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# SUPERIORITY OF MICROWAVES OVER CONVENTIONAL HEATING TO PRESERVE SHELF-LIFE AND QUALITY OF KIWIFRUIT PUREE

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**Abstract:** The effect of both microwave (1000 W-340 s) and conventional heating (97 ºC-30 s) on the quality and shelf-life of kiwifruit puree was investigated. The growth of microorganisms and the evolution of enzyme activity, colour, pH, bioactive compounds and antioxidant activity in the product during storage at 4, 10 and 22 ºC were checked. The storage temperature had a significant ($p<0.05$) impact on both the shelf-life and the nutritional and functional value of the samples: the higher the temperature, the significantly ($p<0.05$) faster the rate of both the sample spoilage and the loss of the bioactive compounds. On the other hand, thermal processing significantly ($p<0.05$) reduced the growth of microorganisms and the degradation rate of some bioactive compounds in a 12-59%, as well as leading to enzyme and colour stabilization. A longer shelf-life (123 days at 4ºC) and a superior preservation of colour ($\Delta E_{SE}$=6.54) and bioactive compounds (57-67%) were obtained when microwave heating was the technology selected to process the kiwifruit puree. Microwave heating

was considered a suitable means of preserving kiwifruit puree that might be successfully employed as an innovation tool with which to help safe, high-quality and minimally processed kiwifruit based-products reach the market.

**Keywords:** microorganism spoilage, *Listeria monocytogenes*, enzymes, bioactive compounds, antioxidant activity, colour.

## 1. INTRODUCTION

A wide variety of minimally processed fruit-based products, such as fresh-cut fruits, fresh-squeezed fruit juices, fruit juice and milk mixture beverages, fruit purees and smoothies, are being marketed in response to the recent increase in demand for convenient, easy-to-preserve, health-promoting foods (Elez-Martínez, Soliva-Fortuny, & Martín-Belloso, 2006). Nevertheless, many fruits which are both appreciated for their sensory and nutritional value and possess a great potential for industrial exploitation, e.g. kiwifruit, still seem to be mostly limited to the fresh market outlet, ignoring their surplus production (Barboni, Cannac, & Chiaramonti, 2010).

Microwave heating has been reported to provide superior quality fruit-based products with an extended shelf-life, representing a good alternative to conventional preservation processes (Landl, Abadias, Sárraga, Viñás, & Picouet, 2010). Given the particular way of heating which takes place during microwave processing (volumetric heating), this technology leads to higher penetrative power, faster heating rates, higher thermal efficiency and shorter processing times compared to conventional heating methods. All these facts seem to result in better organoleptic, nutritional and functional properties preservation, with a particular effect on colour (Huang, Sheng, Yang, & Hu, 2007; Vadivambal & Jayas 2007). Similarly to other novel technologies in the field of food

innovation, microwaves might be a key factor either in the successful differentiation of products (Deliza, Rosenthal, Abadio, Silva, & Castillo, 2005) or in finding new uses for some fruits by helping to develop novel ways with which to process them. To this end, many comparative studies of the effect of microwave and conventional heating on various quality aspects of fruits have been conducted (Barrett & Lloyd 2012), pointing out the advantages of microwave heating (Huang et al., 2007). However, it should be taken into consideration that despite published data on the effect of microwaves on safety and quality being available for different food systems, to date, little seems to be known of the impact of microwaves on the shelf-life and post-processing quality loss of fruit products. The marketing of these products frequently implies a storage step, which might also relevantly contribute to their final quality. For this reason, the evolution of their properties and the growth of microorganisms during shelf-life is an important issue to study (Rodrigo et al., 2003).

A few studies have focused on the evaluation of the shelf-life of microwaved foods of animal origin. Of theese (i) Aziz, Mahrous, and Youssef (2002) studied the impact of microwave and gamma-ray processing on the shelf-life of beef when stored at 5°C, (ii) Göksoy, James, and Corry (2000) assessed the effect of short-time microwave energy exposures on several pathogens inoculated in chicken and the shelf-life of the product, (iii) Hebbar, Nandini, Lakshmi, and Subpramanian (2003) studied the shelf-life of microwaved and infrared-heated honey and its quality during storage and (iv) Paterson, Cranston, and Loh (1995) investigated how microwave processing helped to extend the shelf-life of beef under cold storage.

Despite the existence of one article dealing with microbial, enzymatic, physical and nutritional issues during the short storage (14 days) of an apple-based product subjected to minimal microwave processing (Picouet, Landl, Abadias, Castellari, & Viñas, 2009)

and several studies evaluating the evolution of physicochemical, nutritional and functional properties during the storage of both microwaved and conventionally-heated grapefruit (Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2010, 2011 and 2013), no published study has been found comparing the effect of an alternative microwave process with that of conventional heat pasteurisation on the shelf-life and quality of a fruit-based product.

The aim of this study was to investigate the influence of microwave and conventional thermal pasteurization processes and storage at various temperatures (22, 10 and 4°C) on the pH, colour, enzyme activity, bioactive compounds and antioxidant activity of kiwifruit puree, as well as to determine the shelf-life of the product based on its microbial stability at 4°C.

## 2. MATERIAL AND METHODS

### 2.1. Sample preparation

Kiwifruit (*Actinida deliciosa* var. Hayward) produced in Italy was purchased from a local supermarket. Fruit pieces selected on the basis of a similar soluble solid content (13-16°Brix) and apparent fruit quality 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. The obtained puree was preserved in ice-water until further usage.

### 2.2. Treatments

Processing conditions were chosen based on preliminary experiments to simulate equivalent pasteurization treatments in terms of the degree of enzyme and microbial inactivation they achieved (Benlloch-Tinoco, Igual, Rodrigo, & Martínez-Navarrete,

2013; Benlloch-Tinoco, Pina-Pérez, Martínez-Navarrete, & Rodrigo, 2014). Requirements for pasteurization of fruit juices or similar fruit-based products are: (i) at least 5 $\log_{10}$ cycle inactivation of the most relevant pathogen microorganism (FDA, 2004) and (ii) no less than 90% of enzyme inactivation (Gonçalves, Pinheiro, Abreu, Brandao, & Silva, 2010). In a previous study (data not shown), several power-time combinations for microwave heating (200-1000 W and 60-340 s) and temperature-time combinations for conventional heating (90-97 °C and 30-60 s) were assayed. Those reaching the required level of peroxidase (POD) inactivation and *Listeria monocytogenes* reduction but causing the minimum nutritional and functional value deterioration were selected to carry out the present work (described below).

### *2.2.1. Microwave treatment*

A microwave oven (3038GC, NORM, China), provided with a glass turntable plate, was used to treat the kiwifruit puree. A sample of 500 g was tempered to an initial temperature of 25°C and then heated in the microwave oven in a standard size glass beaker (9 cm inner diameter and 12 cm length) (BKL3-1K0-006O, Labbox, Spain) at 1000W for 340s. The temperature of the sample in the coldest and hottest spots, previously identified (data not shown), was continuously recorded by means of a fibre-optic probe (CR/JP/11/11671, Optcom, Germany) which was connected to a temperature datalogger (FOTEMP1-OEM, Optcom). The treated samples were immediately cooled in ice-water until the puree reached 35°C.

### 2.2.2. *Conventional thermal treatment*

The conventional thermal treatment consisted of heating the sample to 97°C for 30 s in a circulating thermostatic water bath (Precisterm, Selecta, Spain). After the kiwifruit

was triturated, 20 g of puree was placed in TDT stainless steel tubes (1.3 cm inner diameter and 15 cm length) and closed with a screw stopper. A thermocouple, connected to a datalogger, was inserted through the sealed screw top in order to record the time temperature history of the sample during the treatment. Prior to this heating step, the samples were preheated to 25°C to shorten and standardize the come-up time (150 s). The treated samples were immediately cooled in ice-water until the puree reached 35°C.

### 2.3. Storage study

Both the heat-treated and the non-treated kiwifruit purees were packaged in clean, sterile plastic tubes (1.7 cm inner diameter and 11.8 cm length) (ref. 525-0153, VWR, Spain) and then stored in darkness at 4, 10 and 22°C for a maximum of 188, 58 and 23 days, respectively. The purpose of storage at 10 and 22°C was to observe the changes that may take place in the samples in the case of a partial, or total, rupture of the cold chain, respectively, during the shelf-life of the product.

### 2.4. Analytical determinations

The treated samples, as well as a non-treated sample used as control, were analysed as described below. Measurements were performed in triplicate at time 0 and at regular time intervals for each storage temperature tested.

#### *2.4.1. Chemicals and standards*

Unless otherwise stated, all chemicals employed were from Sigma-Aldrich (Germany) and they were of analytical quality or superior.

151 *2.4.2. Colour, pH, enzyme activity and antioxidant activity*

152 Colour of kiwifruit puree samples was measured using a Minolta CM 3600D
153 spectrocolorimeter (Konica Minolta Sensing, Inc., Japan). The colour coordinates were
154 obtained and results were expressed according to CIE L*a*b* uniform colour space
155 (10° observer and D65 illuminant), where: the L* value is a measure of lightness (from
156 0 to 100); a* is a measure of chromaticity on a green (−) to red (+) axis and b* of
157 chromaticity on a blue (−) to yellow (+) axis. Colour differences caused by treatment
158 and storage effects ($\Delta E_{TE}$ and $\Delta E_{SE}$, respectively) were calculated (see Table 1). To
159 obtain $\Delta E_{TE}$, the colour of microwave and conventionally treated samples were
160 compared with that of the non-treated sample, while for $\Delta E_{SE}$, the colour of the treated
161 or untreated samples at the end of their shelf-life was compared with that of the newly-
162 processed samples. To determine the pH, a digital pH-meter Basic 2 was used (Crison,
163 Spain). Peroxidase (POD) and polyphenoloxidase (PPO) activity were determined
164 spectrophotometrically and the $DPPH^{\bullet}$ radical scavenging capacity of kiwifruit extracts
165 was measured to determine antioxidant activity (AOA) of the samples. More details
166 about these methodologies appear in Benlloch-Tinoco, Varela, Salvador, and Martínez-
167 Navarrete (2012) and Benlloch-Tinoco et al. (2013).

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169 *2.4.3. Bioactive compounds*

170 The vitamin C (Vit. C) and total phenol (TP) content was measured as previously
171 described by Igual, García-Martínez, Camacho, and Martínez-Navarrete (2010). Briefly,
172 ascorbic acid and total vitamin C (ascorbic acid + dehydroascorbic acid) were
173 determined by HPLC (Jasco, Italy). The procedure employed to determine total vitamin
174 C was the reduction of dehydroascorbic acid to ascorbic acid, using DL-dithiothreitol as

175 the reductant reagent. Total phenols were quantified by using the Folin–Ciocalteu
176 method.

177 Total flavonoids (TF) were measured spectrophotometrically, following the method
178 described by Djeridane, Yousfi, Nadjemi, Boutassouna, Stocker, and Vidal (2006),
179 based on the formation of a flavonoid-aluminium complex. The extraction of TF
180 consisted of homogenising 35 g of the sample (T25 Janke and Kunkel turrax) for 5 min
181 with 40 ml of methanol, 10 ml of chlorhydric acid and sodium fluoride to inactivate
182 polyphenoloxidases and to prevent phenolic degradation. The homogenate was
183 centrifuged (11,872 *xg*, 10 min, 4 ºC) (P-Selecta Medifrigar BL-S, Spain) to obtain the
184 supernatant. For total flavonoid quantification, 1 mL of the extract was mixed with 1
185 mL of 20g/L $AlCl_3$ methanolic solution. After incubation at room temperature for 30
186 min in darkness, the absorbance of the reaction mixture was measured at 430 nm using
187 a UV-visible spectrophotometer (Thermo Electron Corporation, USA). The TF content
188 was expressed as mg of rutin equivalents (RE) per 100g of sample, using a standard
189 curve range of 0-0.05 mg of rutin/mL.
190
191 *2.4.4. Microbiological analysis*
192 The survival of *L. monocytogenes* was evaluated as described by Benlloch-Tinoco, et
193 al. (2014) with some modifications. Briefly, the kiwifruit puree subjected to both
194 microwave and conventional heat processing and the fresh kiwifruit puree used to
195 assess the growth of *L. monocytogenes* in the sample at various temperatures were
196 previously inoculated with a mean value (and standard deviation) of $1 \cdot 10^7$ ($2 \cdot 10^6$) and
197 $2.8 \cdot 10^2$ ($1.5 \cdot 10^1$) CFU/g, respectively. The total mesophilic bacteria (TMB) and yeast
198 and mould (Y&M) counts were examined by diluting the uninoculated samples in 0.1%
199 (w/v) sterile peptone water (Scharlab Chemie S.A., Spain) and enumerating the viable

cells in Plate Count Agar (PCA, Scharlab Chemie S.A.) and Potato Dextrose Agar (PDA, Scharlab Chemie S.A.) acidified with tartaric acid (10%), by adding 1mL of tartaric acid per 10mL of PDA, respectively. The selected dilutions were incubated at 30°C for 48 h in the case of TMB and at 25°C for 5 days in that of Y&M.

### 2.5. Kinetic modelling degradation

The results of $L^*$coordinate, Vit. C, TP and AOA obtained for kiwifruit puree were plotted vs. time for all temperatures studied to obtain the kinetic parameters explaining the colour changes and the degradative loss of bioactive compounds and AOA in the treated and untreated kiwifruit puree during storage. Reaction order was determined by fitting experimental data to second-order, first-order and zero-order models. Zero-order kinetic (Equation 1) resulted to be the one that best fitted experimental data. The same was observed by (Gonçalves, Abreu, Brandão, & Silva, 2011; Zheng & Lu, 2011). The time for the concentration of a compound to fall to half its initial value (half-life, $t_{1/2}$) was also determined (Equation 2).

$$C = C_0 - k \cdot t \quad (1)$$

$$t_{\frac{1}{2}} = \frac{C_0}{2k} \quad (2)$$

Where

C: concentration of the compound at t ($mg \cdot 100g^{-1}$);

$C_0$: concentration of each compound at time zero ($mg \cdot 100g^{-1}$);

k: zero-order rate constant ($mg \cdot 100g^{-1} \cdot days^{-1}$);

t: storage time (days);

$t_{1/2:}$ the half time of the compound (days).

224 On the other hand, the temperature dependence of the degradation of these attributes
225 was studied by employing the Arrhenius equation (Equation 3). In every case, the
226 goodness of fit between the experimental and predicted data was assessed by means of
227 the adjusted regression coefficient (adj-$R^2$) (Equation 4), considering that the higher the
228 adj-$R^2$ value, the better the fit.

229 $$k = k_0 \cdot e^{\frac{-E_a}{R \cdot T}} \quad (3)$$

230 Where

231 k: rate constant ($days^{-1}$);

232 $k_0$: the pre-exponential factor;

233 $E_a$: activation energy ($kJ \cdot mol^{-1}$);

234 R: gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$);

235 T: absolute temperature (K)

236 $$Adjusted - R^2 = \left[ \frac{(m-1)(1 - \frac{SSQ_{REGRESSION}}{SSQ_{TOTAL}})}{(m-j)} \right] \quad (4)$$

237

238 Where

239 m: number of observations;

240 j: number of model parameters;

241 SSQ: sum of squares.

242

243 **2.6. Statistical analyses**

244 The assumptions of normality and equality of variance were tested by normality plots
245 and box-plots, respectively. Linear mixed models correlating each one of the attributes

evaluated in the present study with the type of sample (fresh, conventionally heated, microwaved), storage temperature and storage time were developed using the SPSS Statistics 19 software program (IBM SPSS, Inc., USA). A p-value of 0.05 (2-sided) was assumed to reflect statistically significant differences. Following significant Fisher-F tests, post-hoc tests (Bonferroni's) were conducted. Non-linear and linear regression analyses, based on the Levenberg–Marquardt estimation method, were carried out in order to estimate the kinetic parameters using the SPSS Statistics 19 software program (IBM SPSS). Furthermore, a correlation analysis was run between all the studied components with a 5% significance level.

## 3. RESULTS AND DISCUSSION

### 3.1. Shelf-life determination based on microbial stability

In order to evaluate the impact of processing and storage on the microbial stability of kiwifruit puree, the survival of *L. monocytogenes*, taken as the pathogen of greatest concern in the product (Benlloch-Tinoco et al., 2014) was investigated (Figure 1). At the same time, TMB and Y&M flora were also followed in the fresh (F), microwaved (MW) and conventionally-heated (C) samples during storage at various temperatures (22, 10 and 4°C) (Figures 2 and 3).

Microwave and conventional thermal treatments lead to a 5.8 (0.4) and 5.1 (0.3) $\log_{10}$ cycle reduction in the count of *L. monocytogenes*, a 2.1 (0.0) and 1.1 (0.2) $\log_{10}$ cycle reduction in the count of TMB and a 2.10 (0.10) and 0.95 (0.13) $\log_{10}$ cycle reduction in the count of Y&M, respectively. While both the microwave and conventional thermal treatments lead to equivalent *L. monocytogenes* inactivation (no significant differences), the microwave process was significantly more effective at inactivating TMB and Y&M.

After such a reduction in the bacterial counts brought about by processing, a growth of microorganisms was observed during storage. Linear mixed models were used to evaluate the effect of storage temperature, storage time and type of sample on their growth. Significant statistical differences were found in the counts of *L. monocytogenes*, TMB and Y&M due to all these factors and their interactions. As expected, the higher temperature led to a significantly faster growth of these microorganisms and the longer storage time to significantly higher counts (Figures 1, 2 and 3). Similarly, Rivas, Rodrigo, Martínez, Barbosa-Cánovas, and Rodrigo (2006) reported faster growth rates of microorganisms in orange and carrot juice when stored at 12°C than at 2°C. On the other hand, F and MW samples showed by far the fastest and slowest growth rate of microorganisms at any of the temperatures studied (22, 10, 4 °C), respectively, while C sample exhibited intermediate behaviour. These differences between samples were found to be more evident at 22°C and 10°C than at 4°C. In this regard, while the untreated kiwifruit puree was rapidly spoiled by microorganisms reaching 3.2, 4.0 and 4.2 $\log_{10}$ CFU/g for *L. monocytogenes*, TMB and Y&M after 74 days at 4°C, respectively, the treated samples (MW, C) stored at 4°C kept microbial loads below 1 $\log_{10}$ CFU/g for 74 days.

The shelf-life of treated samples was determined at 4°C, taking into account the acceptable limit established by EU legislation (*L. monocytogenes* $\leq$ 2.0 $\log_{10}$ CFU/g and TMB and Y&M $\leq$ 3.0 $\log_{10}$ CFU/g) (EU, 2005). On this basis, the shelf-life of C and MW treated puree was found to be 81 and 123 days, respectively (Figures 1, 2 and 3). These results are in the range of those published by other authors working on different fruits subjected to conventional thermal processes. The shelf-life of heat-pasteurized orange and carrot juice (98°C for 21s) stored at 2°C, thermally pasteurized pomegranate (90°C for 5 s) stored at 5°C and conventionally heat-pasteurized orange juice (90°C for

295 50s) stored at 4°C was found to be 70, 120 and 105 days, respectively (Leizerson &
296 Shimoni, 2005; Rivas et al., 2006; Vegara, Martí, Mena, Saura, & Valero, 2013). On the
297 other hand, Picouet et al., (2009) reported that an apple-puree preserved by gentle
298 microwave heating (652W-35s) had a shelf-life of at least 14 days under refrigeration
299 conditions.

300 Bearing in mind the results obtained in the present study, microwave heating seems
301 to provide greater microbial stability than conventional heat processing, allowing for a
302 better preservation of kiwifruit puree. In a similar way, other authors have found a better
303 microbiological shelf-life for various fruit-based products when preserved by novel
304 technologies, such as pulsed electric fields, than when heat pasteurization is used
305 (Sampedro, Geveke, Fan, Rodrigo, & Zhang, 2009; Walkling-Ribeiro et al., 2010). This
306 superiority of microwaves can be supported taking into account the results of another
307 study in which a further comparison between microwave and conventional heating
308 processes was performed by means of Pasteurization Units (PU), which allow treatment
309 severity to be quantified in terms of thermal load (Benlloch et al., 2014). Obtained PU
310 (80ºC) were 0.53 (0.05) min at the coldest spot and 19 (2) min at the hottest spot for
311 MW, and 19.27 (0.13) min for C sample. According to these data, kiwifruit puree was
312 not subjected to a more severe treatment when was processed under microwave heating.
313 In other words, superiority of microwaves cannot be explained by faster heating rates or
314 greater temperature achieved. Like it has been reported by other authors, however, this
315 superiority might indicate the possibility of some enhanced effects associated with
316 microwaves (Banik, Bandyopadhyay, & Ganguly, 2003; Tajchakavit, Ramaswamy, &
317 Fustier, 1998).

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### 3.2. Effect of process on enzyme activity. Stability during storage

The impact of processing and storage on kiwifruit puree enzymes was assessed by investigating the evolution of POD and PPO activity in F, MW and C samples during storage at 22, 10 and 4°C (Figures 4 and 5). The enzyme activity was significantly reduced by both microwave and conventional heat treatments. The mean value (and standard deviation) of inactivation caused by these processes was 96% (2) and 95.7% (1.1) of POD and 82% (2) and 43% (4) of PPO, respectively. Despite the fact that the reduction of POD activity under microwave and conventional heat treatments was found to be equivalent (no significant differences), the microwaved kiwifruit puree exhibited a significantly higher PPO inactivation.

On the other hand, the effect of factors, such as temperature, time and type of sample, on the evolution of enzyme activity during storage was checked by using linear mixed models. The statistically significant differences observed in POD and PPO values were caused by the storage time, type of sample and their interactions. In general terms, F sample showed a significant drop in POD and PPO activity during storage, which may be attributed to a decrease of the substrates concentration available within the kiwifruit puree over time. Additionally, PPO is believed to be irreversibly inactivated during the oxidation of substrate to product due to a free radical-catalyzed fragmentation of one or more of the six histidine residues that bind the two coppers at the active site (Whitaker, Voragen, & Wong, 2003).

On the contrary, treated samples (MW, C) exhibited a slighter variation of POD and PPO activity over time. On the one hand, the residual POD activity remained mostly constant in treated purees at 4°C, there being no observed significant differences between MW and C samples (Figure 4). While the main fall of POD in F puree took place after 44 days when stored at 4°C, varying from 8.6 (1.3) to 1.9 (0.2) $Abs \cdot min^{-1} \cdot g^{-1}$,

C and MW samples stored at 4°C maintained the POD activity below 1.5 $Abs \cdot min^{-1} \cdot g^{-1}$ for all 144 and 182 days, respectively. On the other hand, although the treated samples exhibited lower residual PPO activity than F puree over time, PPO inactivation was shown to be reversible (Figure 5). Some reactivation of this enzyme in both the MW and C samples stored for 74 days at 4°C was observed, PPO activity subsequently, remaining mainly constant. Other authors have reported enzymes, e.g. peroxidase, recovering their activity after heating treatments, especially in high-temperature-short-time processed fruit and vegetables (Thongsook & Barrett, 2005).

From the results obtained, it can be seen that significantly lower residual activity and a markedly smaller variation of POD and PPO enzymes was found to take place during the storage of treated samples; this is especially true in the case of POD, one of the enzymes which most relevantly contributes to the deterioration in the colour and nutritive value of kiwifruit (Fang, Jiang, & Zhang, 2008), a fact that can be taken as an indicator of the stability provided by processing. In this respect, and taking into account the widely recognized detrimental effects of these enzymes, the microwave and conventional thermal treatments applied to pasteurized kiwifruit puree may be considered to make a meaningful contribution to the preservation of the product quality by minimizing the degradative effect of the enzyme during its shelf-life.

### 3.3. Effect of process on pH and colour. Stability during storage

The changes in the pH and $L^*$, $a^*$, $b^*$ colour coordinates of the kiwifruit puree brought about by processing and storage were studied. Table 1 summarises these values, together with the colour changes caused by treatment and storage effects, for treated samples, both those non-stored (storage 0 days) and those stored until the end of their shelf-life.

From the statistical analysis, it can be stated that the pH of the kiwifruit puree was not significantly affected by processing, but it significantly decreased in all the samples during storage, irrespective of the temperature, probably due to a significant growth of the microbial flora (Figures 1, 2 and 3), fact also observed by Elez-Martínez et al. (2006).

From Table 1 it can be seen that processing had a significant impact on the colour of the product, being the samples more luminous and changing to a less greenish hue after treatment. Greater colour changes were observed when the puree was conventionally heated than when microwaved. Similarly, Chandrasekaran, Ramaanathan, and Basak (2013) observed that microwaving preserves colours better than other conventional thermal techniques. The statistical analysis pointed out that the storage time, type of sample and their interactions brought about significant statistical differences in $L^*$ and $b^*$. However, $a^*$ and $\Delta E_{SE}$ values were exclusively affected by the storage time and the type of sample, respectively. In the F sample, $L^*$ values decreased from 38.48 to 34.33 and $a^*$ values increased from -5.18 to 1.12 for the first 44 days of storage at 4°C; thereafter, both of them remained mostly constant. However, $b^*$ values did not show a clear trend. The colour of the treated samples changed in a similar way during storage although to lesser extent than the F sample, leading to a lower degree of luminosity and a redder hue angle in every case. The potential degradative impact of POD and PPO enzymes on the colour of kiwifruit puree was investigated by means of a correlation statistical analysis (Pearson's correlation) in both the treated and untreated samples. A significant correlation between $L^*$ and POD ($R^2$ = -0.3244) and PPO ($R^2$ = -0.3226) was found, which indicated that a loss of luminosity over time might be attributable to the detrimental activity of POD and PPO enzymes. In this respect, colour stabilization

observed in MW and C samples could be attributed to the enzymatic stability provided by processing (Figures 4 and 5).

On the other hand, the kinetics of variation of the colour coordinates and colour differences during storage was investigated. However, the evolution of $L^*$ was the only colour coordinate that properly fitted zero-order kinetics. The values of the kinetic rate constant (k) and half-destruction time ($t_{1/2}$) calculated for the F, MW and C samples at 22, 10 and 4°C are given in Table 2. To determine the effect of temperature on the studied parameters, the obtained rate constants were fitted to the Arrhenius equation. The obtained activation energies ($E_a$) are also shown in Table 2. In order to describe the effect of both the treatments and temperature on the rate of decrease in $L^*$, it was considered that the lower the $t_{1/2}$ and the higher the k values, the faster the variation of the $L^*$ coordinate. Additionally, a higher value of activation energy means a greater dependence of the kinetic rate constant on the storage temperature. In general terms, the storage temperature had a greater impact on the rate of luminosity reduction than the treatment applied, observing that the higher the storage temperature, the more quickly the $L^*$ values decreased. Althoug microwave heating was the only one having a positive effect decreasing the rate at which $L^*$ changed in the sample irrespective of the temperature, MW puree was the sample requiring the lowest temperature increase in order to achieve the same increase in the rate of $L^*$ reduction. Despite the fact that microwave heating leads to a greater sensitivity of the k parameter to temperature changes during storage, from the viewpoint of luminosity this technology may be preferred as a means of preserving the kiwifruit puree, since it clearly leads to the lowest rate of $L^*$ variation at any of the temperatures studied.

### 3.4. Effect of process on the bioactive compounds and AOA. Stability during storage

The influence of processing and storage on the nutritional and functional value of kiwifruit puree was investigated by checking the changes in the amount of vitamin C, total phenols and total flavonoids, as well as the antioxidant activity of the puree samples during storage. Its evolution in the F, MW and C samples stored at 4°C is included in Figure 6.

Although processing did not provoke significant losses in the Vit.C, TP and AOA of the product, it did significantly affect the TF, reducing its content by 28.80% (0.003) and 42.38% (0.02) when the puree was microwave and conventionally treated, respectively. As the storage temperature-time and the type of sample also affected these compounds and AAO, the corresponding degradation kinetics was studied. However, the loss in TF was not appropriately described by zero-order kinetics. The total flavonoid content decreased in kiwifruit samples during storage, the higher the storage temperature, the faster the degradation rate (data not shown). Processing clearly allowed for a better maintenance of TF during storage, especially when kiwifruit puree was microwaved (Figure 6). Decrease of TF over time can be explained by the detrimental activity of PPO, since these compounds are widely known to be common substrate of this enzyme (Whitaker et al., 2003). Not only a smaller decrease was observed in the MW and C samples during storage, but also an increase in the total flavonoid content. In this way, despite the losses caused by processing, from day=16 onwards, the total flavonoid content was higher in the treated samples than in the untreated ones. Likewise, Kevers, Falkowski, Tabart, Defraigne, Dommes, and Pincemail (2007) reported that the total flavonoid content of apricot, yellow pepper, plum and green grape remained stable, or even increased, during storage.

As far as the degradation kinetics of Vit. C, TP and AOA is concerned, the values of the kinetic rate constant (k) and half-destruction time ($t_{1/2}$) for the F, MW and C samples stored at 22, 10 and 4°C are presented in Table 2. From the $t_{1/2}$ values obtained, whereas vitamin C may be considered to be the compound which most easily suffers degradation during storage at 22°C, the total phenols demonstrated they were the most stable. On the other hand, both the $t_{1/2}$ and the k values corroborated the fact that a higher storage temperature meant a faster degradation of bioactive compounds and a decrease in AOA in every sample. As expected, the processing of kiwifruit seemed to improve the stability of TP and AOA when stored at 22°C, leading to reduced degradation rates. However, no positive effect of pasteurization treatments (MW, C) was observed in the total phenol content of the samples stored at 10 and 4°C. Unlike conventional heating, microwave processing reduced the degradation rate of Vit. C at 10 and 4°C. On the other hand, lower rates of AOA decrease were found in the MW (10 and 4°C) and C purees (10°C). Considering that the MW sample exhibited similar or higher $t_{1/2}$ and lower k values for Vit. C and AOA than both the F and C samples, it can be pointed out that the nutritional and functional value of the kiwifruit puree was equally well or better preserved during storage when the kiwifruit puree was processed by means of microwave technology. In addition, vitamin C was the compound showing the highest activation energy values for every sample, which means that the Vit. C degradation rates exhibited greater thermal sensitivity than TP and AOA in the treated and untreated kiwifruit puree. Moreover, the MW sample required a smaller temperature increase to achieve the same increase in the rate of Vit.C and AOA reduction than the F and C purees, while conventional heating reduced the heat sensitivity of TP and AOA with respect to the F sample.

467 Despite the fact that the degradation of bioactive compounds may be explained in
468 many different ways, as a matter of fact, enzyme activity considerably contributes to the
469 quality loss frequently observed in fruits and vegetables during storage. As has
470 previously been mentioned (section 3.2.), POD and PPO enzymes may lead to the
471 oxidation of polyphenolic compounds to quinines that then polymerize to dark melanin
472 pigments, which is commonly known as enzymatic browning (Friedman, 1996). As a
473 result, not only the colour, but also the functional value of the product, is affected. In
474 this respect, a correlation statistical analysis (Pearson's correlation) was carried out so
475 as to improve the understanding of the potential connection of colour changes with the
476 loss in bioactive compounds and AOA observed in kiwifruit samples during storage. As
477 expected, TP and TF were negatively correlated with $\Delta E_{SE}$ ($R^2$ = -0.5940 and $R^2$ = -
478 0.3208, respectively) and TP were positively correlated with $L^*$ ($R^2$ = 0.3296). In other
479 words, when total phenols and total flavonoids gradually decreased (Figure 6), the
480 luminosity of the product was reduced and greater colour differences were detected
481 (data not shown), a fact that could be taken as an indicator of the detrimental activity of
482 kiwifruit enzymes.
483 On the other hand, the nutritional and functional value of the microwave and
484 conventionally treated kiwifruit puree at the end of their shelf-life was compared (Table
485 1). Despite the fact that the MW sample was stored for a longer period of time, it
486 showed significantly higher Vit. C and TP, but lower TF, after 123 days at 4°C. As for
487 AOA, no differences were observed. The variation of the components brought about by
488 both processing and storage was calculated as the difference between each compound in
489 the treated puree at the end of its shelf-life related to the fresh puree and referred to
490 100g of fresh puree. In this respect, losses of 43%, 23% and 62% in vitamin C, total
491 phenols and total flavonoids were found for the MW sample (123 days at 4°C) while

492 losses of 61%, 58 and 56% were observed in vitamin C, total phenol and total flavonoid
493 content of the C sample (81 days at 4°C), respectively. However, AOA was reduced by
494 62% in both cases. The results obtained clearly indicate the superiority of microwaves
495 when it comes to preserving the nutritional and functional value of the product by
496 equating or reducing the post-processing loss in bioactive compounds and AOA. In the
497 same way, Igual et al. (2010) reported that microwave pasteurized grapefruit juices
498 stored at -18°C better preserved both the total phenols and antioxidant capacity when
499 compared with fresh or conventionally pasteurized ones. Furthermore, Igual et al.
500 (2011) found that the use of microwaves led to a greater retention of individual
501 grapefruit juice flavonoids during storage (4 and -18°C) than when conventional heating
502 was used.
503

504 **4. CONCLUSIONS**

505 Microwave heating may be considered a suitable means of processing kiwifruit
506 puree and preserving the safety and quality of the product during storage. This
507 technology led to a greater or equal degree not only of microbial and enzyme
508 inactivation but also of the preservation of colour, bioactive compounds and antioxidant
509 activity in comparison with conventional heating. Microwave-pasteurized kiwifruit
510 puree s not only exhibited a longer shelf-life (123 days at 4°C) than the conventionally
511 heated one (81 days at 4°C), but also superior colour, vitamin C and total phenol
512 maintenance over time. Accordingly, microwave technology might be successfully
513 employed as an innovation tool with which to help safe, high-quality and minimally
514 processed kiwifruit based-products reach the market.
515

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634 pasteurized pineapple juice. *LWT-Food Science and Technology, 44,* 1273-1281.
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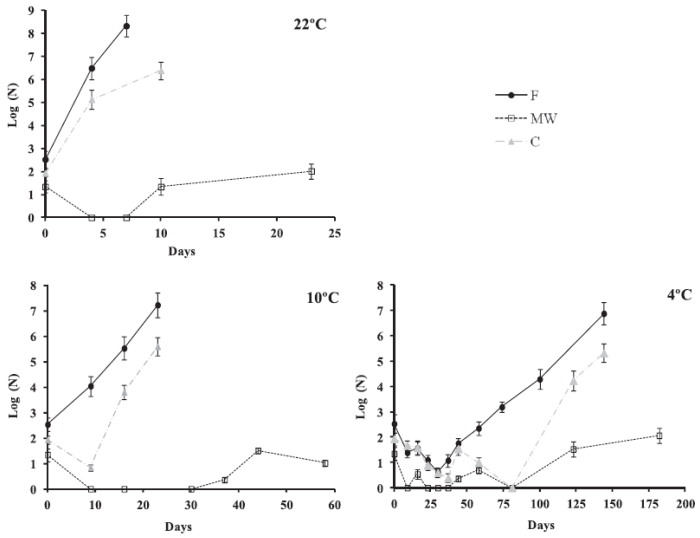



**Figure 1.** Survival of *Listeria monocytogenes* in the kiwifruit puree (F: fresh, MW: microwaved and C: conventionally thermal treated) during storage at 22, 10 and 4°C. The plotted values and error bars represent the average of three replicates and the corresponding standard deviation.

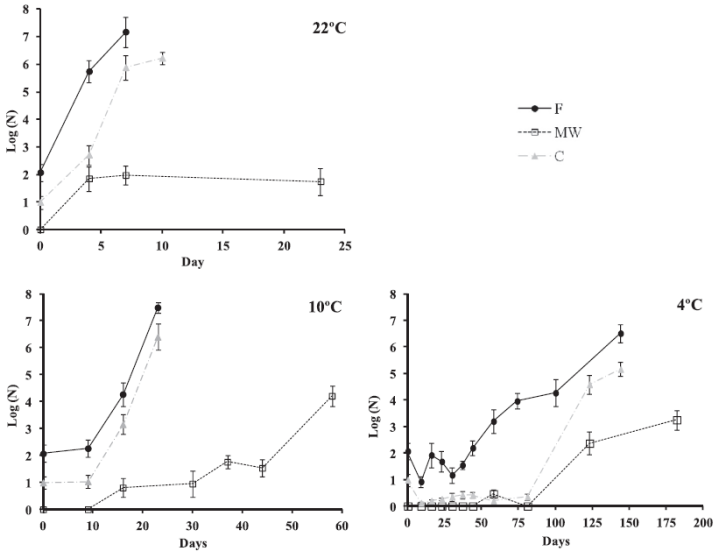



**Figure 2.** Survival of total mesophylic bacteria in the kiwifruit puree (F: fresh, MW: microwaved and C: conventionally thermal treated) during storage at 22, 10 and 4 ˚C. The plotted values and error bars represent the average of three replicates and the corresponding standard deviation.

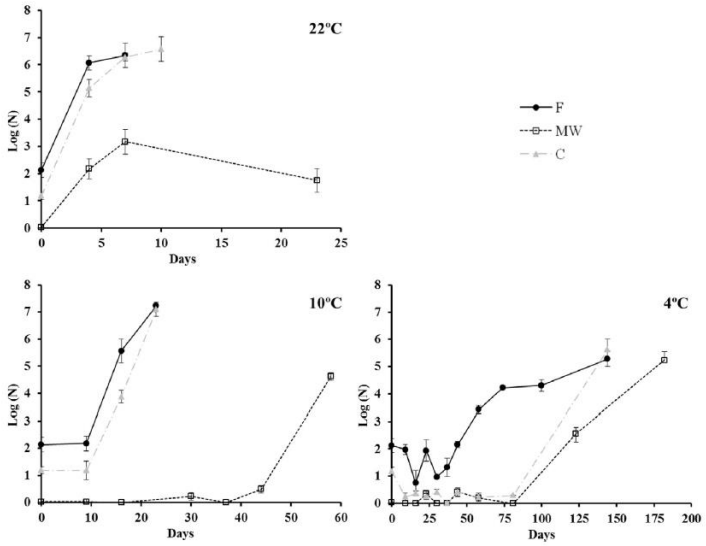
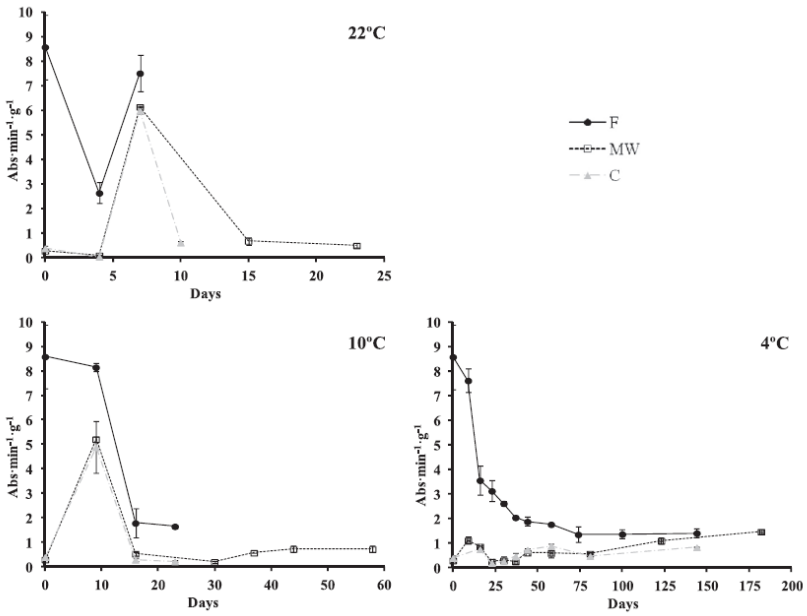
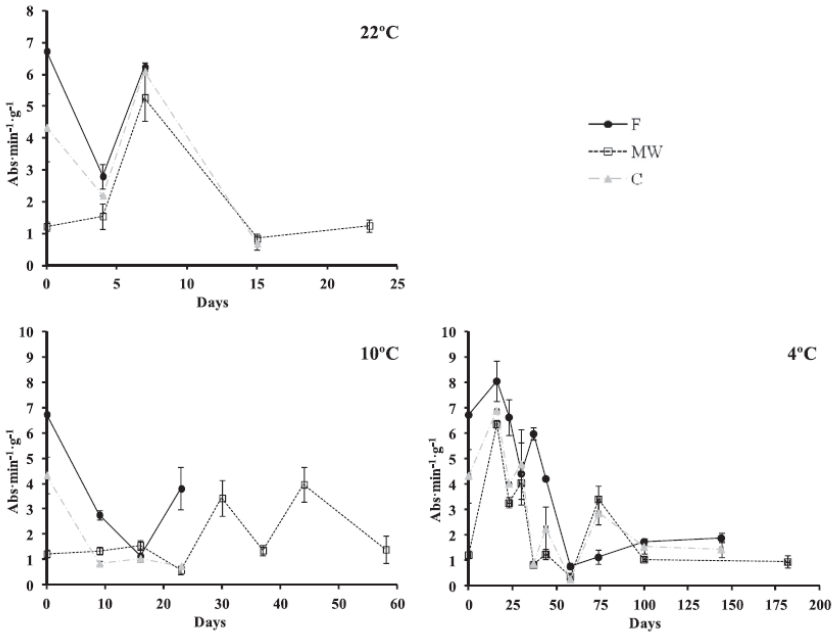



**Figure 3.** Survival of yeast and mould in the kiwifruit puree (F: fresh, MW: microwaved and C: conventionally thermal treated) during storage at 22, 10 and 4 ˚C. The plotted values and error bars represent the average of three replicates and the corresponding standard deviation.



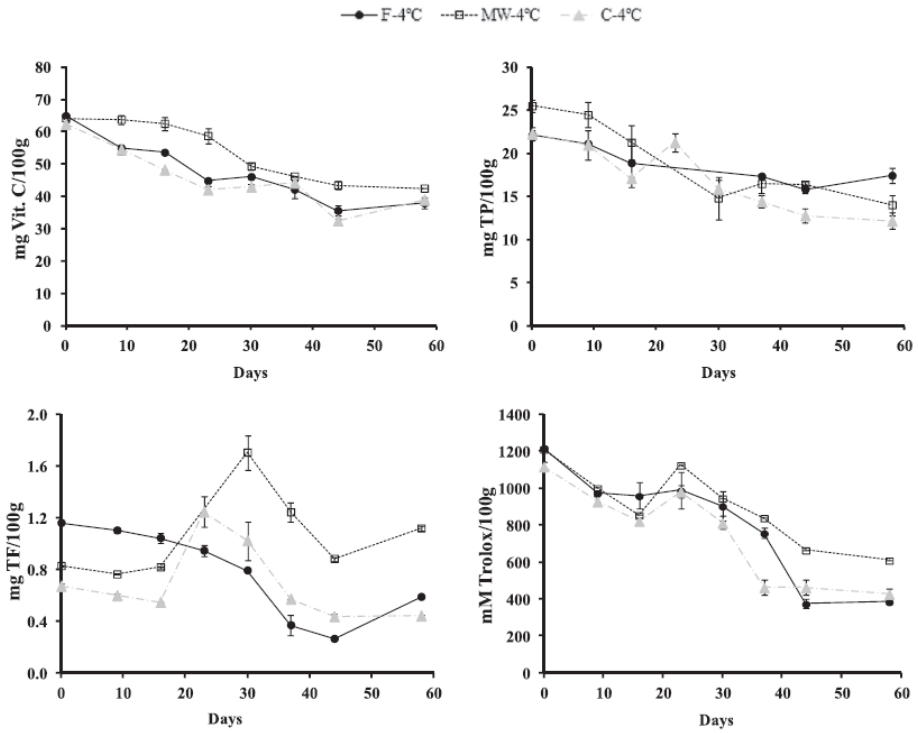

658
659 **Figure 4.** Peroxidase activity (POD) in the kiwifruit puree (F: fresh, MW: microwaved
660 and C: conventionally thermal treated) during storage at 22, 10 and 4°C. The plotted
661 values and error bars represent the average of three replicates and the corresponding
662 standard deviation.

663




664
665 **Figure 5.** Polyphenoloxidase activity (PPO) in the kiwifruit puree (F:fresh, MW:
666 microwaved and C: conventionally thermal treated) during storage at 22, 10 and 4 ºC.
667 The plotted values and error bars represent the average of three replicates and the
668 corresponding standard deviation.

669




670
671 **Figure 6.** Vitamin C (Vit. C), total phenols (TP) and total flavonoids (TF) content and
672 antioxidant activity (AOA, expressed as mM Trolox) in the kiwifruit puree (F: fresh,
673 MW: microwaved and C: conventionally thermal treated) during storage at 4ºC. The
674 plotted values and error bars represent the average of three replicates and the
675 corresponding standard deviation.

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677

678

**Table 1.** Mean values (and standard deviation) of vitamin C (Vit. C, mg/100g), total phenols (TP, mg GAE/100g) and total flavonoids (TF, mg RE/100g) content and antioxidant activity (AOA, mM Trolox/100g), pH, colour coordinates ($L^*$, $a^*$ and $b^*$) and colour difference due to processing ($\Delta E_{TE}$) and storage ($\Delta E_{SE}$) of microwaved (MW) and conventionally heated (C) kiwifruit puree, at the beginning and end of their shelf-life (4°C).

| | **Beginning of shelf-life** | | **End of shelf-life** | |
|---|---|---|---|---|
| | *0 days* | | *123 days at 4ºC* | *81 days at 4ºC* |
| | **MW** | **C** | **MW** | **C** |
| Vit. C | 64.2 (0.7)a | 62.3 (0.7)a | 37.2 (0.6)b | 25.4 (1.5)c |
| TP | 25.50 (0.07)a | 22.2 (0.3)b | 13.92 (0.08)c | 9.3 (0.3)d |
| TF | 0.825 (0.004)a | 0.67 (0.02)b | 0.437 (0.013)c | 0.505 (0.010)d |
| AOA | 1211 (37)a | 1117 (27)b | 478 (35)c | 463 (41)c |
| pH | 3.85 (0.14)a | 3.75 (0.13)a | 3.35 (0.02)b | 3.15 (0.02)a |
| $L^*$ | 43.90 (0.02)a | 44.81(0.03)b | 39.76 (0.02)c | 43.67 (0.03)d |
| $a^*$ | -1.11 (0.02)a | -1.71 (0.02)b | 1.183 (0.012)c | 0.19 (0.03)d |
| $b^*$ | 26.81 (0.03)a | 22.63 (0.02)b | 24.083 (0.012)c | 27.06 (0.05)d |
| $^{(*)}\Delta E_{TE}$ | 7.06 (0.02)a | 7.54 (0.02)b | - | - |
| $^{(*)}\Delta E_{SE}$ | - | - | 6.54 (0.02)a | 7.80 (0.02)b |

Three replicate samples were used to calculate each mean value and the corresponding standard deviation. Different letters in rows, indicate statistical significant differences ($p<0.05$) according to Bonfferoni test when the effect of time was evaluated.

$^{(*)}\Delta E_{TE} = \sqrt{(a_F^* - a_{T_0}^*)^2 + (b_F^* - b_{T_0}^*)^2 + (L_F^* - L_{T_0}^*)^2}$ $\Delta E_{SE} = \sqrt{(a_{T_0}^* - a_{T_{Si}}^*)^2 + (b_{T_0}^* - b_{T_{Si}}^*)^2 + (L_{T_0}^* - L_{T_{Si}}^*)^2}$

Where: the L* value is a measure of lightness (from 0 to 100); a* is a measure of chromaticity on a green (−) to red (+) axis and b* of chromaticity on a blue (−) to yellow (+) axis colour coordinate. Subscripts refer to fresh puree (F), newly treated puree ($T_0$) and treated puree after i days of storage ($Ts_i$).

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**Table 2**
Times of half destruction ($t_{1/2}$: days), mean values (and standard error) of the degradation rates ($k$: mg 100 $g^{-1}$ $day^{-1}$) and the activation energy ($E_a$: kJ $mol^{-1}$) of luminosity ($L^*$), vitamin C (Vit. C), total phenols (TP) and antioxidant activity (AOA) of fresh ($F$), microwaved (MW) and conventionally heated ($C$) kiwifruit puree during storage at 22, 10 and 4 °C. Adjusted regression coefficient ($R^2$-aj.).

| | $T$ (°C) | $L^*$ | | | Vit. C | | | TP | | | AOA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $F$ | MW | $C$ | $F$ | MW | $C$ | $F$ | MW | $C$ | $F$ | MW | $C$ |
| $t_{1/2}$ | 22 | 38.56 | 69.24 | 49.02 | 2.95 | 2.92 | 2.83 | 8.44 | 23.61 | 16.86 | 5.69 | 7.21 | 5.94 |
| $k$ | | 0.50 (0.07) | 0.317 (0.003) | 0.457 (0.004) | 11 (2) | 11 (2) | 11 (2) | 1.31 (0.13) | 0.54 (0.04) | 0.66 (0.03) | 107 (3) | 84 (8) | 94 (5) |
| $R^2$-aj. | | 91.966 | 99.962 | 99.961 | 79.231 | 79.231 | 79.231 | 94.160 | 96.934 | 99.185 | 99.500 | 95.000 | 98.500 |
| $t_{1/2}$ | 10 | 94.79 | 359.81 | 119.17 | 36.06 | 54.40 | 32.81 | 56.34 | 31.87 | 41.21 | 41.42 | 56.67 | 60.08 |
| $k$ | | 0.20 (0.03) | 0.061 (0.007) | 0.188 (0.002) | 0.9 (0.2) | 0.59 (0.06) | 0.95 (0.08) | 0.196 (0.013) | 0.40 (0.07) | 0.27 (0.02) | 14.7 (1.5) | 12 (2) | 9.3 (1.7) |
| $R^2$-aj. | | 89.189 | 86.998 | 99.886 | 82.170 | 93.113 | 91.232 | 95.831 | 83.528 | 92.006 | 95.000 | 81.000 | 86.400 |
| $t_{1/2}$ | 4 | 223.74 | 438.97 | 203.67 | 55.01 | 80.24 | 47.22 | 96.93 | 55.43 | 58.56 | 41.42 | 56.07 | 35.36 |
| $k$ | | 0.086 (0.008) | 0.050 (0.05) | 0.110 (0.007) | 0.59 (0.05) | 0.40 (0.04) | 0.66 (0.05) | 0.114 (0.012) | 0.23 (0.02) | 0.19 (0.02) | 14.7 (1.2) | 10.8 (1.2) | 15.8 (1.4) |
| $R^2$-aj. | | 70.911 | 76.951 | 84.563 | 79.876 | 84.712 | 84.989 | 73.594 | 76.456 | 76.055 | 86.200 | 72.800 | 80.900 |
| $E_a$ | | 64.5 (1.3) | 73 (2) | 53.4 (0.2) | 115 (3) | 131 (4) | 111 (3) | 94 (2) | 30.3 (1.3) | 47.6 (0.4) | 64.5 (1.3) | 73 (2) | 53.5 (0.2) |
| $R^2$-aj. | | 94.859 | 87.660 | 99.789 | 90.868 | 88.534 | 88.534 | 89.610 | 77.248 | 98.964 | 97.900 | 87.700 | 99.800 |

Three replicate samples were used to calculate each mean value and the corresponding standard deviation.

696