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Optimization of low thermal treatments to increase hydrophilic phenols in the Alperujo liquid fraction

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SUMMARY: Hydrophilic phenols are the main bioactive compounds in alperujo. Among them, 3,4-Dihydroxyphenylglycol (DHPG), Hydroxytyrosol (HT) and Tyrosol (Ty), are the most relevant and deeply studied. These compounds exhibit high antioxidant capacity and a wide range of health benefits as well as technologically promising properties. Given that, their recovery represents an attractive opportunity to valorize this by-product. In this work low thermal treatments were applied to alperujo in order to obtain phenol-enriched liquid fractions. Optimization assays combining different levels of temperature (30 to 90 ºC), time (60 to 180 min) and water content (70 to 90%), followed by response surface methodologies were performed. The results indicated that by applying optimal conditions, is possible to obtain theoretical yields of Total phenols, DHPG, HT and Ty of 2.4, 957.8, 3.4 and 6.4 times greater, respectively, than raw dry alperujo. Interestingly, all the evaluated conditions can be reproduced with low investment in a standard olive oil industry.

KEYWORDS: *Alperujo; Hydroxytyrosol; Olive mill by products; Phenolic compounds; Thermal treatments; TPOMW.*

RESUMEN: ***Optimización de tratamientos a baja temperatura para incrementar fenoles hidrofílicos en la fracción líquida de Alperujo.*** Los fenoles hidrofílicos representan los principales compuestos bioactivos del alperujo. Los más relevantes son 3,4-Dihidroxifenilglicol (DHFG), Hidroxitirosol (HT) y Tirosol (Ti). Estos compuestos presentan alta capacidad antioxidante, beneficios para la salud e importantes propiedades tecnológicas, por ello su recuperación representa una alternativa para la valorización de este subproducto. En este trabajo, se aplicaron al alperujo tratamientos térmicos para obtener fracciones líquidas enriquecidas con compuestos fenólicos. Se realizaron ensayos combinando niveles de temperatura (30 ºC a 90 ºC), tiempo (60 min a 180 min) y humedad del alperujo (70 % a 90 %), seguidos de metodologías de superficie de respuesta. Los resultados indicaron que, mediante la aplicación de las condiciones óptimas, es posible obtener rendimientos teóricos de fenoles totales, DHFG, HT y Ti, 2.4, 957.8, 3.4 y 6.4 veces superiores a los obtenidos a partir del alperujo inicial. Es destacable que las condiciones establecidas, se pueden reproducir con bajo costo en una industria olivícola estándar.

PALABRAS CLAVE: *Alperujo; Compuestos fenólicos; Hidroxitirosol; Subproductos olivícolas; Tratamientos térmicos.*

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1. INTRODUCTION

Argentina is the main olive oil producer among South American countries. Olive production in Argentina is mainly located in the central western region in the provinces of San Juan, Catamarca, La Rioja, Mendoza and Córdoba (Gómez del Campo *et al.*, 2010). Olive oil extraction in Argentina is generally carried out with continuous two-phase centrifugation systems. These systems generate a semisolid waste made of olive pulp, stone and vegetation water commonly called "alperujo" or two-phase olive-mill waste (TPOMW) (Alburquerque *et al.*, 2004).

Different technologies have been developed in Mediterranean countries to dispose or reuse alperujo. Secondary oil extraction followed by electrical energy cogeneration represents the main reuse alternatives in leading olive oil producing countries. Nevertheless, other technologies such as composting, gasification, anaerobic digestion, extraction of added-value products, animal feed and soil amendment also present a promising horizon (Morillo *et al.*, 2009; Roig *et al.*, 2006).

The total amount of alperujo generated in the San Juan province amounts to 60.000 to 80.000 t per year (Monetta *et al.*, 2019), however, it is still considered as a residue. Different reports have evaluated the effect of alperujo as olive soil amendment under local conditions (Monetta *et al.*, 2012) which gave rise to recommendations under conservationist management. This practice represents a simple alternative to disposing olive mill by-products, but still presents several limitations and it is not a way to take advantage of all alperujo constituents.

Virgin olive oil is well known for its nutraceutical qualities, most of them provided by the presence of phenolic bioactive compounds. Hydrophilic phenols are the most abundant bioactive compounds of olives and virgin olive oil, although tocopherols and carotenes are also present. The prevalent classes of hydrophilic phenols found in virgin olive oil are phenolic alcohols and acids, flavonoids, lignans and secoiridoids (Servili *et al.*, 2009).

Interestingly, due to their hydrophilic properties, during the malaxation of milled olives, only 2% of phenolic compounds pass through the oil, with the remaining 98% being retained in the alperujo and usually discarded with it (Owen *et al.*, 2003). Among the phenols found in alperujo, 3,4-Dihydroxyphenylglycol (DHPG), Hydroxytyrosol (HT) and Tyrosol (Ty), are the most relevant and deeply studied. These compounds exhibit high antioxidant capacity (Rubio-Senent *et al.*, 2013) and a wide range of health benefits like anti-inflammatory, anti-modulatory, anti-platelet, anti-cancer, anti-viral, anti-microbial or phyto-regulatory properties, among others (Fernández-Prior *et al.*, 2021; Lama-Muñoz *et al.*, 2021) as well as technologically promising properties which aid in the formulation of safer and healthier foods (Balzan *et al.*, 2021; Bartella *et al.*, 2021; Bermúdez-Oria *et al.*, 2019; Munekata *et al.*, 2020). Therefore , they are generally required as additives and inputs in cosmetic, pharmaceutical, agricultural, nutraceutical, and animal feed and food industries.

Different reports describe the extraction of hydrophilic phenolic compounds from olive by-products by employing multistage technologies. The methods employed usually consist of initial pretreatments to enhance the solubilization of water-soluble phenols (Lama-Muñoz *et al.*, 2011; Niknam *et al.*, 2021), followed by a second stage focused on the recovery of the phenolic fraction (Fernández-Prior *et al.*, 2020; Gil and Tuberoso, 2021; Rubio-Senent *et al.*, 2017). Among different approaches, high-pressure thermal treatments have been implemented at industrial scale (Lama-Muñoz *et al.*, 2019). By this technology, alperujo is exposed to high-pressure steam (150 - 170 ºC, 1.2 MPa) during short time periods (15 to 90 minutes). This procedure serves to relax the structure of organic matter, induce the auto-hydrolysis of complex molecules and favor the solubilization of simple phenolic compounds. After thermal treatment, a solid-liquid separation is performed by three-phase centrifugation systems, and a phenol-enriched liquid fraction is obtained. This procedure has been reported as highly efficient to obtain phenol-enriched liquid fractions, although the investment required for applying high temperatures and pressure levels represents an obstacle for scaling up the process at industrial scale in Argentina, as well as in other regions where the alperujo-associated industry is not as developed as in main olive oil producing countries.

Based on the same principles of hydrothermal treatments described, but with the aim of finding a solution to the high investment required, the aim of the present work was to determine the optimal conditions required to obtain high quality phenol-enriched liquid fractions applying low thermal treatments. Treatments were performed combining temperature (30 to 90 ºC), time (60-180 min) and alperujo water content (70 to 90 %). Liq-

uid fractions were obtained by centrifugation, and then total phenolic compounds, DHPG, Ty and HT levels were determined. A two-stage experimental program was developed. A factorial assay towards the selection of relevant variables was carried out, followed by response surface methodologies to determine the optimum operating conditions required for the recovery of total as well as individual phenols of interest.

2. MATERIALS AND METHODS

2.1. Origin and characterization of raw material

For experimental assays, 20 kg of fresh alperujo were obtained from a continuous two-phase centrifugation system (Oliomio 200 Eco, Toscana Enologica Mori, Italy) placed in the olive oil extraction plant of INTA EEA San Juan, Argentina. Milled olive fruits were from the Arbequina cultivar at a maturity index of 4. The raw material was homogenized and stored in individual pots of 1 kg at -20 °C. Before starting the assays, 3 individual samples of alperujo were subjected to the following chemical determinations: pH, water content, organic matter content, total soluble phenols, DHPG, HT and Ty.

2.2. Analytical determinations

Raw alperujo was characterized according to the following determinations: Water content was measured gravimetrically at 70 ºC (Martinez *et al*., 2021); pH was determined by potentiometric determination in 1:5 (w/v) water extract (Martinez *et al*., 2021); total organic matter and ashes were determined by loss on ignition at 550 ºC for 24 h (Martinez *et al*., 2021). Total organic carbon was calculated from organic matter according to (Navarro *et al*., 1993). The phenolic content in alperujo was determined in methanolic extract (methanol:water 80:20 (v/v) using 2 mL per gram of fresh alperujo) by a modified version of the method described by (Singleton and Rossi, 1965) with Folin-Ciocalteu reagent and measured in a Shimadzu 1240 UV–Visible spectrophotometer at 725 nm. The results were expressed as mg of caffeic acid per kg of initial sample. The individual phenols of interest (DHPG, HT and Ty) were measured in methanolic extract (explained above) by high-performance liquid chromatography (HPLC Hewlett-Packard series 1100), using a Kinetex® EVO 5 µm C18 (250 × 4.6 mm) Phenomenex® column. The analysis was performed at room temperature, the elution was at a flow rate of 1.0 mL/min, with a mobile phase A of acetonitrile and B ultrapure water, using the following gradient over a total run time of 55 min: 95% A initially, 75% A at 30 min, 50% A at 45 min, 0% A at 47 min, 75% A at 50 min, 95% A at 52 min until the run was completed. The chromatograms were analyzed at 280 nm by integration peaks at different wavelengths, according to calibrations performed with external standards. The results were expressed in mg/Kg or mg/L.

For determinations in liquid fractions, total soluble solids were determined by a portable refractometer (Atago, JPN), and expressed as Brix degrees. Total soluble phenols were determined in 1/10 water dilutions by the colorimetric method described above. The results were expressed as mg of caffeic acid/L of liquid fraction. The individual phenols of interest (DHPG, HT and Ty) were measured following the protocol described above.

2.3. Selection of relevant variables

In order to evaluate the relevant conditions required to increase the total phenolic concentration in the alperujo liquid fraction a factorial assay was performed. Closed plastic tubes containing 40 g of alperujo were positioned in thermostatic baths at two temperature levels (30 and 70 ºC) and two exposure times (60 and 180 min). In addition, a control treatment of untreated alperujo was included. Three replicates of each treatment were performed in parallel. Once treatments were applied, samples were centrifuged at 3500 g for 20 min to separate the solid and liquid phases. After centrifugation, weight, total soluble solids and total soluble phenols were determined in liquid phases. The percent of phenols recovered was calculated by the following equation:

$$\%\ Phenols\ recovered = \frac{T\ liquid\ phase\ PC\ \left(\frac{mg}{Kg}\right) \cdot\ T\ liquid\ phase\ weight\ (g).100\ \%}{Control\ liquid\ phase\ PC\left(\frac{mg}{Kg}\right) \cdot Control\ liquid\ phase\ weight\ (g)}$$

Where: "T liquid phase PC" represents the phenolic content in the liquid phases obtained after treatment application. "T liquid phase weight" represents the weight of the liquid phase obtained after treatment application. "Control liquid phase PC" represents the phenolic content in liquid phases obtained without treatment application. "Control liquid phase weight" represents the weight of the liquid phase obtained without treatment application.

2.4. Optimization of variables

For the development of optimization studies, Box-Behnken experimental design followed by response surface methodology were applied. Box-Behnken is a second-order design based on three-level incomplete factorial designs. It is used to measure the combined effect of the factors under study, reducing the number of experiments, improving the performance of time and resources (Box and Behnken, 1960). The response surface methodology (RSM) is a technique to model and analyze statistical problems. The main objective is to optimize the response surface which is influenced by various parameters, quantifying the relationship among them and plotting the response (Montgomery and Wiley, 1984). A total of fifteen experimental trials combining three factors at three levels were analyzed: temperature (50 , 70 and 90 ºC), exposure time (30 , 75 and 120 min) and alperujo water content (70 , 80 and 90%). Trials were performed in closed plastic tubes containing 100 g of alperujo in thermostatic baths. As performed in a previous stage, once trials were conducted, phase separation was accomplished by centrifugation at 3500 g for 20 min. Liquid phases were weighed, and total phenols, DHPG, HT and Ty concentrations were determined. Then the content of phenols recovered was calculated considering both concentration in liquid phase and amount of liquid phase recovered by the following equation:

$$Phenols\ recovered\ (mg) = liquid\ phase\ phenolic\ content\ \left(\frac{mg}{Kg}\right) \cdot\ liquid\ phase\ weight\ (Kg)$$

2.5. Statistical analysis and experimental design

Relevant variable selection was performed under factorial design. Analysis of variance (ANOVA) was achieved using Infostat Professional Software 2.0 version (UNC). For comparison of means LSD Fisher ($p \leq 0.05$) was employed.

Variable optimization was performed in the Box-Behnken design according to Response Surface methodology, using Design Expert software 7.0.0 (Stat-Ease Inc., Minneapolis, USA). Statistical differences were established at $p \leq 5\%$. Model significance, polynomic equation, data normality and surface response graphs were determined for each variable. Model efficiency was determined by R2 and adjusted R2. The response surface statistical model responded to the equation adjusted by three factors (temperature, water content and time) and four response variables (Total phenols, DHPG, HT and Ty). The optimal levels of each response variable were obtained and the polynomic equation was determined.

3. RESULTS AND DISCUSSION

3.1. Raw material characterization

As shown in Table 1, the chemical composition of the alperujo employed in the assays was similar to others employed in different reports (Alburquerque *et al.*, 2004; Morillo *et al.*, 2009). It was characterized by high water content and slightly acidic pH. Regardless of the water content, it was mainly composed of organic matter and a minor proportion of mineral ashes. Among organic constituents, the total phenolic content (7233.8 mg/Kg) was in the lower limit compared to other reports, presenting values ranging from 6000 to 26000 mg/Kg of raw dry alperujo (Alburquerque *et al.*, 2004; Rubio-Senent *et al.*, 2017). As stated, the amount and profile of phenolic compounds present in olives is highly variable and dependent mainly on cultivar and maturity stage as well as agronomic and climatic conditions (Obied *et al.*, 2008). In the present work, the alperujo employed belonged to the Arbequina cultivar, grown under intensive drip irrigated system, harvested at a maturity index of 4 and immediately milled. Arbequina represents one of the most abundant olive cultivars in Argentina (Gómez del Campo *et al.*, 2010) and generally the olive oils obtained with this cultivar are characterized by low phenolic content (Monasterio *et al.*, 2017). Regarding the individual phenols of inter-

Table 1. Main chemical parameters of raw alperujo

Parameter	Mean value *
pH	5.04 ± 0.05
Water content (%)	65.6 ± 0.4
Organic matter (%)	32.9 ± 2.0 (95.6)
Total Organic Carbon (%)	17.2 ± 1.1 (48.2)
Ash (%)	1.5 ± 0.5 (4.4)
Total phenols (mg/kg)	2485.0 ± 318.2 (7233.8)
DHPG (mg/kg)	0.3 ± 0.1 (0.9)
HT (mg/kg)	472.2 ±18.4 (1372.7)
Ty (mg/kg)	80.5 ± 8.5 (234.0)

* Mean value ± SD of three independent determinations. Numbers between brackets indicate values in raw dry alperujo.

est, Hydroxytyrosol was the main hydrophilic phenolic compound observed representing almost 20% of the total phenol content, while tyrosol and DHPG together did not reach 4%. These results agree with previous data (Rubio-Senent *et al.*, 2013) and support the significance of applying pre-treatments in order to induce phenolic compound hydrolysis and increase the total amount of hydrophilic forms.

3.2. Relevant variables selection

Table 2 shows the effect of thermal treatments performed on alperujo samples (40 g) on the weight of liquid fraction recovered as well as the total soluble solids and total phenols. As shown, the weight of the liquid fraction reached its highest value when thermal treatments were performed at 70 ºC, without presenting statistical differences between 60 and 180 minutes. On the other hand, the weight of the liquid fraction obtained by treatments achieved at 30 ºC did not present differences compared to untreated control samples. In addition, the level of total soluble solids and total phenols presented a similar pattern, showing an increase when thermal treatments were performed at 70 ºC and remaining similar to untreated alperujo in treatments carried out at 30 ºC (Table 2). These results agree with previous data which suggest that thermal treatments provoke the relaxation of organic matter constituents allowing the exit of intracellular water and favoring the hydrolysis of complex molecules and the solubilization of lower compounds such as phenols, sugars, organic acids and proteins, among others (Rodríguez *et al.*, 2007).

Taken together, both the increase in liquid fraction weight and the total phenol content in these fractions, the amount of recoverable phenols increased to 136-138% with respect to the control when thermal treatments at 70 ºC where applied (Figure 1). The observed increase in the total amount of recoverable phenols is lower than that observed in other reports where thermal treatments were performed at higher temperatures (Lama-Muñoz *et al.*, 2011; Rubio-Senent *et al.*, 2013). As these authors explain, the importance of the application of a heat treatment is to achieve a solubilization of high value-added components, such as phenols, and to enable phase separation. Obviously, the higher the treatment temperature, the higher

Table 2. Thermal treatments applied and main parameters of liquid fractions obtained

Sample	Temperature (ºC)	Exposure Time (min)	Weight of liquid fraction (g)*	Total soluble solids (ºbrix)*	Total phenols (mg/kg)*
Alperujo	RT	untreated	14.5 ± 2.3 a	7.0 ± 0.1 a	4046 ± 261 a
Alperujo	30	60	14.6 ±1.6 a	7.2 ± 0.2 b	4522 ± 271 b
Alperujo	30	180	14.4 ±1.2 a	7.2 ± 0.1 b	4215 ± 337 ab
Alperujo	70	60	16.7 ±0.5 b	7.4 ± 0.2 c	4867 ± 299 c
Alperujo	70	180	16.4 ±0.8 b	7.5 ± 0.2 c	4898 ± 219 c

* Mean values ± SD of three independent determinations. ANOVA analysis was performed. Significant differences in the same column are indicated by different letters. LSD Fisher ($p \leq 0.05$) was used for comparison of means.

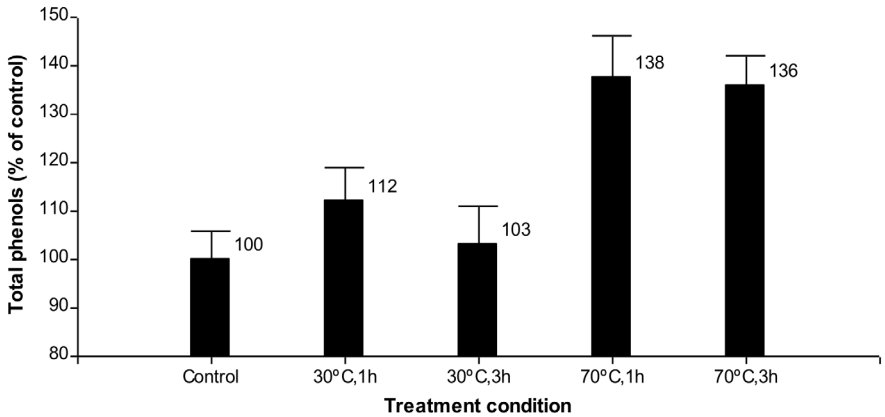

Figure 1. Total phenol content in liquid fractions of alperujo obtained by each treatment. Results are expressed as % of control treatment. Numbers in each column indicate average value of three independent assays. Error bars indicate standard deviation.

the solubilization of phenols and the better the phase separation, but also the higher the formation of degradation metabolites, such as furfurans from sugars, and the higher the investment and operating costs. For this reason, the application of thermo-malaxation is sought to improve the use of alperujo, a first step being the use of temperatures of up to 70 ºC which solubilize phenols and allow an adequate separation of phases, even if a quantity of solids in suspension is reached, which implies an intermediate sedimentation step prior to the extraction of phenols from the liquid phase (Fernández-Prior *et al.*, 2020).

3.3. Optimization of variables

Table 3 summarizes the experimental design conditions applied as well as the values of response variables (Total phenols, DHPG, HT and Ty) expressed as mg in liquid fractions obtained from 100 g of initial sample. Response surface methodology was used to optimize each response variable considering the linear, quadratic, and cross-product interactions of the independent variables at the 95% confidence level. The analysis of the variance (ANOVA) for each response variable is shown in Table 4. As shown, Total phenols, DHPG and Hydroxytyrosol described quadratic response surface models with high R2 and R2 adjusted values. Temperature, water content and the quadratic effect of water content were highly desirable (p-value ≤ 0.05) for Total phenols and HT, while Temperature and its quadratic effect were significant for DHPG. On the other hand, the interaction between independent variables and Tyrosol was explained by a linear model, with temperature and water content as the significant variables.

The polynomial that describes the relationships between the process variables and each response variable are represented by the following equations:

$$\text{Total phenols (mg)} = -6077.87870 - 1.77437*T + 1.87793*t + 163.71542*WC + 0.018556*T*t + 0.027625*T*WC - 0.010944*t*WC - 0.00414583*T^2 - 0.015436*t^2 - 1.05683*WC^2$$

$$\text{DHFG (mg)} = 52.40351347 - 2.171123359*T - 0.08614005*t + 0.479013698*WC + 0.000984705*T*t + 0.011767141*T*WC - 0.000356229*t*WC + 0.010037259*T^2 + 0.000388239*t^2 - 0.008143276*WC^2$$

$$\text{HT (mg)} = -1626.094951 - 3.87111763*T - 0.870032674*t + 47.36861411*WC + 0.007666309*T*t + 0.013617516*T*WC + 0.013152993*t*WC + 0.021685848*T^2 - 0.005201345*t^2 - 0.316310484*WC^2$$

$$\text{Ty (mg)} = -41.12670915 + 0.220703203*T + 0.010423401*t + 0.432320791*WC$$

Where: "T" represents temperature; "t" represents time; "WC" represents water content.

Table 3. Experimental assay and response variables of Box Behnken design. Response variables indicate the contents in total phenols, DHPG, HT and Ty, in liquid fractions obtained after each run

Experimental design				Response variables				
Run	Sample weight (g)	Temperature (ºC)	Time (min)	Water content (%)	Total phenols (mg)	DHPG (mg)*	HT (mg)*	Ty (mg)*
1	100	70	75	80	349.0	0.7	62.6	14.1
2	100	70	75	80	348.5	0.5	87.0	11.4
3	100	90	75	70	286.9	6.6	72.9	7.6
4	100	90	120	80	384.4	12.1	101.6	16.9
5	100	50	75	70	269.2	1.3	55.0	3.8
6	100	50	75	90	190.5	ND	26.2	11.3
7	100	70	120	70	256.2	1.0	36.0	6.2
8	100	90	30	80	323.9	8.4	88.0	15.1
9	100	70	75	80	357.2	0.2	76.1	7.3
10	100	70	120	90	143.4	0.2	19.2	13.8
11	100	70	30	90	182.9	0.2	18.2	ND
12	100	90	75	90	230.3	ND	55.0	16.3
13	100	50	30	80	286.3	0.2	58.9	3.6
14	100	70	30	70	276.0	0.4	58.7	2.7
15	100	50	120	80	280.0	0.4	44.8	1.9

* DHPG, HT and Ty indicate Dihydroxyphenylglycol, Hydroxytyrosol and Tyrosol, respectively.

Table 4. Analysis of variance (ANOVA) for phenolic compounds in the liquid fractions of alperujo from optimization assays

Source	Sum of Squares	gl	Mean Square	F ratio	P value	Model	R^2	R^2 adj.
Total phenols								
A-Temperature	4975.0	1.0	4975.0	7.8	0.0380*	Quadratic	0.95	0.87
B-Time	3.3	1.0	3.3	0.0	0.9457			
C-Water content	14552.2	1.0	14552.2	22.9	0.0049*			
AB	1115.6	1.0	1115.6	1.8	0.2423			
AC	122.1	1.0	122.1	0.2	0.6793			
BC	97.0	1.0	97.0	0.2	0.7119			
A^2	10.2	1.0	10.2	0.0	0.9043			
B^2	3607.7	1.0	3607.7	5.7	0.0628			
C^2	41239.3	1.0	41239.3	65.0	0.0005*			
Total error	47.7	2.0	23.9					
Dihydroxyphenylglycol								
A-Temperature	99.5	1.0	99.5	210.6	0.0007*	Quadratic	0.99	0.97
B-Time	2.5	1.0	2.5	5.4	0.1031			
C-Water content	0.3	1.0	0.3	0.6	0.4904			
AB	3.1	1.0	3.1	6.7	0.0818			
AC	7.4	1.0	7.4	15.6	0.0289			
BC	0.1	1.0	0.1	0.2	0.6726			
A^2	40.7	1.0	40.7	86.2	0.0026*			
B^2	1.6	1.0	1.6	3.3	0.1666			
C^2	1.7	1.0	1.7	3.5	0.1562			
Total error	0.1	2.0	0.1					
Hydroxytyrosol								
A-Temperature	2200.6	1.0	2200.6	19.6	0.0069*	Quadratic	0.94	0.82
B-Time	61.0	1.0	61.0	0.5	0.4944			
C-Water content	1354.8	1.0	1354.8	12.1	0.0178*			
AB	190.4	1.0	190.4	1.7	0.2497			
AC	29.7	1.0	29.7	0.3	0.6292			
BC	140.1	1.0	140.1	1.2	0.3149			
A^2	277.8	1.0	277.8	2.5	0.1766			
B^2	409.6	1.0	409.6	3.6	0.1145			
C^2	3694.2	1.0	3694.2	32.9	0.0023*			
Total error	298.8	2.0	149.4					
Tyrosol								
A-Temperature	155.9	1.0	155.9	20.1	0.0012*	Linear	0.79	0.73
B-Time	1.5	1.0	1.5	0.2	0.6704			
C-Water content	126.4	1.0	126.4	16.3	0.0024*			
Total error	23.3	2.0	11.6					

* Statistically significant (p-value ≤ 0.05)

As can be seen in Figure 2, all the response variables increased with temperature. Regarding time and water content, these factors presented particular patterns for each response variable. Total phenols and HT showed maximum levels when time and water content were set at central points (Figure 2 A, B, E, F). Tyrosol was greater when the water content was set at the lowest level without being highly affected by time (Figure 2 G, H), while DHPG was not highly affected by time or water content (Figure 2 C, D). Based on the different models and equations, the optimal conditions and the higher values for the different response variables are summarized in Table 5.

Table 6 shows the theoretical levels of the different compounds evaluated (total phenols, DHPG, HT and Ty) expressed as concentrations in liquid fractions obtained and in raw dry alperujo. As can be observed, the concentration of total phenols solubilized reached 3804 mg/kg liquid fraction, and 17434 mg/kg raw dry alperujo. The values obtained are interesting and similar to those obtained experimentally by other authors (Lama-Muñoz *et al.*, 2011). The comparison of theoretical values obtained for each response variable (Table 6) with the initial alperujo content (Table 1) indicates increases of 2.4, 957.8, 3.4 and 6.4 folds in initial total phenols, DHPG, HT and Ty, respectively, from raw dry alperujo. These results confirm that applying low thermal treatments to alperujo is possible to obtain phenol-enriched fractions. The observed increase in the content of hydrosoluble phenols supports the hypothesis that thermal treatments provoke the hydrolysis of complex hydrophobic phenolic compounds and favor the solubilization of hydrophilic molecules. The results show that the extraction of phenols is temperature and time dependent, and that for the tested conditions the solubilized amount of the main phenols in free form can justify their extraction as mentioned by other authors (Fernández-Prior *et al.*, 2020). Likewise, at these temperatures it is necessary to continue studying the solubilization of polymerized or more complex phenols that could increase with subsequent chemical or enzymatic hydrolysis processes by the addition of acids or enzymes or by the action of those already present during a certain storage time, which will also help to separate suspended solids that hinder the application of chromatographic processes to obtain final phenolic extracts.

4. CONCLUSIONS

In this work low thermal treatments were applied to alperujo in order to obtain phenol-enriched liquid fractions. All temperatures employed were under 90 ºC with the aim to evaluate conditions that could potentially be reproduced with low investment in a standard olive oil industry. The relevant variable selection stage indicated that the treatment of alperujo at 70 ºC during 1 to 3 h was effective to obtain liquid fractions with higher total soluble solids and phenolic contents than raw alperujo. Furthermore, the optimization tri-

Table 5. Theoretical optimal conditions for each response variable

Response variable	Temperature (ºC)	Time (min)	Water content (%)	Value* (mg)	D - Value
Total phenols	90.0	87.2	78.18	380.4	0.98
Dihydroxyphenylglycol	89.9	117.7	85.74	12.3	1.00
Hydroxytyrosol	90.0	82.0	78.52	101.5	1.00
Tyrosol	88.2	81.1	88.37	17.4	1.00

*Values obtained from 100 g of initial sample.

Table 6. Theoretical content of total phenols, Dihydroxyphenylglycol, Hydroxytyrosol and Tyrosol for optimal conditions obtained by response surface methodology

Compound	Liquid fraction (mg/Kg)	Raw dry alperujo (mg/Kg)
Total phenols	3804	17434
Dihydroxyphenylglycol	123	862
Hydroxytyrosol	1015	4727
Tyrosol	174	1495

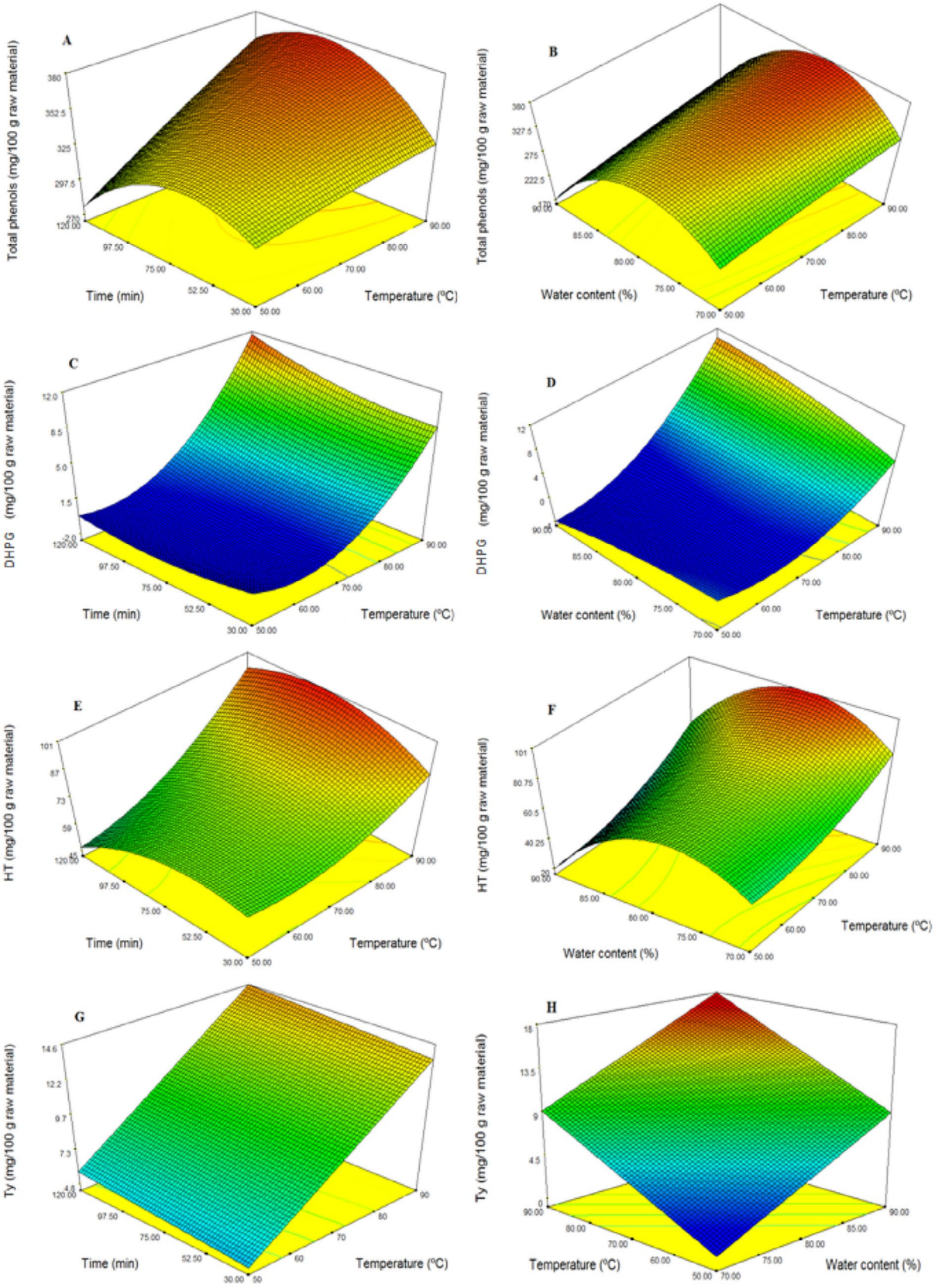

FIGURE 2. Response surface methodology graphs (temperature/time and temperature/water content) for each response variable. (A-B) Total phenols, (C-D) Dihydroxyphenylglycol, (E-F) Hydroxytyrosol, (G-H) Tyrosol.

al indicated that regarding the phenols of interest, not only temperature, but also alperujo water content and time were significant to obtain higher yields of each particular compound. Theoretical equations showed that by applying optimal conditions, it is possible to obtain yields of total phenols, DHPG, HT and Ty of 2.4, 957.8, 3.4 and 6.4 times greater with respect to raw dry alperujo. Therefore, the application of a thermal treatment adaptable to the olive oil industry promotes the obtaining of a liquid fraction from which it is possible to extract high added-value components, such as phenols, and thus improve the use of the main by-product from this industry.

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