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ScienceDirect
Physics Procedia 3 (2010) 169–178
Physics Procedia
www.elsevier.com/locate/procedia

International Congress on Ultrasonics, Universidad de Santiago de Chile, January 2009

Ultrasonically assisted Extraction of bioactive principles from Quillaja Saponaria Molina

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Abstract

A study of ultrasonically assisted extraction of bioactive principles from Quillaja Saponaria Molina (Quillay) is presented. To address the problem it was studied the effects that could influence the extraction process through a two-level Factorial Design. The effects considered in the Experimental Design were: Granulometry, Extraction time, Acoustic Power and Acoustic Impedance.
The production of the quillaja extracts is done with an aqueous extraction and the process is assisted by an ultrasonic field; no other solvents are used in its production. The final product only incorporates natural ingredients and raw materials, authorized for their use in food manufacturing processes.
The principal factors affecting the ultrasonic extraction process were: Granulometry and Extraction time. The enhanced of ultrasonic assisted extraction ratio was measuring the increasing yield of extracted components, the extraction ratio was increased by ultrasonic effect and a reduction in extraction time was verified. In addition the process can be carried out at temperatures lower than the traditional way.
The influence of ultrasound on the quality of bioactive principles was examined by HPLC technique and no influence of ultrasound on natural components was found.

Keywords: bioactive principles, high power ultrasound, process.

1. Introduction

1.1. Quillaja Saponaria Molina trees

Quillaja Saponaria trees grow naturally and extensively in Chile. Quillaja extracts contain mainly saponins but also polyphenols, tannins, salts and sugars in small quantities. Quillaja saponins consist on a triterpenic nucleus with two sugar chains. These sugar chains give saponins a hydrophilic property and the triterpenic nucleus is

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doi:10.1016/j.phpro.2010.01.024

hydrophobic. For these, it is an anphoter molecule. This characteristic gives saponins the condition of a non-ionic tensoactive, allowing the reduction of surface tension and the solubilization of hydrophobic products in aqueous solutions or the formation of microemulsions, all these properties obtained at very low product concentrations.

Saponins are natural foaming agents and emulsifiers widely used as: foaming agents in food, beverages and beer; surfactant in cosmetics, mining and photographic industry; additive in agriculture, animal feed and waste treatment; diary products low in cholesterol; adjuvant in animal vaccines [1].

The production of the quillaja extracts is done with an aqueous extraction; no other solvents are used in its production. The final product, either a crude or purified extract, in liquid or powder formulation only incorporates natural ingredients and raw materials, authorized for their use in food manufacturing processes.

1.2. Ultrasound Assisted Extraction

The employment of the ultrasound to assist extraction of bioactive principles from plants materials has been considered in numerous studies. Many reports on the ultrasound extraction have been published since 1950, decade in which it was started exploring, with great force, the employment of the ultrasounds in diverse processes. Nowadays is accepted that the ultrasounds can stimulate diverse processes of extraction. These processes go from the extraction of medicinal tinctures [2-6] to the industry of the biodiesel [7].

The extraction enhancement by ultrasound process has been attributed to the propagation of high level pressure waves producing cavitation phenomenon. The extraction mechanism involves two types of physical phenomena: Diffusion through the cell walls and washing out the cell contents once the walls are broken. Both phenomena are significantly affected by ultrasonic radiation. The Ultrasonically induced cavitation was shown to increase the permeability of the plants tissues and increase the mass transfer. In addition, the ultrasound improves the extraction of those compounds sensitive to heat due to the use of lower temperatures [8-12].

The objective of this work was to develop the engineer basis for ultrasonic assisted extraction of saponins from quillaja saponaria molina establishing an ultrasonic extraction protocol allowing the obtention of total saponin present in the treated material. For this it was necessary to study the factors that can influence the bioactive plants materials extraction. This task is more easily done by means of a two-levels Factorial Design.

2. Experimental

2.1. Material and methods

The device used for ultrasonically assisted extraction is an experimental system that consist of an ultrasonic probe operating at 20 kHz. The piezoelectric acoustic stepped horn was made of stainless steel**.** For this research, a special ultrasonic treatment chamber was designed and constructed. The chamber was build using a borosilicate cylindrical tube. One of cylinder ends was closed by a stainless steel reflector fitted with the chamber by a Politetrafluoroethylene (PTFE) ring. An electro acoustic network (EA coupling) was used to fit the amplifier with the transducer in the other end of the chamber it is placed the ultrasonic transducer fitted with two PTFE rings.

The experimental set up allows the feed current measurement in the transducer (it correlates quite well with the transducer tip displacement). Both, the treatment chamber and the transducer were mounted in alignment systems to keep parallel the transducer radiant face and the back reflector as it is shown in figure 1. It is fundamental to use agitation allowing that all the chips face the cavitation field improving the rate off mass transfer.

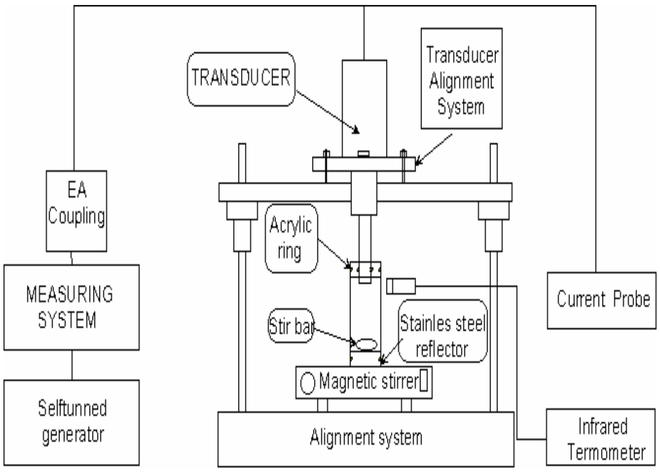

Fig 1. Experimental setup for ultrasonic extraction in aqueous phase.

2.1.1. Study Effects

The most important aspect of this ultrasound assisted extraction study is to establish the influence parameters of the process. To characterize the extraction process it was decided the study of the factors that can inside in the response of the systems (transducer-chamber-solution). The studied variables are shown in the following box.

The variables of the problem were	The experiment outputs were
System Impedance Acoustic Power Concentration Treatment Time Granulometry.	Extract Saponin Efficiency Extract Solids Efficiency.

A precise analysis technique of reverse phase HPLC has been considered to determine saponins and solids concentration [13]. Special parameter like Purity was used to evaluate the efficiency of the process

In the first stage of the investigation there was foreseen the accomplishment of a series of preliminary tests to establish the most suitable experimental levels to asses the potentials of the ultrasound extraction process of bioactive principles from Quillay. In table 1 the total experimental matrix with the tested values of the different variables it is shown.

Table 1. Experimental matrix to Extraction Process

Parameter	Levels				
Acoustic Power (W)	20				
Treatment Time (min)	10	15	20	25	30
Concentration (liq/sol)	1:6	1:10	1:15		
Granulometry (mm)	0.2	0.32	6	10	

2.1.2. Factorial Design

Once the results from table 1 were evaluated it was decided to study the influence of the most important effects. The experiments were planned using Factorial Design to assess the influence of the different parameters in the process with a 95% confidence interval. An analysis of variance provides a significance test for the main effect of each variable in the design. To validate the model a normal probability residues graph of the effects was done [14-15]. The statistical analysis was done using a statistical commercial program.

The extraction temperature keeps constant at 20º C, during all the experiences. The experimental matrix is shown in table 2.

Table 2. Experimental matrix to Extraction Process studied with Factorial Design

Parameter	Levels					
Acoustic Power (W)	3	5	20	30	50	75
Treatment Time (min)	1	5				
Impedance (Ω)	80	800				
Granulometry (mm)	6	10				

2.1.3. System Impedance

To characterize System Impedance factor it is necessary to study the ultrasonic system "*transducer - camera - solution*". The system allows the variation of water chamber length producing different impedances measured in transducer terminals [16]. The impedance values of the system present strong differences according the chamber length, for instance: a length of 70 mm (approximately λ), the impedance value measured is 800 Ω, for a chamber length of 85 mm (about $5\lambda/4$) the impedance value reach a minimum of about 80 Ω. In the figure 2 a curve representing the different values of impedances for different chamber length is shown.

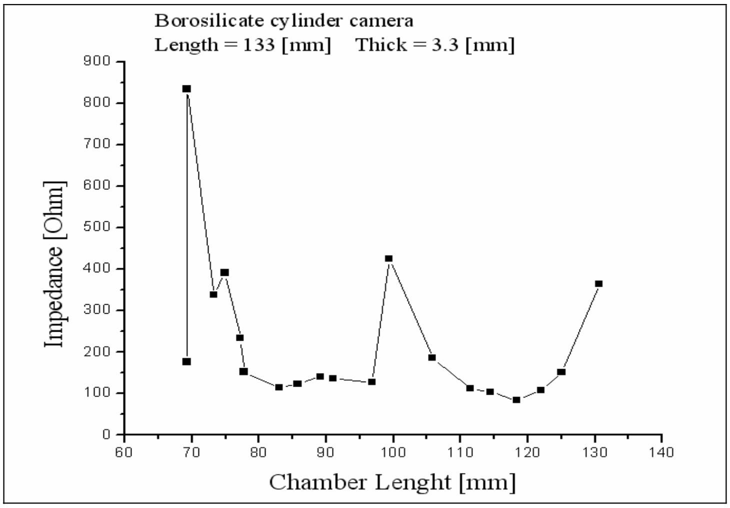

Fig.2.Curve of acoustic impedance vs. water column height.

3. Results and discussion

Vegetal tissue consist of cell surrounded by walls (figure 3). Ultrasound extraction process improves the contact solid-liquid due to the acoustic cavitation wave's phenomenon. The vegetal cell structure bombed by supersonic jets from bubble implosions this micro cavitation bubbles open spaces (capillaries) improving cell hydration. The cavitation bubbles implode near to the solid-liquid interface, the fluid jet produced hits the cell surface generating macro turbulence , particles collisions and distortions in the biomass pores, causing damage to the cell wall and subsequently his rupture. All this improve the diffusion and therefore the extraction process.

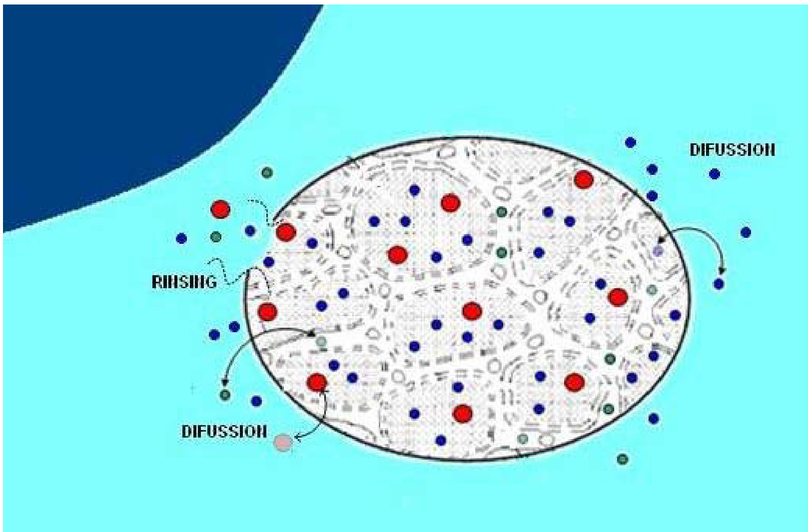

Fig. 3. "Schematic diagram of a vegetal cell ultrasonic extraction"

With the experimental data obtained from table 1 the influence of extraction process of Treatment time, Granulometry and Concentration were established. The extraction temperature was 20º C, during all the experiences.

3.1.1. Treatment time

The extractions yields increased significantly with the sonication period from 10 to 30 minutes. The yields of 20 minutes at 20º C in the ultrasound-assisted extraction were comparable to those achieved by the conventional method (batch) 3 hours extraction at 60º C. In figures 4 and 5 the amount of useful substances extracted vs. different treatment time it is shown, the time dependence is clear.

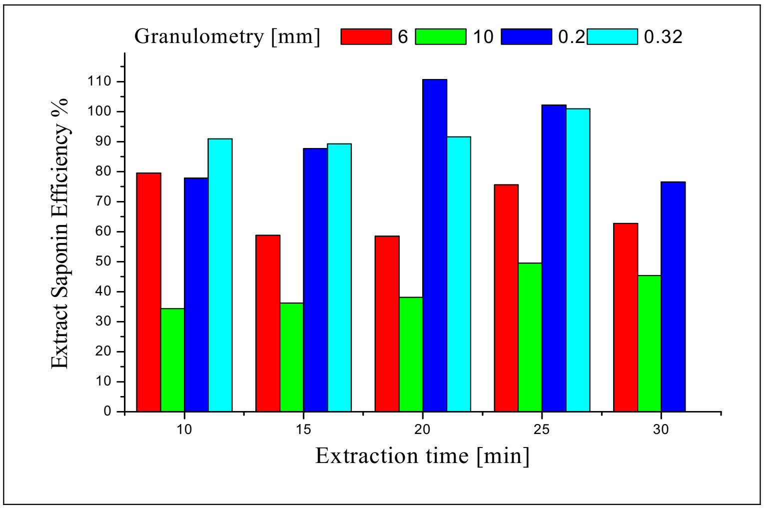

Fig. 4. Extract saponin efficiency v/s Extraction time

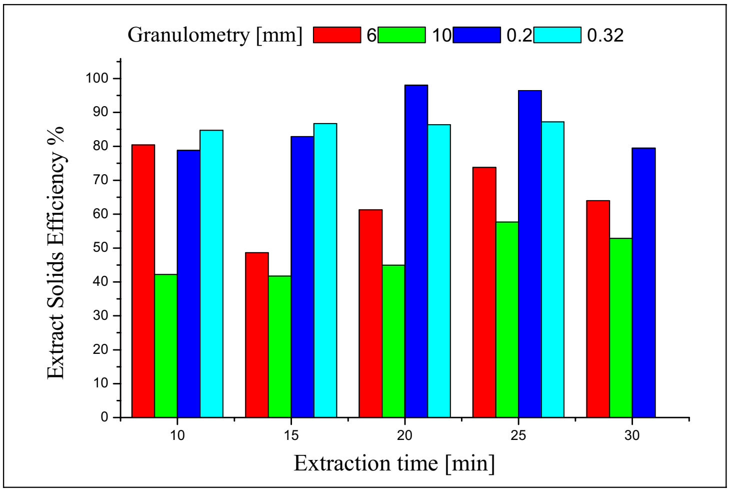

Fig. 5. Extract solid efficiency v/s Extraction time.

3.1.2. Concentration

To major liquid/solid concentration (relation 15/1) increases the percentage of Saponins and Solid presents in the extracted solution. This could be due less chips concentration favored cavitation phenomenon. The figures 6 and 7 shown the result of the concentration study at all time levels when the acoustic power were fixed at 20[W] at 20º C.

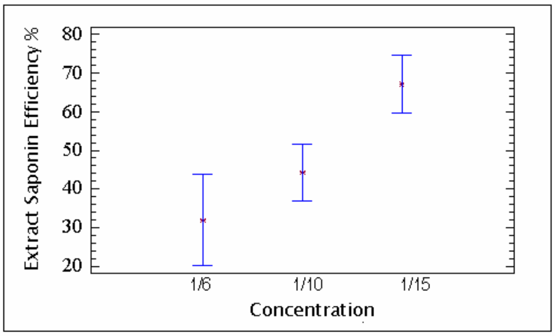

Fig. 6. Extract saponin efficiency v/s concentration

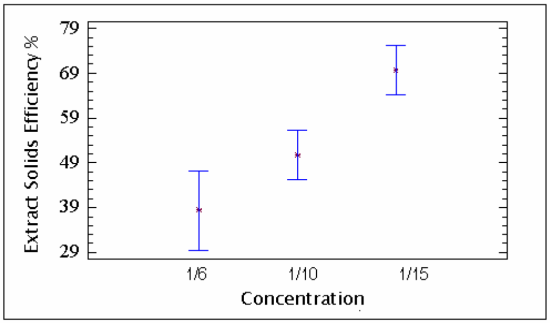

Fig. 7. Extract solids efficiency v/s Concentration

3.1.3. Granulometry

The extraction increases for smaller grains, in general due to the fact that the exposed chip surface is larger for smaller particles. This effect turns out to be of great interest, because suggest the existence of an optimum grain size to obtain the greater efficiency considering the energy comsumption in the grinding and in the extraction. The influence of the grain size in the two responses studied it is shown in figures 8 and 9. The statistical program used for this analysis it gives a confidence interval of 95%.

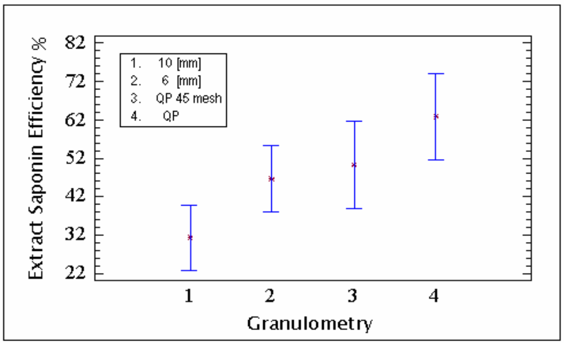

Fig. 8. Extract saponin efficiency v/s Granulometry.

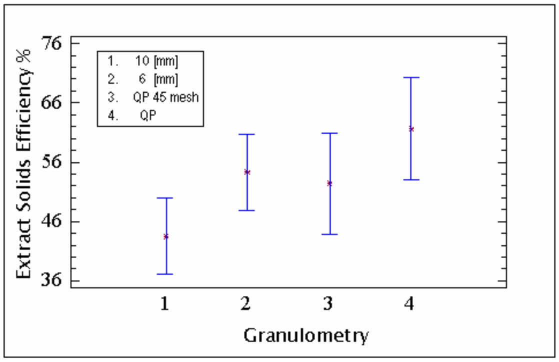

Fig. 9. Extract solids efficiency v/s Granulometry.

3.2. Factorial Design study

3.2.1. Impedance

The Impedance effect in its pressure and speed field (maximum and minimal impedance respectively), didn't show statistical significant effects. Nevertheless, it was expected that in the maximum impedance regimen, the ultrasonic field chips exposure increase, due to the chip spreading effect, therefore the ultrasonic radiation would enter to zones not visited in the normal process promoting the cavitation phenomenon and the extractive process. In the figures 10 and 11 chip disposition photography in both regimes it is shown.

Fig.10**.** Minimum Impedance chip agglomeration.

Fig.11. Maximum Impedance chip disgregation.

3.2.2. Acoustic Power

The Power applied in the studied levels does not offer a statistically significant change in the experimental region between 30 and 70 [W], this is shown in figures 12 and 13. The study of the influence of this factor was done with a statistical program with a confidence interval of 95%.

The transition from a non-cavitating to a cavitating regimen was done. In figure 10 and 11 was established that when the cavitation is not present not improvements in the extraction process was detected, this results are in agreement with those obtained by Vinatoru et all [10,11,12].

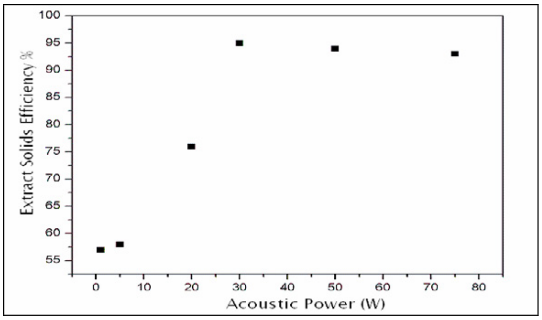

Fig.12. Extract solids efficiency v/s Acoustic Power.

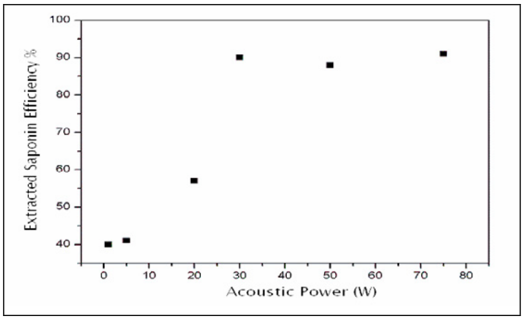

Fig.13. Extract saponin efficiency v/s Acoustic Power

4. Conclusions

Ultrasound can enhance the extraction process of bioactive principles from Quillaja Saponaria Molina.

The statistically significant variables that influence the Ultrasonic Extraction Process are: *Granulometry*, *Concentration and Treatment Time*. In the experiences realized, there wasn't improvements detected in the Extraction Process from current major to 400 [mA] in transducer terminals. For the experimental conditions used in this research this means that displacements of the radiant face transducer greater than 9 [μm] does not produce any significant effects.

The cavitation phenomena it is the responsible of enhancement in the extraction process, this can suggest that the supersonic jets from bubble implosions open spaces (capillaries) improving cell hydration. Also, it's well known that the saponins possesses a hydrophobic character, this can suggest, that the bubbles created during the cavitation phenomena actuate as collectors agents saponins.

The statistical significance of the granulometry effect reflects a relation between the chip size and the acoustic field defined in the reactor. It is suggested to study the efficiency of the process depending on the chip size and morphology especially to explore other wavelengths.

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