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Additional Information

1     **IMPACT OF TEMPERATURE ON LETHALITY OF KIWIFRUIT**  
2     **PUREE PASTEURIZATION BY THERMAL AND MICROWAVE**  
3                     **PROCESSING**

4             María Benlloch-Tinoco<sup>1</sup>, Nuria Martínez-Navarrete<sup>1</sup>, Dolores Rodrigo<sup>2\*</sup>

6     <sup>1</sup>*Universitat Politècnica de Valencia, Food Technology Department, Food Investigation*  
7     *and Innovation Group, Camino de Vera s/n, 46022 Valencia, Spain*

8     <sup>2</sup>*Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Avda. Agustín*  
9     *Escardino 7, 46980 Paterna, Valencia, Spain*

10    \**Corresponding author. Tel.: +34 963 90 0022x 2218. E-mail address:*  
11    *lolesra@iata.csic.es (D. Rodrigo).*

13    **Abstract:**

14    The use of pasteurization units (PU) as a measure of the processes lethal effect was  
15    proposed with the aim of comparing both conventional and novel thermal technologies.  
16    Kiwifruit puree was subjected to microwave (1000 and 900 W) and conventional (97  
17    °C) heating. Processing conditions of the treatments were selected to cause a 90% of  
18    kiwifruit peroxidase inactivation. The temperature profiles of the samples during  
19    processing were registered at different positions. The coldest and hottest spot of the  
20    product were identified and the associated PU numbers were calculated. Significantly  
21    (p<0.05) higher thermal load was necessary in order to inactivate the target level of  
22    peroxidase under conventional (19.27 min) than microwave heating mode (0.22-1.8  
23    min) at any of the studied conditions. Higher effectiveness of microwave heating could  
24    be attributed to non-thermal effects associated to this technology.

26 **Keywords:** Microwaves, thermal treatment, kiwifruit, temperature, accumulated  
27 lethality.

28

## 29 1. INTRODUCTION

30 Microwave heating (MW) appears to be a promising novel technology for food  
31 preservation (Cañumir et al., 2002; Vadivambal & Jayas, 2010). During the last  
32 decades, many studies have been carried out on the evaluation of MW benefits respect  
33 to conventional heat treatments. Its suitability for pasteurization, sterilization and  
34 dehydration processes as well as its capacity of producing safe and better quality  
35 products has been widely demonstrated (Igual, García-Martínez, Camacho, & Martínez-  
36 Navarrete, 2010; Huang, Sheng, Yang, & Hu, 2007). Although MW could potentially  
37 replace conventional heat processes for some specific applications (Awuah,  
38 Ramaswamy, & Economides, 2007), there are still problems that are inherent to this  
39 technology, such as non-uniform product temperature distribution (Salazar-González,  
40 San Martín-González, López-Malo, & Sosa-Morales, 2012), that contribute delaying the  
41 exploitation of MW to its fullest potential in the food industry.

42 On the other hand, improper comparison between treatments, because of inadequate  
43 control of processing parameters such as sample temperature exposure, roughly selected  
44 exposure periods or poor kinetic data accommodation, may be generating doubts and  
45 causing conflicting opinions regarding the superiority of this technology against the  
46 conventional heat treatments. Some authors have proposed different ways of comparing  
47 microwave and conventional treatments: (i) to select processing conditions to get equal  
48 heating rates (°C/min) (Fujikawa, Ushioda, & Kudo, 1992), (ii) to reach similar  
49 temperature profile in samples under both technologies (Welt, Tong, Rossen, & Lund,  
50 1994) and (iii) to carry out kinetic studies (Latorre, Bonelli, Rojas, & Gerschenson,

51 2012; Matsui, Granado, Oliveira, & Tadini, 2008; Tajchakavit, Ramaswamy, & Fustier,  
52 1998). This lack of homogeneity in comparison procedures can result in mistaken  
53 interpretations and hinders the contrast of different research works.

54 In the present study, the concept of accumulated lethality ( $F_0$ ), parameter  
55 traditionally employed to evaluate conventional heat treatments, is proposed as a tool  
56 for both conventional and novel thermal technologies comparison. The process lethal  
57 effect is determined in base of the time-temperature history of the product and it is  
58 expressed as a numerical value in arbitrary units. The pasteurization unit (PU) was  
59 proposed by Shapton, Lovelock and Laurita-Longo (1970) as a measure of accumulated  
60 lethality but more specifically adapted for pasteurization processes.

61 The objective of the present research work was to assess the suitability of PU  
62 parameter to compare the thermal load of microwave and conventional kiwifruit puree  
63 pasteurization treatments.

64

## 65 **2. MATERIAL AND METHODS**

### 66 **2.1. Sample preparation**

67 Kiwifruit (*Actinida deliciosa* var. Hayward) was purchased in a local supermarket. Fruit  
68 pieces were peeled and finally triturated in a Thermomix (TM 21, Vorwerk, Spain),  
69 using the fourth power level for one minute. The physicochemical characteristics of  
70 kiwifruit puree (water content, soluble solids, water activity and pH) were determined in  
71 order to control the fruit which was used as raw material (data not shown).

72

### 73 **2.2. Treatments**

74 Processing conditions were chosen based on preliminary experiments to simulate a  
75 pasteurization treatment (Benlloch- Tinoco, Pina-Pérez, Martínez-Aguirre, Rodrigo, &

76 Martínez- Navarrete, 2012). The treatments selected inactivated 90% of peroxidase  
77 enzyme and reduced more than 5 log<sub>10</sub> cycles of the most important pathogenic  
78 microorganism (*Listeria monocytogenes*) (data not shown). These data correspond to  
79 the global inactivation achieved in the samples. Three replicates of each treatment were  
80 run.

#### 81 *2.2.1. Microwave treatment*

82 A household microwave oven (3038GC, Norm, China) was used to treat the kiwifruit  
83 puree. For each treatment, a sample of 500 g was tempered to an initial temperature of  
84 25° C and then heated in the microwave oven in a standard size glass beaker (BKL3-  
85 1K0-006O, Labbox, Spain). Two microwave treatments, based on different power-time  
86 combinations, were carried out: 1000W-200s and 900W-300s. Processing conditions  
87 were selected based on preliminary experiments to cause approximately a 90% of  
88 peroxidase inactivation (Benlloch-Tinoco, Pina-Pérez, Martínez-Aguirre, Rodrigo, &  
89 Martínez-Navarrete, 2012). Three replicates of each treatment were run. The microwave  
90 oven was provided with a probe (CR/JP/11/11671, Enelec, Spain) which was connected  
91 to a fiberoptical thermometer (FOTEMP1-OEM, Enelec, Spain) to continuously register  
92 the time-temperature history of the sample during the microwave treatments. Because  
93 MW has been traditionally associated with non-uniform heating, the coldest and the  
94 hottest spots were identified and the temperature at these points was recorded.

95

#### 96 *2.2.2. Conventional thermal treatment*

97 The selected treatment consisted in heating the sample at 97 °C for 30 s in a  
98 thermostatic water bath (Precistern, Selecta, Spain). After kiwifruit was triturated, 20 g  
99 of puree were introduced in TDT stainless steel tubes (13 mm inner diameter and 15 cm  
100 length) and closed with a screw stopper. A thermocouple which was connected to a data

101 logger was introduced through the sealed screwed top in order to register the time-  
102 temperature history of the sample during the treatment. Three replicates were carried  
103 out to define an average temperature profile of the process. Previously, samples were  
104 preheated at 25 °C to shorter and standardise the come-up time (150 s).

105

### 106 **2.3. Peroxidase enzyme determination**

107 Peroxidase activity (POD) was measured in all the treated samples (microwaved and  
108 conventionally heated ones) and also in the non-treated sample, which was used as  
109 control, according to the method proposed by De Ancos et al. (1999) with the following  
110 modifications. For enzymes extraction pH 6.5 was used, centrifugation was done for 20  
111 min and filtration step was avoided. Extracts were made in duplicate. Enzyme extract  
112 (0.050 mL) was used for enzymes activity measurement and pH 6.5 was fitted. A  
113 solution containing all the components except the enzyme extract, which was replaced  
114 by 0.050 mL of sodium phosphate buffer, was used as a blank. One unit of POD was  
115 defined as the amount of enzyme that caused an increase of one in the absorbance per  
116 min ( $\text{Abs}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ ), calculated from the linear part of the obtained curve. The  
117 percentage of enzyme inactivation (I) was calculated by using Eq. (1).

$$118 \quad I = \frac{A_F - A_T}{A_F} \times 100 \quad (1)$$

119

120 Where:

121  $A_F$ : enzyme activity of fresh kiwifruit puree

122  $A_T$ : enzyme activity of treated kiwifruit puree.

123

### 124 **2.4. 2L. monocytogenes inactivation study**

125 *L. monocytogenes* is recommended by the National Advisory Committee on  
126 Microbiological Criteria for Foods to be used as a target microorganism for products of  
127 similar characteristics. Kiwifruit puree, prepared as described above, was inoculated by  
128 adding 1 mL of a *L. monocytogenes* (CECT 4032, Spanish Type Culture Collection)  
129 inoculum to give a final concentration of 10<sup>7</sup> CFU/g. Kiwifruit puree was blended for  
130 30 s with the aim of ensuring a homogeneous initial content of the bacterium. After  
131 processing, serial decimal dilutions of both treatments and the untreated one were  
132 performed in 0.1% (w/v) sterile peptone water (Scharlab Chemie S. A., Barcelona,  
133 Spain). The enumeration medium used for viable cells was Tryptic Soy Agar (TSA)  
134 (Scharlab Chemie S. A., Barcelona, Spain). The selected dilutions were incubated at 37  
135 °C for 48 h.

136

## 137 **2.5. Pasteurization units calculation**

138 The pasteurization units corresponding to the microwave and conventionally treated  
139 samples were calculated using Eq. (2) (Heinz, Toepfl, & Knorr, 2003; Lau & Tang,  
140 2002) with a reference temperature of 80°C and a z-value of 13.62 °C, previously  
141 determined for *Listeria monocytogenes* in a kiwifruit puree under thermal processing.

142

$$143 \quad PU = \int_0^t 10^{\left(\frac{T(t)-T_{ref}}{z}\right)} dt \quad (2)$$

144

145 Where,

146 t: Treatment time (s);

147 T(t): Product temperature at each treatment time;

148 T<sub>ref</sub>: 80°C;

149 z: Temperature sensitivity (°C) for *Listeria monocytogenes*.

150

## 151 **2.6. Statistical analyses**

152 Significant differences were evaluated by means of the corresponding analysis of  
153 variance (ANOVA) using Statgraphics Plus 5.1. Differences of  $p < 0.05$  were considered  
154 to be significant.

155

## 156 **3. RESULTS AND DISCUSSION**

157 Microwave and conventional heating comparison has been the base of many studies  
158 dealing with MW processes applications, such as those performed by Cañumir et al.  
159 (2002), Gentry & Roberts (2005) or Igual et al. (2010). The difficulty of comparing both  
160 technologies lies in the particular way of heating which takes place during MW  
161 treatments (Banik, Bandyopadhyay, & Ganguly, 2003). While in conventional heating  
162 holding period is expected, in the case of MW, non-isothermal heating takes exclusively  
163 place (Latorre et al., 2012). Additionally, fixing those parameters affecting the heating  
164 process such as (i) the heating rate, (ii) the range of temperatures at which the samples  
165 are exposed or (iii) providing appropriated sample homogenization, is not usually  
166 possible. In this way, products conventionally and microwave treated are not normally  
167 subjected to equivalent temperature-time combinations and comparing the effect of both  
168 technologies on the product quality may result complicated.

169 Given the different nature of heating processes taking place under conventional and  
170 microwave modes, the temperature control should not be limited to the initial and the  
171 final point of the process, but the whole temperature history of the product should be  
172 taken into account. In this context, the PU parameter offers the possibility of evaluating  
173 the complete thermal load of the heating processes at any reference temperature, as if it  
174 had taken place under isothermal conditions. This implies that the product is considered



175 to instantaneously reach the reference temperature (Matsui et al., 2008), so the effect of  
176 processing factors that could be promoting differences in the nature of the heat  
177 transference such as (i) product characteristics including consistency, solid/liquid ratio  
178 and thermophysical properties, (ii) sample quantity and (iii) container type, size and  
179 shape (Augusto & Cristianini, 2011; Awuah et al., 2007), is avoided.

180 The concept of accumulated lethality has been previously used in relation to  
181 microwaves in order to validate the lethal effect of a formerly established preserving  
182 treatment (Chen, Campanella, & Peleg, 2011; Wang, Wig, Tang, & Hallberg, 2003). It  
183 has also been employed as a tool for assessing the effect of a conventional and a  
184 combined microwave-conventional pasteurization process on the nutritional and sensory  
185 quality of asparagus by calculating C-value (Lau & Tang, 2002). However, up to date,  
186 PU has still not been used with the aim of evaluating the thermal load of various  
187 conventional and novel heating processes to perform comparison.

188 In the present study the temperature profiles of various kiwifruit puree samples  
189 subjected to some microwave and conventional thermal treatments were registered in  
190 order to compare the different processes lethal effects (Figure 1). Although the  
191 conventionally treated and the microwaved samples showed the same level of POD  
192 inactivation (90%) and *L. monocytogenes* reduction (>5 log<sub>10</sub>-cycles), noticeable  
193 differences in the temperature-time profiles can be observed in Fig. 1. The thermal load  
194 associated to each treatment was calculated through the PU parameter. Mean value (and  
195 standard deviation in brackets) of PU numbers obtained for all the assayed treatments  
196 are presented in Table 1. As expected, substantial differences were found in the thermal  
197 load received by the product at the two locations studied during the microwave  
198 treatments. The PU obtained at the hottest spot was considerably higher than the PU  
199 obtained at the coldest spot. On the other hand, the conventional heating mode required

200 a significantly ( $p < 0.05$ ) higher thermal load to achieve the pre-set level of POD  
201 inactivation in the kiwifruit puree than any of the microwave treatments studied,  
202 irrespective of whether the comparison was carried out at the coldest or hottest spot of  
203 the sample. When the microwave treatments were compared, 900W-225 s was the one  
204 showing the highest and lowest thermal load at the hottest and coldest spot,  
205 respectively. However, significant differences ( $p < 0.05$ ) were only observed in relation  
206 with the hottest spot. The greater effectiveness of MW with respect to conventional  
207 heating treatments for food stabilization has been widely reported by various authors,  
208 such as Matsui et al. (2008) and Soysal and Söylemez (2005). Although differences  
209 observed in MW and conventional heating processes have traditionally been attributed  
210 to the faster heating rates of MW (El-Abassy, Donfack, & Materny, 2010), in our case  
211 this premise cannot be accepted to explain the differences observed, because the PU  
212 data were calculated as if the treatments had taken place under isothermal conditions.  
213 Consequently, they might indicate the possibility of some contributory non-thermal  
214 effects associated with MW. Although other authors have reported similar findings  
215 (Banik et al., 2003), in-depth research work on this area is considered necessary.

216

#### 217 **4. CONCLUSIONS**

218 The pasteurization unit seem to be an adequate parameter to evaluate the thermal  
219 load associated to conventional and microwave heating processes. This parameter can  
220 be taken as a common base to compare the effect of different heating technologies on  
221 the products quality and stability. Microwave heating required lower thermal load than  
222 conventional heating to pasteurize the product any of the power levels studied, which  
223 might be attributed to some contributory non-thermal effects associated with this  
224 technology.

225

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229

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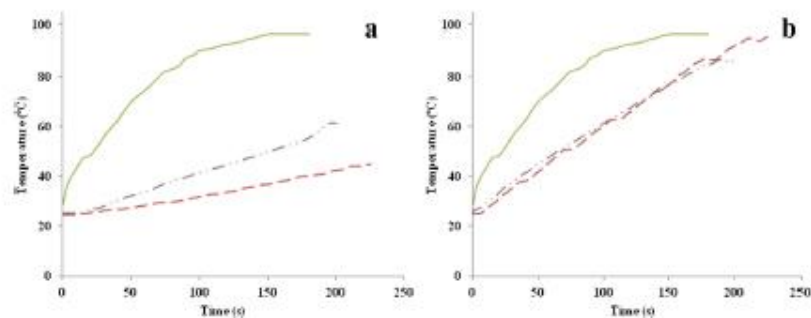


Fig. 1. Temperature profile of kiwifruit puree subjected to conventional thermal (—) and microwave (1000 W, --- and 900 W, - - -) processing at the coldest spot (a) and the hottest spot (b) of the product.

234

**Table 1**

Mean values and standard deviation (in brackets) of pasteurization units (PU) calculated at the coldest and the hottest spot of the kiwifruit puree under conventional and microwave (1000 and 900 W) heating. The same superscript letters in columns (x, y for coldest spot data and a, b, c for the hottest spot data) indicate homogeneous groups established by the ANOVA ( $p < 0.05$ ) when the different treatments are compared.

Treatment	PU (min)	
	Coldest spot	Hottest spot
Conventional heating	19.27 (0.13) <sup>bc</sup>	
Microwave heating		
1000 W-200 s	0.046 (0.007) <sup>f</sup>	1.79 (0.02) <sup>a</sup>
900 W-225 s	0.0027 (0.0014) <sup>z</sup>	7.9 (1.4) <sup>b</sup>