processes

MDPI

Article

Optimizing the Extraction Process of Value-Added Products from Olive Cake Using Neuro-Fuzzy Models

Emilio J. Lozano, Gabriel Blázquez *, Mónica Calero, María Ángeles Martín-Lara, Salvador Pérez-Huertas * and Antonio Pérez

Department of Chemical Engineering, University of Granada, Avenida Fuente Nueva s/n, 18071 Granada, Spain; emiliolp@ugr.es (E.J.L.); mcaleroh@ugr.es (M.C.); marianml@ugr.es (M.Á.M.-L.); aperezm@ugr.es (A.P.)
* Correspondence: gblazque@ugr.es (G.B.); shuertas@ugr.es (S.P.-H.)

Abstract: The use of olive cake, an abundant residue in the olive oil industry, has been studied by developing a biorefinery scheme. The aim was to develop a novel, efficient, and environmentally friendly strategy for the valorization of olive cake, contributing to sustainable agriculture. A special extraction procedure based on a combination of hydrothermal treatments with liquid/liquid extractions was designed to produce value-added products, along with solids that can be used for energy or adsorbent production. The optimal extraction conditions were determined by exploring the influence of the operating variables (temperature, extraction time, solvent type, solvent/extract ratio, extraction stages, and pH) on the extraction yield. The decision about the optimal conditions was made by adjusting the experimental results to a neuro-fuzzy model. Glucose and inositol showed similar response surfaces, allowing simultaneous concentration in a single process. Under optimal extraction conditions, the concentration of inositol increased by up to 70%, while glucose and fructose increased by 70 and 30 times, respectively, compared to the initial feed. The proposed methodology successfully extracted significant amounts of bioactive polyols (mainly inositol) (1126 mg/L), saccharides (15,960 mg/L glucose, 385 mg/L xylose, 5550 mg/L fructose, 165 mg/L lactose, and 248 mg/L sucrose), and polyphenols (4792 mg/L) under mild conditions, i.e., 30 °C and 30 min. Thus, olive cake extracts have a great unexploited potential for application in several industrial sectors, including, but not limited to, food and pharmaceuticals.

Keywords: olive cake; extraction; sugars; inositol; polyphenols; neuro-fuzzy models

Citation: Lozano, E.J.; Blázquez, G.; Calero, M.; Martín-Lara, M.Á.; Pérez-Huertas, S.; Pérez, A. Optimizing the Extraction Process of Value-Added Products from Olive Cake Using Neuro-Fuzzy Models. *Processes* **2024**, *12*, 317. https://doi.org/10.3390/pr12020317

Academic Editor: Agnieszka Zgoła-Grześkowiak

Received: 20 December 2023
Revised: 24 January 2024
Accepted: 27 January 2024
Published: 2 February 2024

1. Introduction

Virgin olive oil is a high-quality vegetable oil extracted from the fruits of olive trees (*Olea europaea* L.). For centuries, olive oil has played an essential role not only in Mediterranean cuisine but also in its culture, due to its pleasant organoleptic properties and proven health advantages [1]. Olive oil production is indeed one of the largest agri-food industries throughout the Mediterranean basin, expanding to other countries, such as the USA and Argentina. However, this industry generates a large quantity of by-products and residues, for which integral use has not yet been considered, and its management represents a considerable challenge [2]. One of these by-products is alperujo, which is the aqueous phase resulting from olive oil extraction (≈60%). Alperujo is characterized by its high moisture content and is primarily used to obtain pomace oil. The latter process also generates a solid residue known as olive cake (≈20–25%), which is considered the major waste product of the olive oil extraction process [3]. Different approaches have been proposed to minimize the harmful environmental impacts of these by-products. For instance, olive cake can be used as an agricultural biofertilizer [4], an additive in animal feed [5], or as a source for bio-oil production [6]; however, these applications have certain limitations that have hindered their widespread success [7]. Thus, further research is required to explore novel technological approaches for profitable utilization.

On the other hand, there is an emerging trend in the use of bioactive compounds in the food, nutraceutical, and cosmetic industries, driven by their recognized health benefits [8]. These bioactive compounds are currently obtained via synthesis reactions (generally from glucose) and laboratory-scale methods. Olive cake is primarily composed of polyalcohols, sugars, and polyphenols, making it a valuable natural source of antioxidants. Therefore, the recovery and isolation of bioactive compounds from olive cake could be an interesting ecological and economical alternative to provide this by-product with commercial value. Additionally, olive cake is considered an exploitable biofuel source due to its high energy content [9]. In light of this, developing a biorefinery scheme could offer significant advantages in obtaining commercially valuable products and enhancing the efficiency and sustainability of energy production from olive cake. Industrial implementation of this process would yield both socioeconomic and technical benefits. In short, this scheme must begin with a simple, cost-effective, and environmentally friendly extraction stage, such as hydrothermal treatment, where only water is used as the extracting agent, and the temperature serves to enhance the extraction process. The resulting solid can then be subjected to more complex processes, e.g., chemical or high-pressure treatments, to obtain other products and solids with a high calorific value [10]. Furthermore, certain fermentable compounds can be obtained for the subsequent production of bioalcohols [11]. Consequently, the integral exploitation of this waste will require the design of an optimal extraction stage that allows a high-yield recovery of value-added products from olive cake and improves the energy characteristics of the resulting solid for its subsequent energy utilization.

It is worth noting that the present research group has conducted several studies exploring different hydrothermal treatments to obtain sugars and antioxidants from olive cake, reporting interesting results [10,12]. In those works, a novel and ecofriendly approach was proposed to extract glucose, xylose, polyphenols, and oligomers from olive cake and enhance the calorific value of the resulting solid residue. This approach involves a specific hydrothermal pretreatment of olive cake, followed by an autohydrolysis treatment of the resulting solid. In these works, the influence of the extraction process parameters, i.e., temperature, time, particle diameter and solid/liquid ratio in the glucose, xylose, polyphenols, and oligomers on the extraction yield was explored. To our knowledge, the extraction procedure performed in these studies has not been previously studied by other authors. Hence, this study aims to develop a special extraction procedure that combines hydrothermal treatments with liquid/liquid extractions to achieve maximum yield recovery of value-added products from olive cake within a biorefinery scheme. The hydrothermal treatment was conducted at temperatures and times lower than those used in previous studies [12], where temperatures reached up to 90 °C, and treatment times extended up to 120 min. In this way, it is intended to enhance the energetic properties of olive cake and obtain highly value-added products under mild conditions. The target products were saccharides, (i.e., glucose, fructose, xylose, lactose, and sucrose), polyphenols, and inositol, with a special focus on the latter. Inositol is an organic compound belonging to the polyol family. It is relatively scarce, but of great functional importance. For instance, inositol supplements are frequently used to treat anxiety and stress while also exhibiting beneficial effects in other disorders. Despite some authors having reported the extraction of inositol from natural sources, such as mung beans and lettuce [13,14], its potential extraction from olive cake has not been previously studied by other authors. The extraction process was optimized by identifying the most suitable solvent and exploring the influence of the operating variables (i.e., temperature, time, solvent/extract ratio, number of extraction stages, and pH) on the extraction yield. Finally, the optimal extraction conditions were determined by adjusting the experimental results to a neuro-fuzzy model, which is one of the most powerful and precise prediction tools for process modelling [15].

2. Materials and Methods

2.1. Raw Material and Work Scheme

The olive waste, i.e., olive cake, was provided by a company located in Granada (Spain). The olive cake was ground and sieved to eliminate coarse particles and impurities. The selected particle size ranged from 2 to 0.25 mm, which, according to previous findings, is the optimal diameter for effective hydrothermal extraction [12]. Prior to the hydrothermal extraction, the olive cake was dried in an oven at 80 °C for 24 h (final moisture content ≈ 0%).

To address the aim of this study, the work consisted of four phases: (i) Determination of the optimal hydrothermal conditions; a series of washings was performed on olive cake under different working conditions to maximize the extraction of the compounds of interest. (ii) Determination of the most efficient solvent; the liquid phase resulting from the optimal hydrothermal treatment was subjected to several liquid/liquid extractions to extract and enrich the compounds of interest using different solvents. The extraction efficiencies of the target compounds were assessed to determine the most efficient solvent. (iii) Extraction process variables analysis; the influence of the process variables, i.e., extraction times (t), solvent/extract ratio (r), number of extraction steps (e), and pH (p), on the extraction of polyphenols, sugars, and polyols was explored. (iv) Data curation and process optimization; the concentrations of the extracted compounds and their variations after solvent treatment were determined. A neuro-fuzzy model was developed to fit the calculated data and predict non-experienced data. Finally, the response surfaces were plotted to determine the optimal extraction conditions.

2.2. Hydrothermal Treatment (Washing)

A series of isothermal hydrothermal treatments were carried out to assess the impact of the washing conditions on the extraction of the target compounds. The extraction process parameters were defined based on prior studies [12], i.e., temperatures of 20, 25, and 30 °C; processing times ranging from 30 min to 1 h; particle diameter from 2–0.25 mm, and solid residue/water ratio of 1:3. Thus, a total of six experiments were carried out. The hydrothermal treatment was conducted in a 1 L jacketed glass reactor, equipped with double two-bladed turbine impellers, and connected to a temperature-controlled thermostatic bath. At the end of each extraction, the solid residue was recovered through filtration and subsequently washed with distilled water to determine the gravimetric yield. Aliquots of the liquid fractions were taken to determine the contents of saccharides, polyols (mainly inositol), and polyphenols extracted from the olive cake using HPLC. Figure 1 depicts a schematic of the hydrothermal extraction process for olive cake.

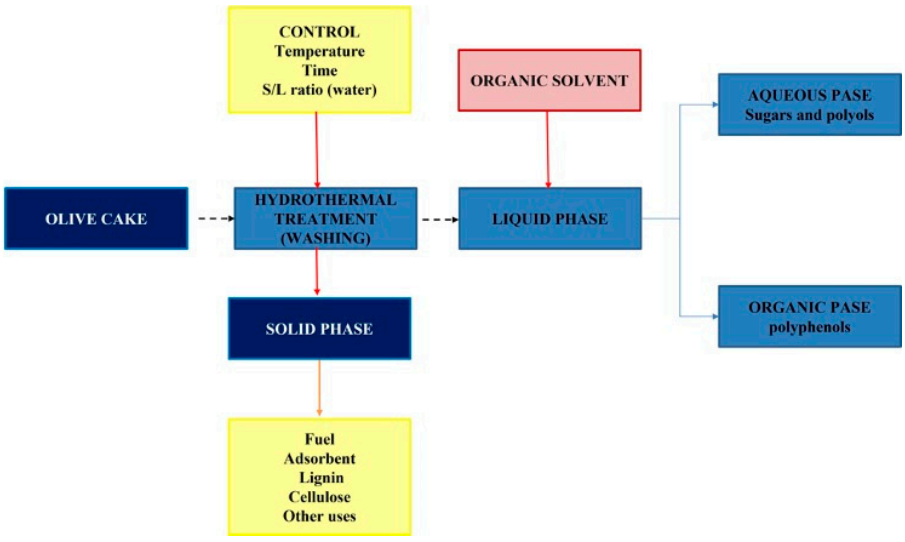


Figure 1. Schematic of the extraction process.

2.3. Liquid/Liquid Extraction

A special procedure was performed to enrich and extract the compounds of interest present in the liquid phase. This procedure was based on a simple and optimized liquid/liquid extraction separation technique. Firstly, polyphenols were separated using different organic solvents, i.e., ethyl acetate, hexane, and dichloromethane. These solvents have low polarity and are thus poorly miscible with water, which facilitates the extraction of polyphenols into an organic phase [16,17]. It is important to note that solvent selection was carried out in accordance with the Spanish Ministry of Health, Consumption and Social Welfare, the Spanish Agency for Food Safety and Nutrition (AECOSAN), and Royal Decree 1101/2011, of 22 July [18], which regulates the use of some solvents for food production. In order to determine the most suitable solvent, a series of liquid/liquid extractions was carried out with different solvent/extract ratios, i.e., 1:1, 1:2, and 1:3. Thus, each solvent was subjected to three experiments, each involving three steps. In stage I, 100 mL of solvent and 33.3 mL of liquid phase (extract) were added to the first beaker, 100 mL of solvent and 50 mL of extract were added to the second beaker, and 100 mL of solvent and 100 mL of extract were added to the third beaker. Then, the beakers were shaken for 24 h. Subsequently, the organic phase (rich in polyphenols) was separated from the aqueous phase (rich in polyols and sugars) by means of decanting process. A sample from each organic phase was collected and stored in a refrigerator for later analysis, while the aqueous phase proceeded to stage II. In stage II, the same procedure was followed using the aqueous phase from stage I. Aqueous phase I was mixed with the solvent, and after stirring, a sample from the organic phase was taken and stored in a refrigerator. The aqueous phase from stage II was subjected to subsequent experiments. In the last stage, the same procedure was repeated and samples from both the organic and aqueous phases were collected and stored in a refrigerator for later analysis. Therefore, four samples of each solvent (i.e., organic phases I, II, and III; aqueous phase) were collected to determine the most efficient solvent. This was achieved by determining the total amount of phenols present in each aqueous sample using the Folin–Ciocalteu method [19]. A low or zero phenol content in the aqueous phase (resulting from stage III) would indicate that the polyphenols have mostly moved to the organic phase, thereby confirming the effectiveness of the solvent.

Liquid/Liquid Extraction: Optimization Extraction Process

A factorial experimental design was performed based on the approach described in [20]. Briefly, the model was designed with four input variables (number of extraction steps (e), extraction time (t), solvent/extract ratio (r), and pH (p)), one response variable (compound concentration), and a central point. Each variable was assigned three levels, namely low (−1), intermediate (0), or high (1). The number of required experiments was determined using the following equation:

$$n = 2^{k-p} + 2k + n_c \quad (1)$$

where n is the number of experiments, n_c the number of central points, p is the constant for values of k, and k is the number of independent variables (if $k < 5$; $p = 0$, if $k > 5$, $p = 1$), so:

$$n = 2^{5-1} + 2 \times 5 + 1 = 27$$

Therefore, a total of 27 experiments were required to elucidate the operation and to predict the outputs. These experiments were conducted following a similar procedure to the previous extraction tests, but in this case, varying the extraction times, number of extraction stages, solvent/extract ratio, and pH (Figure 2). The latter variable is particularly important because the concentrations of polyphenols and sugars vary depending on pH [21].

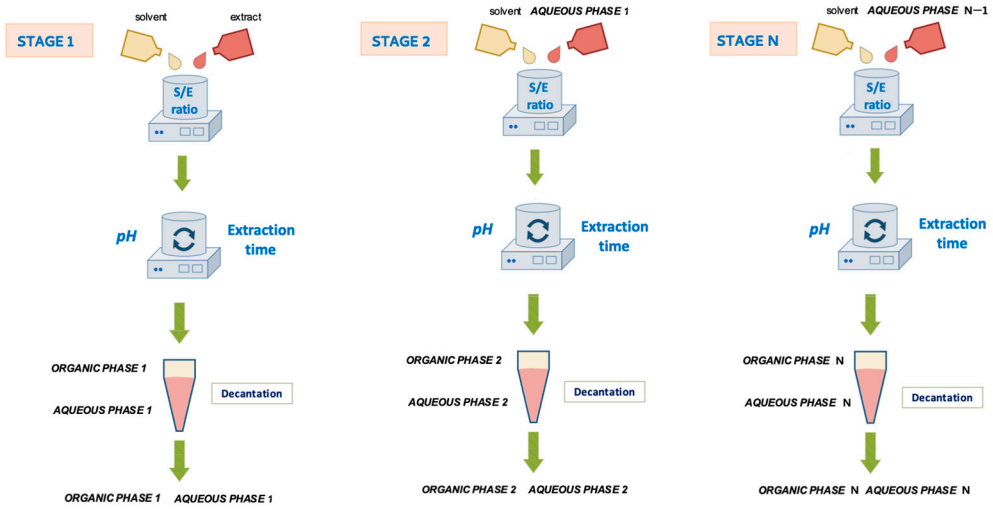


Figure 2. Schematic representation of the procedure followed for the optimization of the extraction process.

First, the organic solvent was mixed with the liquid phase (extract) according to the defined solvent/extract ratio (stage 1). The solution was then stirred for the specified extraction time, and the pH was adjusted to the defined level. Subsequently, the organic and the aqueous phases were separated by means of decanting process. In stage 2 (and subsequent stages), the aqueous phase obtained from the previous stage was mixed with the solvent and stirred under the same conditions as the previous stage. Then, the organic and liquid phases were separated by decanting. This sequence was repeated based on the predetermined number of extractions. Finally, the resulting aqueous phase was stored in the refrigerator until subsequent chromatographic analysis. Table 1 lists the operating variables assigned for the identification of the most effective extraction conditions.

Table 1. Extraction operating conditions with factors.

Time (t)	S/E Ratio (r)	No. Stages (e)	pH (p)	Code
30 min	100/100	2	3.5	−1
75 min	75/100	3	4	0
120 min	50/100	4	4.5	+1

It is important to note that these data are derived from a thorough analysis of results from several dozen experiments, as well as data curation from prior studies [10,12]. For instance, extractions conducted with a solvent/extract ratio lower than 1:2 were not suitable for the present purpose. Similarly, performing more than four extraction stages or extractions for longer than 120 min did not improve the concentration of the target compounds. Consequently, ratios ranging from 1:1 to 1:2 or extractions shorter than 120 min were further considered. The factor parameters were coded by the values −1 and +1, representing the maximum and minimum values within the defined domain, respectively. Parameter 0 represents the central value, with experiments 10, 17, and 27 being the central points. Table 2 presents the 27 experiments conducted for the designed model.

Table 2. Experimental extraction conditions designed for the mathematical model.

Exp.	Time min	S/E Ratio	Number Stages	pH	Exp.	Time min	S/E Ratio	Number Stages	pH
1	30	100/100	2	4.5	15	75	75/100	4	4
2	30	100/100	2	3.5	16	75	75/100	2	4
3	30	75/100	3	4	17 *	75	75/100	3	4
4	30	50/100	4	4.5	18	120	50/100	4	4.5
5	30	50/100	4	3.5	19	120	50/100	4	3.5
6	30	100/100	4	3.5	20	120	75/100	3	4
7	30	100/100	4	4.5	21	120	50/100	2	4.5
8	30	50/100	2	4	22	120	50/100	2	3.5
9	30	50/100	2	3.5	23	120	100/100	4	3.5
10 *	75	75/100	3	4	24	120	100/100	4	4.5
11	75	75/100	3	4.5	25	120	100/100	2	4.5
12	75	75/100	3	3.5	26	120	100/100	2	3.5
13	75	50/100	3	4	27 *	75	75/100	3	4
14	75	100/100	3	4					

* Central points.

2.4. Neuro-Fuzzy Model

A neuro-fuzzy model was used with the aim of fitting the data. This mathematical model combines the advantages of fuzzy logic systems and neural networks, offering a powerful prediction tool [22]. It is based on the following equation, with two independent variables, the use of rules (μ), a constant, and a Gaussian dependence function:

$$y_e = \frac{\sum_{l=1}^{m} y^l \cdot \left[\prod_{i=1}^{n} \mu_{F_i}^l \left(x_i, \theta_i^l\right)\right]}{\sum_{l=1}^{m} \left[\prod_{i=1}^{n} \mu_{F_i}^l \left(x_i, \theta_i^l\right)\right]} \quad (2)$$

where y_e is the estimated value of the property to be modelled; μ represents a fuzzy rule; x_i, θ_i indicate the values of time (t); solvent/extract ratio (r); number of stages (e); pH (p). A Gaussian dependence function with three levels (low, medium, and high) was used for one of the variables and a Gaussian dependence function with two levels (low and high) for the other three. Thus, with 4 variables, n was 4, and m the number of fuzzy rules. Taking this into account, the numerator and denominator would contain 24 terms, respectively. The Gaussian dependence function would be as follows:

$$\mu(\text{low}) = \exp\left(-0.5 \times \left(\frac{c - c_{\text{low}}}{L}\right)^2\right) \quad (3)$$

$$\mu(\text{medium}) = \exp\left(-0.5 \times \left(\frac{c - c_{\text{medium}}}{L}\right)^2\right) \quad (4)$$

$$\mu(\text{high}) = \exp\left(-0.5 \times \left(\frac{c - c_{\text{high}}}{L}\right)^2\right) \quad (5)$$

where c is the absolute value of the variable and L is the width of its Gaussian distribution. The parameters and constants of the above equations were estimated using the ANFIS (Adaptive Neural Fuzzy Inference System) Edit tool. Finally, the rates of increase/in of compounds concentrations were fitted to a neuro-fuzzy model and the response surfaces were prepared to better understand the influence of the variables in the extraction.

Relative Value and Relative Increase

Based on the poor correlation between the response variable (concentrations of the compounds of interest) and the operating ones (time (t), S/E ratio (r), number of stages (e) and pH (p)) found in previous works [10,12], the data obtained were adjusted before performing the fit. For this purpose, relative values and relative increments were calculated

using the concentration of each component in the extract. Relative values (Equation (6)) were calculated for compounds that were present in the extract and relative increments (Equation (7)) for the compounds not extracted in the hydrothermal treatment.

$$\text{Relative value} = \frac{(C_{ext} - C_h)}{C_h} \quad (6)$$

$$\text{Relative increase} = \frac{(C_{ext} - Cmin_{ext})}{(Cmax_{ext} - Cmin_{ext})} \quad (7)$$

where C_{ext} is the concentration of the compound present in the aqueous phase after L/L extraction, C_h is the concentration of the compound present in the extract after hydrothermal extraction, and $Cmín_{ext}$ and $Cmax_{ext}$ are the minimum and maximum concentrations of the compound after L/L extraction, respectively. The parameters and constants of Equation (2) were estimated using a Gaussian dependence function with three levels (low, medium, and high) for one independent variable and Gaussian dependence functions with two levels (low and high) for three operational variables. We tested which combination of levels provides the most similar values to the experimental ones with higher R^2. The estimation was determined using ANFIS Edit tool. It was found that the variable with three levels makes the model more effective. Outliers were discarded.

2.5. Determination of Sugars and Oligomers

Chromatographic determination was performed to quantify the sugars, i.e., glucose, xylose, fructose, lactose, sucrose, and inositol, content using an HPLC 940 professional IC Vario (Metrohm, Herisau, Switzerland) equipped with a conductivity meter as detector, and a column Metrosep Carb 2—250/4.0., under the following conditions: mobile phase composed of 100 mM NaOH and 10 mM NaAc; a flow rate of 0.500 mL/s; and an operating temperature of 30 °C. The retention times were determined using the reference chemical standard of each compound. The retention times for each target compound are given in Table 3.

Table 3. Retention times of each measured compounds.

Compound	Retention Times (min)
Inositol	5.242
Glucose	16.284
Xylose	17.183
Fructose	19.381
Lactose	27.923
Sucrose	33.357

The chromatography analyses were repeated four times for each sample, and the results were reported as arithmetic means. Any results that deviated by 5% or more from the mean were excluded.

2.6. Determination of Polyphenols: Folin–Ciocalteu Method

The Folin–Ciocalteu method measures the total phenolic compounds present in vegetable products by assessing the ability of phenols to react with oxidizing agents. It is based on the capability of phenolic compounds to react with Folin–Ciocalteu reagent at basic pH, producing a blue color that can be measured spectrophotometrically at 765 nm. The reagent contains molybdate and sodium tungstate which react with phenols to form phosphomolybdic–phosphotungstic complexes. At a basic pH, the phosphomolybdic–phosphotungstic complexes are reduced to deep blue chromogenic oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) through electron transfer. The intensity of the resulting color is directly proportional to the number of hydroxyl groups present in the molecule. The concentration of total phenols is expressed as milligrams of Gallic Acid Equivalents

(GAE) per unit weight. The following reagents were used: 98% w/v, Na_2CO_3, (Panreac, Castellar del Vallès, Spain), Folin–Ciocalteu reagent 2N (Sigma-Aldrich, St. Louis, MO, USA), ethanol, and gallic acid.

The gallic acid standard curve (see Figure S1 in the Supplementary Materials) was established by dissolving 0.5 g of gallic acid in 10 mL of ethanol and then diluting it to 100 mL with distilled water. Solutions with concentrations of 50, 100, 150, 250, 500, 1000, and 2000 mg/L of gallic acid were prepared from this solution. The total phenols content was determined using the following steps. Initially, 100 µL of the sample was mixed with 8 mL of distilled water. Subsequently, 500 µL of Folin's reagent was added, and the resulting solution stirred using a vortex. Then, 1.5 mL of carbonate solution was added, and the solution was stirred again. Finally, the solution was placed in an oven at 40 °C for 30 min. After cooling, the absorbance at 765 nm was measured.

3. Results and Discussion

3.1. Hydrothermal Treatment

Table 4 presents the concentrations of inositol and saccharides obtained from the liquid fractions of various hydrothermal treatments of the olive cake.

Table 4. Results of hydrothermal treatment of olive cake at different conditions.

Temperature °C	Time min	Inositol mg/L	Glucose mg/L	Xylose mg/L	Fructose mg/L	Sucrose mg/L
20	30	645.6	257.9	51.7	182.5	162.2
25	30	657.1	218.3	43.1	188.1	186.1
30	30	661.8	228.3	-	185.6	168.7
20	60	627.7	208.7	-	298.3	220.1
25	60	661.1	218.3	-	310.7	215.9
30	60	655.2	213.1	-	297.9	203.5

Optimal operating conditions were found to be 30 °C and 30 min, as they produced the highest inositol concentration. Furthermore, analysis of the liquid fractions revealed the presence of saccharides, such as glucose, sucrose, fructose, and to a lesser extent xylose. In any case, the extraction yield of the main component (inositol) was larger than that of all saccharides (Table 4). Therefore, the hydrothermal treatment proposed in this study proved to be more efficient and cost-effective than that in previous studies [12], where higher temperatures (50, 70, and 90 °C) and longer treatment durations (60–120 min) were needed to extract the compounds of interest (e.g., sugars and antioxidants). The liquid phase resulting from optimal hydrothermal treatment was subjected to a special procedure to assess the potential for further enrichment of these compounds (see Figure 2).

3.2. Liquid/Liquid Extraction

Table 5 shows the phenol concentrations in several aqueous phases resulting from liquid/liquid extractions with different solvents.

Table 5. Total phenol concentration in different aqueous phases. Study of the most suitable solvent.

Solvent	S/E Ratio	Absorbance	Phenol Concentration mg/L
None		0.681	4792
Ethyl acetate	100/100	0.25	1713
Ethyl acetate	100/50	0.444	3099
Ethyl acetate	100/33.3	0.468	3270
Dichloromethane	100/100	0.332	2299
Dichloromethane	100/50	0.636	4470
Dichloromethane	100/33.3	0.66	4642
Hexane	100/100	0.351	2435
Hexane	100/50	0.662	4656
Hexane	100/33.3	0.695	4892

It was found that the lowest phenol concentration, i.e., 1713 mg/L, was obtained using ethyl acetate as solvent. Consequently, ethyl acetate was the most efficient solvent for the present purpose. As mentioned above, these solvents are poorly miscible with water, which facilitates the extraction of polyphenols into the organic phase. After mixing the liquid fraction resulting from hydrothermal treatment with these solvents, two phases appeared: an organic phase (enriched in polyphenols) and an aqueous phase (enriched in inositol and saccharides). Since the phenol concentration of the native aqueous phase was 4792 mg/L (Table 5), a decrease in phenol concentration in the aqueous phase indicates the migration of polyphenols to the organic phase. Thus, the lower the phenol content, the more efficient the solvent. Regarding the S/E ratio, the phenol content tended to increase as the S/E ratio decreased. The highest phenol concentrations were found at an S/E ratio of 1:3, regardless of the solvent used. Consequently, the optimal conditions involve extraction using ethyl acetate with an S/E ratio of 1:1.

A series of liquid/liquid extractions were carried out to explore the influence of the number of extraction stages, pH, S/E ratio, and extraction time on phenol concentration. The extractions were conducted using ethyl acetate as the solvent. Table 6 shows the extraction conditions and phenol concentrations in the resulting aqueous phase.

Table 6. Additional experiments, total phenol concentrations.

Exp.	S/E Ratio	Stage	Time h	pH	Absorbance	[Phenol] mg/L
1	100/100	1	1	5.18	0.417	2906
		2	1	5.19	0.404	2813
		3	1	5.35	0.381	2649
2	100/33.3	1	1	5.37	0.42	2927
		2	1	5.54	0.422	2942
		3	1	5.73	0.473	3306
3	100/100	1	3	5.07	0.501	3506
		2	3	5.15	0.419	2920
		3	3	5.28	0.44	3070
4	100/33.3	1	3	5.3	0.425	2963
		2	3	5.48	0.443	3092

Increasing the extraction time from 1 to 3 h did not decrease the phenol concentration, regardless of the number of stages performed or the S/E ratio. At S/E ratios of 1:1 (exp. 1 and 3), the aqueous phase exhibited the highest concentration of phenols (indicating lower phenol extraction) in the first extraction stage. In contrast, at the S/E ratio of 1:3 (exp. 2 and 4), the highest phenol concentration was observed in the last extraction stage. Therefore, extractions using S/E ratios of 1:1 improve when performing two or three stages, as opposed to extractions using S/E ratios of 1:3, which worsened after the first extraction stage. The lowest concentration of phenols (2649 mg/L) was found after performing three stages of 1 h using an S/E ratio of 1. Finally, the pH increased after each extraction, regardless of the S/E ratio or extraction time. Subsequent extractions were conducted based on these findings.

3.3. Study of the Variables of the Extraction Process

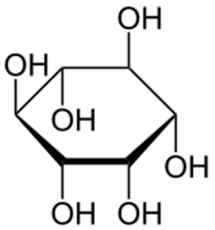
This section presents the proposed models designed to optimize the extraction process and enrich target compounds. Neuro-fuzzy models were validated using the coefficient of correlation (R^2). The response surfaces were used to determine the optimal output values (compound concentrations) and related input values (extraction time, number of extraction stages, pH, and solvent/extract ratio).

3.3.1. Inositol

Table 7 shows the results of the experiments conducted to optimize of the inositol extraction process. A total of 27 experiments were designed based on previous findings (see Table 2). The experimental data were introduced in the ANFIS Edit Tool to estimate the parameters and constants for Equation (2), which are also given in Table 7.

Table 7. Experimental results of inositol extractions, estimated ANFIS values and model constants.

Exp.	Experimental mg/L	Relative Value	Estimation ANFIS	% Error	Exp.	Experimental mg/L	Relative Value	Estimation ANFIS	% Error
1	641	−0.03	−0.03	1.14	15	719	0.09	0.09	0.92
2	561	−0.15	−0.15	0.54	16	524	−0.21	−0.21	0.29
3	823	0.24	0.25	1.81	18	698	0.05	0.06	15.01
4	916	0.38	0.38	0.54	19	671	0.01	0.01	5.64
5	618	−0.07	−0.07	0.52	20	703	0.06	0.04	31.82
6	962	0.45	0.45	0.19	21	685	0.03	0.04	16.32
7	690	0.04	0.04	1.80	22	630	−0.05	−0.05	3.31
8	794	0.20	0.20	1.08	23	754	0.14	0.14	0.02
9	823	0.24	0.24	0.02	24	644	−0.03	−0.03	2.78
11	652	−0.02	−0.02	0.24	25	700	0.06	0.06	0.37
12	588	−0.11	−0.11	0.43	26	694	0.05	0.05	0.49
13	608	−0.08	−0.08	0.23	10 * 17 * 27 *	637.49	−0.04	−0.03	8.40

Constants				Variables		Value L	Molecule
a1	0.314	a13	0.051	t (min)	30	19.1097	
a2	0.205	a14	0.512		75	19.1097	
a3	−0.132	a15	0.167		120	19.1097	
a4	0.407	a16	0.848	r	0.5	0.2298	
a5	−0.326	a17	−0.059		1	0.1261	
a6	−0.104	a18	0.063	e	2	0.7648	
a7	0.641	a19	0.022		4	0.8928	
a8	−0.055	a20	0.063	p	3.5	0.2778	
a9	−0.150	a21	0.049		4.5	0.4594	
a10	−0.366	a22	0.042	**Coefficient of determination R^2**			
a11	−0.172	a23	0.184	0.999			
a12	0.061	a24	−0.096				

* Central point.

The data from the 27 experiments were fitted to a Gaussian dependence of 3 × 2 × 2 × 2. As shown, there was a minimal difference between the experimental and ANFIS estimated values, showing a low error rate with an R^2 coefficient of 0.999. An error rate equal to or lower than 5% is considered acceptable, indicating that ANFIS can be effectively employed to provide a reliable and precise prediction model. The response surface was plotted as a function of pH, number of stages, extraction times, and solvent/extract ratios. The inositol concentration was expressed in relation to that obtained in the hydrothermal treatment, i.e., 661 mg/L (Table 4). The surface plot from the neuro-fuzzy fitting of the optimal conditions to obtain inositol is presented below (Figure 3).

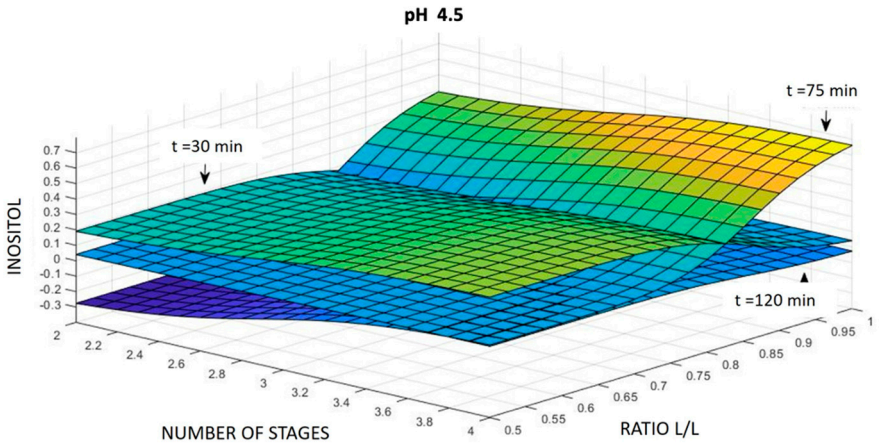


Figure 3. Surface plot of the most effective inositol extractions as a function of the studied variables.

Figure 3 displays the three-dimensional illustration of the effect of each input (number of stages, solvent/extract ratio, and the extraction time) on the output (inositol concentration) at pH 4.5 found by the neuro-fuzzy model. This shows that a higher amount of inositol was obtained (50–70%) after conducting more than three stages of 75 min with ratios exceeding 0.75. The inositol concentration also increased (20%) when using an extraction time shorter than 75 min. Under these conditions, neither the number of stages nor the ratio influenced the extraction yield, as the inositol concentration remained virtually constant (Figure 3). Additionally, extraction times longer than 75 min were not effective, as they resulted in lower inositol yields compared to those obtained in the hydrothermal treatment, i.e., 562 and 661 mg/L, respectively.

The response surface obtained at pH 4 was similar to that obtained at pH 4.5 (see Figure S2). A higher inositol concentration (1057 mg/L) was achieved by conducting four extraction stages of 75 min at a ratio of 1. Moreover, extraction for 30 min increased the inositol concentration by up to 20%, while extraction for 120 min reduced it. At pH 3.5, the most enriched inositol extract was achieved by performing extractions for 30 min, either with more than three stages and ratios higher than 0.75 (up to 962 mg/L), or with less than three stages and ratios lower than 0.5 (up to 823 mg/L) (Table 7). Performing longer extractions was not effective, as the inositol concentration remained virtually constant with respect to that obtained in the hydrothermal treatment (Figure S2).

Consequently, the optimal conditions for the extraction of inositol involved a solvent/extract ratio of 1, pH 4.5, extraction time of 75 min, and four extraction stages. Extractions under these conditions led to a significant increase in inositol concentration of up to 70%, i.e., 1126 mg/L (Figure 4). Zuluaga et al. [14] obtained inositol from different lettuce types using a microwave-assisted extraction process. The highest inositol concentration (5.42 mg/g dry sample) was obtained using a liquid–solid ratio of 100:1, performing one extraction for 30 min at 40 °C, with an ethanol–water mixture as the solvent. Ruiz-Aceituno et al. [23] developed a pressurized liquid extraction method to obtain inositol from pine nuts and reported an inositol concentration of 5.7 mg/g. Optimal conditions were as follows: 50 °C, 18 min, three cycles of 1.5 mL water each, at 10 MPa. Therefore, the extraction process developed in this study has proven to be an efficient and cost-effective strategy to obtain enriched extracts of inositol from olive cake waste under mild conditions.

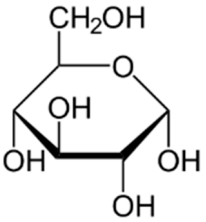
3.3.2. Glucose

Table 8 shows the experimental results for the optimization of the glucose extraction process, parameters, constants, and the ANFIS model fit.

Table 8. Experimental results of glucose extractions, estimated ANFIS values, and model constants.

Exp.	Glucose mg/L	Relative Value	Estimation ANFIS	% Error	Exp.	Glucose mg/L	Relative Value	Estimation ANFIS	% Error
1	755	2.31	3.04	31.59	15	10,029	42.93	42.93	0.01
2	672	1.94	1.88	3.20	16	6679	28.26	28.24	0.06
3	1031	3.52	3.48	1.14	18	9376	40.07	40.03	0.09
4	12,798	55.06	55.42	0.67	19	7720	32.81	32.50	0.94
5	8582	36.59	36.58	0.04	20	7901	33.61	34.43	2.45
6	13,078	56.28	56.34	0.09	21	8983	38.35	38.29	0.13
7	9351	39.96	39.95	0.01	22	8284	35.28	34.86	1.21
9	11,368	48.79	48.79	0.01	23	9692	41.45	41.47	0.03
11	8900	37.98	37.98	0.01	24	8144	34.67	34.66	0.04
12	8040	34.21	33.97	0.71	25	9228	39.42	39.42	0.01
13	8316	35.43	34.41	2.88	26	8353	35.59	35.58	0.00
14	8954	38.22	38.22	0.00	10 * 17 * 27 *	7779	33.08	34.41	4.03

Constants				Variables		Value L	Molecule
a1	50.548	a13	46.5		30	19.1097	
a2	−735.60	a14	14.997	t (min)	75	19.1094	
a3	35.061	a15	36.887		120	19.1097	
a4	127.85	a16	7.227	r	0.5	0.2221	
a5	−10.921	a17	35.357		1	0.0737	
a6	119.23	a18	37.515	e	2	0.888	
a7	67.376	a19	31.718		4	0.7697	
a8	29.409	a20	38.647	p	3.5	0.4412	
a9	27.995	a21	34.806		4.5	0.2602	
a10	56.012	a22	41.797	**Coefficient of determination R^2**			
a11	41.325	a23	43.996	0.999			
a12	79.067	a24	33.736				



* Central point.

The data from the 27 experiments were fitted to a Gaussian dependence of 3 × 2 × 2 × 2. Similar to the previous case, a good fit with a low error rate (<5%) was obtained. The coefficient of determination was R^2 = 0.999. The response surface was plotted as a function of pH, number of stages, extraction times, and S/E ratios. The glucose concentration was expressed in relation to that achieved during the optimal hydrothermal treatment, i.e., 228 mg/L (Table 4). The surface plot from the neuro-fuzzy fitting of the optimal conditions to obtain glucose is shown in Figure 4.

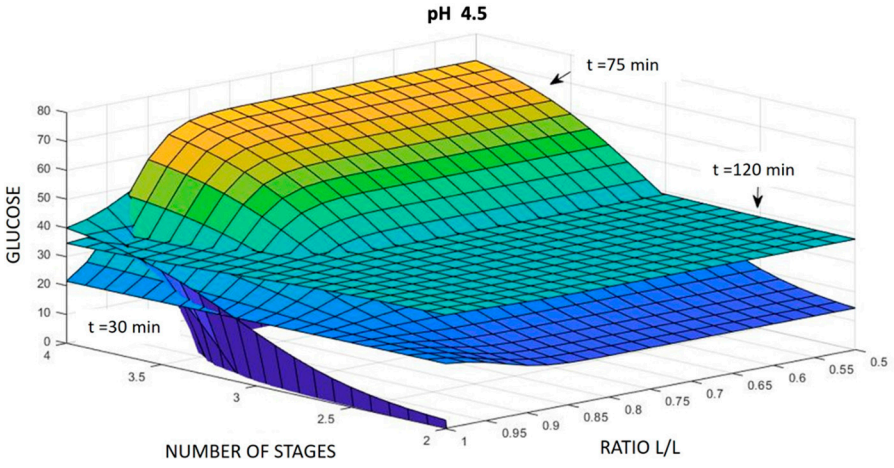


Figure 4. Surface plot of the most effective glucose extractions as a function of the studied variables.

A higher concentration of glucose was achieved (45–70-fold increase) by performing more than three stages with extraction times of 75 min, a ratio lower than 0.9, and a pH of 4.5 (Figure 4). Additionally, extractions longer than 75 min resulted in an extract with a concentration 35 times greater than that obtained in the hydrothermal treatment, regardless of the number of stages or solvent/extract ratio used.

The response surfaces obtained at pH 3.5 and 4 were similar (Figure S3). The most enriched glucose extracts (13,078 and 12,210 mg/L) were achieved after four stages of 30 min and a ratio of 1 at pH levels of 3.5 and 4, respectively. Extractions longer than 30 min resulted in a significant increase in the glucose concentration (40-fold), which remained almost constant with respect to the number of stages and solvent/extract ratio.

Therefore, the most efficient extraction conditions included a solvent/extract ratio ranging from 0.9 to 0.5, a pH of 4.5, and four extraction stages of 75 min. These specific conditions resulted in an extract containing 15,960 mg/L of glucose, indicating that glucose was concentrated by approximately 70 times compared to the initial feed. The glucose concentration obtained from olive cake was larger than that obtained from other materials using similar extraction techniques. For instance, López et al. [24] conducted sugar extraction from sunflowers stalks using autohydrolysis, and the resulting liquor contained 960 mg/L of glucose, whereas Casas-Godoy et al. [25] reported lower concentrations of reduced sugars extracted from blue agave bagasse using water as the solvent (680 mg/L). Furthermore, Zoubiri et al. [26] obtained 8.14 mg/mL of fructose from apricot waste through hydrolysis at 80 °C for 30 min. These differences can be attributed to the distinct nature of the precursors. It is worth noting that glucose exhibited a similar response to inositol, with the optimal extraction conditions being quite similar for both compounds. This provides the opportunity for their simultaneous concentration within a single process.

3.3.3. Xylose, Fructose, Lactose, and Sucrose

The surface plots from the neuro-fuzzy fitting of the optimal conditions to obtain xylose, fructose, lactose, and sucrose are given in Figure 5.

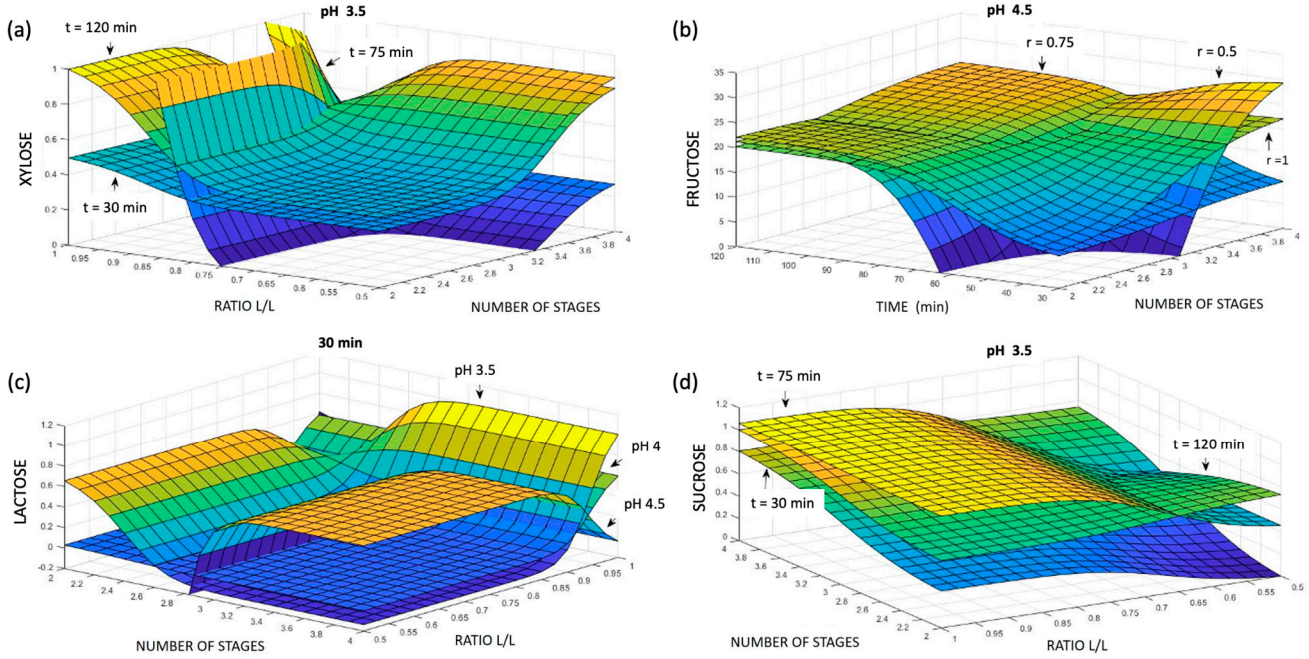


Figure 5. Surface plot of the most effective extractions of (**a**) xylose, (**b**) fructose, (**c**) lactose, and (**d**) sucrose as a function of the studied variables.

Generally, a strong fit with high coefficient of determination, i.e., R^2 = 0.999–0.983, was obtained for these saccharides (see Tables S1–S4). The surface plot from the neuro-fuzzy

fitting of xylose extractions at a pH of 3.5 is shown in Figure 5a. The xylose concentration was expressed in relation to the relative increments calculated from Equation (7). It was found that a greater xylose content was achieved by conducting less than four stages, with extraction times longer than 75 min, and employing a ratio larger than 0.75 at a pH of 3.5 (up to 385 mg/L). At pH 3.5 and an extraction time of 30 min, the solvent/extract ratio did not exhibit a significant influence on the response variable, as it remained nearly constant. This was also observed at pH values of 4 and 4.5, with extractions of 120 min (Figure S4). It is worth noting that no xylose content was found in the liquid phase resulting from the hydrothermal treatment (see Table 4), but it was detected after liquid/liquid extraction using ethyl acetate as the solvent. This could be attributed to several factors, e.g., a potentially low extracted concentration that might fall below the detection limit of the chromatograph or the possible degradation of xylose during the extraction process. Additionally, hydrothermal extraction conditions might not have been suitable for the effective extraction of this compound (monomer), necessitating the use of an organic solvent for its extraction. Similar findings have been reported in other studies, where no xylose content was found after hydrothermal treatment of several woods but was subsequently identified in the liquid phase after post-treatment [24]. In conclusion, the optimum conditions to obtain extracts enriched in xylose were to perform two stages of 75 min with a ratio of 1 at pH 3.5.

Figure 5b shows the surface plot from the neuro-fuzzy fitting of the optimal conditions to obtain fructose. The fructose concentration was expressed in relation to that achieved during the optimal hydrothermal treatment, i.e., 185 mg/L (Table 4). A higher amount of fructose was achieved when performing four stages with an extraction time either shorter than 75 min and a ratio of 0.5 at pH 4.5 (30-fold increase) (Figure 5b), or longer than 75 min, and a ratio of 0.75 at pH 3.5 (25-fold increase) (Figure S5). The minimum values of fructose were obtained when fewer than three steps were performed with times shorter than 75 min, regardless of pH. Therefore, the optimum conditions included a solvent/extract ratio of 0.5, pH 4.5, extraction times of 30 min, and four stages, resulting in an extract containing 5550 mg/L fructose. These findings are interesting because a low quantity of solvent, low acidification rate (4.5 pH of the extract), and short extraction times can result in a substantial increase in the fructose concentration (approximately 30 times greater).

Similar to xylose, lactose was not detected in the extract resulting from the hydrothermal treatment (Table 4). Lactose is a dimer resulting from the union of glucose and galactose molecules, and hydrothermal treatment may not be suitable for its formation [27]. However, the L/L extraction process probably facilitated their bonding, leading to the formation of lactose molecules during this process. This may explain why lactose was not detected in the extract of some experiments (Table S3). Figure 5c shows the effect of the number of stages, solvent/extract ratio, and pH on the lactose concentration for 30 min extractions determined with the neuro-fuzzy model. In this case, the resulting response surfaces were very similar to each other (Figure S6). This indicates that the lactose concentration remained almost constant for extraction times of 30, 75, and 120 min. At pH 4.5, a higher lactose content was achieved by performing three to four stages with a ratio ranging from 0.5 to 0.75, regardless of the extraction time. At pH 3.5, a higher lactose concentration was obtained by conducting more than three stages using ratios lower than 0.8. Thus, the optimal conditions included performing four extraction stages for 30 min with a solvent/extract ratio of 1.

Figure 5d displays the surface plot from the neuro-fuzzy fitting of the optimal sucrose extraction conditions. At pH 3.5, higher extraction of sucrose was achieved by performing extractions for 75 min with ratios larger than 0.75, regardless of the number of stages. Extractions of 120 min were also effective when conducting four stages with a ratio of 1. The response surfaces for extractions at pH levels of 4 and 4.5 were nearly identical; thus, the sucrose content remained constant at these pH levels. In both cases, higher sucrose extraction was obtained after four stages of 30 min at a ratio of 0.5 (Figure S7). For longer extraction times, a higher amount of sucrose was extracted by either performing two stages with a ratio of 0.5 (75 min extraction times) or four stages with a ratio larger than 0.8

(120 min extraction time). The optimum conditions included a ratio of l, pH 3.5, extraction times of 75 min, and two extraction stages.

3.3.4. Polyphenols

The data were fitted to a Gaussian dependence of 3 × 2 × 2 × 2. The experimental data and the ANFIS estimates were practically identical; thus, a strong fit with a low error rate (<5%), and a high coefficient of determination (R^2 = 0.999) was achieved (Table S5). In this case, the optimal extraction conditions involved the lowest phenol concentration. It means that the polyphenols migrated to the organic phase, resulting in an aqueous phase enriched in inositol and saccharides. The response surface was plotted as a function of pH, number and duration of extraction stages, and solvent/extract ratios. The polyphenols concentration was expressed in relation to that obtained in the liquid phase resulting from the hydrothermal treatment, i.e., 4792 mg/L (Table 4). Figure 6 shows the surface plot resulting from the neuro-fuzzy fitting of the least effective polyphenol extractions.

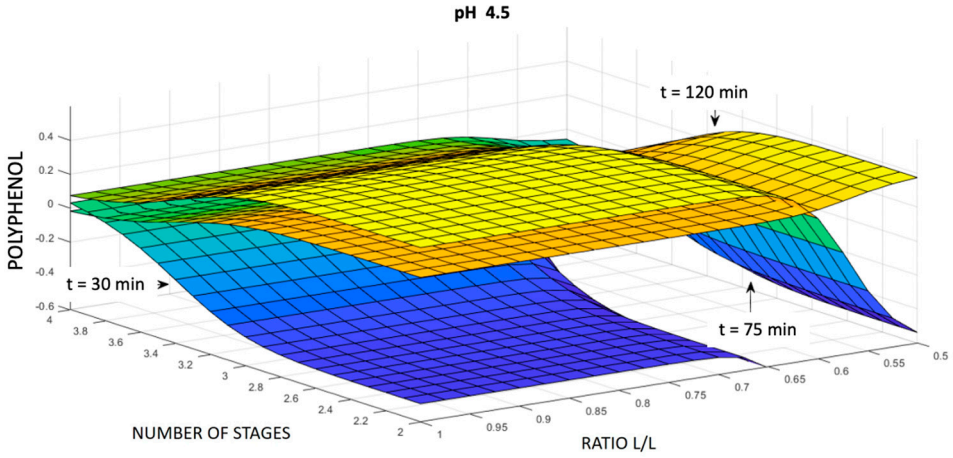


Figure 6. Surface plot of the polyphenol extractions as a function of the studied variables.

Generally, the extraction time was the most influential variable, whereas the pH had almost no influence. The minimum values were obtained by performing fewer than three stages of 30 min with ratios greater than 0.65. Additionally, a low concentration of phenols was achieved by performing two to three stages of 75 min with a ratio of 0.5. Conducting four stages with ratios larger than 0.65 was the most efficient conditions for longer extractions. In any case, the optimum polyphenol extraction conditions (lowest concentration of polyphenols, 2865 mg/L) involved performing two stages of 30 min with a ratio of 0.65, regardless of the pH.

4. Conclusions

The use of olive cake was studied by developing a biorefinery scheme. The aim was to produce value-added products of potential food and pharmaceutical interest, such as saccharides, polyphenols, and polyols, as well as solids that can be used for energy or adsorbent production. All experimental results were perfectly fitted to neuro-fuzzy models. The optimal inositol extraction conditions involved a solvent/extract ratio of 1, pH 4.5, extraction time of 75 min, and four extraction stages. Glucose and inositol showed similar response surfaces, allowing simultaneous concentration in a single process. The designed extraction conditions allowed the recovery of a significant amount of the target compounds, i.e., inositol—1126 mg/L, glucose—15,960 mg/L, xylose—385 mg/L, fructose—5550 mg/L, lactose—165 mg/L, sucrose—248 mg/L, and polyphenols—4792 mg/L, under mild conditions. The results indicate that olive cake waste is a promising source of bioactive compounds with potential applications as ingredients in functional foods or nutraceuticals. However, research covering extract purification would be desirable since the potential applications of the final products are determined by their purity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr12020317/s1, Figure S1: Gallic acid standard curve Folin-Ciocalteu method (λ = 765 nm); Figure S2: Surface plot of inositol extractions at pH of 3.5 (left) and 4 (right) as a function of the studied variables; Figure S3: Surface plot of glucose extractions at pH of 4 (left) and 3.5 (right) as a function of the studied variables; Figure S4: Surface plot of xylose extractions at pH of 4.5 (left) and 4 (right) as a function of the studied variables; Figure S5: Surface plot of fructose extractions at pH of 3.5 as a function of the studied variables; Figure S6: Surface plot of lactose extractions for 75 min (left) and 120 min (right) as a function of the studied variables; Figure S7: Surface plot of sucrose extractions at pH of 4.5 (left) and 4 (right) as a function of the studied variables; Figure S8: Surface plot of polyphenols extractions at pH of 4 (left) and 3.5 (right) as a function of the studied variables; Table S1: Experimental results for the optimization of the xylose extraction process, parameters, constants, and the ANFIS model fit; Table S2: Experimental results for the optimization of the fructose extraction process, parameters, constants, and the ANFIS model fit; Table S3: Experimental results for the optimization of the lactose extraction process, parameters, constants, and the ANFIS model fit; Table S4: Experimental results for the optimization of the sucrose extraction process, parameters, constants, and the ANFIS model fit; Table S5: Experimental results for the optimization of the polyphenols extraction process, parameters, constants, and the ANFIS model fit.

Author Contributions: Conceptualization, A.P. and G.B.; methodology, E.J.L.; software, A.P.; validation, A.P., S.P.-H. and G.B.; formal analysis, A.P.; investigation, E.J.L.; resources, M.C.; writing—original draft preparation, E.J.L. and S.P.-H.; writing—review and editing, S.P.-H., M.Á.M.-L. and M.C.; supervision, G.B.; project administration, M.Á.M.-L. and M.C.; funding acquisition, M.Á.M.-L. and M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Spanish Ministry of Economy, Industry and Competitiveness (Project CTM2016-75977-R). S.P.-H. is funded by a Juan de la Cierva Fellowship (FJC2021-048044-I, funded by MCIN/AEI/10.13039/501100011033 and the EU "NextGenerationEU/PRTR").

Data Availability Statement: The data presented in this study are available on request from the corresponding author (S.P.-H.) upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Oliveras-López, M.J.; Berná, G.; Jurado-Ruiz, E.; de la Serrana, H.L.G.; Martín, F. Consumption of extra-virgin olive oil rich in phenolic compounds has beneficial antioxidant effects in healthy human adults. *J. Funct. Foods* **2014**, *10*, 475–484. [CrossRef]
2. Souilem, S.; El-Abbassi, A.; Kiai, H.; Hafidi, A.; Sayadi, S.; Galanakis, M. *Olive Oil Production Sector: Environmental Effects and Sustainability Challenges*; Galanakis, C., Ed.; Olive Mill Waste; Academic Press: Cambridge, MA, USA, 2017; pp. 1–28.
3. Melgar, R.; Benitez, E.; Nogales, R. Bioconversion of wastes from olive oil industries by vermicomposting process using the epigeic earthworm Eisenia andrei. *J. Environ. Sci. Health Part B* **2009**, *44*, 488–495. [CrossRef] [PubMed]
4. Jeguirim, M.; Goddard, M.L.; Tamosiunas, A.; Berrich-Betouche, E.; Azzaz, A.A.; Praspaliauskas, M.; Jellali, S. Olive mill wastewater: From a pollutant to green fuels, agricultural water source and bio-fertilizer. *Renew. Energy* **2020**, *149*, 716–724. [CrossRef]
5. Al-Harthi, M.A. The efficacy of using olive cake as a by-product in broiler feeding with or without yeast. *Ital. J. Anim. Sci.* **2016**, *15*, 512–520. [CrossRef]
6. Demirbas, A. Producing bio-oil from olive cake by fast pyrolysis. *Energy Sources Part A Recovery Util. Environ. Eff.* **2007**, *30*, 38–44. [CrossRef]
7. Rajpoot, L.; Tagade, A.; Deshpande, G.; Verma, K.; Geed, S.R.; Patle, D.S.; Sawarkar, A.N. An overview of pyrolysis of de-oiled cakes for the production of biochar, bio-oil, and pyro-gas: Current status, challenges, and future perspective. *Bioresour. Technol. Rep.* **2022**, *19*, 101205. [CrossRef]
8. Ko, K.; Dadmohammadi, Y.; Abbaspourrad, A. Nutritional and Bioactive Components of Pomegranate Waste Used in Food and Cosmetic Applications: A Review. *Foods* **2021**, *10*, 657. [CrossRef]
9. Caballero, B.M.; López-Urionabarrenechea, A.; Pérez, B.; Solar, J.; Acha, E.; de Marco, I. Potentiality of "orujillo" (olive oil solid waste) to produce hydrogen by means of pyrolysis. *Int. J. Hydrogen Energy* **2020**, *45*, 20549–20557. [CrossRef]
10. Gálvez-Pérez, A.; Pérez, A.; Calero, M.; Martín-Lara, M.A.; Blázquez, G. Integral exploitation from olive cake for energy production in a biorefinery scheme. *Process Saf. Environ. Prot.* **2019**, *131*, 135–143. [CrossRef]
11. Lemes, A.C.; Álvares, G.T.; Egea, M.B.; Brandelli, A.; Kalil, S.J. Simultaneous production of proteases and antioxidant compounds from agro-industrial by-products. *Bioresour. Technol.* **2016**, *222*, 210–216. [CrossRef]

12. Pérez, A.; Blázquez, G.; Iáñez Rodríguez, I.; Osegueda, O.; Calero, M. Optimization of the sugar hydrothermal extraction process from olive cake using neuro-fuzzy models. *Bioresour. Technol.* **2018**, *268*, 81–90. [CrossRef]
13. Yoshimitsu, U.; Akira, H.; TSuneki, T. Isolation of 0-methyl-styllo-inositol from mung bean seeds. *Carbohydr. Res.* **1973**, *29*, 520–521.
14. Zuluaga, A.M.; Mena-García, A.; Chito-Trujillo, D.; Rada-Mendoza, M.; Sanz, M.L.; Ruiz-Matute, A.I. Development of a microwave-assisted extraction method for the recovery of bioactive inositols from lettuce (Lactuca sativa) byproducts. *Electrophoresis* **2020**, *41*, 1804–1811. [CrossRef]
15. Nauck, D.; Rudolf, K. Neuro–Fuzzy Systems. In *Handbook of Fuzzy Computation*; CRC Press: Boca Raton, FL, USA, 2020.
16. Rubio-Senent, F.; Fernández Bolaños, J.; García-Borrego, A.; Lama Muñoz, A.; Rodríguez Gutiérrez, G. New phenolic compounds hydrothermally extracted from the olive oil by product alperujo and their antioxidative activities. *J. Agric. Food Chem.* **2012**, *60*, 1175–1186. [CrossRef] [PubMed]
17. Rubio-Senent, F.; Fernández Bolaños, J.; García-Borrego, A.; Lama Muñoz, A.; Rodríguez Gutiérrez, G. Chemical characterization and properties of a polymeric phenolic fraction obtained from olive oil waste. *Food Res. Int.* **2013**, *54*, 2122–2129. [CrossRef]
18. Real Decreto 1101/2011, de 22 de Julio, Por el Que se Aprueba la Lista Positiva de los Disolventes de Extracción Que se Pueden Utilizar en la Fabricación de Productos Alimenticios y de Sus Ingredientes. Available online: https://www.boe.es/buscar/pdf/2011/BOE-A-2011-14223-consolidado.pdf (accessed on 1 October 2023).
19. Folin, O.; Ciocalteu, V. On tyrosine and tryptophane determinations in proteins. *J. Biol. Chem.* **1927**, *73*, 627–650. [CrossRef]
20. Montgomery, D.C. *Diseño y Análisis de Experimentos*, 2nd ed.; Wiley: México, 2004.
21. Rubio-Senent, F.; Fernández Bolaños, J.; García Borrego, A.; Lama Muñoz, A.; Rodríguez Gutiérrez, G. Influence of pH on the antioxidant phenols solubilised from hydrothermally treated olive oil by-product (alperujo). *Food Chem.* **2017**, *219*, 339–345. [CrossRef] [PubMed]
22. de Campos Souza, P.V. Fuzzy neural networks and neuro-fuzzy networks: A review the main techniques and applications used in the literature. *Appl. Soft Comput.* **2020**, *92*, 106275. [CrossRef]
23. Ruiz-Aceituno, L.; Rodríguez-Sánchez, S.; Sanz, J.; Sanz, M.L.; Ramos, L. Optimization of pressurized liquid extraction of inositols from pine nuts (*Pinus pinea* L.). *Food Chem.* **2014**, *153*, 450–456. [CrossRef] [PubMed]
24. López, F.; García, J.C.; Pérez, A.; Feria, M.J.; Zamudio, M.A.M.; Gil, G. Chemical and energetic characterization of species with a high-biomass production: Fractionation of their components. *Environ. Prog. Sustain.* **2010**, *29*, 499–509.
25. Casas-Godoy, L.; Campos-Valdez, A.R.; Alcázar-Valle, M.; Barrera-Martínez, I. Comparison of Extraction Techniques for the Recovery of Sugars, Antioxidant and Antimicrobial Compounds from Agro-Industrial Wastes. *Sustainability* **2022**, *14*, 5956. [CrossRef]
26. Zoubiri, F.Z.; Rihani, R.; Bentahar, F. Golden section algorithm to optimise the chemical pretreatment of agro-industrial waste for sugars extraction. *Fuel* **2020**, *266*, 117028. [CrossRef]
27. Gagić, T.; Perva-Uzunalić, A.; Knez, Ž.; Škerget, M. Hydrothermal treatment of sugars to obtain high-value products. *J. Serbian Chem. Soc.* **2020**, *85*, 97–109. [CrossRef]