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THERMALLY-TREATED STRAWBERRY EXTRUDATE: A RICH SOURCE OF ANTIOXIDANT PHENOLS AND SUGARS.

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Abstract

Strawberries have antioxidant, cardiovascular, and antiproliferative properties. The agroindustrial production of strawberry concentrate generates a food waste after extrudation that is usually landfilled. This strawberry extrudate is a rich source of valuable bioactive compounds such as phenols and sugars. In the present study, industrial thermal treatments currently in use for the valorization of other agricultural wastes were determined to be suitable for the treatment of strawberry extrudate. Thermal treatment conditions in the range of 90 ºC to 200 ºC were studied. Thermal treatment at 150 ºC for 60 minutes without acid addition was the most efficient process based on the solubilization of sugars and phenols as well as the antioxidant capacity of the liquid phase produced. Instead of sending this residual fraction to landfill, such treatment would permit the use of strawberry extrudate as a source for the recovery of valuable bioactive compounds.

Keywords:

Strawberry extrudate, thermal treatments, antioxidant, phenol, sugar, valorization.

1. INTRODUCTION

The global strawberry industry produced 11 million tons of strawberries in 2014. The United States is the world's major producer with 12.2 % of world production, followed by Mexico, Turkey, and Spain (FAO, 2017). The strawberry is a popular seasonal fruit due to its unique flavour and aroma. The strawberry is considered a functional food that offers multiple health benefits, including antioxidant, cardiovascular, antihypertensive, and antiproliferative effects (Basu, Nguyen, Betts, & Lyons, 2014). These health effects are due to strawberry's unique combination of nutrients, phytochemicals, and fibres, which play a synergistic role in characterizing strawberries as a functional food (Basu et al., 2014). Moreover, strawberries are an important source of B-group vitamins, vitamin C, vitamin E, potassium, folic acid, carotenoids, and specific flavonoids, such as pelargonidin, quercetin and catechin (Giampieri, Tulipani, Alvarez-Suarez, Quiles, Mezzetti, & Battino, 2012) or the tiliroside that is a glycosidic flavonoid and possesses anti-inflammatory, antioxidant, anticarcinogenic and hepatoprotective activities (Goto et al., 2012).

The high antioxidant capacity of strawberries is mainly due to the presence of ascorbic acid, ellagitannins and anthocyanins (Basu et al., 2014). Anthocyanins give the fruit its characteristic red color. Anthocyanins present in the strawberry have been investigated and identified as cyanidin and pelargonidin glycosides (Cerezo, Cuevas, Winterhalter, Garcia-Parrilla, & Troncoso, 2010), and pelargonidin-3-glucoside is the main anthocyanin in the strawberry (Cerezo et al., 2010). Other polyphenols, such as glucosides and glucuronides of quercetin and kaempferol, are also present (Cerezo et al., 2010; Cruz-Atonio, Saucedo-Pompa, Martinez-Vázquez, Aguilera, Rodríguez, &Aguilar, 2010). There is particular interest in determining strawberries' ellagic acid

content due to its possible chemopreventive effects (da Silva Pinto, Lajolo, & Genovese, 2008). This compound may exist in a free form, as a glycoside, or bound as glucose esterified ellagitannins, like agrimonin as a ellagitannin dimer (Maas, Galletta, & Stoner, 1991). It is also remarkable the content of ellagitanins with antioxidant and cancer chemopreventive activities that might contribute to health benefits in humans (Cerdá, Tomás-Barberán, & Espín, 2005). Other important group of phenols with bioactives properties are the tannins, divided into condensed tannins (proanthocyanidins) and hydrolyzable tannins (Skrovankova, Sumczynski, Mlcek, Jurikova, & Sochor, 2015).

In addition to strawberry production and commercialization, the strawberry sector includes the manufacture of derived products. For example, around 21% of the total production of strawberries is used for the elaboration of products such as yogurt, juices, jams, etc. (Serrano, 2015). These products are generally elaborated from a strawberry concentrate. During the industrial process to obtain the strawberry concentrate, strawberries are extruded by several sieves with different mesh sizes. The residual fraction formed of the fibrous part and the achenes is retained in the sieves and named strawberry extrudate. Strawberry extrudate represents 7% of the weight of processed strawberries (Serrano, Siles, Chica, & Martin, 2014). The strawberry extrudate contains most of the beneficial components found in the whole strawberry. Therefore, an interesting revaluation and management option, instead of sending this residual fraction to landfill as in current practice, would be the recovery of bioactive compounds with a high economic interest from strawberry extrudate. Extracts rich in bioactive compounds could be used in a wide range of novel applications because of their proven health effects on long-term consumption. The phenols present in strawberry, and therefore in the strawberry extrudate, have been widely studied and their remarkable antioxidant

properties (Banerjee, Singh, Vijayaraghavan, MacFarlane, Patti, & Arora, 2017) make them suitable for use as additives in food formulation to prevent oxidation (Balasundram, Sundram, & Samman, 2006). Thus, their extraction would not only increase the economic revalorization of this waste but also permit the further degradation of the remaining waste since phenolic compounds have been reported as potential inhibitors of downstream digestive bioprocesses (Borja, Alba, & Banks, 1997; Chen, Cheng, & Creamer, 2008).

The aim of this study was to evaluate different thermal treatment conditions in order to maximize the recovery of bioactive compounds with a high economic interest from strawberry extrudate. The temperature, pressure, time and addition of reagents for each condition used were chosen based on the industrial thermal treatments widely in use for other agroindustrial products or by-products. An organic extraction with ethanol, commonly used in the food industry, has been used as a control. All the conditions tested in the present study have been previously tested for the recovery of added valuable compounds from olive oil waste, secondary date varieties, asparagus waste, or cocoa husk (Rubio-Senent, Rodríguez-Gutíerrez, Lama-Muñoz, & Fernández-Bolaños, 2012; Mrabet, Jiménez-Araujo, Fernández-Bolaños, Rubio-Senent, Lama-Muñoz, Sindic, & Rodríguez-Gutiérrez, 2016; Fuentes-Alventosa et al., 2012; Hernández-Hernández, Viera-Alcaide, Morales-Sillero, Fernández-Bolaños, & Rodríguez-Gutiérrez, 2017).

The thermal treatment consists of an autohydrolytic that results in the solubilization of the strawberry extrudate (Fernández-Bolaños, Rodríguez, Lama, & Sánchez, 2010). Autohydrolysis takes place when acetic acid from acetyl groups is formed because of the high temperatures (Jönsson, & Martín, 2016). As mentioned, this type of treatment has already been applied to the valorization of other organic waste such as olive mill

solid waste (Rubio-Senent et al., 2012). Among the bioactive compounds extracted from olive mill solid waste are polyphenols, which can be used as preserving agents and/or antioxidants due to their ability to eliminate free radicals and prevent oxidation reactions in food (Banerjee et al., 2017).

In the present study, thermal treatments were tested which was shown to favour the disruption of fibrous material (Fernández-Bolaños et al., 2004). Low-temperature thermal treatments mainly induce the de-flocculation of macromolecules with minimal solubilization of the lignocellulosic matter (Jain, Jain, Wolf, Lee, & Tong, 2015); whereas high-temperature thermal treatments (150 ºC–180 °C) mainly induce the solubilization of the lignocellulosic matter, first the hemicelluloses and shortly after the cellulose and lignin (Hendriks & Zeeman, 2009). For steam explosion treatments, after applying a high pressure and temperature, strawberry extrudate is exposed to atmospheric pressure by a quick-opening ball valve, which makes the material undergo an explosive decompression in an expansion chamber. The temperature, pressure, and time of steam explosion treatments range between 160–260° C, 0.69–4.83 MPa, and from several seconds to a few minutes (Fernández-Bolaños et al., 2004). Under steam explosion conditions, hemicellulose is hydrolysed into its component sugars, and lignin is highly degraded (Fernández-Bolaños et al., 2004).

2. MATERIALS AND METHODS

2.1. Strawberry extrudate

The strawberry extrudate obtaining from strawberry fruit by twin-screw extruder in could conditions (below 6 ºC) was collected in HUDISA S.A, (Huelva, Spain) in 2017 season and immediately stored at -20 ºC to avoid any fermentation or degradation.

2.2. Thermal treatments

Low, medium and high severities of treatment were tested with and without the addition of an acid catalyst (0.5% glacial acetic acid) by duplicate:

Low-temperature thermal treatments (90 ºC–120 °C) were carried out by heating in a laboratory stove and autoclaving at 90 ºC and 120 ºC, respectively. For the 90 ºC treatment, 0.4 kg of strawberry extrudate with 0.74 L of distilled water were introduced into a Pyrex bottle and kept at 90 ºC for 90 minutes in a laboratory stove (J.P. Selecta*)*. For the 120 ºC treatment, 0.3 kg of sample with 0.3 L of distilled water were introduced into a Pyrex bottle and kept in an autoclave (Trade Raypa Steam Sterilizer) at 120 ºC for 60 minutes. After each treatment, the wet solid was centrifuged (Pacisa, Milan, Italy) at 7155 g for 10 min to separate the solid and liquid phases.

High-temperature thermal treatments were carried out using a steam treatment reactor with 100 L of capacity, which can reach temperatures up to 190 °C and a maximum pressure of 1.2 MPa. Heating of the strawberry extrudate was performed by direct steam injection. Extrudate samples (6 kg) were treated at 150 ºC and 170 ºC in the reactor for 60 minutes. After the treatment period, the sample was cooled to 50 °C and then centrifuged at 4700 g/1450 rpm (Comteifa, S. L., Barcelona, Spain) to separate the liquid and solid phases.

Steam explosion treatments were performed in a pilot-scale reactor (Nusim, S.A., Madrid, Spain). The reactor is equipped with a stainless steel deposit with 2 L of capacity. The steam explosion reactor was loaded with 250 g of strawberry extrudate which was heated at a temperature of 180 ºC–240 °C with high-pressure saturated steam (with a corresponding pressure of 0.78–4.27 MPa) for 2 and 5 min. An electronic computing device controls the time and the temperature in a pre-programmed manner. After each treatment, the wet solid was filtered in a Buchner funnel using Whatman

144 filter paper discs to separate the solid and liquid phases. A concentration of 0.5% glacial
145 acetic acid was used in each thermal treatment to study the effect of acid addition in the
146 solubilization of phenols and sugars.
147
148 **2.3. Chemicals**
149 The chemicals trifluoroacetic acid (TFA), anthrone, Folin-Ciocalteu's phenol reagent,
150 hydroxymethylfurfural and 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma-
151 Aldrich (Madrid, Spain). Sodium bicarbonate (Na_2CO_3), ethanol, and methanol were
152 from Panreac Quimica S.A. (Barcelona, Spain). Concentrated acetic acid was purchased
153 from Fluka (Switzerland). Acetonitrile was of HPLC-grade purity (Romyl,
154 Teknokroma, Barcelona, Spain).
155
156 **2.4. Preparation of liquid and solid phases**
157 The scheme of the extractions is showed in Figure 1. To extract the soluble compounds
158 from the liquid phase, the liquid phase was centrifuged again to remove solids in
159 suspension and microfiltered with 0.45 μm nylon microfilters.
160 A technique widely used for composting analysis and based on water extraction
161 (Thompson, Leege, Millner, & Watson, 2001) was applied to quantify the soluble
162 compounds in the solid phase. To 20 g of solid fraction, 160 g of distilled water was
163 added. After stirring for 24 hours, the mix was centrifuged and microfiltered with 0.45
164 μm nylon microfilters.
165
166 **2.5. Solid phase characterization**
167 The determination of pH, chemical oxygen demand (COD), soluble COD (sCOD), total
168 solids (TS), mineral solids (MS), and total volatile solids (VS) to characterize the solid

169 phase were developed following the recommendations of the Standard Methods of
170 APHA (2005), as previously described (Serrano, Fermoso, Alonso-Fariñas, Rodríguez-
171 Gutierrez, Fernández-Bolaños, & Borja, 2017).
172
173 **2.6. Analytical methods**
174 *Hydroxymethylfurfural (HMF).* The identification of HMF was performed by high
175 performance liquid chromatography (Hewlett-Packard model 1100, Palo Alto, CA,
176 USA) equipped with an array detector monitoring at 280 nm and a C18 reverse-phase
177 column (Spherisorb ODS-2; 250 x 4.6 mm i.d. and 5 μm particle size) supplied by
178 Teknokroma (Barcelona, Spain) kept at 25 °C with a C18 guard column. All aliquots of
179 liquors from hydrothermal treatments were filtered through 0.45 μm membranes and
180 injected directly into the HPLC instrument. A flow rate of 1.0 mL/min and an injection
181 volume of 20 μL were used. Separation was achieved using two solvents: solvent A
182 (Milli-Q water, pH 2.5 adjusted with 20 mM TFA) and solvent B (acetonitrile). A linear
183 gradient analysis was used as follows: Starting with 95% solvent A and 5% solvent B,
184 this was increased to 25% solvent B over 30 min. The system was equilibrated between
185 runs for 5 min using the starting mobile phase composition. The identification of HMF
186 was based on the comparison of retention times with those of reference compound and
187 the UV spectrum was recorded in the range of 200–360 nm. Quantification was
188 performed using regression curves in triplicate for four different concentrations ($r^2 \geq$
189 0.99).
190 *Total phenols.* In order to obtain total phenols, 20 ml of methanol-water (v/v, 80/20)
191 was added to 10 g of the solid or liquid fraction and incubated for 1 hour at 70° C in a
192 water bath then microfiltered with 0.45 μm nylon microfilters. The total phenol content

193 was determined by the Folin-Ciocalteu spectrophotometric method and expressed as
194 grams of gallic acid equivalents (Singleton & Rossi, 1965).
195 *Sugars and uronic acids.* Total sugars were analyzed by the Anthrone colorimetric
196 method (Witham, Blaydes, & Devlin, 1971) using a spectrophotometer (BIO-RAD
197 iMark Microplate Reader, USA). Uronic acids were measured by the m-
198 hydroxydiphenyl method, as described by Blumenkrantz & Asboe-Hansen (1973), and
199 expressed as grams of galacturonic acid per kg of fresh strawberry extrudate.
200 *Individual neutral sugars.* Individual neutral sugars were analyzed from duplicate
201 samples of solubilized fractions with and without initial TFA hydrolysis prior to
202 reduction, acetylation, and analysis by gas chromatography (GC), using a method
203 described by Lama-Muñoz, Rodríguez-Gutiérrez,Rubio-Senent, &Fernández-Bolaños,
204 (2012).
205

206 **2.7. Antioxidant determinations**

207 *Antiradical activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH).* The antioxidant activity of
208 each liquid fraction obtained after thermal treatments and the untreated control was
209 determined by the free radical scavenging capacity, using the DPPH method as
210 previously described (Rodríguez, Fernández-Bolaños, Rodríguez, Guillén, & Jiménez,
211 2007). The antioxidant capacity of each liquid fraction was expressed as EC50
212 (effective concentration, mg/mL), as calculated for each antioxidant from a calibration
213 curve using linear regression.
214 *Reducing power.* The method described by Rodríguez et al., (2007) was used to
215 determine the reducing power of each liquid fraction after thermal treatment. Briefly,
216 the solutions were treated by L−1 $FeCl_3$ in citric acid. The mix was measured in a
217 microplate reader in quadruplicate, including a blank without $FeCl_3$. The microplate was

incubated during 20 min at 50º C, and after a prewarmed dipyridyl solution in trichloroacetic acid was added, read at 490 nm. Reducing power (RP) was expressed as quercetin equivalents (g/L QE) from the equation as determined from linear regression: RP = 0.2172 × *A*490 − 0.018.

2.8. Statistical analysis

Results were expressed as mean values ± standard deviations. To assess the differences between samples, a comparison was performed using the Statgraphics Plus program version 2.1. Multivariate analysis of variance (ANOVA) followed by Duncan's comparison test was performed. Results were considered statistically significant for $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Solubilization of sugars

The addition of acid increased the concentration of solubilized total sugars in the liquid fraction separated after thermal treatments in most cases (Figure 2A). The exception was steam explosion treatment for 5 minutes, which caused a significant degradation of sugars due to the high temperatures used. The best solubilization of total sugars from the liquid fraction was obtained with treatment at 90 ºC with acid addition (0.5% glacial acetic acid) (65 g/kg of fresh raw material). The concentration obtained at 90 ºC with acid addition indicated that sugars were released easily by the de-flocculation of macromolecules and hemicellulose solubilization. Sugar solubilization from lignocellulosic biomass at 90º C was also previously observed by Mosier et al. (2005). Interestingly, the concentration of solubilized sugars at 150 ºC and 170 ºC (53–57 g/kg) were only slightly lower than at 90 ºC, indicating that even at high temperatures, sugar

243 solubilization was the main process happening. The solubilized sugars in the liquid
244 fraction could be used as a fermentable source for the production of wine or vinegar
245 (Hornedo-Ortega, Álvarez-Fernández, Cerezo, Garcia-Garcia, Troncoso, & Garcia-
246 Parrilla, 2017) or for the production of ethanol or ethanol derivatives.
247 The total values of acid sugars in the liquid fraction, expressed as grams of uronic acids
248 per kg of strawberry extrudate, were directly proportional to the pectin substances
249 (Figure 2B). Pectins are widely used in the food industry as gelling or thickening agents
250 (Chamorro & Mamani, 2015). The treatment at 150 ºC without acid addition produced
251 the highest yield of acid sugars (8.9 g/kg). Temperatures above 150 ºC seemed to cause
252 not only the solubilization of acid sugars but also their degradation, with the exception
253 of treatment at 200 ºC with acid addition for two minutes. Uronic acids are released
254 from hemicellulose at high pressure and temperatures (Jönsson, & Martín, 2016) like
255 the treatment at 200 ºC in which the lower reaction time seems to be crucial to avoid
256 this degradation. Therefore, except in the latter treatment, the addition of acetic acid did
257 not improve the concentration of acid sugars solubilised from the liquid fraction of raw
258 thermally-treated strawberry extrudate.
259 Next, the composition of individual sugars and their distribution in monosaccharides
260 and oligosaccharides, as well as the HMF concentration, as a degradation product of
261 sugars (hexoses), were quantified for the liquid and solid phases of all thermally-treated
262 samples (Table 1). HMF is neither present in the raw material nor formed at 90 ºC. The
263 maximum concentration of HMF was obtained with treatment at 170 ºC for 60 minutes
264 with acid addition. These HMF values were higher than those obtained using steam
265 explosion, which subjected the sample to higher temperatures but for shorter periods of
266 time. HMF is commonly present in food processing products in which a thermal
267 treatment has been applied. HMF has recently been identified within natural extract

with high antioxidant properties (Mabret et al., 2016) that can be used to prevent the oxidation of edible oils, enhancing the commercial life up to four times for sunflower oil. However, the presence of HMF in the liquid phase is controversial since it has been shown to have an inhibitor activity against microorganisms (Ghasimi, Aboudi, de Kreuk, Zandvoort, & van Lier, 2016), which could complicate the use of further downstream bioprocesses for the utilization of the liquid phase.

The glycoside composition (Table 1) shows that the solubilized individual sugars were extracted mainly in the liquid phase. The major sugar present in the liquid phase was glucose, followed by mannose, in all conditions tested. No significant amounts of sugars linked to the solid fraction were found except for the sample treated at 120 ºC, in which around 40% of the quantified sugars were retained in the solid fraction. Most probably this treatment enhanced the linked formation between the solubilized sugars from hemicellulose with the rest of the cell wall material. The maximum concentrations of mono- and oligosaccharides were obtained by treatment at 90 ºC with acid addition, whereas the same treatment conditions without acid addition yielded 2-fold lower concentrations. In conditions without acid addition, the concentration of sugars increased at higher temperatures, reaching a maximum concentration of oligosaccharides at 150 ºC, 21% of which were monosaccharides. In contrast to the result obtained for treatment at 90 ºC, the use of acid at 150 ºC did not significantly increase the solubilization of sugars. In addition, the concentration of HMF in the liquid phase treated at 150 ºC increased compared to the sample treated at 90 ºC. Batch thermal treatments between 90 ºC and 170 ºC obtained 26–41 g of monosaccharides/kg of fresh strawberry extrudate, whereas in a continuous thermal treatment, it would be possible to obtain a liquid source with a much higher concentration of fermentable sugars, over 100 g/L, because the condensed water is lower.

293

294 **3.2. Solubilization of phenols**

295 The total phenols transferred in the liquid phase after thermal treatment, representing
296 the simple and complex phenols that are soluble in a mix of ethanol/water were
297 quantified (Figure 3A). The concentration of total phenols in the raw material (5.55 g/kg
298 of fresh raw material) was lower than that present in the liquid fractions following
299 treatment at 150 ºC and 170 ºC without acid addition (10.0 mg/kg). The concentration
300 of total phenols in the liquid fractions at 150 ºC and 170 ºC were much higher than for
301 the other tested conditions (Figure 3A), suggesting that phenols required not only a high
302 temperature to be solubilized but also a longer reaction time, over one hour. The
303 addition of acid at 150 ºC led to the solubilization of 9.68 mg/kg, a value not
304 significantly different than the maximum value determined without acid addition. The
305 steam explosion treatment was not efficient at releasing a high concentration of phenols,
306 most probably due to the short reaction time. Thus, the maximal recovery of phenols
307 from strawberry extrudate was obtained with conditions in the industrial range (150 ºC–
308 170 ºC), similar to those currently in use to treat other agroindustrial wastes (Rubio-
309 Senent, Rodríguez-Gutiérrez, Lama-Muñoz, & Fernández-Bolaños, 2013).
310 In comparison with the phenols soluble in ethanol/water, less than half of the total
311 phenols in the liquid fraction (Figure 3A) were soluble in water (Figure 3B); therefore,
312 these compounds must be complex phenols. The total phenolic content (Figure 3C) and
313 the concentration of phenols soluble in water (Figure 3D) in the solid fraction were
314 much lower than those contained in the liquid fraction (Figures 3A and 3B).

315

316 **3.3. Antioxidant assays**

317 The ability of each liquid fraction to scavenge the free radical DPPH was expressed as
318 the concentration of extract (in mg per mL) necessary to decrease the initial
319 concentration of DPPH• by 50% (IC_{50}). Low IC_{50}values represent high antioxidant
320 activity (Rodríguez et al., 2007). All the treatments at temperatures above 120 ºC
321 produced liquid fractions with significantly higher antioxidant activity than the raw
322 material (Figure 4A), because the higher solubilisation of phenols and the formation of
323 other antioxidant compounds like HMF. No correlation was observed between the
324 antioxidant activity of the samples and their phenolic concentration, unlike the results
325 reported for other agroindustrial wastes after thermal treatment (Rubio-Senent et al.,
326 2012). However, there was a positive correlation with the antioxidant activity when the
327 phenolic concentration was considered together with the HMF concentration, which
328 also has antioxidant activity (Mabret et al., 2016). When also considering the HMF
329 concentration, the highest antioxidant activity was found for the liquid fraction
330 produced by treatment at 150 ºC without acid addition for 60 minutes, almost 5 mg/mL,
331 although this antioxidant activity was lower than those reported for extracts obtained
332 with the same conditions from dates (0.3 mg/mL) or from olive oil waste (1.7mg/L)
333 (Mrabet et al., 2016; Rubio-Senent et al., 2013). In the cases of other agroindustrial
334 waste, the extracts were further purified; hence the antioxidant activity of the liquid
335 fraction of strawberry extrudate could potentially be improved by purification.
336 Reducing power results are expressed as Trolox equivalents in mg/mL and high
337 concentrations of Trolox equivalents indicate high reducing power activity. All liquid
338 fractions showed higher reducing activity than the control, with the maximum activity
339 detected for the liquid fraction obtained after treatment at 90 ºC without acid addition
340 (Figure 4B). Reducing activity decreased with increasing temperature for no acid
341 addition conditions. Addition of acid resulted in a lower reducing activity for all

conditions tested compared to the non-acid addition conditions, with the exception of steam explosion treatment at 200 ºC for 5 minutes. The reducing power values for thermally-treated strawberry extrudate show the potential use of the phenolic extracts that could be obtained from the liquid fraction. However, these values correspond to half the activity of the liquid fraction obtained from one of the most active phenolic sources, olive oil waste, after thermal treatment at 160 ºC for 60 minutes (Rubio-Senent et al., 2013).

The phenolic compounds present in the liquid fraction obtained from strawberry extrudate showed two of the most important mechanisms on which antioxidant activity is based: the ability to inhibit the formation of free radicals and the ability to scavenge any free radicals formed (Fraga, Galleano, Verstraeten, & Oteiza, 2010).

3.4. Physicochemical characterization of the solid phase

The solid phases obtained after each thermal treatment were analyzed in order to determine their potential use as biomass for further bioprocess applications. The use of the solid phase in industrial processes such as anaerobic digestion could be complementary not only for the final stabilization of strawberry waste, but also to obtain an energy source (methane) necessary to power the thermal treatment reactor.

After thermal treatments, the pH values of the solid fraction increased slightly due to the addition of water during direct heating, even without the addition of acid (Table 2). On average, 91% of total solids were volatile solids. The low concentration of mineral solids was mainly due to the presence of salts and/or carbonates. The humidity increased after thermal treatment because the capacity of the cell material to retain water also increased (Fernández-Bolaños et al., 2004). The highest humidity corresponded to the solid fractions obtained from the steam explosion reactions, in

367 which the severity for lignocellulosic materials is higher than the other tested conditions
368 (Fernández-Bolaños et al., 2004). The total chemical oxygen demand (COD)
369 concentration of the solid phase reached up to 87% for the treatments at 150 ºC and 170
370 ºC compared to the raw material (Table 2). The high temperature and the long reaction
371 time led to the solubilisation of desirable compounds into the liquid fraction, with a high
372 organic content remaining in the final solid residue. The addition of acetic acid did not
373 improve the COD in any of the conditions tested (Table 2).
374 For all the treatments tested, the values of sCOD/COD ratio were close to zero because
375 most of the organic matter was solubilised (Table 2). The values demonstrate the
376 potential of the solid phase of thermally-treated strawberry extrudate to be used as a
377 source rich in organic matter for the application of anaerobic digestion or other
378 bioprocesses for the complete valorization of this agroindustrial waste.
379

380 **3.5. Mass balance**

381 Based on the sugar and phenolic solubilization as well as the antioxidant capacity of the
382 liquid phase, the two most appropriate thermal treatments for the valorization of
383 strawberry extrudate were 150 ºC and 170 ºC for 60 minutes without acid addition. The
384 phenolic and sugar solubilization were roughly the same for both treatments, while the
385 acid sugar solubilization was higher at 150 ºC. The DPPH assay showed a high
386 antioxidant activity for the liquid phase obtained at 150 ºC, while the reducing power
387 method did not show any difference between the two treatments. The concentration of
388 undesirable compounds of sugar degradation like HMF was lower for the treatment at
389 150 ºC. Thus, the use of treatment at 150 ºC for 60 minutes led to better results than
390 treatment at 170 ºC, in addition to the consequent energy savings. Figure 5 summarizes
391 the mass balance of thermal treatment at 150 ºC for 60 minutes in a discontinuous

392 system to valorize the strawberry extrudate. From 1 kilogram of extrudate, a liquid
393 fraction that was rich in sugars (57 g) and phenols (10 g) was produced, plus 0.4 kg of
394 final solid that could potentially be used for methane production by anaerobic digestion
395 to produce the energy necessary to power the thermal treatment reactor. About the HMF
396 content (1.7 g/kg of SE), the dietary human intake of HMF has been estimated in a
397 range of 2.1-23 mg/day (Rufian-Henares & De la Cueva, 2008). Therefore, the
398 maximum consumption should be 13.5 g/day of SE treated at 150 ºC, 1 hour, being this
399 quantity high because the antioxidants are used in food in a very low concentration.
400 Besides, the concentration of HMF could be diminished in the SE by economic system
401 such as chromatographic columns commonly used in the food industry (Lama-Muñoz et
402 al., 2012).
403 It is important to note that treatments at 150 ºC in this study were carried out in a
404 discontinuous reactor without a proper preheating, hence a high amount of water
405 condensed during the reaction. In contrast, nowadays, most industrial thermal
406 treatments are carried out in more efficient continuous reactors (Fernández-Bolaños et
407 al., 2010), such as those used for olive oil waste treatment, in which the water added by
408 steam condensation only increases the initial humidity of the sample by 1%. The volume
409 of liquid generated during the reaction under industrial conditions is expected to be lower,
410 and thus the concentration of sugars and phenols solubilized in the liquid phase of
411 strawberry extrudate is expected to be even higher than the values reported in this study,
412 as is the extract's antioxidant activity.
413
414 **4. CONCLUSIONS**
415

The application of thermal treatment at 150 ºC for 60 minutes can be used at an industrial scale to obtain a liquid fraction with a high concentration of phenols (10 g/kg of fresh strawberry extrudate), 57 g/kg of total sugars, 8.9 g/kg of which are sugar acids, and high antioxidant activity, even without any purification (DPPH value of 4.96 g/L and reducing power of 0.121 g/L Trolox equivalent).

The treatment at 150 ºC for 60 minutes also produced 0.40 kg of final solid per kg of raw material, with the solid phase having high values of volatile solid, COD and sCOD (of 201 g/kg, 258 gO_2/kg and 9.5 gO_2/L, respectively).

After thermal application, it is necessary to extract the phenols and the hydroxymethylfurfural to facilitate the further application of bioprocesses for the total bio-depuration of all phases in order to purify valuable bioactive compounds or use the extracts for energy production processes. Thus, we propose the combination of thermal treatment and a subsequent extraction process, followed by anaerobic digestion to permit the simultaneous valorization of strawberry extrudate for agronomic purposes and the obtention of energy to power the extrudate's own thermal treatment.

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568 **Figure Captions**

569

570 **Figure 1**. Scheme of the solid and liquid extraction using water and ethanol for the
571 phenolic, sugars and uronic acid determination.

572

573 **Figure 2**. Grams of sugars (A) and uronic acids (B), which can be extracted from 1 kg
574 of fresh strawberry extrudate sample using all the thermal treatments tested, with and
575 without acid addition. Untreated strawberry extrudate (Raw SE) was used as control.
576 Means with the same letter were not significantly different, $p<0.05$.

577

578 **Figure 3**. The quantity in milligrams of total phenols (expressed as gallic acid) in the
579 liquid phase soluble in ethanol (A) or soluble in water (B) and in the solid phase
580 extracted with ethanol (C) or water (D) that can be extracted from 1 kg of fresh
581 strawberry extrudate using the thermal treatment is shown. Untreated strawberry
582 extrudate (Raw SE) was used as control. Means with the same letter were not
583 significantly different, $p<0.05$.

584

585 **Figure 4**. Antioxidant activities determined by DPPH (A) and reducing power (B)
586 methods of each liquid fraction obtained after the thermal treatments. Untreated
587 strawberry extrudate was used as control (Raw SE). Means with the same letter were
588 not significantly different, $p<0.05$.

589

590 **Figure 5.** Mass balance for the treatment at 150 ºC for 60 minutes of the strawberry
591 extrudate. HMF: hydroxymethylfurfural.

FIGURES

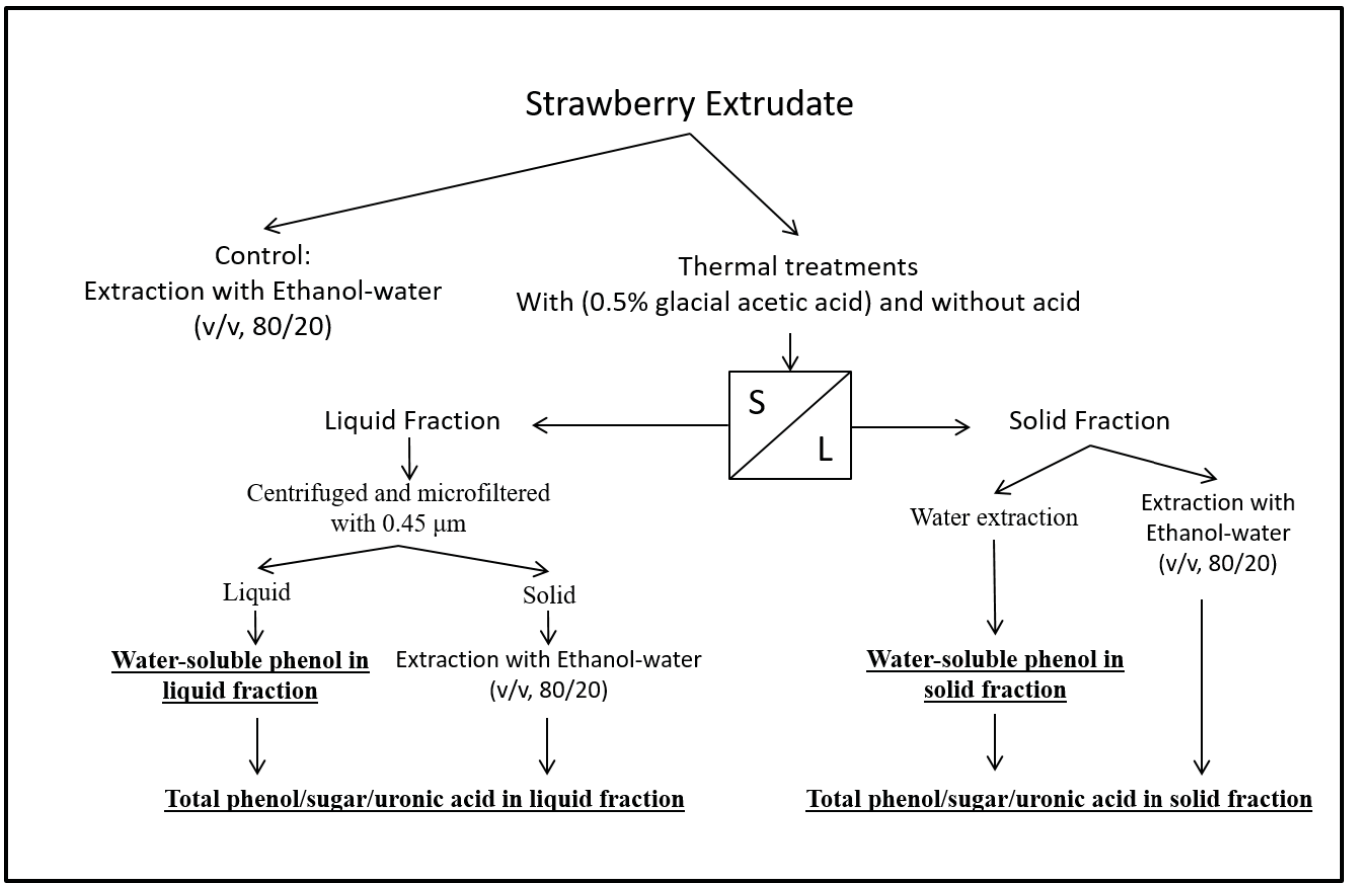

Figure 1.

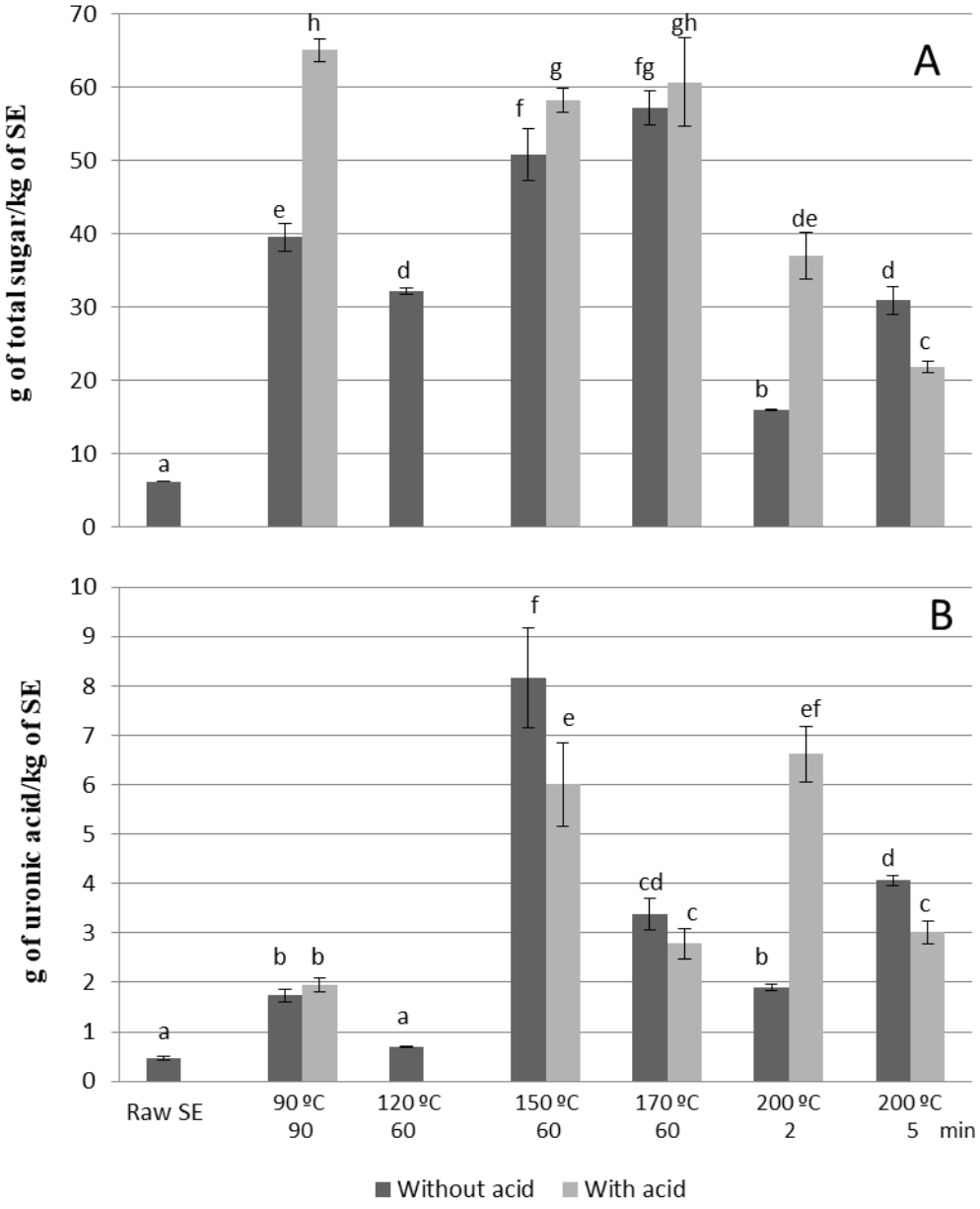
g of total sugar/kg of SE
A
g of uronic acid/kg of SE
B
Raw SE
90 ºC 90
120 ºC 60
150 ºC 60
170 ºC 60
200 ºC 2
200 ºC 5 min
Without acid
With acid

Figure 2.

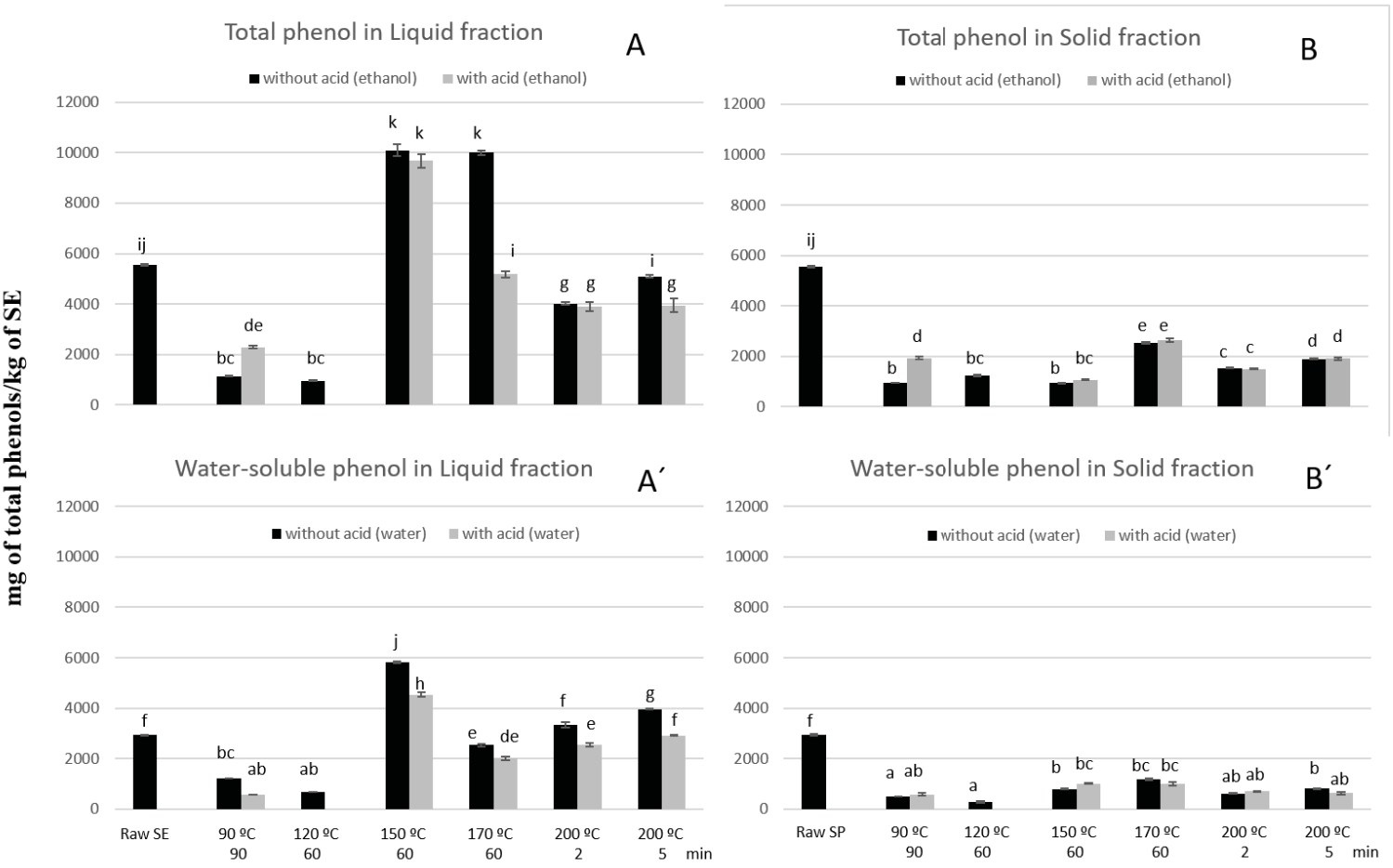
Total phenol in Liquid fraction
A
without acid (ethanol)
with acid (ethanol)
Total phenol in Solid fraction
B
Water-soluble phenol in Liquid fraction
A´
without acid (water)
with acid (water)
Water-soluble phenol in Solid fraction
B´
mg of total phenols/kg of SE
Raw SE
90 ºC 90
120 ºC 60
150 ºC 60
170 ºC 60
200 ºC 2
200 ºC 5
min
Raw SP

Figure 3.

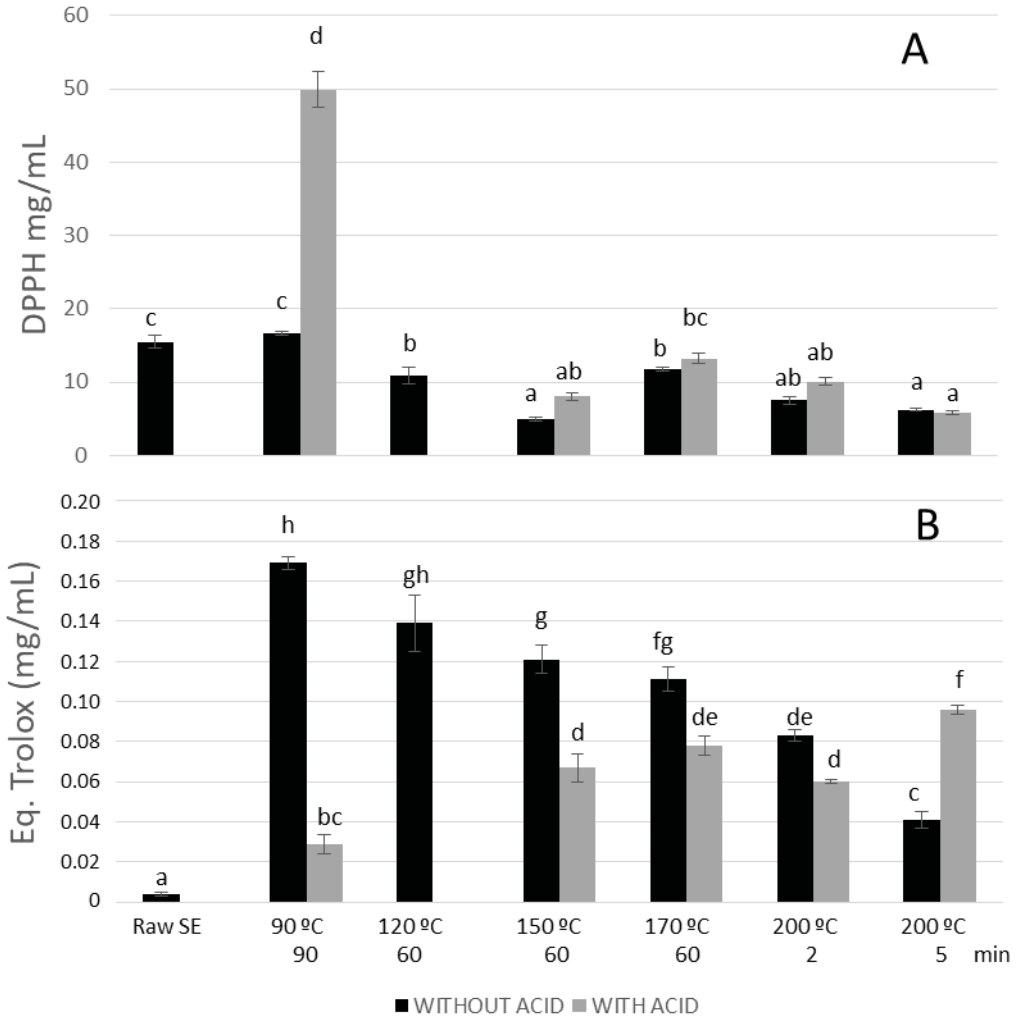
A
DPPH mg/mL
60
50
40
30
20
10
0
c
c
d
b
a
ab
b
bc
ab
ab
a
a
B
Eq. Trolox (mg/mL)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
a
h
bc
gh
g
d
fg
de
de
d
c
f
Raw SE
90 ºC 90
120 ºC 60
150 ºC 60
170 ºC 60
200 ºC 2
200 ºC 5 min
WITHOUT ACID
WITH ACID

Figure 4.

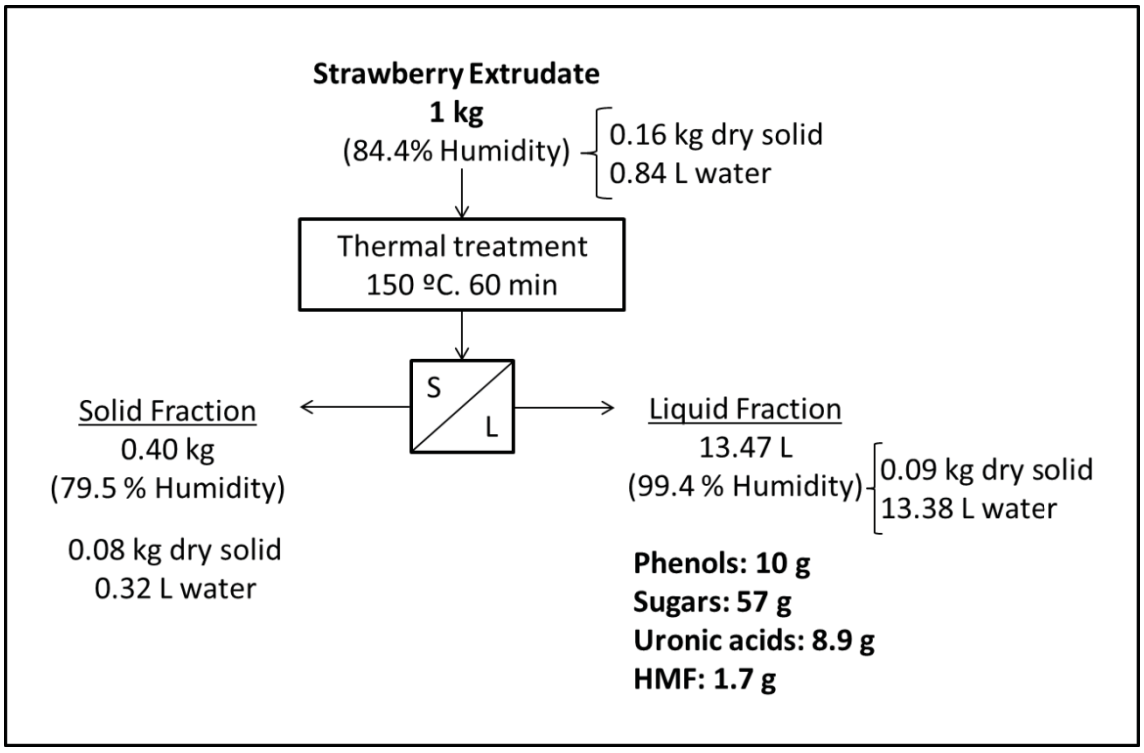
Strawberry Extrudate
1 kg
(84.4% Humidity)
0.16 kg dry solid
0.84 L water
Thermal treatment
150 ºC. 60 min
S
L
Solid Fraction
0.40 kg
(79.5 % Humidity)
0.08 kg dry solid
0.32 L water
Liquid Fraction
13.47 L
(99.4 % Humidity)
0.09 kg dry solid
13.38 L water
Phenols: 10 g
Sugars: 57 g
Uronic acids: 8.9 g
HMF: 1.7 g

Figure 5.

Supplementary material.

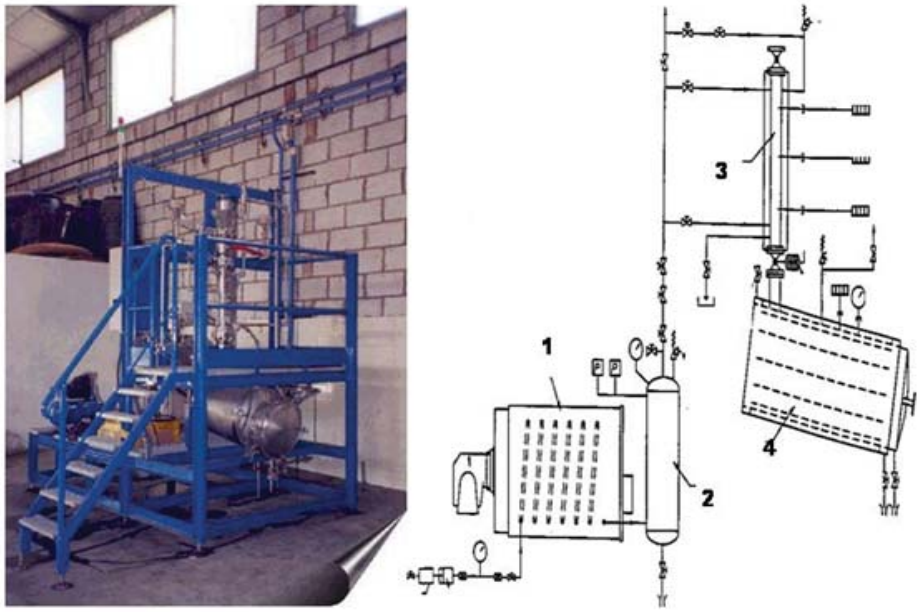

Figure S1. Steam explosion reactor used for the strawberry extrudated treatment at 180 ºC–240 °C with high-pressure saturated steam. 1) Steam generator. 2) Steam accumulator. 3) Reactor chamber (2 L). 4) Expander deposit (120 L).

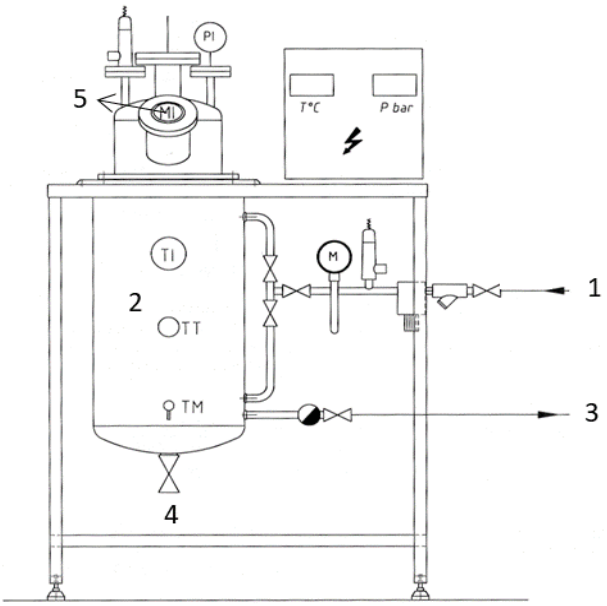

Figure S2. *Steam treatment reactor used for the strawberry extrudated treatment at 150 ºC–170 °C during 60 minutes. 1) Steam entrance. 2) Reactor chamber (100 L).3) Steam purge.4) Discharge valve. 5) Supply valve.*

TABLES

Table 1. Glycoside composition (mg/kg of fresh strawberry extrudate), total monosaccharides (Total MS) and total oligosaccharides (Total OS) and hydroxymethylfurfural (HMF) of the liquid phase (LP) and the solid phase (SP) extracted by alcoholic solution (SF) for all the thermally treated samples and the untreated strawberry extrudate (Raw SE) as a control. The analysed sugars are Rhamnose (Rha), Fucose (Fuc), Arabinose (Ara), Xylose (Xyl), Mannose (Man), Galactose (Gal) and Glucose (Glu). n.d. non detected. Traces: values □ 0.005* .0.00 meaning value between 0.001 and 0.004. Standard deviations in brackets.

Treatment		% Acid	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	Total OS	Total MS	HMF
Raw SE			0.50 (0.01*)	n.d.	0.17 (0.01)	0.65 (0.02)	8.42 (0.11)	0.10 (0.01)	24.72 (0.96)	4.16 (0.05)	38.37 (1.17)	n.d.
90 ºC, 60 min	LP	0	0.12 (0.00)	n.d.	0.20 (0.01)	0.37 (0.02)	5.72 (0.20)	n.d.	15.45 (0.77)	1.12 (0.24)	21.87 (0.90)	n.d.
		0.5	0.21 (0.01)	n.d.	0.15 (0.01)	0.73 (0.01)	10.36 (0.33)	n.d.	30.16 (1.42)	1.96 (0.16)	41.61	n.d.
	SP	0	traces	n.d.	traces	0.01 (0.00)	0.13 (0.00)	n.d.	0.38 (0.00)	0.09 (0.00)	0.53 (0.01)	n.d.
		0.5	traces	n.d.	traces	0.02 (0.00)	0.28 (0.00)	0.01 (0.00)	0.85 (0.00)	0.10 (0.01)	1.16 (0.01)	n.d.
120 ºC, 60 min	LP	0	0.17 (0.01)	n.d.	0.18 (0.01)	0.53 (0.06)	5.99 (1.25)	n.d.	21.41 (1.87)	1.88 (0.15)	28.28 (3.20)	362.88 (12.41)
	SP	0	traces	n.d.	traces	0.22 (0.00)	2.59 (0.66)	n.d.	8.57 (0.16)	0.88 (0.22)	11.39 (0.72)	n.d.
150 ºC, 60 min	LP	0	0.16 (0.02)	n.d.	0.29	0.58 (0.01)	7.83 (0.08)	0.11 (0.01)	23.06 (0.34)	6.74 (0.13)	32.03 (0.46)	1667.09 (4.64)
		0.5	0.14 (0.01)	n.d.	0.25 (0.02)	0.54 (0.01)	8.64 (0.06)	n.d.	22.78 (0.20)	4.16 (0.43)	32.35 (0.30)	3083.09 (9.82)
	SP	0	traces	n.d.	traces	traces	0.03 (0.00)	n.d.	0.06 (0.00)	0.04 (0.00)	0.09 (0.01)	n.d.
		0.5	traces	n.d.	traces	traces	0.03 (0.00)	n.d.	0.08 (0.00)	0.05 (0.00)	0.12 (0.01)	n.d.
170 ºC, 60 min	LP	0	0.28 (0.01)	0.09 (0.00)	0.80 (0.01)	0.53 (0.02)	7.22 (0.27)	0.18 (0.00)	18.93 (0.68)	4.22 (0.23)	28.04 (0.99)	7651.81 (4.56)
		0.5	0.29 (0.00)	0.11 (0.01)	0.67 (0.01)	0.51 (0.01)	6.77 (0.01)	0.22 (0.01)	17.61 (0.05)	4.51 (0.29)	26.17 (0.10)	9370.97 (46.22)
	SP	0	0.01 (0.00)	traces	0.01 (0.00)	0.01 (0.00)	0.13 (0.00)	Traces	0.31 (0.01)	0.09 (0.01)	0.47 (0.02)	232.83 (2.02)
		0.5	traces	traces	0.01 (0.00)	0.01 (0.00)	0.068 (0.00)	traces	0.18 (0.00)	0.07 (0.00)	0.27 (0.01)	191.82 (10.28)
200 ºC, 2 min	LP	0	0.09 (0.00)	0.03 (0.00)	0.12 (0.00)	0.16 (0.00)	1.88 (0.00)	0.03 (0.00)	5.79 (0.06)	1.92 (0.00)	8.10 (0.07)	351.93 (37.71)
		0.5	0.18 (0.00)	0.07 (0.01)	0.26 (0.01)	0.40 (0.02)	5.56 (0.11)	0.09 (0.00)	16.33 (0.69)	5.05 (0.24)	22.88 (0.84)	1045.51 (3.58)
	SP	0	traces	traces	traces	traces	0.04 (0.00)	traces	0.11 (0.01)	0.05 (0.00)	0.16 (0.02)	n.d.
		0.5	traces	n.d.	traces	traces	0.05 (0.00)	n.d.	0.14 (0.00)	0.04 (0.00)	0.20 (0.01)	n.d.
200 ºC, 5 min	LP	0	0.21 (0.01	0.09 (0.00)	0.37 (0.01)	0.384 (0.00)	4.42 (0.06)	0.12 (0.00)	12.94 (0.18)	3.55 (0.28)	18.54 (0.26)	3950.11 (7.51)
		0.5	0.16 (0.00)	0.07 (0.00)	0.28 (0.00)	0.259 (0.00)	3.06 (0.02)	0.09 (0.00)	8.59 (0.18)	3.28 (0.24)	12.51 (0.20)	3310.86 (5.66)
	SP	0	traces	traces	0.01 (0.00)	0.01 (0.00)	0.07 (0.00)	traces	0.18 (0.00)	0.07 (0.00)	0.26 (0.01)	10.07 (1.18)
		0.5	traces	traces	traces	traces	0.05 (0.00)	traces	0.13 (0.00)	0.03 (0.00)	0.18 (0.00)	2.35 (0.65)

Table 2. Physicochemical characterization of untreated strawberry extrudate (Raw SE) and different solid fractions obtained after the thermal treatments (Chemical Oxygen Demand (COD), soluble COD (sCOD), total solids (TS), mineral solids (MS), humidity (H) and total volatile solids (VS)). Standard deviations in brackets.

Treatment	% Acid	pH	TS (mg/Kg)	MS (mg/Kg)	VS (mg/Kg)	%H	COD (mg O_2/kg)	sCOD (mg O_2/L)	sCOD/COD ratio
Raw SE	-	3.3 (0.1)	156356 (916)	7482 (87)	148874 (834)	84.4 (0.5)	200953 (6793)	56460 (2498)	0.28
90 ºC, 60 min	0	4.0 (0.1)	138847 (6158)	4850 (161)	133997 (6047)	86.1 (3.8)	142898 (4564)	20768 (616)	0.15
	0.5	3.8 (0.1)	120644 (1474)	4313 (101)	116332 (1568)	87.9 (1.1)	126533 (5403)	22977 (453)	0.19
120 ºC, 60 min	0	3.6 (0.1)	171287 (1996)	6078 (129)	165209 (2109)	82.9 (1.0)	196287 (7137)	27955 (442)	0.14
150 ºC, 60 min	0	4.0 (0.1)	205296 (2615)	4235 (234)	201.062 (2381)	79.5 (1.0)	258084 (2368)	9510 (476)	0.04
	0.5	3.7 (0.1)	194085 (4526)	3855 (66)	190229 (4464)	80.6 (1.9)	261723 (3245)	12141 (341)	0.05
170 ºC, 60 min	0	3.6 (0.1)	205787 (3040)	5278 (80)	200508 (3116)	79.4 (1.2)	307863 (9058)	24260 (1058)	0.08
	0.5	3.4 (0.1)	283920 (12645)	6898 (476)	277022 (12170)	71.6 (3.2)	376426 (7871)	27020 (1011)	0.07
200 ºC, 2 min	0	4.5 (0.1)	91622 (2151)	1874 (330)	89748 (1887)	90.8 (2.1)	123355 (2519)	16097 (802)	0.13
	0.5	4.1 (0.00)	102035 (2751)	1736 (172)	100299 (2579)	89.8 (2.4)	90104 (2120)	7050 (160)	0.08
200 ºC, 5 min	0	4.4 (0.1)	73039 (2438)	1879 (11)	71160 (2427)	92.7 (3.1)	83715 (3233)	32872 (1620)	0.39
	0.5	4.1 (0.1)	75585 (3557)	1573 (130)	74012 (3648)	92.4 (4.4)	108518 (3283)	7119 (350)	0.07