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Additional Information

1 ADVANTAGES OF SOUS-VIDE COOKED RED CABBAGE:

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STRUCTURAL, NUTRITIONAL AND SENSORY ASPECTS

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8 ABSTRACT

The comparison between equivalent cooking treatments should be applied in a systematic way. This study 9 10 proposes a methodical way to provide cooked samples with similar firmness using two cooking treatments. In 11 addition, the structural, nutritional and sensory properties of red cabbage cooked with sous-vide treatment in 12 comparison with traditional cooking (boiling water) was evaluated. Changes in texture, color and anthocyanin 13 content were measured in samples cooked with traditional cooking (for different times) and sous-vide 14 (modifying time and temperature according to a Response Surface Methodology). Consumers described 15 sensory properties and preferences between samples. Cryo-scanning electron microscopy was used to study 16 the samples microstructure.

The firmness of samples, traditionally cooked for 11 min and preferred by consumers, was achieved in samples cooked with sous-vide treatment by optimizing of the cooking conditions (87 °C/50 min or 91 °C/30 min). Sousvide treatment was preferred to traditional cooking by consumers. Sous-vide samples were more purple, more aromatic and tastier than traditionally cooked ones. The loss of anthocyanins in traditional cooking was twice that in sous-vide samples. Micrographs from different treatments showed different degrees of cell wall damage. Sous-vide treatment could be recommended as a treatment for the catering industry providing better guality products.

24

25 **Keywords**: firmness, color, response surface methodology, sensory analyses, anthocyanins.

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1. INTRODUCTION

The incorporation of red cabbage (*Brassica oleracea* convar. capitata var. capitata f. rubra) in the diet is beneficial to the consumer because of its high-water, fiber and antioxidant content, such as anthocyanins (Halvorsen et al., 2002; Van Duyn & Pivonka, 2000).

The red cabbage is traditionally cooked in boiling water (around 100 °C according to the atmospheric pressure) for several minutes. This habitual treatment is drastic as it applies high temperatures. Therefore, the beneficial compounds, such as anthocyanins, could be destroyed by heat.

Considering other cooking methods, sous-vide treatment is based on raw materials or raw materials with intermediate foods that are cooked under controlled conditions of temperature and time inside heat-stable vacuum pouches (Schellekens, 1996; Baldwin, 2012). The use of sous-vide was widely applied in restaurants and caterings. To assure the microbial safety during its use despite the risk related to the use of low temperatures, practical manuals for its use were published (Light & Walker, 1990; Ghazala, 1998; Gould, 1999; Baldwin & Nutridox, 2010). The sous-vide method has now become a popular and safe treatment used in the catering industry (Dodgshun, Peters, & O'Dea, 2011).

40 The nutritional benefits of sous-vide have been studied (Petersen, 1993; Trejo-Araya et al., 2009; Chiavaro, 41 Mazzeo, Visconti, Manzi, Fogliano, & Pellegrini, 2012). In cooking treatments, time and temperature are the 42 main factors. Kinetic models (considered primary models) characterize the changes (such as firmness and 43 color) according to time. In environmental conditions, other factors such as temperature are commonly modeled using a secondary model. Primary and secondary models could be combined in differential equations 44 45 permitting the description of a process under dynamic conditions. Experimental design, such as response surface methodology (RSM) could be also useful for modeling. RSM has been developed to explore 46 47 relationships between several variables and one or more responses. This permits selection of an adequate 48 combination of conditions to achieve an optimal or desired response (Box & Hunter, 1957).

49

Sensory evaluation is important for developing products, but the cost of the study and the quantity of products
used mean that the process has to be as efficient as possible. The use of instrumental texture measurements,

52 such as the Kramer cell test, puncture test and Warner Bratzler test (McKenna & Kilcast, 2004), have been 53 shown to correlate with sensory evaluation (Bourne, 2002). Therefore, they can replace sensory tests for 54 assessing products in the first steps of development new products (Walter, Truong, & Espinel, 2002; Meullenet, 55 Lyon, Carpenter, & Lyon, 1998).

56 Sensory quality is one of the prime concerns in the catering industry which applies the sous-vide to minimize 57 the workload during services and to produce dishes using second-class cuts of meat and poultry with 58 extraordinary tenderness and texture (Dodgshun, Peters, & O'Dea, 2011). Therefore, it is important to understand how cooking techniques, cooking time and temperature affect the sensory quality. Different tests 59 have been applied to discern the opinion and the perceptions of the consumers, such as JAR (Just About Right) 60 61 scale and FP (Flash Profiling). The JAR (Just About Right) scale permits the measurement of the intensity of 62 specific attributes linked to hedonic assessment by consumers (Gacula, Rutenbeck, Pollack, Resurreccion, & Moskowitz, 2007), while FP facilitates sensorial descriptions by reducing the training time of assessors (Dairou 63 & Sieffermann, 2002). 64

With the aim to find equivalent cooking conditions providing a similar firmness between two treatments reducing as much as possible the number of sensory tests, the present study proposes a methodical way based on the Response Surface Methodology combining instrumental and sensory analysis. In addition, the study evaluated the structural, nutritional and sensory features of red cabbage cooked with sous-vide treatment and traditional cooking.

70 2. MATERIALS AND METHODS

71 2.1. MATERIALS

Red cabbage (*Brassica oleracea* convar. capitata var. capitata f. rubra) purchased from a local company (Reypama, Spain) was used for the tests. Samples were harvested a week before the experiments and stored at 4 °C until their use. The leaves were washed and cut into discs (20 mm diameter) using a manual cylinder cutter.

76 **2.2. COOKING METHODS**

77 Two cooking methods were applied: the traditional cooking (with time modifiable and temperature around 78 100 °C -boiling water at atmospheric pressure-) and the sous-vide treatment (with modifiable time and 79 temperature).

Traditional cooking was carried out using a stainless steel saucepan for times of 30 seconds (blanching), 7 min, 11 min or 15 min with a constant product weight:water volume ratio of 1 : 40. After the cooking treatment, all samples were rapidly cooled in a water-ice bath for a minute as usually doing by professional cooks, and then vacuum packaged in pouches (Cryovac[®] HT3050) applying vacuum conditions (98% vacuum) with a vacuum packaging machine (EV-25, Technotrip, Spain). The pouches were stored at low temperature at 3-4 °C for 24 h, before the instrumental measurements to simulate conditions in the catering industry.

For the sous-vide treatment, the raw red cabbage discs were vacuum sealed in thermoresistant pouches (Cryovac® HT3050) applying vacuum conditions (98% vacuum) with a vacuum packaging machine (EV-25, Technotrip, Spain). The heat treatment was conducted in a water bath cooker (GD 120, Grant Instruments, Cambridge, UK) at atmospheric pressure. Table 1 shows the time and temperature of the cooking conditions. After the cooking treatment, all pouches containing sous-vide samples were rapidly cooled in a water-ice bath for a minute as usually doing by professional cooks. The pouches were stored at low temperature at 3-4 °C for 24 h, before the instrumental measurements to simulate conditions in the catering industry.

93 2.3. SENSORY ANALYSES

A just about right (JAR) test was used to evaluate firmness of samples cooked with traditional cooking (100 °C) at three different times (7 min, 11 min and 15 min). Consumers (n = 65) evaluated the firmness of cooked red cabbage using a 5-point just about right (JAR) scale (1 = too soft, 3 = just about right; 5 = too hard) (Gacula, Rutenbeck, Pollack, Resurreccion, & Moskowitz, 2007).

98

Paired tests were carried out following ISO standards (ISO, 2005). Two paired test were used in this study. The
first one was used to analyze the perceptive differences between sous-vide samples cooked with two different

101 combinations of factors (time and temperature). In this test consumers (n=47) were questioned about 102 firmness, purple color, aroma, taste and preference.

103 The second one was carried out to compare treatments (sous-vide and traditional cooking). The purpose was 104 to discern the preference and differences perceived in attributes (firmness, purple color, aroma and taste) by 105 consumers (n=92). Also questions related to global preference and the most important attribute for the choice 106 of preferred sample were added.

107

Flash profiling (FP) was used to obtain information about characteristics perceived by consumers related to 108 109 different cooking treatments and cooking conditions (Dairou & Sieffermann, 2002). FP was used to describe 110 the samples cooked by five treatments based on traditional cooking (7 min, 11 min and 15 min) and sous-vide 111 (87 °C/50 min and 91 °C/30 min). Consumers received 6 samples at the same time, of which two samples were from the same treatment to validate the study (91 °C/30 min) and check the performance of consumers 112 according to a cluster test applied to the coordinates for each samples provided by the Generalized Procrustes 113 114 Analysis (GPA). 28 non-trained consumers participated in the test and the performance to describe the intensity of attributes was verified. After applied the Generalized Procrustes Analysis, the coordinates of the 115 116 position of each samples according to the perceptions of each consumers has been obtained. To each 117 consumer, the coordinates has been analysed with a cluster analysis. 10 consumers have been ruling out due 118 to the lack of consistency in his criteria because the samples from the same treatment were not grouped or 119 perceived as similar (Veinand, Godefroy, Adam, & Delarue, 2011; Varela & Ares, 2012).

120 2.4. INSTRUMENTAL TEXTURE ANALYSIS

121 The texture of the red cabbage discs was measured in a Kramer shear cell using a Texture Analyser TA-XT2 122 (Texture Technologies Corp., Scardale, NY, USA). The test speed was 1.6 mm/s using a stainless steel 5-blade 123 probe (HDP/KS5) with a load cell of 5 kN. 10.0(0.5) g of samples covered the entire surface of the test cell. The 124 test was carried out until total penetration of the samples was achieved. The peak force (N) determined the 125 firmness. The measurement was repeated four times for each treatment.

126 **2.5. COLOR MEASUREMENT**

127 Color was measured using a Minolta CM3600d colorimeter (Minolta Corp., Ramsey, NY, USA). The instrument 128 was calibrated against a ceramic reference, illuminant C, before use. Samples were placed on a white tile, 129 previously verifying that samples were not translucent. Results were given in the CIELab system for illuminant 130 D65 and a 10° angle of vision. Registered parameters were L* (brightness), a* (redness) and b* (blueness), 131 from these parameters, h*_{ab} (hue) and C*_{ab} (chroma) were obtained. For each treatment, ten samples were 132 used to measure the color of the leaves.

133 2.6. DETERMINATION OF TOTAL MONOMERIC ANTHOCYANIN CONTENT

134 The determination of total monomeric anthocyanins was based on the pH differential method (Lee, 2005). The preparation of samples consisted of chopping 40 g of cooked red cabbage. Next 2 g of the chopped product 135 136 was homogenized for 30 seconds with 20 mL of methanol (Panreac, Barcelona, Spain) and 0.1 mL of hydrochloride acid (37% HCl, Panreac, Barcelona, Spain). The homogenate stored for 24 hours at 4 °C in dark 137 138 conditions was then centrifuged (10.000 rpm, 10 min, 4 °C) to obtain the supernatant. Aliquots of 0.4 mL were 139 added to 3.6 mL of pH 1.0 and pH 4.5 buffers, prepared as suggested by Lee (2005). After waiting for at least 20 140 min, but not more than 50 min, samples were evaluated at λ = 700 and 530 nm in a spectrometer (Helios Zeta UV-VIS, Thermo Fisher Scientific, UK). The anthocyanins pigment concentration, expressed as cyanidin-3-141 142 glucoside equivalents, was calculated as follows:

143 Anthocyanins pigment (_{cyanidin-3-glucoside equivalents, mg/L}) =
$$\frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

where A = $(A_{530nm}-A_{700nm})_{pH1.0} - (A_{530nm} - A_{700nm})_{pH4.5}$; MW (molecular weight) 449.2 g/mol for cyanidin-3glucoside (cyd-3glu); DF = dilution factor; 10^3 = factor for conversion from g to mg; ε =26900 molar extinction coefficient, in L × mol⁻¹ × cm⁻¹, for cyd-3-glu; and 1= path length in cm. The total monomeric anthocyanin content was expressed as mg of cyanidin-3-glucoside equivalents per 100 gram of cooked samples. Each sample was analyzed in quadruplicate.

149 2.7. EXPERIMENTAL DESIGN

150 Response Surface Methodology (RSM) was used to determine the experimental design with the sous-vide 151 treatment (Table 1) and to establish the optimal time and temperature conditions to provide a target value of 152 firmness, following a similar procedure of a previous work (Iborra-Bernad, Tárrega, García-Segovia, & Martínez-153 Monzó, 2013a). Statgraphics Centurion (Statistical Graphics Corp., Herndon, VA, USA) was employed to 154 generate the experimental design, and to conduct the statistical analyses and regression models. A five-coded 155 level, two-factor, rotatable, orthogonal and central composite design was employed (Kuehl, 2000; Myers & 156 Montgomery, 2002) to study the effect of the two independent variables or factors (time and temperature) on 157 the response: firmness, redness (a^*) and hue (h^*_{ab}).

158 2.8. CRYO SCANNING ELECTRON MICROSCOPY (CRYO-SEM)

159 The microstructure of the sample was examined using cryo-scanning electron microscopy (CRYO-SEM) with a 160 JEOL JSM-5410 microscope (Jeol, Tokyo, Japan). Samples were cut into rectangular pieces 4 x 1.5 x 5 mm. The 161 samples were frozen by immersion in Slush Nitrogen (-210 °C) and were then fractured, etched (at -90 °C, 10⁻⁵ Torr vacuum, for 15 min), and gold coated before being viewed in the cold-stage scanning electron microscope. 162 163 Using this technique, the fractured surface of the frozen sample was viewed directly at -150 °C or lower. Micrographs were analyzed after day 1 of storage at 4 °C. The micrographs were taken at 750 and 200 164 165 magnifications. Samples observed were raw samples, ones blanched for 30 s (100 °C), others cooked for 11 166 minutes with traditional treatment (100 °C), and samples cooked with sous-vide treatment 91 °C/30 min.

167 2.9. STATISTICAL ANALYSIS

The data of firmness, color coordinates and anthocyanins were analyzed with Statgraphics 5.1 plus (STSC, Rockville, MD, USA). ANOVA with LSD post-hoc analysis was used to compare the means of the cooking treatments. The significant differences were fixed at $p \le 0.05$.

Just about right scale results were analyzed estimating the below and above deviation from point 3 on the scale (JAR) according to Gacula *et al.* (2007). For each sample, the mean of values below JAR point 3

ACCEPTED MANUSCRIPT corresponded to the negative deviation values (too little of the attribute), while the mean of values above JAR 173 174 point 3 corresponded to the positive deviation value (too much of the attribute). 175 To analyze the data obtained with the paired test comparisons (sensory test), the significant differences in 176 preferences and sensory properties were established for α =0.05 (ISO, 2005). 177 The software XLSTAT 2010 (Addinsoft, USA) was used to analyze FP applying Generalized Proscrutes Analysis 178 (GPA) (Gower, 1975). The consensus between the assessors sensory maps and the instrumental data (firmness, 179 CIE L*a*b* coordinates and anthocyanins) was obtained with the GPA. The performance of the consumers has 180 been verified with the application of a cluster analysis in the coordinates of the position of each sample according to the perceptions of each consumer. It was applied the nearest neighbor method and the squared 181 182 Euclidean distance as a measure of dissimilarity, and dendrograms were used to check if the samples from the 183 same treatment were grouped together.

184 3. RESULTS AND DISCUSSION

185 **3.1. FIRMNESS AND COLOR OF RED CABBAGE.**

186 **3.1.1. TRADITIONAL COOKING.**

187 Table 2 shows the instrumental data for cooked red cabbage for 30 s (blanching), 7 min, 11 min and 15 min. 188 Firmness decreased with an increase in cooking time, the firmness ranged from 598 to 145 N. Compared with 189 blanched samples (30 seconds at 100 °C), the firmness decreased by 54% at 7 min and the loss of firmness 190 decreased (from 274 to 145 N) between 7 and 15 minutes. During the first minutes, the loss of cell turgor was 191 the reason of the rapid decay in softening, while the main reason in the second stage was the degradation of 192 pectic substances, the main polymers in the middle lamella (De Belie, Herppich, & De Baerdemaeker, 2000; De 193 Belie, Laustsen, Martens, Bro, & De Baerdemaeker, 2002; Greve, Shackel, Ahmadi, McArdle, Gohlke, & 194 Labavitch, 1994).

195 Regarding color, cooking time affected the color coordinates. Therefore, samples with longer cooking times (11 196 and 15 min) provided significantly lighter samples (L*) ($p \le 0.05$), while blueness (b*) showed no significant

differences. An increase in the immersion time of samples caused a loss of redness (a*), a change of hue (h_{ab}^*) and an increase in lightness (L*). These changes could be related to contact time with boiling water which increases the leaching of anthocyanins into the water. The concentration of this antioxidant was reduced significantly (p<0.05) with the increase in cooking time (Table 2)

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201 **3.1.2. SOUS-VIDE.**

202 Table 3 shows the changes in firmness produced by different cooking conditions of sous-vide. It was observed 203 that firmness decreased when both time and temperature increased. Firmness values were significantly 204 decreased from 559 N for sous-vide treatment at 78 °C/40 min to 126 N for sous-vide treatment at 92 °C/40 min (p≤0.05). The treatments with higher firmness were 78 °C/40 min and 80 °C/30 min, while the lower firmness 205 206 was reported for treatments at 90 °C/50 min and 92 °C/40 min. To better understand the effect of time and 207 temperature, a second-order polynomial depending on time and temperature was fitted to the measured 208 firmness values with coefficients Bi and Bij (Table 4). According to the F-value, temperature (B1) had more 209 weight (higher F-value) in the model, followed by the linear time term (B2). The linear coefficients were 210 negative, the firmness reduced as time and temperature increased. Nevertheless, the quadratic coefficient of 211 temperature (B11) and interaction coefficient (temperature x time, B12) were both significant and positive. 212 This explains the rapid loss of firmness at lower time (less than 40 min) and temperature (less than 85 °C), and 213 the slow change in firmness at high levels of each factor (more than 40 min and above 85 °C).

214

Color coordinates (Table 3) were also measured for each combination of conditions. Lightness (L*) showed no 215 216 differences between treatment conditions and its values ranged between 24 and 26, unlike the change observed in traditional cooking. Regarding the proportion of a* (+, redness) and b*(-, blueness) in samples, 217 results suggested more reduction of redness (from 8 to 4) than blueness (from -10 to -8.9). For chroma (C^*_{ab}), 218 219 the values ranged between 10 and 13, being higher in less aggressive treatments (78 °C/40 min, 80 ° C/30 min 220 and 85 °C/26 min). Concerning hue (h_{ab}^*), values ranged between 308 (more purple) and 294 (more blue). 221 Color coordinates were modeled, but only redness (a*) and hue (h*_{ab}) were considered in this study because of 222 their higher coefficient of determination (R^2). Redness values were fitted to a second order model: $a^* = 5.1$ -

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1.17 x Temperature - 0.82 x Time + 0.45 x Temperature ² (R^2 adjusted for df =0.831. P-value (lack of fit)
=0.548). Both linear terms were significant and with negative coefficients, and temperature had more weight
(higher F-value) in the reduction of redness, as was observed for firmness. Quadratic terms of temperature and
interaction terms were significant and with positive coefficients. The behavior of redness according to the
paring conditions is similar to the firmness model. A rapid loss of redness was observed when the levels of
factors were lower, such as 80 °C/30 min; while the reduction of redness was slower when both factors
increased, such as 90°C/50 min. Concerning hue (h_{ab}^*), values were also fitted to a second order model:
$h_{ab}^*=298.5 - 4.6 \times Temperature - 2.5 \times Time (R^2 adjusted for df =0.783. P-value (lack of fit) =0.768).$ In this
case, only linear terms had significant coefficients. Therefore, change in hue fitted a linear equation where the
temperature had more weight (higher F-value) in the model. Linear terms had both negative coefficients,
changing samples towards a bluish color with the increase of temperature and cooking time.

234

235 3.2. SENSORY AND NUTRITIONAL PROPERTIES OF COOKED RED CABBAGES.

236 **3.2.1. JUST ABOUT RIGHT TEST.**

To establish the preferred firmness of cooked red cabbage by consumers, samples cooked for 7, 11 and 15 min with traditional cooking were evaluated using Just About Right (JAR) tests (n = 65). The lower the deviation on the JAR scale the greater the preference. The samples with least deviation (≤0.30) from the optimal firmness were cooked for 11 min (0.30 and 0.28 deviation for too soft and too firm, respectively), while samples cooked 7 minutes had deviation values of 0.16 and 0.58 for too soft and too firm, respectively, and samples cooked 15 minutes had deviation values of 0.51 and 0.20 for too soft and too firm, respectively.

Results presented in this section suggested that the most suitable firmness for cooked cabbage corresponded to a value of instrumental firmness near to 180 N (Table 2). This instrumental value of firmness was considered as the target firmness (TF).

The next step was to determine cooking conditions with sous-vide treatment to provide samples with TF (180 N). The fitted model (Table 4) was plotted to find the range of conditions (temperature and time) which

predicted firmness values near to TF (180 N). Fig. 1 shows a wide range of combinations of possible times and 248 249 temperature between 87 °C/50 min (+a) and 91°C/30 min (+b). These conditions were chosen to compare with 250 samples cooked with traditional cooking. This procedure to optimize the cooking conditions was based on 251 sensory analyses combined with instrumental measurements, although in some vegetables is possible to 252 optimize the cooking conditions with only instrumental data, such as done in a previous study with green 253 beans described by Iborra-Bernad et al. (2013b). The present procedure seems more recommendable to 254 compare cooking treatments because the cooked samples have similar firmness, which is determined by 255 consumers. In addition, it could be applied in vegetables which color coordinates do not change according to a second-order polynomial, such as in the case of carrots (Iborra-Bernad, Tárrega, García-Segovia, & Martínez-256 257 Monzó, 2013a), and it permits to choose the conditions in a wide range of temperatures and times.

258 **3.2.2. ANTHOCYANIN CONTENT.**

Anthocyanins are the main flavonoid in red cabbage (Bhagwat, Gebhardt, Haytowitz, Holden, & Harnly, 2011), being the cyanidin the principal one.(Wu, Beecher, Holden, Haytowitz, Gebhardt, & Ronald, 2006; Dyrby, Westergaard, & Stapelfeldt, 2001). Fig. 2 shows the monomeric anthocyanin content expressed in cyanidin-3glucoside equivalents per 100 gram of cooked samples, for five different treatments: traditional cooking at 7, 11 and 15 min and sous-vide at 87°C/50 min and 91°C/30 min.

264 Comparing traditional cooking and sous-vide anthocyanin content, better retention was observed in sous-vide 265 treatments ($p \le 0.05$), which contents is almost the double. These treatments avoided contact between the red 266 cabbage discs and the water since the product is cooked in a sealed bag, while traditional cooked samples are immersed in water during cooking increasing the probability of compound leakage. Volden et al. (2008) 267 268 reported that blanching, boiling and steaming resulted in losses of 59%, 41% and 29% respectively in the 269 anthocyanin content of red cabbage. In addition to the heat sensitivity of these compounds (Patras, Brunton, 270 O'Donnell, & Tiwari, 2010), leakage could be the main phenomena that explains different losses of 271 anthocyanin content between treatments.

3.2.3. FLASH PROFILING TEST OF RED CABBAGE COOKED WITH TRADITIONAL

273 COOKING AND SOUS-VIDE TREATMENT.

A flash profile test (FP) was carried out to compare the sensory properties of red cabbage samples cooked with traditional cooking (for 7 min, 11 min and 15 min) and sous-vide (at 87 °C/50 min and 91 °C/30 min). The sample treated at 91 °C/30 min with sous-vide was presented twice in the test to verify the performance of consumers (Veinand, Godefroy, Adam, & Delarue, 2011; Varela & Ares, 2012). A total of 6 samples were compared. Sensory and instrumental tests were used to represent the data in two dimensions.

279 Fig. 3a shows the positioning of the samples cooked with different treatments in a sensory consensus map 280 with 87.89% of information summarized in two dimensions. 69.90% of the information is explained with the 281 horizontal axis (F1) and 17.99% is represented by the vertical axis (F2). The samples treated with the same 282 conditions (91°C/30 min, A and B) were placed close together, indicating that consumers perceived similar 283 attributes. Sous-vide samples (87 °C/50 min and 91 °C/30min) were placed on the positives values of the F1 284 axis, but only the shorter treatments (91 °C/30 min, A and B) were also located in the positive values of the F2 285 axis. For traditional cooked samples, all coordinates were negative for the F1 axis, while for the F2 axis values 286 moved from positive values for 7 min and 11 min treatments to negative values for 15 min treatments. To 287 understand the relationship between the position of the samples and the meaning of each axis it is necessary to compare Fig. 3a with a descriptor term biplot generated with consumers and instrumental data (Fig. 3b). 288

289 Fig. 3b shows the summarized representation on a two axes plot of instrumental data and consumer descriptor 290 terms. According to the instrumental data, the F1 axis seems to be related to the color and anthocyanin 291 content, probably because anthocyanins are the main pigments contained in red cabbage (He & Giusti, 2010). 292 Positive values might be associated with more purple samples (higher values of h_{ab}^*) and higher retention of 293 anthocyanins (such as showed in Fig. 2), where sous-vide samples are situated. According to the location of 294 descriptor terms, sous-vide samples were in the same region of descriptors related to more purple hue (h^{*}_{ab} 295 values around 300) and descriptors such as purple color. Negative values of the F1 axis seem to be related to 296 lighter (high values of L*), bluer (negative values of b*) samples and with more vivid or saturated color (high 297 values of C*_{ab}). Traditional cooked samples were located on this area presumably due to the degradation and

the leakage of their anthocyanins (Patras, Brunton, O'Donnell, & Tiwari, 2010; Volden, Borge, Bengtsson, 298 299 Hansen, Thygesen, & Wicklund, 2008), which induced samples lighter in color than sous-vide ones perceived 300 by consumers. The coordinates of b* (blueness) were placed near traditional cooked samples probably due to 301 changes favored by higher temperatures in the anthocyanins molecular specie (Andrés-Bello, Barreto-Palacios, 302 García-Segovia, Mir-Bel, & Martínez-Monzó, 2013; Dyrby, Westergaard, & Stapelfeldt, 2001). Redness (a*) is 303 not well explained in the map because of the short distance to the origin of both axes (F1 and F2). 304 Several aroma descriptors were close to the sous-vide samples underlining that samples retained more 305 aromatic volatile components than samples treated with traditional cooking. This trend has been reported in other studies which described samples cooked with traditional cooking, sous-vide and other cooking 306

treatments, being broccoli florets, green beans and carrots cooked with sous-vide perceived as the most aromatic samples by the consumers (Petersen, 1993; Iborra-Bernad, Tárrega, García-Segovia, & Martínez-Monzó, 2013a; Iborra-Bernad, C., Philippon, D., García-Segovia, P., & Martinez-Monzo, J., 2013b). In the case of carrots and Brussels sprouts, the sous-vide treatment provided samples with different volatile profiles compared to the cooking with steam (Rinaldi et al., 2012). Their results were mainly ascribed to the presence of a vacuum pouch which retained some aromatic molecules and reduced some reactions related to the presence of oxygen.

In Fig. 3a, on the F2 axis, positive values seemed related to the firmer samples. In Fig. 3b, textural descriptors and firmness instrumental data are mainly placed on the positive F2 axis. As Fig.3a shows, the position of points corresponding to traditional cooked samples gradually decreased across the F2 axis from positive to negative values according to the cooking time, as described in Table 2. Sous-vide samples with a longer treatment time (87 °C/50 min) were placed as softer than sous-vide samples treated for a shorter time (91 °C/30 min).

320 **3.2.4. COMPARISON BETWEEN SOUS-VIDE TREATMENTS.**

The sensory properties of sous-vide samples treated at 91 °C/30 min and 87 °C/50 min were compared by consumers (n=47). Fig. 4a shows the results obtained by the paired comparison tests of preference, purple color, aroma, firmness and taste for sous-vide samples treated at different conditions. For these attributes,

differences were not significant (p>0.05) (ISO, 2005). Preference firmness did not significantly differ between 324 samples suggesting that the application of response surface methodology with instrumental measurements 325 was a successful approach to preliminary selection of cooking conditions providing samples with similar 326 327 texture. Results of paired comparison test suggested the two treatment conditions produced samples that did 328 not differ in aroma and taste. But as was discussed before, the results of the FP test seem to show that this 329 kind of analysis discriminates the consumer perception of the product better. However, this test did not show 330 the distance from which consumers perceived two products as being significant different (p<0.05). The sousvide treatment at 91 °C/30 min was selected to be compared with traditional cooking as a shorter cooking time 331 332 is preferable due to practical criteria.

333 **3.3.5. COMPARISON BETWEEN SOUS-VIDE AND TRADITIONAL COOKING** 334 **TREATMENT.**

A paired comparison test was carried out to compare samples of both treatments (sous-vide and traditional cooking) in selected conditions (n=92). Fig. 4b shows the results obtained by the paired comparison tests of preference, purple color, aroma, firmness and taste for sous-vide and traditional cooked samples. Sous-vide samples were preferred to the traditional cooked ones (p≤0.05). By design, the difference in firmness was not significant between the treatments. According to consumers, sous-vide treatment provided tastier, more purple and more aromatic samples than traditional cooked ones.

As Fig. 4b shows, sous-vide treatment was perceived by consumers as a treatment that produces samples that retain more aroma and taste than traditional cooked treatment ($p \le 0.05$). Similar results were observed in the flash profile test (Fig. 3a and 3b). The main reason could be that sous-vide products are not in contact with the cooking water. Therefore, hydrophilic components do not leach into the water. This retention possibly increased the preference for this treatment ($p \le 0.05$), as taste was selected by 65% of consumers as the most important attribute for choosing the preferred sample according to the last question of the questionnaire. Firmness (12%), firmness and taste (10%) and color (7%) were the other answers most selected in the test.

348 3.4. MICROSTRUCTURE OF CELL WALL ON THE RED CABBAGE.

Observation with the cryo-SEM permitted comparison of four samples of red cabbage: raw (Fig.5a, 5A), 349 350 blanched (100 °C/30 s) (Fig. 5b, 5B), traditional cooked (100 °C/11 min) (Fig. 5c, 5C) and sous-vide (91 °C/30 min 351 (Fig. 5d, 5D) samples. Some differences were notices between raw and treated samples. The most surprising 352 feature of raw samples is the higher number of detached cells (Fig. 5a, 5A) compared with cut cells in treated 353 samples. The different way of debonding underlines the composition of intercellular gaps (labeled G in Fig. 5a, 354 5A) which could be filled with air in raw samples allowing cell-to-cell debonding as wall cells are only 355 connected by the middle lamella, plasmodesmata connections and cell-to-cell contact (Harker, Stec, Hallett, & 356 Bennett, 1997). In contrast, blanched, traditional cooked and sous-vide treated cells were fractured suggesting 357 intercellular gaps filled by intracellular water from the cytosol.

358

359 After fracturing the samples, the water was sublimed. Therefore, lines of crystallized solutes were drawn and 360 the number of lines was higher and denser inside the raw cells (Fig. 5a), indicating a higher concentration of solutes and water (more turgor). In contrast, heat treated samples (Fig. 5b, 5c, 5d) showed a continuous 361 362 presence of lines inside cells and in intercellular gaps. Nevertheless, the density of these lines inside the cells 363 was lower than in raw ones. This means lower cellular turgor and a higher degree of shrinkage (Prestamo & 364 Arroyo, 2007). In addition, the separation between cell membranes and cell walls could underline the loss of 365 turgor. In Fig. 5b, c and d two separation points can be seen between membranes and walls. External cells 366 showed a gap between cell membrane and cell wall (S_1) of about 1, 5 and 1.5 μ m for blanched, traditional 367 cooked and sous-vide samples, respectively. For internal cells, separations (S₂) between membrane and cellular walls were around 3, 4 and 1.5 µm for blanched, traditional cooked and sous-vide samples, respectively. These 368 369 distances suggested that traditionally cooked samples suffered more loss of turgor than the other treatments. 370 Besides, an increase of gaps between cell walls is observed in traditional cooking (labeled J in Fig. 5c) at 100 °C, 371 while sous-vide samples (Fig. 5d, 5D) did not show marked gaps between cells despite a higher cooking time 372 (30 min) and a lower temperature (91 °C). Besides different cooking conditions (time and temperature), sous-373 vide treatment was also different to traditional cooking as a slight overpressure was created by saturated

374 steam inside the vacuum bag and samples were not in contact with the cooking medium. On one hand, sous-375 vide treated tissues were subjected to a pressure which favored the better conservation of their structure (cell 376 wall contact) and presence of some organelles (labeled by O on Fig. 5d). The presence of these cellular 377 compartments in blanched and sous-vide samples suggested both treatments were less aggressive than 378 traditional cooking.

379 4. CONCLUSION

The comparison of samples with similar firmness cooked with traditional cooking and sous-vide was possible with the combination of sensory and instrumental tests. Instrumental firmness was well related to firmness perceived by consumers and RSM was a practical methodology to optimize instrumental firmness.

In sous-vide treatment, time and temperature conditions significantly influenced the firmness and the color of cooked red cabbage. Firmness in sous-vide samples followed a second-order model. A range of combined conditions provided similar products. Quadratic and interaction terms of time and temperature were significant in the model which highlighted the importance of applying a multifactorial study in cooking treatments.

The Flash profile test was successfully applied to the characterization of several samples of cooked red cabbage, considering sensory and instrumental data. This test should be accompanied by other sensory tests, such as paired tests, to permit the verification of the significant differences perceived by consumers.

Comparing cooked samples with similar firmness, sous-vide samples preserved better color, taste, aroma and anthocyanin content than traditional cooked samples due to the bag which retained flavor and antioxidant components. Taste was the main reason for consumers to prefer sous-vide samples. Results suggested that sous-vide treatment would increase the sensory and nutritional quality of red cabbage served in the catering industry with the same budget invested in raw materials.

396

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- 500

Fig. 1. Response surface plot of the effects of time and temperature on firmness (N) of red cabbage discs cooked by sous-vide (SV). Axes values coded following Table 1. Temperature: 0 is equal to 80 °C and 1 unit is equivalent to 5 °C. Time: 0 is equal to 40 min and 1 unit is equal to 10 min. Optimal proposed uncoded conditions for SV were (+a) 87 °C/50 min; (+b) 91 °C/30 min.

Fig. 2. Anthocyanin content (mg/ 100g cooked red cabbage) of cooked product with traditional cooking (100 °C) (white bars) and sous-vide treatment at 91.2 °C/30 min (light grey bars) and 87.4 °C/50 min (dark grey bars) and stored one day (4 °C) in vacuum conditions (98% vacuum). Different letter in the bars indicate significant differences between treatments (p<0.05).

Fig. 3. Product biplot (a) and descriptors biplot of the flash profile data (b).

Fig. 4.Sensory 'comparison of cooked red cabbage: (a) 91.2 °C/30 min with sous-vide (light grey bars) Vs. 87.4 °C/50 min with sous-vide (dark grey bars) (n=47); (b) 91.2 °C/30 min with sous-vide (light grey bars) Vs. 100 °C/11 min with traditional cooking (white bars) (n=92). The dotted line indicates the minimum value of response for which the differences is significant to each test (α =0.05).

Fig. 5.Cryo-scanning electron micrographs of red cabbage (magnification: ×750 - lower case letter - and x200 - uppercase letters-). (a,A) raw material; (b,B) blanched red cabbage (100 °C/30 s); (c, C) traditional cooked samples (100 °C/11 min, immersed in water); (d, D) sous-vide cooked samples (91 °C/30 min). G: Gaps between cells; S: Separation between cell membranes and cell wall; O: Intracellular organelles; J: Cellular junctions.

Table 1. Second-order design matrix used to evaluate the effects of temperature (T) and time

(t) on the texture and color of red cabbage.

	Ir	ndepende	ent variable	es		
	Coded	llevels	Original	Originals levels		
RUNS	T (° C)	t (min)	T (° C)	t (min)		
1	-1	-1	80	30		
2	1	-1	90	30		
3	-1	1	80	50		
4	1	1	90	50		
5	-1.414	0	77.9	40		
6	1.414	0	92.1	40		
7	0	-1.414	85	25.9		
8	0	1.414	85	54.1		
9	0	0	85	40		
10	0	0	85	40		
11	0	0	85	40		
12	0	0	85	40		
13	0	0	85	40		
14	0	0	85	40		
15	0	0	85	40		
16	0	0	85	40		

Table 2. Means and standard deviation of firmness (N, Kramer shear test) and CIE L*a*b* color coordinates from cooked red cabbage using traditional cooking (immersed in water, 100 °C) at different cooking time.

Cooking conditions	Firmness (N)	L*	a*	b*	С*	h _{ab}	Anthocyanins (mg/ 100g cooked product)
100 °C/30 sec	598(60) ^c	26 (2) ^a	7.5(1.1) ^b	-11.5(2.2) ^{ns}	14 (2) ^b	303 (5) ^b	70(4) ^d
100 °C/7 min	274(23) ^b	26 (1) ^a	1.2(1.7) ^a	-11.2(1.1) ^{ns}	11 (1) ^a	276 (9) ^a	38(3) ^c
100 °C/11 min	182(32) ^ª	30 (2) ^b	0.4(0.8) ^a	-11.1(1.0) ^{ns}	11 (1) ^a	273 (6) ^a	30(2) ^b
100 °C/15 min	145(14) ^a	29 (3) ^b	1.6(1.8) ^a	-12.2(1.5) ^{ns}	12 (2) ^{ab}	277 (8) ^a	25(1) ^ª

^{a-d}Different letters in columns indicate significant differences (p≤0.05) between treatments.

^{ns}: no significant differences.

Table 3.Means and standard deviation of firmness (N, Kramer shear test) and CIE L*a*b* color coordinates from samples cooked with different conditions of sous-vide (SV) treatment.

SV treatment	Firmness (N)	L*	a*	b*	C*	h* _{ab}
78 °C/40 min	559(36) ^e	24(3) ^{ns}	8(2) ^c	-10(1.6) ^{ab}	13(2) ^b	308(7) ^e
80 °C/30 min	575(42) ^e	24(3) ^{ns}	8(2) ^c	-10(2) ^{ab}	13(3) ^b	308(5) ^e
80 °C/50 min	403(18) ^d	25(2) ^{ns}	5.5(0.6) ^b	-8.9(1.0) ^b	10.5(0.9) ^a	302(4) ^{cd}
85 °C/26 min	435(11) ^d	25(4) ^{ns}	7(2) ^c	-10(1.8) ^a	12.7(1.9) ^b	304(8) ^{de}
85 °C/40 min*	301(39) ^c	24(3) ^{ns}	5.1(1.3) ^b	-9.4(1.4) ^b	10.7(1.6) ^a	299(6) ^{bc}
85 °C/54 min	206(34) ^b	25(3) ^{ns}	4.4(0.5) ^{ab}	-9.6(1.4) ^{ab}	10.6(1.3) ^a	295(4) ^{ab}
90 °C/30 min	202(17) ^b	25(2) ^{ns}	4.5(1.3) ^{ab}	-9.1(1.4) ^{ab}	10(1.8) ^a	296(4) ^{ab}
90 °C/50 min	135(23)ª	24(2) ^{ns}	4.0(1.0) ^a	-9.0(1.7) ^b	10(1.7) ^a	294(6) ^ª
92 °C/40 min	126(19) ^a	26(4) ^{ns}	5(2) ^{ab}	-9.5(1.6) ^{ab}	11(2.0) ^a	296(9) ^{ab}
100 °C/30 sec	637(14)	29(5)	10(2)	-12(3)	16(3)	311(7)

^{a-e}Different letters in columns indicate significant differences (p≤0.05) between

treatments.*The treatment was repeated 8 times (central point of the response surface

design). ^{ns}: no significant differences.

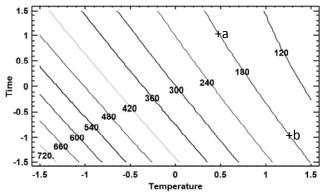
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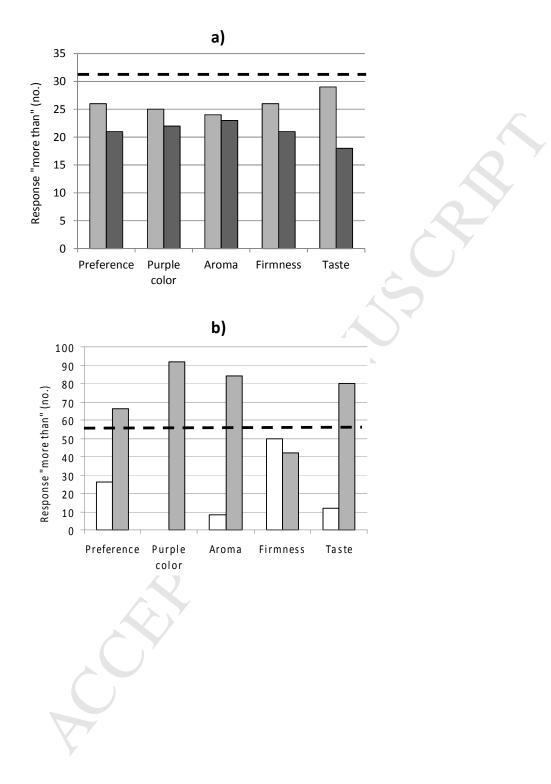
Table 4. Estimated regression coefficients of the fitted equations obtained for firmness of sousvide cooked red cabbage discs by sous-vide treatment depending on temperature (1) and time (2) conditions.

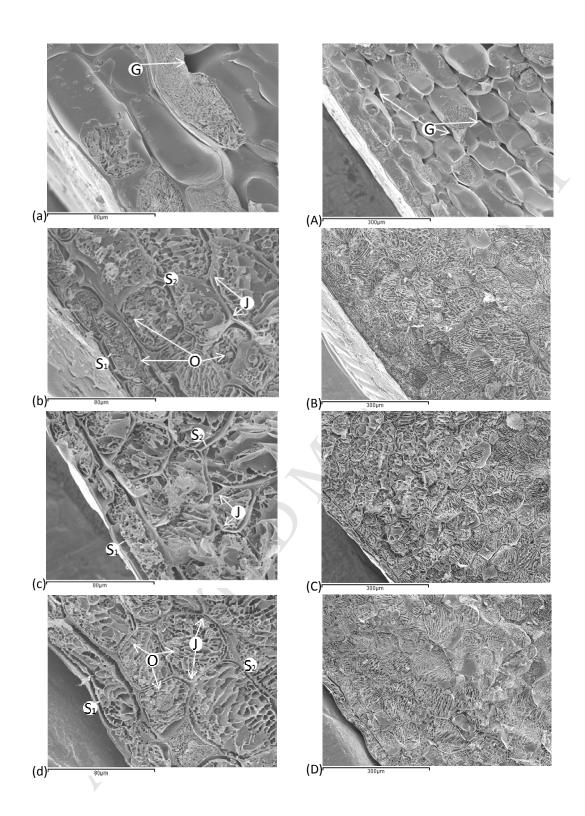
	Coeff	icients	AN	ANOVA		
	Estimated					
Item	value	SE	F-Value	P-Value		
B0	300.800	6.309	_			
Linear						
B1	-156.745	6.309	642.66	<0.001		
B2	-70.418	6.309	129.71	<0.001		
Quadratic						
B11	20.194	6.309	10.67	0.014		
B22	9.094	6.309	2.16	0.185		
Interactions						
B12	26.125	8.923	8.93	0.020	_	
					-	

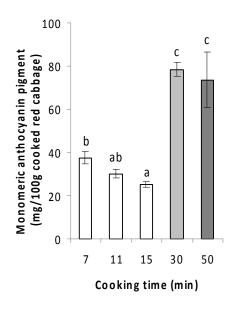
Firmness (N) = 300.8 - 156.7*Temperature - 70.4*Time + 20.2*Temperature² +

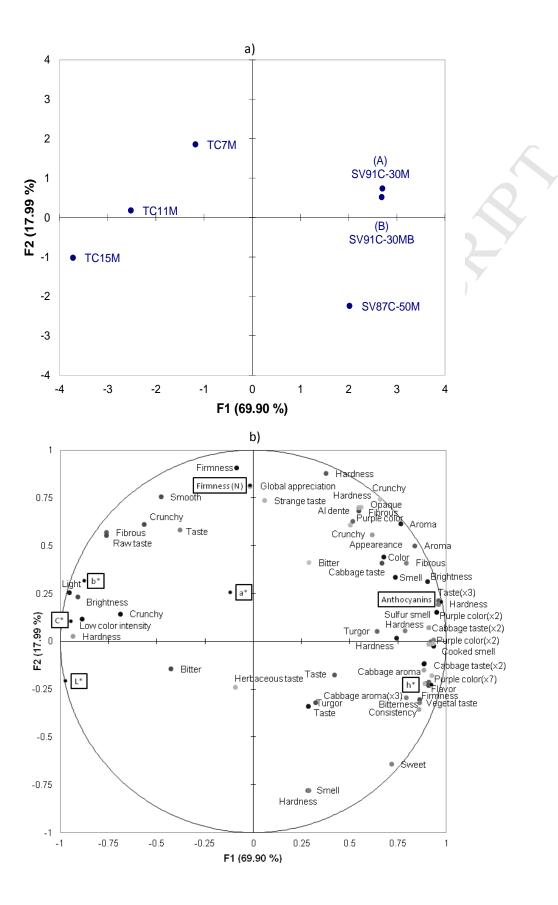
26.1*Temperature*Time. R^2 adjusted for df = 98.059. P-value (lack of fit) = 0.398











ADVANTAGES OF SOUS-VIDE TREATMENT FOR COOKING RED CABBAGE: SENSORY AND NUTRITIONAL ASPECTS

- Instrumental firmness of the suitable texture for cooked cabbage was fixed (TF).
- Changes in firmness and color of red cabbage with sous-vide treatment were modeled.
- Time and temperature pairings for sous-vide (SV) were defined to provide TF.
- SV cooked cabbage was preferred to traditionally cooked one with similar TF.
- SV samples were tastier, more purple and retained more anthocyanins than TC ones.

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