tomás Roig", in Artemisa, Cuba, in July 2011. Herbarium specimens, voucher ROIG 4681, have been deposited in Herbarium at the Experimental Station.

EXTRACTION EXPERIMENTAL DESIGN AND SAMPLE PREPARATION

The fruit were washed with H_2O and 2 % Sodium Hypochlorite solution and milled until size smaller than 200 µm. The extraction of polyphenols was ultrasound-assisted and was carried out in ultrasonic cleaning bath (SAKURA US-5, RETOMED, Cuba), operating at 28 KHz and power of 150 W with thermostat to regulate temperature. The extracting temperature was of 30 ± 2 ºC and solid liquid ratio (g/mL) was fixed in 1/20, conditions similar to the extracts elaborated by maceration process.[11]

In order to optimize the extraction process a factorial design 3^2 (Statgraphics plus, versión 5.1, EUA), was used to identify the effect of two factors (extraction time and alcoholic concentration) and its levels on dry residue, pH and total phenolic compound content.

Nine experiments were designed (table 1). Five gram of sample measured precisely, was put into 250 mL reaction flask and added 100 mL of hydroalcoholic solution. The test was repeated in triplicate.

EXTRACTION STUDY IN THE TIME

Extractions at 15, 30, 60 and 120 min were carried out maintaining the best extraction conditions according to the experimental design. The total phenolic content were determined according to *Rodríguez et al.*[11]

STATISTICAL ANALYSIS

The experimental design, data analysis and optimization procedure were performed using Statgraphic plus 5.1. In the extraction study the statistic significance of differences among results was evaluated by ANOVA. The differences between the means were assessed using Duncan multiple comparisons post test. Results were considered significant when $p < 0.05$.

Table 1. Design experimental conditions

Levels			
Independent variables	Low	Half	High
Extraction time	30 min	75 min	120 min
Alcoholic concentration	30 %	50 %	70 %
Experimental condition			
Experiments	Extraction time (min)	Alcoholic concentration (%)	
1	30	50	
2	75	70	
3	120	50	
4	30	30	
5	75	50	
6	75	30	
7	30	70	
8	120	30	
9	120	70	
10	75	50	
11	75	50	

For measurements, the solution was filtrated and used for determination of dry residue, pH and total phenolic content were determined according to *Rodríguez et al*.[11]

RESULTS

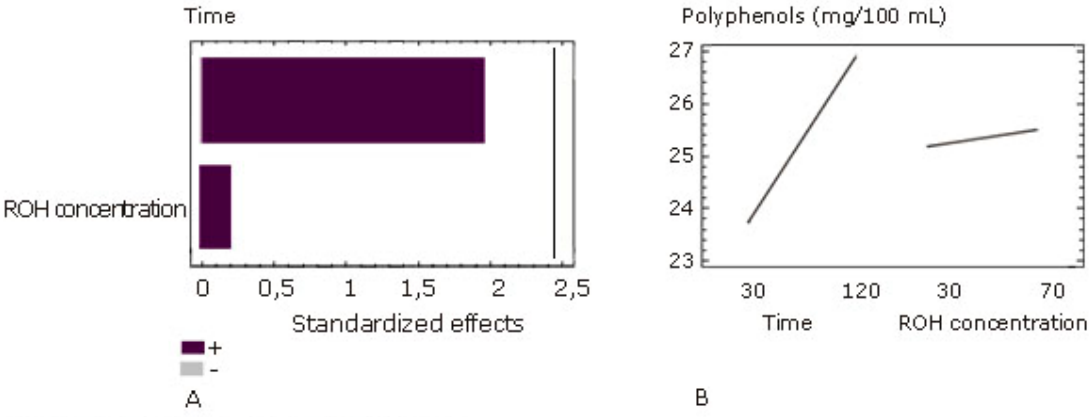
The results show that the employment of the ultrasound doesn't influence on the studied parameters (p= 0.0981 and p= 0.8504 for time of extraction and alcoholic content, respectively) (Fig. 1) (A). These results show that the extraction conditions don't have a significant effect on the independent variables. Therefore the best experiment was defined on the base of polyphenols content.

The results showed that the different indicators of the process did not exhibit the same pattern on the content of polyphenols. The influence of the time of extraction on the polyphenols content is bigger than the influence of the alcoholic concentration (Fig. 1) (B). Nevertheless, the influence of these parameters is not significant.

The application of this design allowed us to study the main and possible interaction effects between the extraction time and alcoholic content on polyphenols content. Table 2 shows the polyphenols content yield results according to the factorial design. Maximum polyphenols content (29.4 %) was recorded under the following experimental condition: extraction time 120 min, solid liquid ratio 1:20 and alcoholic concentration of 50 % (v/v). By applying multiple regression analysis on the experimental data, the variables were related by the following linear equation:

$$\% \text{ polyphenols content} = 22.259 + 0{,}035\ X_1 + 0.008\ X_2$$

where X_1 extraction time (min) and X_2 alcoholic concentration (v/v).



A: pareto chart; B: main effects chart.

Fig. 1. Results of the experimental design 3^2.

Table 2. Results of the experimental design for polyphenols content

Experiments	Extraction time (min)	Alcoholic concentration (%)	Polyphenols content (%)
1	30	50	24,6
2	75	70	24,7
3	120	50	29,4
4	30	30	21,5
5	75	50	27,5
6	75	30	26,0
7	30	70	24,0
8	120	30	25,3
9	120	70	25,0
10	75	50	28,1
11	75	50	27,0

A transparent yellow liquid with fragrant characteristic was obtained to the hydroalcoholic extract. The dry matter content and pH of the extract was 8 ± 0.5 % and 5 ± 0.5**,** respectively. Maximum polyphenolic content according to the experimental design was recorded under the following experimental condition: Extraction time 120 min and 50 % of alcoholic concentration.

Figure 2 show the concentration-time profile of polyphenolic during their ultrasound--assisted extraction at 28 KHz, 30 ± 2 ºC, 150 W of actual power and 50 % of alcoholic concentration. It should be pointed out that the polyphenolic content increases in the time until the 60 min. After this time the extracted concentration is constant.

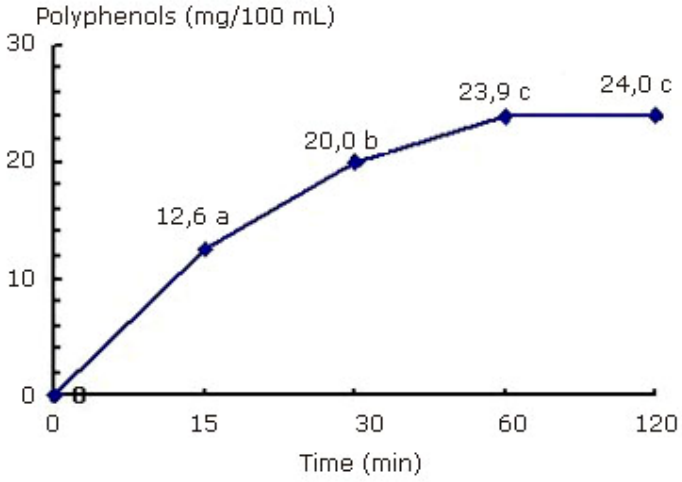


Fig. 2. Extraction process in the time (solid liquid ratio: 1:20 and 30 % alcoholic concentration). Similar letter no significant for $p<0,05$.

ANOVA was used to evaluate the significance difference among results at different times of extraction. The evaluation showed significant differences among the results at different times ($p= 0.0000$). Duncan multiple comparisons test showed significant differences among the mean values to 15 and 30 min, but there are not significant differences among the mean values at superior times (Fig. 2).

DISCUSSION

Extraction of bioactive compounds from vegetable materials is a classical operation applied in many industrial processes. Few studies have made references to processes to obtain extracts from *P. granatum.* This fruit is generally consumed in form of natural or fermented juices.[8-10,15]

In Cuba, numerous studies have shown antiviral property, as well as sequestrator agent of reactive species caused by hydrogen peroxide, a mechanism that allows it to protect the DNA against the lesions provoked by this agent.[5-7] In all these studies, the valued hydroalcoholic extracts were elaborated by maceration process by 15 days according to *Iglesia*.[16]

Ultrasonic assisted extraction has been proven to significantly decreased extraction time and increased extraction yields in many vegetable material.[12] However, few works report the use of this methodology in the extraction processes from *P. granatum*.[13,14]

The results show that the extraction conditions don't have a significant effect on the independent variables. The polyphenols content were also similar to those reported by *Rodríguez et al.*[11]

On the other hand; the alcoholic concentration did not have significant effects on the content of polyphenols obtained. This can be due to the poor solubility of the polyphenols in ethanol.

The pomegranate is a source of phenolic compounds as punicalagin (high molecular weight polyphenol and poor solubility in ethanol); ellagic acid derivatives such as ellagic acid glucoside and ellagic acid (both with low solubility in water); hydrolysable tannic and other.[17,18] Total punicalagins (the sum of punicalagins A + B and punicalin) and ellagic acid content in the pomegranate present the main pharmacological activities attributed to this species.[8-10,19-21] Due to this, the ethanolic extract preparation reported in the literature was elaborated in alcoholic concentration of 50 % (v/v) by maceration process.[11,16,17,19]

The extraction study in the time showed higher values of polyphenolic content starting from 60 min. After this time the values of polyphenolic content were constant. This result is important because the maceration method used in the industry requires 72 h.[11]

In conclusion, the method here reported describes a fast ultrasonic extraction of polyphenolic compounds from *P. granatum* compared with maceration method. According to our results, the optimal extraction conditions of polyphenols were determined as follows: extraction time 60 min, solid liquid ratio 1:20 and alcoholic concentration of 50 % (v/v). The extracts are similar to the extracts obtained by *Peña at al*.[16] and *Rodríguez et al.*[11] elaborated by maceration process.

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