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Optimization of Alternative Techniques for the Extraction of Natural Pigments from Beetroot Waste †

María V. Schultheis [1,2,*], Candela Acuña [1], Vanina Guntero [1,2] and Cristian Ferretti [2]

[1] Group of Natural Products and Materials (ProNaM), Universidad Tecnológica Nacional (UTN)-Facultad Regional San Francisco 2400, Argentina; candelaacu@facultad.sanfrancisco.utn.edu.ar (C.A.); vguntero@facultad.sanfrancisco.utn.edu.ar (V.G.)

[2] Group of Organic Synthesis and Materials (GSOM), Laboratorio Fester-Quimica Orgánica(FIQ), Instituto de Química Aplicada del Litoral (IQAL) (UNL-CONICET), Universidad Nacional del Litoral, Santa Fe 3000, Argentina; cferretti@fiq.unl.edu.ar

* Correspondence: mschultheis@facultad.sanfrancisco.utn.edu.ar; Tel.: +54-9-3564-500428

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Abstract

The increasing demand for natural compounds with functional properties in the food, pharmaceutical, and cosmetic industries highlights the importance of sustainable sourcing. Beetroot, rich in betalains, with significant antioxidant potential, is a prime candidate for valorization, especially considering the considerable waste generated during its processing. Recovering these discarded materials not only reduces waste but also provides a valuable source of bioactive substances that can add significant value to various industries. This study focuses on optimizing ultrasound-assisted aqueous extraction (UAE), a versatile and environmentally friendly technology, for recovering these pigments. UAE offers several advantages, including reduced solvent usage, operational simplicity, and the ability to preserve the biological activity of extracted compounds, making it suitable for industrial implementation. Our experimental approach utilized a full factorial design to evaluate the influence of key variables: solvent type (water, ethanol, and mixture of equals parts ethanol and water), extraction time (20, 30, and 40 minutes), and temperature (30, 40 and 50 °C). Following extraction, a sequential process of filtration, evaporation, and oven drying was performed to separate and purify the bioactive compounds. Results consistently showed extraction yields ranging between 7% and 14% across all samples. These findings underscore the potential of UAE as an efficient method for extracting valuable natural pigments from beetroot waste, contributing to a more sustainable agri-food chain and creating new avenues for high-value product development.

Keywords: ultrasound-assisted extraction; betalains; sustainable valorization; natural pigments

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1. Introduction

Globally, the issue of food waste represents a major challenge in both environmental and economic terms. Due to overproduction and deficiencies in storage, it is estimated that approximately one-third of the food produced for human consumption is not utilized, which corresponds to 1.2 billion kilograms throughout the supply chain [1]. This phenomenon results in the loss of nutrient-rich products such as fruits and vegetables,

whose annual production exceeds 1.5 billion tons [2]. These billions of tons of discarded food waste generated each year have detrimental consequences for the environment, including the emission of greenhouse gases [3].

In order to reduce environmental impact and add value to agro-industrial by-products, the recovery of bioactive compounds present in fruits and vegetables has been proposed, since, as previously mentioned, the disposal of these foods accounts for a large proportion of waste. Bioactive compounds, such as pigments and phenolic compounds, exhibit antioxidant, antibacterial, and anti-inflammatory properties [4]. For this reason, natural pigments play an important role in the production of food, cosmetic, and pharmaceutical materials, offering a natural and non-toxic alternative that is safer than synthetic colorants [5].

Beetroot is an agro-food crop of significant relevance cultivated in various countries, with a global production of 270 million tons per year, which reflects its importance within the agro-industrial chain [6], from which a considerable percentage of waste is inevitably generated. Beetroot is considered one of the most important vegetables in terms of health benefits [7], and it has gained increasing interest as a functional food due to the presence of betalains, which are responsible for its characteristic red coloration and are also attributed with a relevant antioxidant potential [8]. Betalains are hydrophilic pigments composed of two main groups: betacyanins, which exhibit red-violet hues, and betaxanthins, which present yellow-orange tones; both share betalamic acid as their core unit [9]. The extraction of bioactive compounds from underutilized plant resources represents a promising strategy to enhance the sustainability of the agro-food chain [10]. Within this framework, the extraction of these biologically active compounds is being investigated through the application of ultrasound technology.

Ultrasound-assisted extraction in aqueous medium has been established as an emerging technique of remarkable efficiency for the recovery of bioactive compounds from plant matrices. Its innovative nature, together with the ability to intensify processes through acoustic cavitation, positions it as a sustainable alternative to conventional methods, particularly in the context of the integral utilization of natural resources. However, it is a complex process in which numerous variables are involved [11]. Ultrasound waves cause the mechanical disruption of the cell wall, releasing bioactive components, while the localized heating of the solvent enhances extract diffusion, thereby improving mass transfer across the solid–liquid interface [10]. In this study, an evaluation of the operating conditions was carried out to obtain extracts from beetroot by-products using ultrasound-assisted extraction, with the aim of optimizing such conditions.

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2. Materials and Methods

2.1. Materials

The residues studied consisted of beetroot discarded by markets in the city of San Francisco, Córdoba, Argentina, for failing to meet the optimal conditions for commercialization. Throughout this study, the starting beetroot material will be referred to as biomass. Additionally, water (W), ethanol (E), and a water–ethanol mixture (W–E) were used as solvents for the extraction.

2.2. Biomass Characterization

The beetroots were characterized both physically and chemically by analyzing their morphology, weight, diameter, moisture content, and protein content. For the physical

characterization, six units were randomly selected, and each measurement was performed in triplicate to calculate the mean and standard deviation.

Moisture content was determined by drying the samples in an oven at 100–105 °C until constant weight was achieved, recording the sample weight before and after drying.

For protein content analysis, the Kjeldahl method was employed. In this procedure, the sample is subjected to digestion with sulfuric acid, followed by neutralization with an alkaline solution and distillation. The captured ammonia is titrated with a standard acid, and the nitrogen content in the sample is calculated from the volume used in the titration. Finally, the crude protein percentage is determined according to Equation (1).

$$P(\%) = \%N \times 6{,}25 \quad (1)$$

2.3. Experimental Design

To determine the optimal conditions that maximize the yield of beetroot extract obtainable by the ultrasound method, a full factorial design (3^3) was conducted to study the influence of temperature, time, and ethanol–water mixture in different proportions, as well as the interactions between these factors, on the obtained yield, while keeping the biomass-to-solvent ratio constant (1:10). The levels were selected considering that the bioactive compounds of interest can degrade at high temperatures and extended extraction times, and taking into account the use of polar and non-toxic solvents.

Accordingly, the following levels were established: pure water (0% E), water–ethanol mixture (50% E), and pure ethanol (100% E); temperatures of 30, 40, and 50 °C; and extraction times of 20, 30, and 40 minutes. These factors are critical for determining process efficiency and stability, as they are the variables that most significantly influence the response.

The full factorial design was performed to ensure all possible combinations were evaluated, and the results are presented in Table 2.

2.4. Ultrasound-Assisted Extraccion Method

This procedure was carried out in an ultrasonic bath, as shown in Figure 1, under the different operating conditions established according to the experimental design described above. Briefly, a certain amount of crushed beetroot was placed in a beaker with a measured volume of solvent and subjected to the ultrasound equipment. The resulting extract was then filtered, and the solvent was removed using a rotary evaporator.

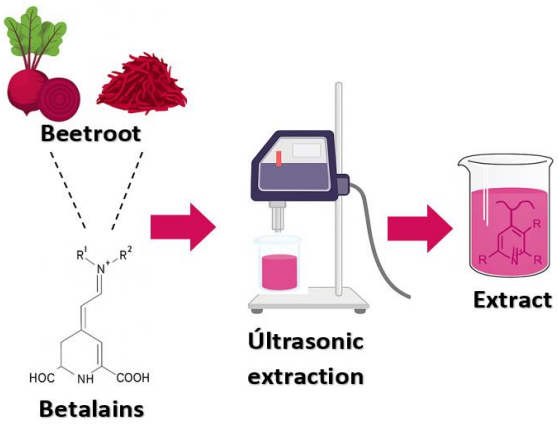


Figure 1. Process extraction ultrasonic.

The composition and functional properties of the obtained extracts are strongly influenced by the extraction parameters applied during the process. Extraction conditions—

namely temperature, extraction time, and solvent polarity—play a crucial role in defining the chemical profile of the obtained extracts. Elevated temperatures and prolonged extraction times generally enhance yield but may also lead to the degradation of thermolabile compounds such as phenolic acids and betalains. Similarly, solvent polarity determines the selectivity of the process: polar solvents, such as water or ethanol–water mixtures, facilitate the recovery of hydrophilic constituents, whereas less polar solvents favor the extraction of lipophilic molecules. In this study, these parameters were carefully optimized to preserve both the chemical stability and the antioxidant potential of the extracts (Cacace & Mazza, 2003; Kujala et al., 2000; López et al., 2021). These findings highlight the importance of selecting appropriate extraction conditions to maximize the recovery of bioactive compounds while maintaining their structural integrity and functional activity.

2.5. Yield Calculation

The yield (R) was expressed as a percentage and calculated based on the mass of the dry extract (m) and the mass of the initial beetroot (mi).

$$R\ (\%) = \frac{m(g)}{m_i\ (g)} \times 100\ \% \quad (2)$$

3. Results

3.1. Biomass Characterization

Beetroot is a root vegetable, and depending on its variety, its shape may be rounded, elongated-globular, conical, or cylindrical. Both diameter and weight can vary, with Dt representing the transverse diameter and Dl the longitudinal diameter. The results are presented in Table 1.

Table 1. Physical characterization.

Samples	Weight (g)	D_t (mm)	D_l (mm)
R1	170.5 ± 0.01	70.36 ± 0.01	74 ± 0.01
R2	95.7 ± 0.01	59.18 ± 0.01	59.3 ± 0.01
R3	92.8 ± 0.01	53.48 ± 0.01	58.76 ± 0.01
R4	145.5 ± 0.01	64.32 ± 0.01	60.46 ± 0.01
R5	169.3 ± 0.01	65.72 ± 0.01	74.62 ± 0.01
R6	77.8 ± 0.01	46.38 ± 0.01	60 ± 0.01

Regarding moisture content and crude protein, the results were 88.22% and 1.92%, respectively.

3.2. Analysis of the Experimental Design

The experimental design included 27 possible combinations, and the yield obtained for each is shown in Table 2.

Table 2. Experimental design and obtained yields.

Assay	T (°C)	Time (min)	Svte.	R (%)
U1	30	20	0	10.10
U2	30	20	100	10.52
U3	30	20	50	9.85
U4	30	30	0	10.61
U5	30	30	100	9.97
U6	30	30	50	12.82
U7	30	40	0	11.54

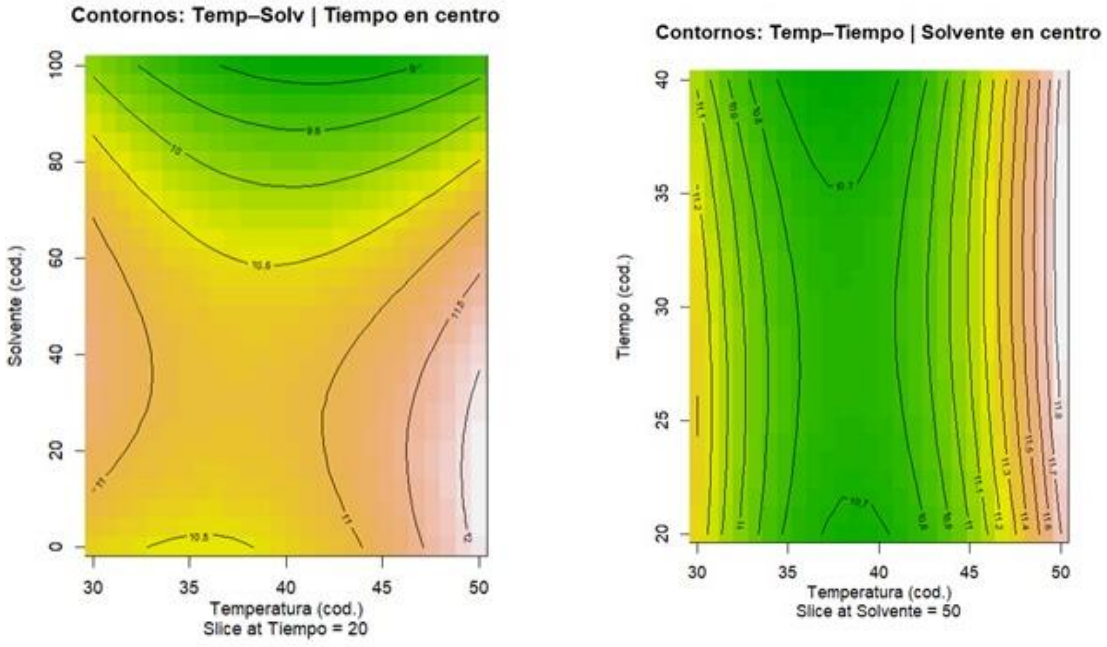
U8	30	40	100	9.06
U9	30	40	50	10.51
U10	40	20	0	11.15
U11	40	20	100	10.00
U12	40	20	50	10.02
U13	40	30	0	9.38
U14	40	30	100	7.03
U15	40	30	50	14.12
U16	40	40	0	9.67
U17	40	40	100	8.56
U18	40	40	50	10.43
U19	50	20	0	12.85
U20	50	20	100	8.90
U21	50	20	50	11.64
U22	50	30	0	12.40
U23	50	30	100	8.74
U24	50	30	50	10.68
U25	50	40	0	12.50
U26	50	40	100	11.45
U27	50	40	50	11.00

Using RStudio, it was possible to carry out an analysis of the three factors under study, that is, the effect of temperature, extraction time, and ethanolic solvent concentration on the extraction yield of bioactive compounds from beetroot was evaluated. Regarding the response variable, extraction yields ranged from 7.03 to 14.13%, with an average of 10.58%. However, some treatments showed higher yields, making it necessary to examine them in greater depth through an analysis of variance (ANOVA), as this suggested favorable experimental conditions.

The results of the analysis of variance show that the solvent is a factor that significantly influences yield, due to the fact that the compounds present different solubility in water and hydroalcoholic mixtures. Nevertheless, significant interactions among the three factors can be observed, which allows us to conclude that the optimal yield depends on the simultaneous combination of the three parameters.

Given this situation, it was necessary to apply a response surface methodology (RSM) followed by a desirability analysis to more precisely identify the optimal operating region with the aim of maximizing extraction yield without compromising the bioactive compounds.

The adjusted response surface model explained approximately 46% of the variability in yield ($R^2 = 0.456$), indicating a moderate fit. On the other hand, none of the individual terms were statistically significant, although a relevant trend was observed for the solvent variable ($p \approx 0.21$), which confirms the importance of the hydroalcoholic composition in the extraction process.



(**a**) (**b**)

Figure 2. Gráficos de contorno de RSM: (**a**) interacción entre temperatura y solvente (tiempo fijo en el centro) sobre el rendimiento de extracción.; (**b**) interacción entre temperatura y tiempo (solvente fijo en el centro) sobre el rendimiento de extracción.

In Figure 2a, the interaction between temperature and solvent can be observed, while keeping the extraction time constant at 20 min. The extraction yield shows an intermediate maximum region around 35–40 °C and at ethanol concentrations of 40–60%, conditions that enhance the release of bioactive compounds. In contrast, at low temperatures and with pure solvent (0% and 100%), the yield decreases significantly, which allows us to conclude that extreme conditions result in lower efficiency of the process.

In Figure 2b, the interaction between temperature (30–50 °C) and time (20–40 min) is analyzed, while keeping the solvent constant at an ethanol concentration of 50%. It can be observed that the extraction yield reaches a maximum in the central region, that is, at approximately 40 °C and 30 min. This behavior suggests that the extraction process is sensitive to the balance between time and temperature; in other words, short extraction times are insufficient to adequately recover all compounds, whereas high temperatures promote their degradation.

Finally, the response surface obtained in Figure 3 confirms that the condition of 100% ethanol as solvent leads to a marked decrease in yield compared to those obtained with water (0%) and mixtures (50%), demonstrating that the latter are more efficient than pure ethanol for the extraction of pigments and bioactive compounds. This three-dimensional representation corroborates the previous ANOVA and multiple comparison results, highlighting that process optimization is achieved under intermediate solvent conditions, while temperature and time exert less decisive effects within the studied range.

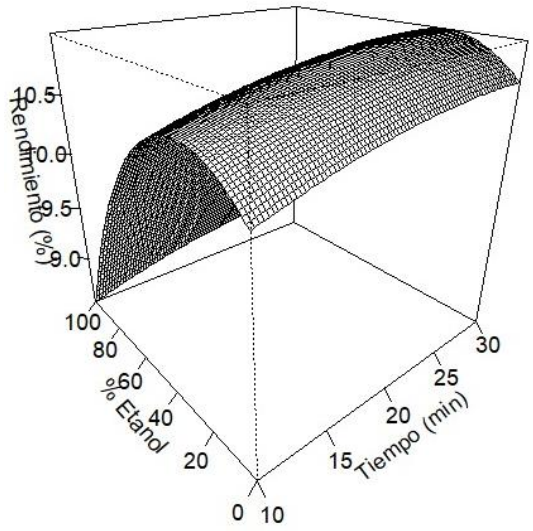


Figure 3. 3D surface of the effect of time-solvent on yield at 40 °C.

4. Conclusions

The results obtained indicate that the extraction process of betalaíns and bioactive compounds from beetroot using ultrasound technology is feasible and exhibits an optimal region with the highest yields under the following conditions: 40 °C, 30 min, and a solvent consisting of an equal mixture of ethanol and water.

This study represents a significant advancement in the optimization of the extraction process of bioactive compounds from beetroot. However, new research lines should be pursued in the future; in particular, it would be advisable to incorporate and control additional variables in the model, such as the pH of the extraction medium, as this factor influences the stability and preservation of betalaíns.

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