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Optimization of the Ultrasound-Assisted Extraction of Citric Acid from Citrus Peels †

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Abstract: Ultrasound-assisted extraction was used in the extraction of citric acid from orange, lemon and lime peels. An experimental design combining five levels of the independent variables time (2–45 min), ultrasonic power (50–500 W), and ethanol proportion (0–100%) was implemented and the extraction process was optimized by response surface methodology (RSM). The citric acid levels were monitored by ultra-fast liquid chromatography coupled to photodiode array detection. The highest citric acid contents were obtained from orange peels, followed by lime. In both cases, the process was maximized by sonicating at medium powers for short time periods, using low ethanol proportions. For lemon peels, citric acid was not detected in most runs, so it was not possible to construct a predictive model. This work provided information on the optimal conditions for the extraction of citric acid as a functional agent and contributes to the valorization of citrus peels.

Keywords: citrus peels; extraction optimization; citric acid; biowaste valorization

1. Introduction

Orange, lemon and lime are citrus fruits widely produced and consumed worldwide [1,2]. These fruits, in addition to being consumed in their fresh form, are widely used for the production of citrus juices, which generates a large amount of industrial waste, such as peels, pomace and seeds [3]. In the particular case of citrus peels, these by-products have been described as a good source of molasses, pectin, and limonene, being currently used mainly for the manufacture of cattle feed. Moreover, these by-products are also rich in nutrients and health-promoting compounds, such as amino acids, dietary fibers, organic acids, and vitamins [3–6]. Since this waste is a renewable, abundant and cheap source of biomass, it is of the industries' interest to promote its recycling and valorization [4].

Currently, there is an increasing consumer perception about the possible harmful effects of artificial food additives massively used in processed foodstuff, which has aroused an increasing demand for foods formulated with natural ingredients, making the development of healthier foods a hot topic of the food research [7]. Organic acids are compounds with preservative capacity found in natural matrices and recognized as safe in the food industry [8]. The effectiveness of citric acid as a preservative in a nutraceutical product was demonstrated by Fernandes et al. [9], which conclude that it has a strong potential to be used as a preservative in the food industry.

Therefore, in order to valorize citrus peels, this work was carried out with the objective of optimizing the ultrasound-assisted extraction (UAE) of citric acid from orange, lemon and lime peels, using the response surface methodology (RSM).

2. Material and Methods

2.1. Plant Material and Samples Preparation

Orange, lemon and lime were purchased from a local supermarket in Bragança, Portugal, and their peels were frozen and lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA), reduced to ~20 mesh particle size, and stored in the dark at −20 °C until use.

2.2. Experimental Design for Extraction Process Optimization

A central composite rotatable design (CCRD) combining five-level of the independent variables X_1 (time, 2–45 min), X_2 (ultrasonic power, 50–500 W), and X_3 (ethanol proportion, 0–100%, *v*/*v*) was implemented to optimize the extraction of citric acid from citrus peels using RSM. The Design-Expert software, Version 11 (Stat-Ease, Inc., Minneapolis, MN, USA) was used to generate the 20 experimental points of the RCCD design by entering the factor ranges in terms of alphas (α = 1.68). These designs included 8 factorial points, 6 axial or star points, and 6 replicated centre points. The 20 experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

2.3. Ultrasound-Assisted Extraction (UAE)

The UAE was performed using an ultrasonic system (Ultrasonic homogenizer, model CY-500, Optic Ivymen Systen, Barcelona, Spain) equipped with a titanium probe. Citrus peel samples (~1 g) were placed in a beaker with 50 mL of solvent (ethanol/water mixtures) and processed according to the experimental design matrix in Table 1. Extractions were performed at 20 g/L at room temperature. The mixtures were then centrifuged at 4000 *g* for 10 min and the supernatant was filtered through Whatman filter paper No. 4 to obtain extract solutions.

2.4. Chromatographic Analysis of Citric Acid

The extract solutions (~1.5 mL) were filtered through 0.22 μm syringe filter disks and analyzed by ultra-fast liquid chromatography, in a system (UPLC, Shimadzu 20A series, Kyoto, Japan) coupled to a photodiode array detector, folowing an analitical method previously described by Barros et al. [10]. Compounds were eluted with sulfuric acid (3.6 mM) in reverse phase on a C18 column (5 μm particle size, 250 × 4.6 mm; Phenomenex, Torrance, CA, USA). Citric acid was identified by comparing its retention time and UV-Vis spectrum with that of the standard (citric acid from Sigma-Aldrich, St. Louis, MO, USA) and quantified based on calibration curves obtained by plotting the peak area recorded at 215 nm against concentration. The results were expressed as g per 100 g of dry peel.

2.5. Extraction Process Optimization by Response Surface Methodology

The citric acid content was the dependent variable used in the extraction process optimization. The response surface models were fitted by means of least squares calculation using the following second-order polynomial equation:

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{\substack{i=1 \\ j>1}}^{n=1} \sum_{j=2}^{n} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{ii} X_i^2 \quad (1)$$

where Y is the dependent (response) variable to be modelled, X_i and X_j define the independent variables, b_0 is the constant coefficient, b_i is the coefficient of the linear effect, b_{ij} is the coefficient of the interaction effect, b_{ii} is the coefficient of the quadratic effect, and n is the number of variables.

Fitting procedures, coefficient estimates and statistical verification were performed using Design-Expert software. The analyses of variance (ANOVA) was used to assess the significance of the models generated and of all the terms that make up these models, as well as the lack-of-fit. The method for testing statistical significance was performed by calculating the *p*-value from the F-value, considering the existence of significance for $p < 0.05$. Only the statistically significant terms were used in models construction. Coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}) and adequate precision, were used to estimate the adequacy of the polynomial equations to the responses [11]. The lack-of-fit measures the quality of the model's fit to the experimental data. Thus, the lack-of-fit must be non-significant ($p > 0.05$) [11].

3. Results and Discussion

RMS is a statistical tool suitable for optimization of extraction processes involving one or more response variables and allows to determine the optimal processing conditions with a reduced number of experimental trials, when compared with conventional one-factor-at-a-time approaches [12]. To develop polynomial models capable of predicting the effects of the process independent variables on a given response, it is necessary to assess the accuracy of their fitting to the experimental data. In this study, the response data in Table 1 were fitted to the Equation (1) polynomial model, but not all parameters were used in the models construction since some coefficients were non-significant (*ns*) (Table 2). For lemon peels, citric acid was not detected in most of the runs, so it was not possible to construct a predictive model. The results of ANOVA and regression analyses are presented in Table 2.

Table 1. Experimental responses obtained under the extraction conditions defined by the RCCD design matrix for the citric acid content.

Run	Experimental Design Matrix			Experimental Responses		
	Time	Power	Solvent	Citric Acid Content (g/100 g dry peel)		
	min	W	% (*v*/*v*)	Orange Peel	Lime Peel	Lemon Peel
1	11(−1)	142(−1)	20(−1)	4.39	1.98	4.74
2	36(+1)	142(−1)	20(−1)	5.71	2.36	5.05
3	11(−1)	409(+1)	20(−1)	5.52	2.71	5.90
4	36(+1)	409(+1)	20(−1)	4.63	2.22	5.96
5	11(−1)	142(−1)	80(+1)	1.51	0	0
6	36(+1)	142(−1)	80(+1)	2.80	0	0
7	11(−1)	409(+1)	80(+1)	2.52	0	0
8	36(+1)	409(+1)	80(+1)	2.52	0	0
9	2(−1.68)	275(0)	50(0)	2.68	2.21	0
10	45(+1.68)	275(0)	50(0)	3.29	2.26	0
11	24(0)	51(−1.68)	50(0)	2.86	0.60	0
12	24(0)	500(+1.68)	50(0)	3.93	0	0
13	24(0)	275(0)	0(−1.68)	6.06	2.46	0
14	24(0)	275(0)	100(+1.68)	1.00	0	0
15	24(0)	275(0)	50(0)	3.20	0.82	0
16	24(0)	275(0)	50(0)	3.60	0.61	0
17	24(0)	275(0)	50(0)	3.86	0.36	0
18	24(0)	275(0)	50(0)	3.58	0.34	0
19	24(0)	275(0)	50(0)	3.69	0.32	0
20	24(0)	275(0)	50(0)	3.72	0.21	0

The parametric values represent the expected change in response per unit change in factor value when all remaining factors are held constant. The higher the parametric value, the more significant is the weight of the variable. A synergistic effect is indicated by a positive sign, while a negative sign indicates an antagonism [13]. The complexity of the extraction trends is thus illustrated by the developed models. In Table 2, the intercept corresponds to the overall average response of all the runs of the RSM design. These values are lower for lime peel and higher for the orange peel.

Table 2. Parametric values of the polynomial equations and statistical information of the model fitting procedure. Parametric superscripted 1, 2 and 3 stand for time, power and solvent, respectively.

Effect		**Orange Peel**	**Lime Peel**
Intercept	b_0	3.55 ± 0.07	0.4 ± 0.1
Linear effects	b_1	0.20 ± 0.08	*ns*
	b_2	0.19 ± 0.08	*ns*
	b_3	−1.42 ± 0.08	−0.98 ± 0.08
Quadratic effects	b_{11}	*ns*	0.61 ± 0.08
	b_{22}	*ns*	*ns*
	b_{33}	*ns*	0.26 ± 0.08
Interactive effects	b_{12}	−0.4 ± 0.1	*ns*
	b_{13}	*ns*	*ns*
	b_{23}	*ns*	*ns*
Statistics			
Model F-value		69.48	48.68
Lack of Fit		*ns*	*ns*
R^2		0.9488	0.9285
R^2_{adj}		0.9351	0.9094
Ad. Precision		29.02	21.04

R^2: coefficient of determination; R^2_{ajd}: adjusted coefficient of determination; Ad. Precision: adequate precision.

All models presented a *ns* lack of fit and an adequate precision greater than 21, which indicates that the model equations adequately describe the effects of the independent variables on the response [14]. The coefficients R^2 and R^2_{adj} were greater than 0.92 and 0.90 in both cases, respectively (Table 2), thus indicating that the response variability can be explained by the extraction variables. Both models were statistically validated and used to predict optimal processing conditions.

Certain features regarding the overall effects of the independent variables on the extraction of citric acid from orange and lime peels can be inferred from the complexity of the polynomial models. For orange peel, the extraction of organic acid was affected mostly through the negative linear effects of the ethanol proportion, which means that by increasing the ethanol proportion, decreases the amount of citric acid extracted (Figure 1A). Negative interactive effects between processing time and ultrasonic power were also noticed; thus, as illustrated in Figure 1B, the extraction yield is higher when processing at high ultrasonic powers for reduced times or at low powers for longer times, a trend that can show that the combination of both factors may not be sufficient to promote extraction (at low levels) or may lead to the degradation of citric acid (at high levels). For lime peel, the ethanol proportion also affected the extraction through negative linear effects (Table 2). Quadratic effects were observed for this citrus by-product, induced by the extraction time followed by the ethanol proportion. No significant interaction effects occurred in this extraction process.

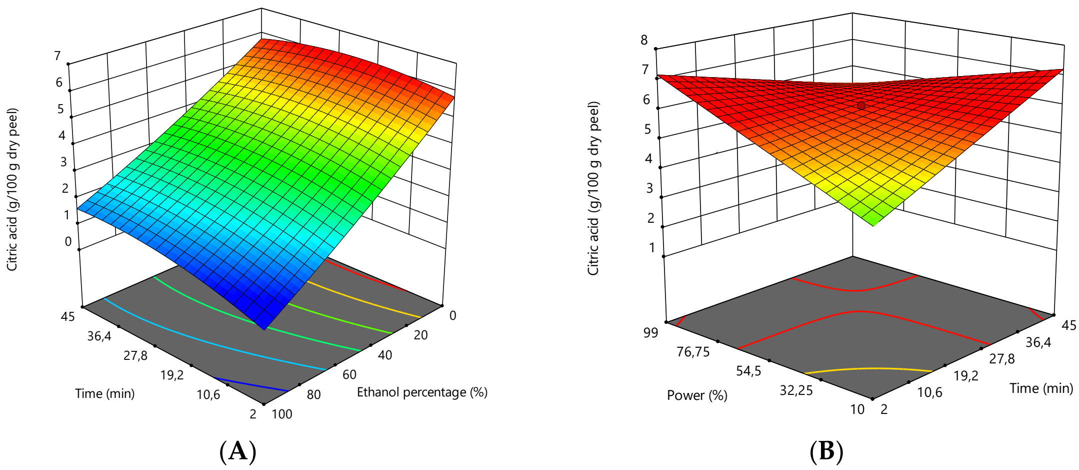

Figure 1. Response surface graph for the effects of the independent variables on the citric acid levels obtained from orange peels. The excluded variable was positioned at its optimal value (Table 2).

As presented in Table 3, the studied by-products proved to be a good source of citric acid, especially the orange peel. When applying the optimal processing conditions (medium ultrasonic power, medium-low time, and low ethanol proportion), it was possible to recover 6.2 g of citric acid per 100 g of dry orange peel and 3.4 g of citric acid per 100 g of dry lime peel. For lime peels, the extraction process was faster, but for orange peels it water was the most suitable extraction solvent.

Table 3. Optimal processing conditions that maximize the extraction of citric acid from citrus peels and predicted responses.

	Optimal HAE Conditions			Optimum
	Time (min)	**Power (W)**	**Solvent (%)**	**(g/100 g)**
Orange peel	35.5	236.2 (46.8%)	0.0	6.2 ± 0.2
Lime peel	5.8	225.9 (44.7%)	9.0	3.4 ± 0.2

There are several studies on the valorisation of citrus peels as a source of bioactive compounds, however they are studies aimed at the extraction of phenolic compounds [15]. Regarding the use of this residue for the extraction of organic acids, studies are scarce, however Montero-Calderon et al. [16], optimized the UAE extraction for ascorbic acid, obtaining at the optimal conditions (400 W, 30 min, and 50% ethanol) yields of 53.78 mg/100g of fresh mass.

4. Conclusions

More consumers try to combine food with their health, showing a preference for foodstuff free of chemical additives. Thus, it is in the interest of the industry and academy to find new sources of natural bioactive compounds and, also, to study efficient extractions for the production of bio-based ingredients, including preservatives. In this work, UAE of citric acid was optimized, combining the effects of three independent variables in order to maximize the recovery of this organic compound from three citrus by-products. Lime and especially orange peels proved to be a good source of citric acid. For lemon peels, citric acid was not detected in most of the runs of the experimental design, so it was not possible to construct a predictive model. The present work contributes to the valorization of citrus by-products through their recycling into a natural ingredient. However, a deeper analysis of these ingredients is necessary to allow the industries to implement sustainable extraction techniques and to use citric acid recovered from citrus as a food ingredient. In future studies, it will also be interesting to experimentally validate the model-predicted processing conditions.

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