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COMPARISON OF MICROWAVES AND CONVENTIONAL THERMAL TREATMENT ON ENZYMES ACTIVITY AND ANTIOXIDANT CAPACITY OF KIWIFRUIT PUREE
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3

4

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13

14

15 **Abstract**

16 Enzymes are naturally present in food and can cause product deterioration. For this
17 reason, most food-processing steps try to reduce the enzymatic activity. The aim of this
18 work was to compare, in terms of both the inactivation of kiwifruit puree peroxidase,
19 polyphenoloxidase and pectinmethylesterase and also the maintenance of the
20 antioxidant capacity of the product, the effect of a microwave treatment with a
21 conventional thermal treatment designed to cause the same level of peroxidase
22 inactivation (90%). The microwave power and process time that best permitted the
23 maximization of both the enzymes inactivation and the antioxidant capacity of the
24 product, was selected by means of the Response Surface Methodology. The results
25 obtained point to microwave heating as an appropriate technology with which to

26 produce a stable kiwifruit puree, since these treatments were more effective at enzyme
27 inactivation and antioxidant capacity retention than the conventional thermal treatment.
28
29 **Keywords:** kiwifruit, microwaves, peroxidase, polyphenoloxidase,
30 pectinmethylesterase, antioxidant capacity.
31
32 1. INTRODUCTION

33 Different food scientists and nutrition specialists consider the consumption of fruit and
34 vegetables as having many health benefits (Antunes, Dandlen, Cavaco, & Miguel, 2010;
35 Du, Li, Ma, & Liang, 2009). Kiwifruit has been attributed with exceptional nutritional
36 and sensory properties, as well as high antioxidant activity comparable to that of
37 mangosteen, avocado, papaya, mango and cempedak (Park, Jung, Kang, Delago-Licon,
38 Katrich, Tashma, Trakhtenberg, & Gorinstein, 2006).
39 In recent years, consumers' food habits have changed towards a greater consumption of
40 ready-to-eat and minimally processed fruit-based products, leading to the marketing of
41 products such as fruit juices, beverages of fruit juices mixed with milk, fruit purees or
42 smoothies (Antunes et al., 2010; Elez-Martínez, Aguiló-Aguayo, & Martín-Belloso,
43 2006; Osorio, Martínez-Navarrete, Moraga, & Carbonell, 2008). This type of products
44 has been traditionally preserved by means of conventional thermal technologies (Osorio
45 et al., 2008; Whitaker, Voragen, & Wong, 2003). However, it usually requires the use of
46 high temperatures combined with long process times which has been widely associated
47 with a marked deterioration in food quality, especially with the development of cooked
48 off-flavours, colour alteration and the loss of thermosensitive compounds (Elez-
49 Martínez et al., 2006; Gonçalves, Pinheiro, Abreu, Brandao, & Silva, 2010; Llano,
50 Haedo, Gerschenson, & Rojas, 2003; Maskan, 2001; Queiroz, Mendes, Fialho, &

51 Valente-Mesquita, 2008). For this reason, alternatives to conventional processing
52 technologies are being explored. Microwave heating has been proposed as a good
53 alternative to conventional heating when the purpose is either drying, pre-cooking,
54 tempering or preserving (Huang, Sheng, Yang, & Hu, 2007; O'Donnell, Tiwari,
55 Bourke, & Cullen, 2010; Vadivambal & Jayas, 2010). Microwave energy (MW) is
56 transported as an electromagnetic wave (0.3 GHZ-300 GHz). When intercepted by
57 dielectrical materials, MW produces an increase in the product temperature associated
58 with dipole rotation and ionic polarization (Schubert & Regier, 2010). This type of
59 technology implies volumetric heating, which means that the materials can absorb
60 microwave energy directly and internally. For this reason, compared to conventional
61 heating methods, microwaves lead to a faster heating rate, thus reducing the process
62 time (Huang et al., 2007; Igual, García-Martínez, Camacho, & Martínez-Navarrete,
63 2010; Queiroz et al., 2008). In this way, the processing cost can be cut and the product
64 may present better preserved sensory, nutritional and functional properties (De Ancos,
65 Cano, Hernández, & Monreal, 1999; Igual et al., 2010).
66 Enzymatic and microbiological control is essential to ensure the quality and safety of
67 fruit-based food products. However, much care has to be taken with antioxidant
68 compounds because they are what is mainly responsible for the benefits to human health
69 commonly attributed to the regular intake of vegetable origin products (Antunes et al.,
70 2010; Du et al., 2009). Enzymes are naturally present in fruit and can cause product
71 deterioration in many ways (Whitaker et al., 2003). Peroxidase (POD) and
72 polyphenoloxidase (PPO) are the enzymes that are principally responsible for the
73 deterioration of the colour and nutritive value of most fruits and vegetables (Gonçalves
74 et al., 2010; Queiroz et al., 2008). POD can be used to evaluate the efficiency of
75 vegetable thermal treatments because of its relatively high thermal stability (Anthon,

Sekine, Watanabe, & Barrett, 2002; Lemmens, Tibäck, Svelander, Smout, Ahrné, Langton, Alminger, Loey, & Hendrickx, 2009). Pectinmethylesterase (PME) can cause changes in the rheological properties of foods by means of pectin desterification (Jolie, Duvetter, Houben, Vandevenne, Van Loey, Declerck, Hendrickx, & Gils, 2010; Osorio et al., 2008).

The following aspects have been considered in order to establish a process optimisation procedure with which to obtain a high quality microwaved kiwifruit puree: (i) the effect of microwave processing factors (power and time) on POD, PPO and PME inactivation as well as on the antioxidant capacity maintenance in a kiwifruit puree; (ii) the necessary treatment conditions with which to obtain the greatest enzyme inactivation and the lowest antioxidant capacity degradation in the product and (iii) a comparison of the effectiveness with which microwave and conventional thermal processing inactivate enzymes without altering the antioxidant capacity of the kiwifruit puree.

2. MATERIALS AND METHODS

2.1. Sample preparation

Kiwifruit (*Actinida deliciosa* var. Hayward) was purchased from a local supermarket. Fruit pieces were peeled, washed with distilled water, cut into slices and triturated with a Thermomix (TM 21, Vorwerk, Spain), using the fourth power level for one minute.

2.2. Treatment of kiwifruit puree

2.2.1. Microwave treatment

100 A household microwave oven (NORM 3038GC, China), provided with a glass turntable
101 plate, was used to treat the kiwifruit puree. To study the effect of microwave power and
102 process time on the inactivation of POD, PPO and PME, as well as on the antioxidant
103 capacity of the product using the minimum number of experimental trials (Beirão-da-
104 Costa, Steiner, Correia, Empis, & Moldão-Martins, 2006; Cano, Hernández, & De
105 Ancos, 1997), an experimental design based on a central composite design was applied
106 (Cochran & Cox, 1957). The power and the time were designed to vary between 300-
107 900 W and 100-300 s respectively, taking into consideration the results of previous
108 experiments that ensure that these microwave process conditions allow kiwifruit puree
109 to be obtained with and without sensorily perceivable significant differences when
110 compared to fresh puree (Benlloch-Tinoco, Varela, Salvador, & Martínez-Navarrete,
111 2012). A total of 16 running factorial points were defined by the experimental design.
112 Samples were tempered to an initial temperature of 35 ºC (no enzymatic degradation
113 has been observed at this temperature (Rodrigo, Jolie, Van Loey, & Hendrickx, 2007;
114 Sampedro, Rodrigo, & Hendrickx, 2008). For each treatment, a sample of 500 g was
115 heated in the microwave oven in a standard size glass beaker (9 cm inner diameter and
116 12 cm length) (BKL3-1K0-006O, Labbox, Spain). Temperature of the sample in the
117 hottest spot, previously identified (data not shown), was continuously recorded by
118 means of a fibre-optic probe (CR/JP/11/11671, Enelec, Spain) which was connected to a
119 temperature datalogger (FOTEMP1-OEM, Enelec, Spain). Treated samples were
120 immediately cooled in ice-water until the puree reached 35 ºC.
121
122 2.2.2. *Conventional thermal treatment*
123 A conventional thermal treatment, which ensured a POD inactivation of 90%, was set
124 up (Elez-Martínez et al., 2006; Llano et al., 2003; Williams, Lim, Chen, Pangborn, &

125 Whitaker, 1986). This level of inactivation was fixed by taking into account the
126 industrial requirements which have to be met in order for the product to be considered
127 as stable (Gonçalves et al., 2010, Zheng & Lu, 2011). The treatment needed to obtain
128 this level of POD inactivation (data not shown) was 97 ºC for 30 s in a thermostatic
129 water bath (Precisterm, Selecta, Spain). After kiwifruit was triturated, 20 g of puree
130 were introduced into TDT stainless steel tubes (13 mm inner diameter and 15 cm
131 length) and closed with a screw stopper. A thermocouple connected to a data logger was
132 introduced through the sealed screwed top to control the process. Prior to the treatment,
133 the samples were preheated at 35 ºC to shorten and standardise the come-up time. Under
134 these conditions, a come-up time of 150 s was needed to reach the treatment
135 temperature. Treated samples were immediately cooled in ice-water.
136

137 **2.3. Analytical determinations**

138 Enzyme activity (POD, PPO and PME) and antioxidant capacity were measured in both
139 all the treated samples (microwaved and conventionally heated ones) and also in the
140 non-treated sample, which was used as control. Additionally, some physicochemical
141 properties were determined in the fresh sample.
142

143 *2.3.1. Physicochemical properties*

144 Water content (x_w) was measured by drying the sample to constant weight at 60 °C in a
145 vacuum oven following the AOAC 934.06 method (2000). Soluble solids were
146 determined by measuring the °Brix in a previously homogenised sample using a
147 portable digital refractometer, Refracto 3PX, at 20 °C (Metler Toledo, Switzerland).
148 Water activity (a_w) was measured by means of a dew point hygrometer (GBX FA-st lab,

France) and pH with a digital pH-meter, Basic 2 (Crison, Spain). Each analysis was carried out in triplicate.

2.3.2. Peroxidase and polyphenoloxidase activities

2.3.2.1. Extraction procedure

The enzymes were extracted using the method of De Ancos et al. (1999), with some modifications. Kiwifruit puree (10 g) was homogenised with 20 mL of 0.2M sodium phosphate buffer (pH 6.5) containing 10 $g{\cdot}L^{-1}$ insoluble polyvinylpolypyrolidone and 10 mL L^{-1} Triton X-100 with external cooling for 3 min with stop intervals every 30 s. The homogenate was centrifuged at 16,000 *xg* and 4 ºC for 20 min and the supernatant was collected. The volume of the obtained extract was measured. Extracts were taken in duplicate.

2.3.2.2. Polyphenoloxidase activity measurement

PPO activity was assayed spectrophotometrically by placing 3 mL substrate, which consisted of a solution of 0.015 M catechol in 0.05 M sodium phosphate buffer (pH 6.5) and 0.050 mL of enzyme extract, in a cuvette (De Ancos et al., 1999). The increase in absorbance at 400 nm and 25 ºC was recorded automatically for 30 min (Thermo Electron Corporation, USA). A solution containing all the components except the enzyme extract, which was replaced by 0.050 mL of sodium phosphate buffer, was used as a blank. One unit of PPO activity was defined as the amount of enzyme that caused an increase of one in the absorbance per min ($Abs{\cdot}min^{-1}{\cdot}g^{-1}$), calculated from the linear part of the obtained curve. The percentage of enzyme inactivation (I) was calculated by using Eq. (1).

174

$$I = \frac{A_F - A_T}{A_F} \times 100 \quad (1)$$

175

176 Where:

177 A_F: enzyme activity of fresh kiwifruit puree

178 A_T: enzyme activity of treated kiwifruit puree.

179

180 2.3.2.3. Peroxidase activity measurement

181 POD activity was measured by spectrophotometry following to the method described by
182 De Ancos et al. (1999). Aliquots of enzyme extract (0.050 mL) were added to a reaction
183 mixture made up of 2.7 mL of 0.05 M sodium phosphate buffer (pH 6.5) with 0.2 mL *p*-
184 phenylenediamine (10 $g \cdot kg^{-1}$) as H-donor and 0.1 mL hydrogen peroxide (15 $g \cdot L^{-1}$) as
185 oxidant. The oxidation of *p*-phenylenediamine was measured at 485 nm and 25 ºC using
186 a Thermo Electron Corporation spectrophotometer (USA). A solution containing all the
187 components except the enzyme extract, which was replaced by 0.050 mL of sodium
188 phosphate buffer, was used as a blank. One unit of POD activity was defined as the
189 amount of enzyme that caused an increase of one in absorbance per min ($Abs \cdot min^{-1} \cdot g^{-1}$),
190 calculated from the linear part of the obtained curve. The percentage of enzyme
191 inactivation was calculated by using Eq. (1).

192

193 *2.3.3. Pectinmethylesterase activity*

194 PME activity was determined following the pH decrease produced by the carboxyl
195 groups released by the hydrolysis of methyl esters of pectin, by means of an automatic
196 pH-stat titrator (Metrohm, Switserland) using the method described by Rodrigo, Cortés,
197 Clynen, Schoofs, Van Loey, and Hendrickx (2006), with some modifications. The
198 sample was placed in a thermostated water bath at 22 °C to control the temperature

199 during the determination. The reaction mixture consisted of 2 mL of kiwifruit puree and
200 30 mL of 0.35% citrus pectin (70-75% esterification, Fluka) containing 0.117M NaCl,
201 which was previously adjusted to pH 6.5 with NaOH. During hydrolysis, the pH was
202 maintained at 6.5 by the addition of 0.02N NaOH using the automatic pH-stat titrator.
203 The consumption of NaOH was recorded for 5 min. One unit of PME activity can be
204 expressed as the amount of enzyme that produces 1µmol of acid per minute at pH 6.5
205 and 22ºC. The enzyme activity (U/mL) and the percentage of enzyme inactivation were
206 calculated by using Eq. (2) and Eq. (1), respectively.
207

208 $$PME\left(\frac{U}{mL}\right)=\frac{V\cdot N\cdot 1000}{V_s\cdot t_r} \quad (2)$$

209 Where:
210 V: added volume of NaOH (mL),
211 N: normality of the NaOH used,
212 V_s: volume of sample (mL),
213 t_r: reaction time (min)
214

215 2.3.4. *Antioxidant capacity measurement*

216 To determine the antioxidant capacity of kiwifruit puree, the DPPH$^\bullet$ radical scavenging
217 capacity of kiwifruit extracts was measured as described by Igual et al. (2010) with
218 some modifications. Kiwifruit puree was appropriately diluted with methanol by mixing
219 with external cooling for 30 s. The homogenate was centrifuged at 11,872 *xg* for 10 min
220 at 4 ºC and the supernatant was collected. Aliquots of kiwifruit extract (0.03 mL) were
221 combined with 3 mL of DPPH$^\bullet$ $6.25\cdot10^{-5}$ M in methanol. A Thermo Electron
222 Corporation spectrophotometer (USA) was used to measure the change in absorbance at
223 517 nm and 25 ºC until the reaction reached a plateau (time at the steady state). A

224 control sample, where the kiwifruit puree extract was replaced by $DPPH^{\bullet}$ $6.25 \cdot 10^{-5}$ M in
225 methanol, was used to measure the maximum $DPPH^{\bullet}$ absorbance. The percentage of
226 antioxidant capacity variation (A) was calculated using Eq. (4).
227
228
$$A = \frac{AC_T - AC_F}{AC_F} \times 100 \quad (4)$$
229 Where:
230 AC_T: Antioxidant capacity of the treated kiwifruit (mM Trolox/mL of kiwifruit);
231 AC_F: Antioxidant capacity of the untreated kiwifruit (mM Trolox/mL of kiwifruit)
232
233 In order to express the antioxidant capacity in terms of millimolar Trolox, a calibration
234 curve was prepared by measuring absorbance at 517 nm of different Trolox solutions in
235 the range of 0.3–3 mM.
236
237 **2.4. Statistical analysis**
238 The statistical analysis carried out to optimise the process consisted of a Multiple
239 Response Optimisation procedure. To relate the experimental data with the independent
240 variables a Response Surface Methodology was applied. Only the terms found to be
241 statistically significant ($p<0.05$) after the analysis of variance of the corresponding
242 regression analysis were included in the final reduced model (Mirhosseini, Tan, Hamid,
243 Yusof, & Chern, 2009). The non-significant lack of fit in all the selected final models
244 ($p>0.05$) confirmed the suitability of the fitted model and the non-significance of the
245 Durbin-Watson statistic proved that there was no significant autocorrelation or serial
246 correlation. The Statgraphics 5.1 plus software program (StatPoint Technologies, Inc.,
247 Warrenton, VA, USA) was used.
248

249 ## 3. RESULTS AND DISCUSSION

250

251 ### 3.1. Kiwifruit puree characterization

252 Enzyme activity (POD, PPO and PME), antioxidant capacity and some physicochemical
253 properties (x_w, a_w, ºBrix and pH) of fresh kiwifruit were determined in order to control
254 the fruit which was used as raw material for microwave and conventional heating
255 treatments. The obtained values (Table 1) coincide closely with those reported by other
256 authors working on this fruit (Antunes et al., 2010; Beirão-da-Costa, Cardoso, Martins,
257 Empis, & Moldão-Martins, 2008; De Ancos et al. 1999; Du et al., 2009; Fúster,
258 Préstamo, & Cano, 1994; Llano et al., 2003; Zolfaghari, Sahari, Barzegar, & Samadloiy,
259 2010).

260

261 ### 3.2. Effect of microwave treatment

262 The inactivation of POD, PPO and PME (mean value with standard deviation in
263 brackets) in kiwifruit puree produced by processing in the desired interval of microwave
264 power (300-900 W) and time (100-300 s) ranged from 43 (6) % to 88.0 (0.7) %, 11.4
265 (0.5) % to 81 (2) % and -19.0 (1.3) % to 57 (6) %, respectively. These results point out
266 that in kiwifruit, PME and POD were the enzymes that were most resistant and labile to
267 MW, respectively. Other authors, such as Beirão-da-Costa et al. (2008) and McFeeters,
268 Fleming, and Thompson (1985) found PME to be a highly heat stable enzyme, as
269 intense heat treatments were necessary to inactivate it. On the other hand, De Ancos et
270 al. (1999) reported that the POD enzyme was more efficiently inactivated than other
271 enzymes in a microwaved kiwifruit puree and Terefe, Yang, Knoerzer, Buckow, and
272 Versteeg (2010) found the same to be true in a thermally treated strawberry puree. This
273 fact could be connected to the characteristic low pH of these fruits. Williams et al.

(1986) reported that POD was less stable at pHs below 4. Despite the fact that POD was the most labile enzyme in this case, it could be considered as an adequate indicator of treatment efficiency since it has been reported to be greatly relevant in kiwifruit because of its high activity and extensive contribution to the quality of this fruit (Fang, Jiang, & Zhang, 2008). Additionally, PME residual activity can be rapidly lost during storage in acid conditions (pH<4) (McFeeters et al., 1985) and PPO activity does not seem to have a very important repercussion on kiwifruit quality in view of the lack of browning in kiwifruit tissues. This could be explained by the low polyphenolic levels and the high ascorbic acid content in this fruit, which could itself prevent the oxidation of many polyphenols (Fúster et al., 1994). On the other hand, the microwave treatment~~s~~ 300W-100s led to a PME activity promotion. This could be related to the low temperature reached in this case by the sample, around 43 ºC, and the short time of exposition. This phenomenon was observed by Beirão-da-Costa et al. (2008), who found a significant increase in PME activity in kiwifruit slices subjected to a mild heat treatment prior to inactivation. The other sample submitted to 300W reached 45 ºC but the corresponding treatment was of 300 s. Under these conditions inactivation of PME was of just 4.3 (0.7) %. The temperature reached by the other samples was in the range 60-100 ºC.

The results obtained from the enzyme inactivation study were analysed by means of the Response Surface Methodology. The models obtained from this analysis, together with the corresponding adjusted R^2 values and the standard error of the estimate, are summarised in Table 2. Adjusted R^2 values indicate the % of the variation in enzyme inactivation produced by microwave power and process time that is explained by the models. According to other authors, it can be considered that models satisfactorily represent the data in the experimental domain when R^2 values range between 70 and 90%, while they can be considered excellent if R^2>90% (Granato, Ribeiro, Castro, &

Masson, 2010; Granato, Castro, Ellendersen, & Masson, 2010; Montgomery, 2009). In this way, the three obtained models can be assumed to be valid predictors of POD, PPO and PME inactivation as a function of microwave power and process time, in the range considered in this study. From equations presented in Table 2, it can be observed that increases in both factors (microwave power and time) produced significant effects, both linear and quadratic ($p<0.05$).

A three-dimensional plot showing POD inactivation is presented in Figure 1. As can be observed, a significant increase in POD inactivation occurs up to 800 W of power, decreasing slightly when a higher microwave power was applied. This behaviour can be explained by the fact that the model provided positive linear and negative quadratic effects (Table 2). De Ancos et al. (1999) observed the inactivation of papaya POD to behave similarly under microwave heating. They reported an increase in peroxidase inactivation when microwave power increased from 285 to 570W at 30 s of processing time. Thereafter, a higher power level (800W) did not increase the POD inactivation. In accordance with other authors, a linear increase in POD inactivation was detected the longer the process lasted (Matsui, Gut, de Oliveira, & Tadini, 2008; Zheng & Lu, 2011).

Figure 2 shows the PPO inactivation behaviour as related to microwave power and process time based on the obtained model. As can be observed, the level of PPO inactivation rose as the microwave power increased, which had significant ($p<0.05$) linear and quadratic effects (Table 2). The negative quadratic effect of this factor leads to the smaller increase in the PPO inactivation observed at greater powers. Process time also had a significant effect, in this case both positive linear and quadratic (Table 2), leading to a greater inactivation of this enzyme. Latorre, Bonelli, Rojas, and Gerschenson (2012) and Matsui et al. (2008) found that there was a greater level of PPO inactivation in red beet and green coconut water, respectively, after longer microwave

324 exposure. De Ancos et al. (1999) observed that PPO inactivation in kiwifruit and
325 strawberry was better controlled by pre-fixing the power rather than the exposure time.
326 In addition, an interactive effect was observed between both independent variables
327 (microwave power and process time) on PPO inactivation. As expected, in samples
328 subjected to longer treatment times, the level of PPO inactivation rose faster as greater
329 microwave power was applied than in kiwifruit puree subjected to shorter treatment
330 times.

331 PME inactivation increased significantly ($p<0.05$) as the microwave power level rose
332 and the processing time got longer (Figure 3). In both cases, only a linear effect was
333 found in PME inactivation (Table 2). In the same way, Tajchakavit and Ramaswamy
334 (1997) reported a linear relationship between time and PME inactivation during
335 microwave heating of orange juice and, when microwaving orange peels, Kratchanova,
336 Pavlova, and Panchev (2004) found that the higher the microwave power, the greater
337 the PME inactivation.

338 The mean value with standard deviation of antioxidant capacity variation caused in
339 kiwifruit puree under microwave processing ranged between 3% (2) and 36% (0.6).
340 Table 2 shows the model found to explain the effect of microwave power and process
341 time on this variation and Figure 4 the corresponding response surface plot based on the
342 obtained model. Although applying intense thermal treatments (T>60 ºC) is usually
343 regarded as undesirable because it can induce oxidative condensation or degradation of
344 thermolabile compounds, the opposite behaviour was found during the present study.
345 When microwave treatments were applied, a positive variation of the antioxidant
346 capacity of treated purees was observed, which is related with an increase in the
347 antioxidant capacity of the product: the more intense the treatments, the lower the
348 antioxidant capacity increase and so the smaller the antioxidant capacity variation

349 (Figure 4). According to literature, the application of thermal treatment can be
350 associated with the dissociation of conjugated forms into free phenolic acid compounds,
351 like gallic, caffeic, ferulic and p-coumaric acids (Dewanto, Wu, Adom, & Liu, 2002;
352 Gallegos-Infante, Rocha-Guzman, Gonzalez-Laredo, & Pulido-Alonso, 2010; Jing,
353 Jing-Feng, Yu-Ying, & Lin-Chun, 2010). Despite the fact that the oxidative and
354 hydrolytic enzymes that can destroy the antioxidant compounds can also be released
355 during processing, they are deactivated by the thermal treatment thus avoiding the loss
356 of phenolic acids (Dewanto et al., 2002). Several authors, such as Randhir, Kwon, and
357 Shetty (2008), Sahin, Topuz, Pischetsrieder, and Ozdemir (2009) and Jing et al. (2010)
358 reported an increase of antioxidant capacity in cereals, carob powder and sweet potatoes
359 after being thermally treated, dried or steamed, respectively. As far as the effect of
360 microwave heating on the antioxidant capacity preservation of treated purees is
361 concerned, a significant repercussion of microwave power and process time was
362 observed ($p<0.05$). The polynomial model obtained from the experimental design
363 explained 90.19% of the data variation caused by these effects (Table 2). Both factors
364 had negative linear and positive quadratic effects on the antioxidant capacity variation
365 of the kiwifruit puree (Table 2). In our experimental conditions, this implied that a
366 greater antioxidant capacity promotion was obtained when lower microwave power and
367 shorter processing times were employed, and when greater microwave power was
368 applied, the repercussion of the length of the processing time seemed to be less relevant.
369

370 **3.3. Process optimisation procedure to obtain an optimum microwaved stable**
371 **kiwifruit puree**

372 In the present research work, the effect of microwave heating on the global quality of
373 kiwifruit puree has been evaluated in a pre-established range of power and time, taking

374 both the enzyme inactivation and the antioxidant capacity preservation as quality
375 indicators. Through the superposition of all the obtained models (Table 2), it is possible
376 to predict which treatment conditions (power and time combination) are better at
377 achieving, simultaneously, the largest enzyme activity reduction (POD, PPO and PME)
378 and the maximum increase in the antioxidant capacity within the studied range. From
379 this multiple response optimisation, the overall optimum condition was achieved by
380 applying 1000 W of power for 340 s. Under this optimum condition, the corresponding
381 predicted response variables for POD, PPO and PME inactivation and antioxidant
382 capacity variation were 90.7%, 97.5%, 77.2% and 13.0 %, respectively. This treatment
383 could be considered the adequate method by which to produce stable kiwifruit puree
384 that is not seriously affected, since 90% of the POD activity was reduced (Gonçalves et
385 al., 2010; Zheng & Lu, 2011) and no loss of antioxidant capacity was caused.
386

387 **3.4. Microwave versus conventional heating**

388 The conventional thermal treatment caused 89.2 (0.9) %, 64.9 (0.7) % and 65 (4) %
389 POD, PPO and PME inactivation, respectively and -11 (8) % antioxidant capacity
390 variation. Although both microwave mode and conventional treatments reached the
391 same level of POD inactivation (≈90%), a considerably greater reduction of PPO and
392 PME enzyme activity, as well as an increase instead of a loss in antioxidant capacity,
393 were observed in the sample subjected to the optimum microwave treatment. These
394 results seem to indicate that microwave heating was more effective at enzyme
395 inactivation and led to a better antioxidant capacity retention in kiwifruit puree than
396 conventional heating. Several authors have reported similar results when working on
397 enzyme inactivation in different fruit products processed by means of microwave
398 technology. Tajchakavit and Ramaswamy (1997) found significantly faster PME

399 inactivation in orange juice in the microwave heating mode than in the conventional
400 heating mode. Matsui et al. (2008) published that the inactivation of PPO and POD
401 during microwave processing of green coconut water was significantly faster in
402 comparison with the conventional processes reported in the literature. Zheng and Lu
403 (2011) found microwave heating to be more effective at inactivating POD and
404 preserving nutritional properties in carrot than the conventional thermal treatment. All
405 these differences could indicate the possibility of there being some contributory non-
406 thermal effects of microwaves, making them more effective at enzyme inactivation than
407 the conventional thermal treatment (Awuah, Ramaswamy, & Economides, 2007; Banik,
408 Bandyopadhyay, & Ganguly, 2003; Tajchakavit & Ramaswamy, 1997; Tajchakavit,
409 Ramaswamy, & Fustier, 1998). Although the mechanism is still unclear, Kermasha,
410 Bisakowski, Ramaswamy, and Van de Voort (1993) proposed that enzyme inactivation
411 under microwave heating may be the result of both the temperature and the interaction
412 of the microwave energy with the enzyme, because the microwave field can affect the
413 polar and/or charged moieties of proteins. This phenomenon has long been under
414 investigation and it was Olsen, Drake, and Bucnh (1966) who were probably the first
415 ones to postulate the non-thermal effects of microwaves. Nowadays, however, it
416 remains necessary to study this matter more thoroughly given that there are still
417 controversial opinions (Awuah et al., 2007). In any case, it has been demonstrated that
418 MW technology is suitable for facing enzyme inactivation (De Ancos et al., 1999;
419 Matsui et al., 2008; Latorre et al., 2012) in a shorter process time in comparison with
420 other conventional technologies, which indicates that stability can be properly ensured
421 and product quality can be effectively preserved (Igual et al., 2010; Zheng & Lu, 2011).
422
423

424 ## 4. CONCLUSION

425 More than conventional heating, microwave technology can be an appropriate means of
426 achieving the required level of enzyme inactivation at which to obtain a stable kiwifruit
427 puree with an improved antioxidant capacity. Nevertheless, microwave power and
428 processing time must be adequately selected as they had a significant influence on all
429 the variables considered in the present research work. Response surface methodology
430 may be used as a suitable tool with which to optimize the process conditions that allow
431 both the enzyme inactivation and the antioxidant capacity of kiwifruit puree to be
432 maximised.

433

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594
595

596 FIGURE CAPTIONS

597

598 **Figure 1.** Response surface plot for the percentage change of peroxidase (POD)
599 inactivation in kiwifruit puree as a function of microwave power and process time.

600 **Figure 2.** Response surface plot for the percentage change of polyphenoloxidase
601 inactivation (PPO) in kiwifruit puree as a function of microwave power and process
602 time.

603 **Figure 3.** Response surface plot for the percentage change of pectinmethylesterase
604 (PME) inactivation in kiwifruit puree as a function of microwave power and process
605 time.

606 **Figure 4.** Response surface plot for the percentage change of the antioxidant capacity
607 variation (A) in kiwifruit pure as a function of microwave power and process time.

Figure 1

Figure 1

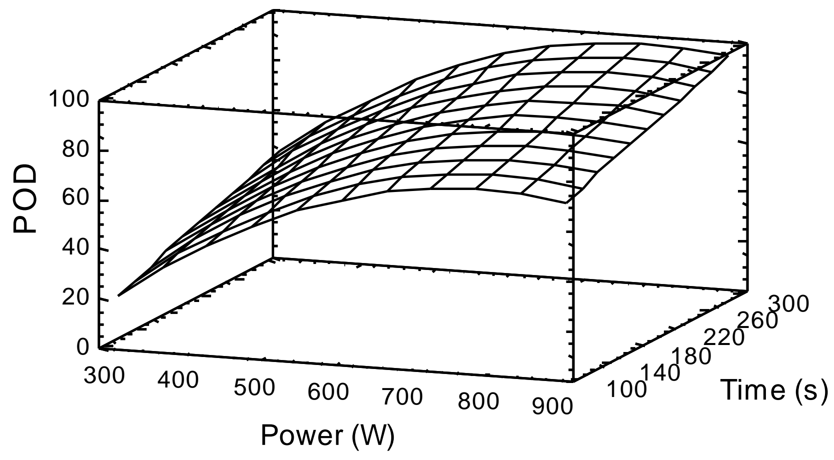

Figure 2

Figure 2

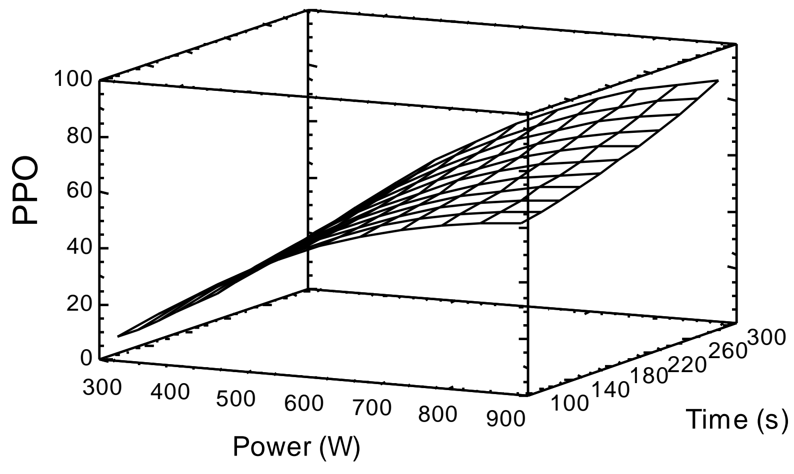

Figure 3

Figure 3

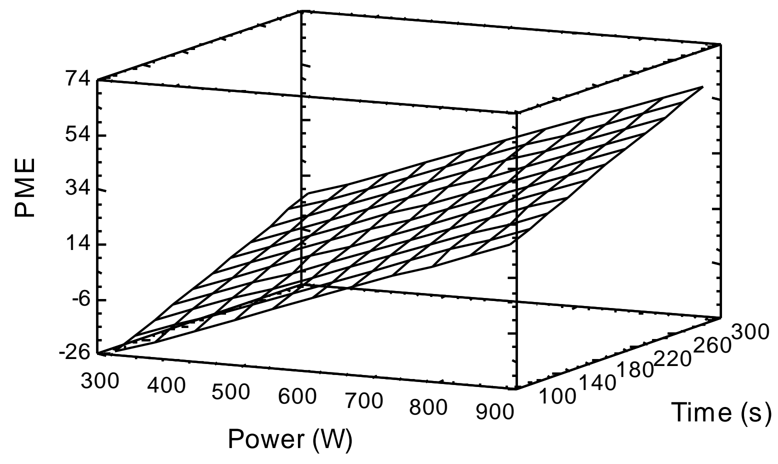

Figure 4

Figure 4

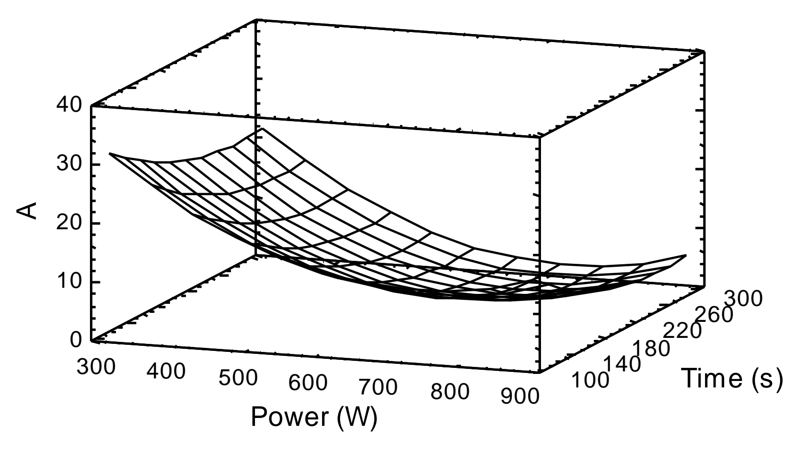

Table 1

Table 1. Characteristics of fresh kiwifruit puree. Mean values and standard deviation (in brackets).

Water content (g water/100 g product)	84.1 (1.0)
Water activity	0.984 (0.003)
ºBrix (g soluble solids/100 g product liquid phase)	14.3 (0.3)
pH	3.40 (0.07)
POD activity (Abs·min^{-1}·g^{-1})	9 (2)
PPO activity (Abs·min^{-1}·g^{-1})	6.0 (1.4)
PME activity (U·g^{-1})	1.1 (0.2)
Antioxidant capacity (mM Trolox·mL^{-1})	13 (2)

POD: peroxidase; PPO: polyphenoloxidase; PME: pectinmethylesterase

Table 2

Table 2. Models explaining peroxidase (POD), polyphenoloxidase (PPO) and pectinmethylesterase (PME) inactivation and antioxidant activity variation. Adjusted R^2 (Adj. R^2) and standard error of estimate (SEE) values. P: Microwave power (W); t: process time (s).

Dependent variable	*Equation*	*Adj. R^2*	*SEE*
PME inactivation	PME = -73.8202 + 0.0916287*P +0.16751*t	75.15	10.36
POD inactivation	POD = -75.772 + 0.347601*P + 0.121953*t – 0.000220345*P^2	82.40	1.49
PPO inactivation	PPO = -48.6351+ 0.235261*P + 0.0802141*t – 0.000133145*P^2 + 0.000116258*P*t+ 0.000271352*t^2	98.64	2.09
Antioxidant capacity variation	A = 86.2719 – 0.153908*P -0.182197*t+ 0.000105077*P^2 + 0.000337186*t^2	90.19	3.30