

## Article

# Solubilization of Phenols and Sugars from Raspberry Extrudate by Hydrothermal Treatments

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**Abstract:** Raspberry extrudate residue has bioactive compounds in its matrix that are considered high-added value compounds. In the present study, different hydrothermal treatments were carried out using different operational systems, temperatures and times to solubilize raspberry extrudate in order to obtain these bioactive compounds (i.e., sugars and phenolic compounds). Hydrothermal treatment conditions were assessed in the range of 60 °C to 210 °C, with increments of 30 °C. The hydrothermal treatment at 210 °C for 5 min with direct steam and decompression was the most efficient process based on the solubilization of sugars and phenols, as well as on the antioxidant capacity of the products recovered in the liquid phase after treatment. Under these conditions, the concentration of soluble phenols was more than double the concentration in the raw raspberry extrudate, with more than 5000 mg phenols per kilogram of raspberry extrudate. The obtained values demonstrate the potential of applying this treatment for recovering valuable bioactive compounds from raspberry extrudate.

**Keywords:** raspberry extrudate; hydrothermal treatments; antioxidant compounds; phenolic compounds

## 1. Introduction

Up to 870,000 tons of raspberries are produced worldwide every year. In Spain, more than 43,000 tons of raspberries were produced in 2018 [1]. Several components of raspberries have been found to provide potential health benefits, including cardiovascular, antihypertensive, antioxidant, antiproliferative, and antidiabetic effects [2,3]. These health effects are due to the synergistic combination of nutrients, minor compounds, and fibres that come from raspberries, which suggests the raspberry could be considered as a functional food [4].

Raspberries are an important source of bioactive compounds such as vitamin C, riboflavin, folic acid, niacin, magnesium, potassium, copper, and iron [2]. The high antioxidant capacity of raspberries is related to the presence of major groups of bioactive compounds such as ellagitannins and anthocyanins, while phenolic acids and flavonoids are the minor phenolic constituents [5]. In addition to their antioxidant capacity, anthocyanins are also responsible for the characteristic red color of the raspberry [6]. The main anthocyanins contained in raspberries are derived from cyanidins in non-acylated forms [7]. Among the ellagitannins, ellagic acid, as well as the anthocyanins, are of high interest due to their possible chemopreventive effects [8,9]. Raspberries also contain a high amount of

sugars and fibres, which are combined to form lignocellulose. Lignocellulose primarily contains lignin, hemicellulose, and cellulose, which are composed of sugars and acid sugars [10]. Pectins, which are composed of acid sugars, have been associated with important health benefits, i.e., preventing diabetes and cardiovascular diseases [11].

Besides the production and marketing of raspberries as fresh fruit, this sector includes the manufacture of derived products such as juices, jams, etc. These products are generally manufactured from a raspberry concentrate, which is industrially obtained by sieving raspberries several times with different mesh size (1.5 and 0.5 mm). During the process, the sieves retain a residual fraction, called residual raspberry extrudate (RE), which is composed of the fibres and achenes that contain most of the bioactive compounds found in raspberry fruit [12]. The achenes contain a wide variety of phenolic compounds with a high antioxidant capacity whereas the fibres are rich in other phenolic compounds [13–15].

Because RE is a by-product that contains bioactive compounds, its high added-value compounds such as phenols, sugars should be valorised instead of wasted. The product market for these compounds has already been envisaged, for example, phenolic compounds can be used as additives in foods to prevent oxidation [16], sugars can be used in fermentation and biorefinery processes [17–19], and pectins can be used as gelling or thickening agents [20].

As reported for other food industry wastes [21–23], hydrothermal treatments are one option for solubilizing and separating the raspberry extrudate into a solid and a liquid phase with high added-value compounds; however, optimal conditions for the thermal pre-treatment, as well as the recovery efficiencies that may be achieved, need to be determined.

Hydrothermal treatments at low temperatures (less than 150 °C) mainly induce the deflocculation of macromolecules with minimum solubilization of lignocellulosic matter [24]. Hydrothermal treatments at medium temperatures (around 150–180 °C) mostly affect hemicelluloses, and shortly thereafter, cellulose and lignin [25]. In severe hydrothermal treatments at high temperatures (more than 180 °C) and pressure conditions with rapid decompression, the cellulose and lignin are more affected than during treatments at medium temperatures. At the same time, treatments at high temperatures can convert part of the hemicellulose into smaller sugars, which can form undesirable compounds such as hydroxymethylfurfural (HMF), or they can enhance the formation of bioactive structures like phenolic glycosides or antioxidant soluble and insoluble fibres [21,26].

The main objective of this work was to evaluate the hydrothermal treatment using a wide range of temperatures (60–210 °C), to find the optimal conditions for the recovery of high added-value compounds such as phenols from raspberry extrudate.

## 2. Materials and Methods

### 2.1. Raspberry Extrudate

Raspberry extrudate is obtained from raspberry fruit, by using a twin-screw extruder with a 0.5 mm mesh and applying a heat treatment for enzymatic inactivation at 65 °C. Raspberry extrudate was collected in the HUDISA S.A factory, (Huelva, Spain) in 2018. After collection, it was immediately stored at −20 °C to prevent any possible fermentation or degradation.

### 2.2. Hydrothermal Treatments

Six hydrothermal treatments were carried out with increases in the temperature of thirty degrees. These hydrothermal treatments were classified into three temperatures ranges: low (60 °C, 90 °C, and 120 °C), medium (150 °C and 180 °C), and high (steam explosion, 210 °C and 16 kg/cm<sup>2</sup>).

Low-temperature hydrothermal treatments (60 °C, 90 °C, and 120 °C) were carried out by indirect heating at 60 and 90 °C in a laboratory stove (J.P. Selecta), and at 120 °C in an autoclave (Trade Raypa Steam Sterilizer). For the low-temperature hydrothermal treatments, 0.35 kg of raspberry extrudate

with 0.65 L of distilled water was added in a Pyrex bottle. The treated RE was centrifuged (Pacisa, Milan, Italy) at  $7155 \times g$  to obtain two phases: a solid phase (SP) and a liquid phase (LP).

Medium-temperature hydrothermal treatments ( $150\text{ }^{\circ}\text{C}$  and  $180\text{ }^{\circ}\text{C}$ ) were carried out with steam in a reactor of 100 L volume, which allow temperatures up to  $190\text{ }^{\circ}\text{C}$  to be obtained and a maximum pressure of 1.2 MPa. The raspberry extrudate was heated by direct steam injection, and indirectly by a heating jacket. Raspberry extrudate samples were processed at  $150\text{ }^{\circ}\text{C}$  (12.59 kg) and  $180\text{ }^{\circ}\text{C}$  (6 kg) in the reactor for 60 min.

Steam explosion treatment was carried out in a reactor at pilot-scale (Nusim, S.A., Madrid, Spain). The reactor was equipped with a stainless-steel deposit of 2 L of volume. The steam explosion reactor was filled with 250 g of raspberry extrudate, which was directly heated at a temperature of  $210\text{ }^{\circ}\text{C}$  with high-pressure saturated steam at  $16\text{ kg/cm}^2$  for a 5 min period. After the reaction time, an explosive decompression occurred from 2 L of reactor chamber up to 120 L of the expansion deposit. An automatic computing device monitored the time and the temperature in a pre-programmed manner. A centrifuge Pacisa 4226, Milan, Italy at  $7155 \times g/8000\text{ rpm}$  was used after the medium temperature and steam explosion treatments to separate the SP and LP [27].

### 2.3. Extraction of Water-Soluble Compounds

After the hydrothermal treatments, each LP was centrifuged again to eliminate solids in suspension and microfiltered with  $0.45\text{ }\mu\text{m}$  nylon microfilters. Then, a method that is extensively used for the analysis of compost samples and based on water extraction [28] was used to determine the amount of the soluble compounds in the RE and each SP. Distilled (160 g) water was added to 20 g of solid phase. After stirring for 24 h at room temperature, the mixture was centrifuged and microfiltered with the above-mentioned microfilters.

### 2.4. Analytical Methods

The determination of the pH, total solids (TS), mineral solids (MS), volatile solids (VS) chemical oxygen demand (COD), and soluble COD (sCOD) to characterize both phases was performed according to the guidelines of the Standard Methods of APHA [29]. Water-soluble sugars were analyzed by the Anthrone photometric method [30] and water-soluble acid sugars were determined by the m-hydroxydiphenyl method [31] using a spectrophotometer (BIO- RAD iMark Microplate Reader, California, USA). Water-soluble individual sugars were analyzed with and without initial TFA hydrolysis prior to reduction, acetylation, and analysis by gas chromatography (GC), using the method reported by Lama-Muñoz et al. [32]. The detection of HMF was accomplished by high-performance liquid chromatography (HPLC) as recently reported by Rodríguez-Gutiérrez et al. [21]. In order to extract phenolic compounds, 20 mL of methanol-water (v/v, 80/20) were added to 10 g of each solid and liquid phase and incubated for 1 h at  $70\text{ }^{\circ}\text{C}$  in a water bath. Then, it was microfiltered with  $0.45\text{ }\mu\text{m}$  nylon microfilters. The phenol concentration was analyzed by the Folin-Ciocalteu spectrophotometric method [33]. All the measurements were carried out at least in triplicate. The mean and standard deviation values were calculated for statistical analysis.

### 2.5. Individual Phenols

Individual phenolic compounds of the soluble RE and liquid phases obtained after the application of the hydrothermal treatments were analyzed following the method proposed by Gil et al. [34] and slightly amended by Pérez et al. [35]. Briefly, quantification of the phenolic compounds was performed in a Beckman Coulter high-performance liquid chromatography (HPLC) system equipped with a System Gold 168 detector, a solvent module 126 and a stainless steel Merck Superspher RP-18 ( $250 \times 4\text{ mm}$ ) column using acidified water (2.5% formic acid) (Solvent A) and acidified methanol (2.5% formic acid) (Solvent B) as mobile phases. The detection was performed at 280, 350 and 510 nm, and the sample injection loop was 20  $\mu\text{L}$ . The purification of the phenolics was carried out by a chromatographic

system under patent [27] using water as an eluent in the first step and ethanol/water (20:80 v/v) in the second step.

## 2.6. Antioxidant Determination

The antioxidant capacity was determined using two different methods, as previously described by Rodríguez et al. [36]. Reducing power ( $\text{FeCl}_3$ ) and antiradical activity (DPPH) were used to determine the reduction and scavenging capacity the free radicals, respectively.

## 2.7. Achenes

In order to quantify and characterise the achenes contained in RE and in each solid phase obtained after each hydrothermal treatment, 10 g of sample was taken and dried for 24 h in a laboratory oven. Then, the achenes were manually separated from the rest of the material and weighed.

# 3. Results and Discussion

## 3.1. Mass Distribution after Hydrothermal Treatments and Separation of Phases by Centrifugation

Table 1 shows the values for the RE mass after each hydrothermal treatment, the mass of the different obtained SP and LP after separation by centrifugation, the mass distribution between phases, and the volume of water added during each hydrothermal treatment.

**Table 1.** General data about the mass distribution after each hydrothermal treatment.

Temperature (°C)	Time (min)	Pressure (kg/cm <sup>2</sup> )	Initial RE Weight (kg)	Incorporated Water (kg)	Phases	Weight of Phases (kg)	Mass Distribution (%)
60	60	1.00	0.35	0.650	SP	0.28	29
					LP	0.68	71
90	60	1.00	0.35	0.650	SP	0.31	34
					LP	0.60	66
120	60	1.20	0.35	0.650	SP	0.31	34
					LP	0.59	66
150	60	5.00	12.59	36.90	SP	4.72	10
					LP	44.77	90
180	60	9.00	6.00	7.14	SP	2.47	19
					LP	10.67	81
210	5	16.00	0.25	2.19	SP	0.34	14
					LP	2.10	86

The volume of incorporated water depended on the hydrothermal treatment and on the characteristics of the reactor used in each case. For temperatures below 120 °C, 0.65 kg of water was incorporated. For temperatures above 120 °C the volume of water incorporated varied due to the need to keep a constant temperature inside the reactor during the hydrothermal treatment (Table 1). Ratios between initial RE weight and incorporated water at temperatures equal and above 120 °C were: 1:1.86 at 120 °C, 1:2.93 at 150 °C, 1:1.19 at 180 °C and 1:8.76 in weight at 210 °C. According to these ratio values, a higher amount of water was introduced in the treatment at 210 °C compared to the other hydrothermal treatments, even though the hydrothermal treatment at 210 °C only lasted 5 min, and not 60 min as occurred with all other hydrothermal treatments. After the hydrothermal treatment and separation of phases by centrifugation, in all cases, most of the mass moved to the LP (Table 1). However, the separation was more effective for the phases obtained at temperatures higher than 120 °C, where the mass percentage of the LP varied from 81% to 90%. On the contrary, LP obtained from hydrothermal treatments with temperatures below 120 °C had lower mass percentages, with values around 70% (Table 1). This difference in the mass distribution may be related to the severity of each hydrothermal treatment, which affects the RE cell wall structures to a different degree, allowing a higher or lower degree of solubilization. In fact, it has been reported that hydrothermal treatments at

temperatures higher than 150 °C can affect lignocellulosic structures, mainly the hemicellulose, and to a lesser extent, the cellulose [25]. On the other hand, Serrano et al. [37] reported that hydrothermal treatments at 65 °C for 1 h had a deflocculating effect on olive mill solid waste, rather than the breakdown of the lignocellulosic fibres.

### *3.2. Characterization of the Untreated Raspberry Extrudate and the Solid and Liquid Phases Obtained after Each Hydrothermal Treatment*

The physicochemical characteristics of untreated RE and the different phases obtained for each hydrothermal treatment after separation by centrifugation are shown in Table 2. After the hydrothermal treatment, the pH values of each phase slightly increased with respect to the untreated RE, although all the phases presented an acid pH, i.e., lower than 5. The increase in pH values may be caused by the incorporation of water during direct heating (Table 2). This addition of water, as well as the ability of the cell material to keep water [21], increased the moisture in the substrates after each hydrothermal treatment. The highest moisture content in the SP was achieved after the steam explosion treatment (210 °C, 5 min, 16 kg/cm<sup>2</sup>), probably due to the high proportion of water added with respect to the initial substrate, i.e., 8.76 L of water per kg of RE, which was several times higher than the water added in the other hydrothermal treatments (Table 1).

In all hydrothermal treatments, it was observed that around 98% of the TS were VS, and the highest amount of VS was retained in the SP, i.e., only about 15% of VS remained in the LP (Table 2). With regard to the organic matter, the measured sCOD/COD ratio indicated that the greatest part of the organic matter was not initially in soluble form, i.e., the sCOD/COD ratio in the untreated RE was 0.09 (Table 2). After the hydrothermal treatments, the solubilization of part of the organic matter resulted in an increase in the sCOD/COD ratio in the sum of SP and LP with values up to approximately 0.13 (Table 2). Although these ratios indicate a difference of around 44% in respect to the untreated RE, most of the COD was not susceptible to being solubilized and remained in a non-soluble form (Table 2). With regard to the distribution of the soluble organic matter in the phases obtained after the thermal treatments, the values of the sCOD/COD ratio in the SP ranged from 0.01 to 0.06, indicating that most of the soluble organic matter was moved to the LP, where the sCOD/COD ratio values were higher than 0.90 in all cases (Table 2).

The organic matter contained in different phases obtained after the hydrothermal treatment, contains high added-value compounds that can be recovered with these treatments as well as other organic compounds that could be extracted by other techniques such as anaerobic digestion, as was recently proposed by Trujillo-Reyes et al. [38].

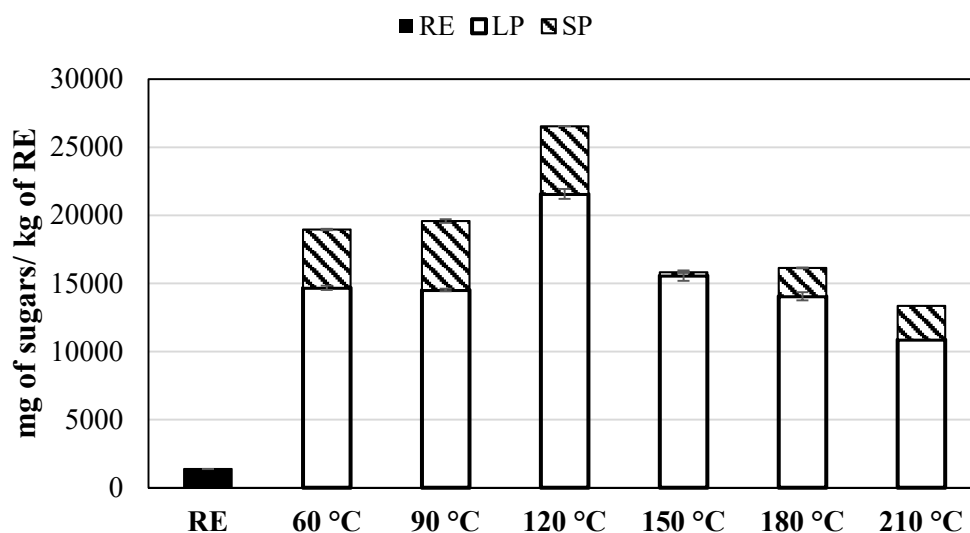
**Table 2.** Physicochemical characterization of untreated raspberry extrudate and different solid and liquid phases obtained by centrifugation after all hydrothermal treatments were carried out: untreated raspberry extrudate (RE), solid phase (SP), liquid phase (LP), chemical oxygen demand (COD), soluble COD (sCOD), total solids (TS), mineral solids (MS), volatile solids (VS) and moisture (Moist.).

Treatment	Phase	pH	TS (mg/kg RE)	MS (mg/kg RE)	VS (mg/kg RE)	%Moist.	COD (mg/kg RE)	sCOD (mg/kg RE)	sCOD/COD ratio	sCOD/COD Ratio of SP + LP
RE		3.3 ± 0.1	232,798 ± 3921	4012 ± 34	228,786 ± 3955	76.7 ± 1.3	328,097 ± 918	29,640 ± 585	0.09	0.09
60 °C, 60 min	SP	3.8 ± 0.1	147,388 ± 5041	2274 ± 237	145,223 ± 4836	81.4 ± 2.8	235,793 ± 4598	11,006 ± 193	0.05	0.14
	LP	3.4 ± 0.1	23,964 ± 971	223 ± 49	23,741 ± 1.021	98.8 ± 4.0	26,609 ± 708	25,451 ± 450	0.96	
90 °C, 60 min	SP	4.0 ± 0.1	233,531 ± 6936	3393 ± 455	230,138 ± 6582	73.3 ± 2.2	286,948 ± 10,904	15,822 ± 328	0.06	0.13
	LP	3.5 ± 0.1	23,863 ± 351	627 ± 410	23,236 ± 60	98.6 ± 1.4	28,762 ± 446	26,146 ± 115	0.91	
120 °C, 60 min	SP	3.9 ± 0.1	180,313 ± 5536	2085 ± 83	178,228 ± 5614	79.3 ± 2.4	294,066 ± 8851	15,010 ± 572	0.05	0.15
	LP	3.3 ± 0.1	29,019 ± 1297	986 ± 202	28,033 ± 1099	98.3 ± 4.4	34,546 ± 1112	33,191 ± 684	0.96	
150 °C, 60 min	SP	4.6 ± 0.1	182,312 ± 3519	1876 ± 97	180,436 ± 3555	51.4 ± 1.0	230,917 ± 7066	2896 ± 125	0.01	0.14
	LP	3.5 ± 0.1	32,088 ± 247	1396 ± 341	30,692 ± 95	99.1 ± 0.8	36,996 ± 518	34,645 ± 364	0.94	
180 °C, 60 min	SP	3.9 ± 0.1	157,138 ± 534	1136 ± 16	156,002 ± 515	61.8 ± 0.2	216,745 ± 7694	7612 ± 109	0.04	0.11
	LP	3.4 ± 0.1	36,891 ± 92	2349 ± 145	34,542 ± 238	97.9 ± 0.2	47,075 ± 330	21,887 ± 85	0.97	
210 °C, 5 min	SP	4.2 ± 0.1	147,245 ± 1664	703 ± 96	146,489 ± 1699	89.3 ± 1.0	291,142 ± 13,393	10,932 ± 459	0.04	0.14
	LP	4.0 ± 0.1	37,970 ± 638	1391 ± 531	36,554 ± 110	99.5 ± 1.7	51,777 ± 161	36,133 ± 325	0.74	

### 3.3. Solubilization of Sugars after the Application of the Hydrothermal Treatments

#### 3.3.1. Water-Soluble Sugars

Figure 1 shows the concentrations of water-soluble sugars at 25 °C, which were quantified and expressed as mg D-glucose equivalent per kilogram of RE, from the RE, SP, and LP after each hydrothermal treatment. In all cases, sugar concentrations were increased after hydrothermal treatments compared to the untreated RE (Figure 1). RE contained a low amount of water-soluble sugars, which could be because during the processing of the raspberries to obtain the raspberry concentrate, most of the sugars were displaced to this concentrate. The application of the different hydrothermal treatments increased the concentration of the water-soluble sugars by several times compared to the untreated RE because the sugars were mainly displaced to the LPs (Figure 1). This increase in sugars could be because hydrothermal treatments favor the solubilization and alteration of the cell wall material contained in RE to lower polymerized oligosaccharides and monosaccharides such as water-soluble sugars [26]. The maximum concentration of water-soluble sugars was achieved in the LP after the hydrothermal treatment at 120 °C, where it obtained a value around 15 times higher than that of the untreated RE, i.e.,  $21,571 \pm 93$  mg/kg RE and  $1378 \pm 10$  mg/kg RE, respectively (Figure 1). The increase in the concentration with respect to the untreated RE may be due to the solubilization of sugars from lignocellulosic matter during the hydrothermal treatments [25,39,40]. However, for temperatures above 120 °C, it is likely that sugars were degraded into other compounds such as hydroxymethylfurfural (HMF), which could explain the decrease in the concentration with respect to the maximum achieved at 120 °C [21,26]. The decrease in the concentration of soluble sugar with the increase in the temperature of the hydrothermal treatments was previously reported for strawberry extrudate by Rodríguez-Gutiérrez et al. [21], who reported a decrease from 50 to 18 g/kg of strawberry extrudate when the treatment temperature increased from 150 °C to 200 °C.



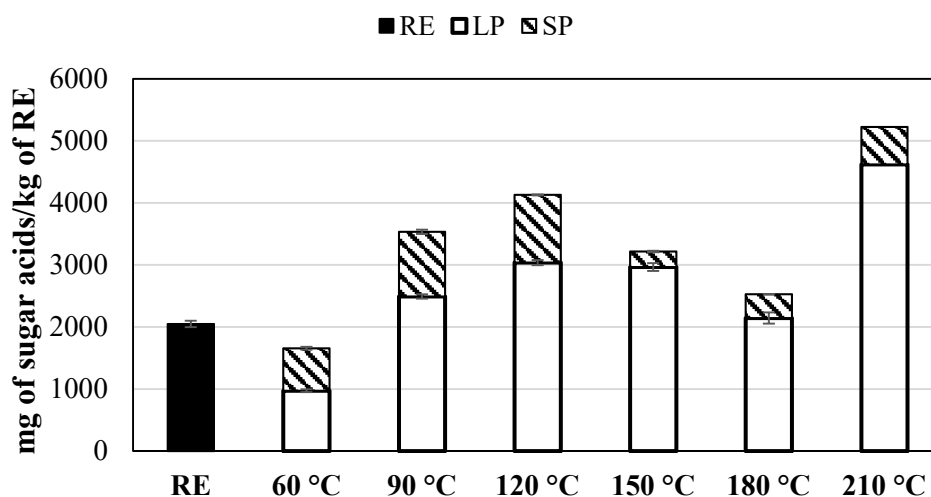
**Figure 1.** Milligrams of sugars (D-glucose equivalents) per kilogram of extrudate for untreated raspberry extrudate (RE) and different fractions obtained after the hydrothermal treatments.

#### 3.3.2. Water-Soluble Acid Sugars

Figure 2 shows the concentration of water-soluble acid sugars at 25 °C, which are expressed as mg of galacturonic acid equivalents per kilogram of RE for the RE, the SP, and the LP after each hydrothermal treatment. After the different hydrothermal treatments, the concentration of acid sugars increased with respect to the untreated RE in all cases, except at 60 °C (Figure 2). Generally, the concentration of acid sugars increased as temperatures increased, except for the hydrothermal treatments at 150 and 180 °C. The highest concentration of water-soluble acid sugars was obtained



with the hydrothermal treatment at 210 °C, with a concentration in the LP that was more than twice the acid sugars in the untreated RE, i.e.,  $4620 \pm 91$  mg/L and  $2084 \pm 51$  mg/L, respectively. It has been reported that an increase in acid sugars is a consequence of the solubilization of higher molecules such as hemicelluloses and pectins [21,41]. Besides, with regard to the LP, there was an inflection point at 120 °C (Figure 2). As described for soluble sugars, the concentration of acid sugars in LP was higher than that found in the SP after each hydrothermal treatment (Figure 2).



**Figure 2.** Sugar acids expressed as grams of galacturonic acid per kilogram of extrudate for untreated RE and different fractions obtained after the hydrothermal treatments.

### 3.3.3. Individual Water-Soluble Sugars

Table 3 shows the glycoside composition of the soluble sugars that comes from the cell wall material and the HMF concentration, which are expressed as milligrams or grams of each individual sugar per kilogram of RE. Untreated RE contained a lower concentration of each individual compound than the thermally treated RE, with the same trend described for water-soluble sugars as shown in Figure 1. In all cases, glucose was the main sugar in the LPs after each hydrothermal treatment, followed by mannose, which was taken as the sum of mannose and mannitol (Table 3). The higher concentration of monosaccharides with respect to the oligosaccharides after the thermal treatments indicates that the treatment conditions favored the hydrolysis of the hemicellulosic and cellulosic structures of the RE.

The application of high temperatures to lignocellulosic substrates has been reported to favor the degradation of sugars into furanic compounds such as the HMF [21,25]. HMF was only found in the LP of hydrothermal treatments performed at temperatures higher than 120 °C (Table 3), which is in line with the increasing severity of the hydrothermal treatments [42]. The higher concentration of HMF was determined after the hydrothermal treatment carried out at 180 °C, i.e.,  $6901 \pm 10$  mg/kg RE (Table 3). The formation of HMF may be of interest since it has been found that it can be used to impede the oxidation of edible oils, thus improving the commercial life of sunflower oils by up to four times [21,43]. However, the formation of HMF could also result in some limitations in the subsequent bioprocessing of the treated RE due to its widely reported microbial inhibitory effect [25].

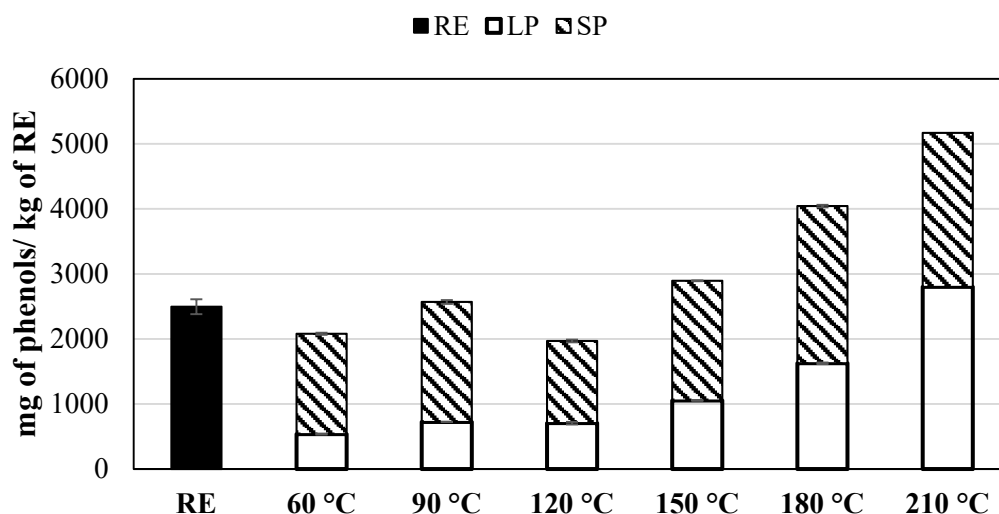


**Table 3.** Glycoside composition (g sugar/kg of raspberry extrudate), total monosaccharides (Total MS) and total oligosaccharides (Total OS) and hydroxymethylfurfural (HMF, mg/kg of raspberry extrudate) of untreated raspberry extrudate (RE), solid phase (SP), liquid phase (LP) and de-phenolized liquid phase (DLP). The analyzed sugars are Rhamnose (Rha), Fucose (Fuc), Arabinose (Ara), Xylose (Xyl), Mannose (Man), Galactose (Gal) and Glucose (Glu). n.d. non-detected.

Treatment	Phase	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	Total OS	Total MS	HMF
RE		0.07 ± 0.00	n.d	0.25 ± 0.00	0.29 ± 0.00	0.11 ± 0.01	0.07 ± 0.00	0.15 ± 0.00	0.98 ± 0.02	0.93 ± 0.01	n.d
60 °C, 60 min	SP	0.17 ± 0.00	n.d	0.60 ± 0.05	0.46 ± 0.01	1.49 ± 0.16	n.d	4.09 ± 0.04	3.25 ± 0.18	6.81 ± 0.17	n.d
	LP	0.07 ± 0.00	n.d	0.26 ± 0.01	0.28 ± 0.01	3.16 ± 0.20	n.d	10.17 ± 0.79	8.01 ± 0.91	13.99 ± 0.81	n.d
90 °C, 60 min	SP	0.22 ± 0.03	n.d	0.35 ± 0.50	0.54 ± 0.02	1.51 ± 0.30	n.d	4.24 ± 0.50	3.53 ± 1.23	6.85 ± 0.77	n.d
	LP	0.08 ± 0.00	n.d	0.25 ± 0.02	0.30 ± 0.00	3.60 ± 0.03	n.d	11.70 ± 0.01	10.60 ± 0.13	15.98 ± 0.04	n.d
120 °C, 60 min	SP	0.23 ± 0.02	n.d	0.77 ± 0.00	0.58 ± 0.03	1.58 ± 0.15	n.d	4.56 ± 0.47	4.19 ± 0.58	7.77 ± 0.50	n.d
	LP	0.12 ± 0.00	n.d	0.59 ± 0.01	0.38 ± 0.01	3.44 ± 0.08	n.d	11.30 ± 0.39	9.89 ± 0.43	15.91 ± 0.40	176 ± 10
150 °C, 60 min	SP	0.01 ± 0.00	n.d	0.07 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	n.d	0.06 ± 0.00	0.31 ± 0.05	0.20 ± 0.00	n.d
	LP	0.09 ± 0.00	n.d	0.73 ± 0.00	0.35 ± 0.00	3.13 ± 0.08	0.13 ± 0.00	10.78 ± 0.44	8.84 ± 0.46	15.21 ± 0.45	864 ± 10
180 °C, 60 min	SP	0.10 ± 0.00	n.d	0.37 ± 0.02	0.27 ± 0.03	0.23 ± 0.04	n.d	0.71 ± 0.14	0.58 ± 0.44	1.73 ± 0.15	n.d
	LP	0.09 ± 0.00	n.d	0.97 ± 0.01	0.95 ± 0.01	1.66 ± 0.01	0.43 ± 0.01	6.39 ± 0.07	5.74 ± 0.16	10.49 ± 0.07	6901 ± 10
210 °C, 5 min	SP	0.12 ± 0.01	n.d	0.49 ± 0.03	0.35 ± 0.02	0.52 ± 0.03	n.d	1.23 ± 0.02	1.35 ± 0.20	2.75 ± 0.05	n.d
	LP	0.07 ± 0.00	n.d	0.73 ± 0.02	0.43 ± 0.02	1.46 ± 0.05	0.17 ± 0.01	4.88 ± 0.15	5.06 ± 0.61	7.74 ± 0.16	518 ± 10

### 3.4. Solubilization of Phenols after the Application of Hydrothermal Treatments

Figure 3 shows the influence of temperature on the phenols extracted in RE, as well as in the different LP and SP obtained after each hydrothermal treatment. As can be seen, two different trends were detected: at temperatures below 120 °C, there is a slight decrease in the concentration of phenolic compounds at increasing temperatures, which may be due to the degradation of thermosensitive compounds [44]. At temperatures above 120 °C, the structure of the RE began to degrade, releasing the phenolic compounds retained in the lignocellulosic structures. The maximum concentration of phenols was achieved after the steam explosion treatment at 210 °C, which was around twice that determined for the untreated RE, i.e.,  $5169 \pm 33$  mg of gallic acid/kg RE and  $2497 \pm 115$  mg of gallic acid/kg RE, respectively (Figure 3). The high concentration of phenols found after the steam explosion treatment could be a consequence of the high severity of the treatment compared to conventional hydrothermal treatments. Steam explosion treatments affect most of the hemicelluloses and celluloses and, even, part of the lignin thanks to the fast pressure reduction, whereas conventional hydrothermal treatments only degrade hemicellulose, and partially degrade the cellulose [45,46]. On the other hand, it was observed that the concentration of phenols in most SPs was higher than those contained in LPs after the hydrothermal treatments. A positive trend in the solubilization of phenols in the LP was observed with the increase in the temperature (Figure 3), similar to that reported in other studies on the treatment of strawberry extrudate [21]. Despite the solubilization of the phenols, the SP had a higher concentration of phenols than the LP after all of the hydrothermal treatments, except for 210 °C, which could be a consequence of the high phenolic content of the achenes that remained in the SP [47].



**Figure 3.** Phenols expressed as milligrams of gallic acid equivalents per kilogram of extrudate for untreated RE and different fractions obtained after the hydrothermal treatments.

Table 4 shows the concentrations of individual anthocyanins and phenolic compounds in the LP after each hydrothermal treatment. As can be seen, the total content of anthocyanins, which corresponds to the sum of each individual anthocyanin, was 26.79 and 19.26 mg/kg RE, after treatments carried out at 60 °C and 90 °C, respectively. However, total anthocyanins were not found at temperatures above 90 °C. The total content of anthocyanins obtained after the application of hydrothermal treatments were very low compared to those obtained for fresh red raspberry fruit, 200–1000 mg/kg fresh fruit [2]. As shown in other studies, anthocyanins are thermosensitive compounds, and therefore, they are susceptible to thermal degradation [48]. This explains the absence of these compounds at temperatures equal to and higher than 120 °C. Otherwise, hydrothermal treatments at 120, 150, 180, and 210 °C resulted in the release of ellagic acid and ellagic acid derivatives (Table 4). The quantified free ellagic acid content was similar to that reported in other studies for fresh red raspberry fruit, i.e., 1–10 mg/kg

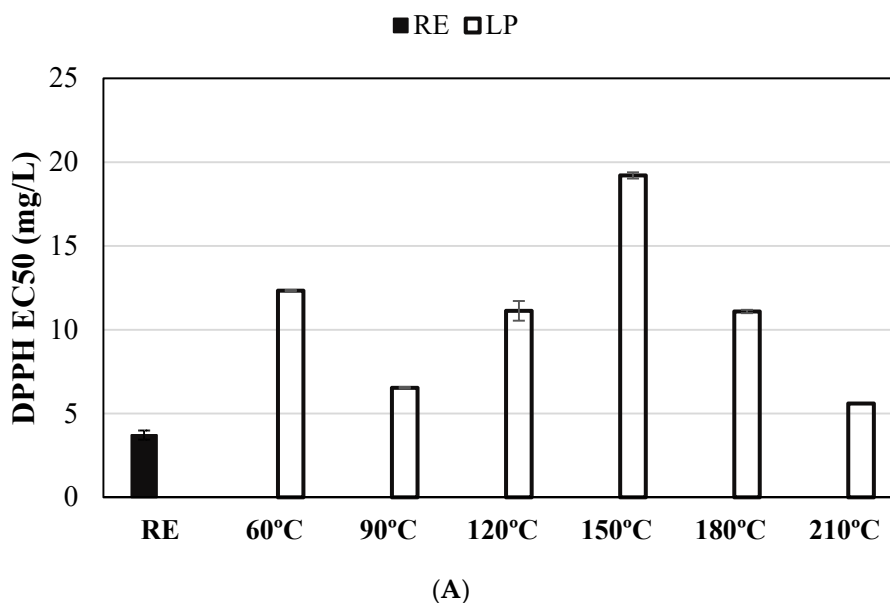
fresh fruit [7,8,49,50]. However, a markedly higher concentration of ellagic acid derivative, probably an oxidized derivative, was determined after these hydrothermal treatments, with the concentration increasing with increasing treatment temperatures (Table 4).

**Table 4.** Concentration of individual phenolic compounds obtained after each hydrothermal treatment.

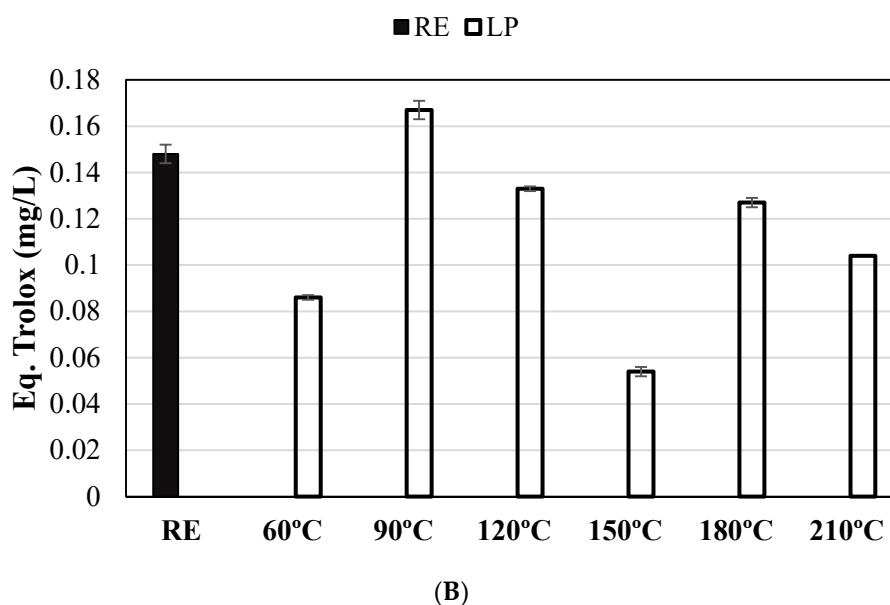
Concentration (mg/kg)	60 °C, 60 min	90 °C, 60 min	120 °C, 60 min	150 °C, 60 min	180 °C, 60 min	210 °C, 5 min
Cyanidin-3-glucoside	20.87	17.17	-	-	-	-
Other anthocyanins	1.53	2.09	-	-	-	-
Quercetin-3-glucoside (350 nm)	4.39	-	-	-	-	-
Ellagic Acid Free (350 nm)	-	-	1.56	0.78	0.71	0.00
Ellagic Acid derivative (RT: 16.7 min)	-	-	27.52	29.87	77.20	85.98

### 3.5. Antioxidant Determination

The antioxidant power of each LP obtained after hydrothermal treatments was determined by the antiradical activity and the power to reduce free radicals. All values for DPPH EC<sub>50</sub> in the LP obtained after each hydrothermal treatment were higher than the value of RE, with the lowest value for the hydrothermal treatment at 210 °C (Figure 4A). This indicates a loss of antioxidant capacity in the treated fractions with respect to the untreated RE. This could be a consequence of the degradation of highly antioxidant thermosensitive compounds, such as the anthocyanins during the hydrothermal treatments [48]. Similarly, most of the fractions showed a lower reducing capacity, expressed as Trolox equivalents in mg/mL, than the untreated RE (Figure 4B). Only the liquid fraction at 90 °C showed higher reducing activity than the RE, i.e.,  $0.167 \pm 0.001$  and  $0.148 \pm 0.004$  mg/mL, respectively.



**Figure 4.** Cont.



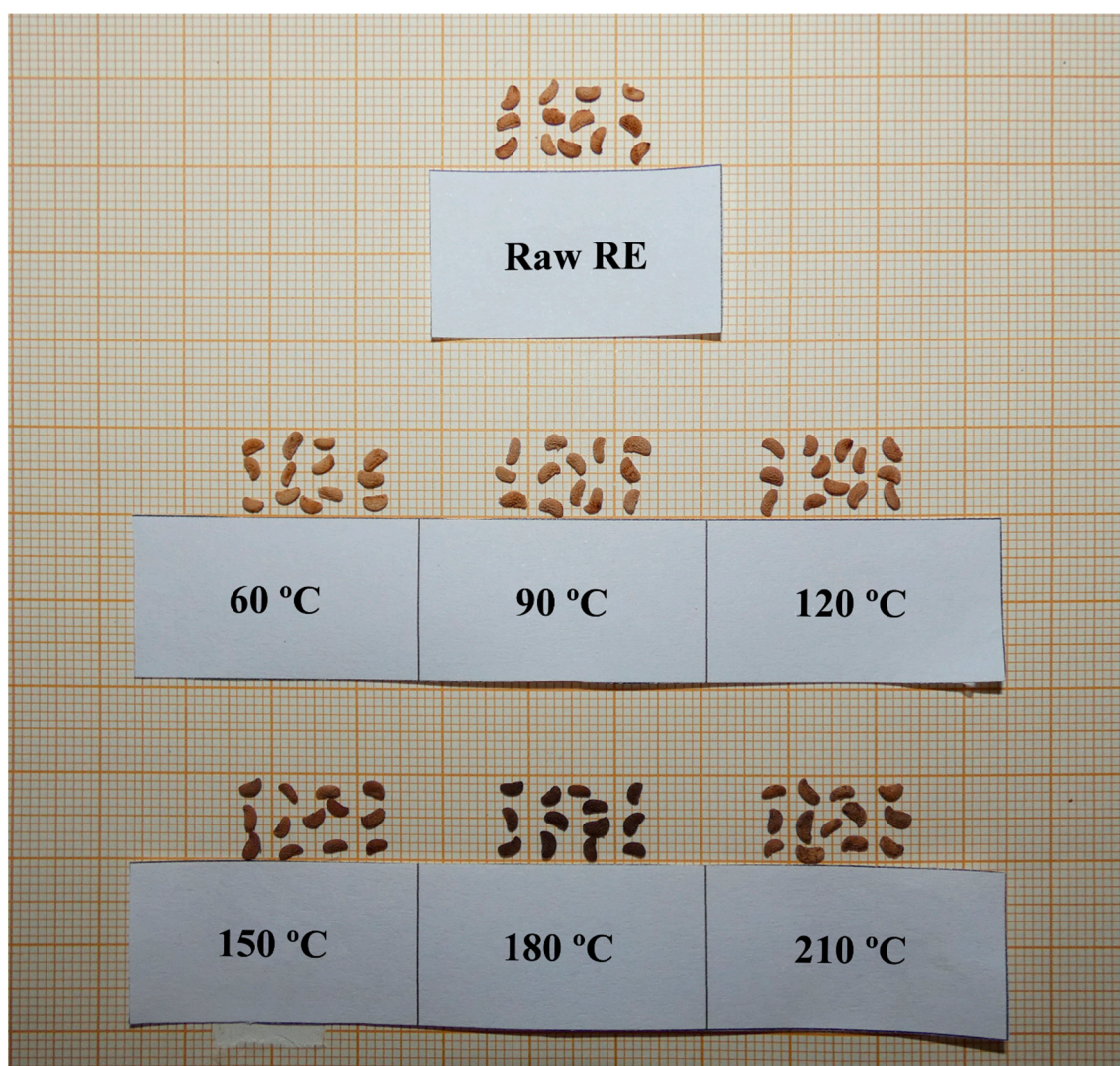
**Figure 4.** Antioxidant capacity determined by antiradical activity (DPPH) (A) and reducing power (B) methods of extrudate for untreated RE and each LP obtained after hydrothermal pre-treatments.

### 3.6. Achenes

The hydrothermal treatments in the studied range did not affect the form and size of the achenes, although a darkening of the achenes was detected at temperatures higher than 120 °C (Figure 5). Achenes from hydrothermal treatment at 180 °C were the darkest, which is probably related to the high severity of the treatment and the high concentration of HMF generated at this temperature (Table 3). Table 5 shows the percentages of achenes in dry weight that were quantified in untreated RE and the different SPs, obtained after each hydrothermal treatment. The percentage of achenes in RE was 89%, while the percentage of achenes after each hydrothermal treatment was more than 92%. The treatment at 180 °C had the highest percentage, with a value of 97% (Table 5). These results show that hydrothermal treatments are not severe enough to break the achenes despite their high presence in the RE. Therefore, further research could consider the implementation of another type of treatments to break the achenes to facilitate the release of the high-added value compounds that are present in the achenes [15].

**Table 5.** Quantification of achenes present in untreated RE and in the different SPs obtained after each hydrothermal treatment (percentage of achenes in dry weight).

Treatment	RE	60 °C, 60 min	90 °C, 60 min	120 °C, 60 min	150 °C, 60 min	180 °C, 60 min	210 °C, 5 min
Achenes (% dry weight)	89.5 ± 1.4	94.1 ± 0.6	95.0 ± 1.2	94.8 ± 1.0	94.5 ± 1.1	97.2 ± 0.8	92.3 ± 1.0



**Figure 5.** Achenes present in the untreated RE and in the different SPs obtained after each hydrothermal treatment (a graph paper with a 1 mm scale has been used as a photographic background).

#### 4. Conclusions

The solubilization of phenols and sugars, and their concentration and composition, from raspberry extrudate is highly dependent on the operational conditions of the applied hydrothermal treatments. Higher temperatures usually result in higher solubilization, as well as better separation of the treated raspberries in the solid and liquid phases. The maximum concentration of water-soluble sugars was achieved in the LP after the hydrothermal treatment at 120 °C, where it was around 15 times higher than that of the untreated RE. Otherwise, the hydrothermal treatment at 210 °C for 5 min and the subsequent decompression resulted in the highest concentration of water-soluble acid sugars. The solubilization of phenolic compounds showed a positive trend with respect to the hydrothermal treatment temperature, reaching a high value of  $5169 \pm 33$  mg of gallic acid/kg RE after the hydrothermal treatment at 210 °C. However, an increase in the hydrothermal treatment temperature above 90 °C resulted in the decomposition of thermosensitive compounds such as anthocyanins. The hydrothermal treatment at 210 °C was considered as optimal because of its high solubilization capacity, with the additional advantage of lower generation of undesirable HMF compared to the other assessed conventional hydrothermal treatments.



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