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PHYSICO-CHEMICAL AND STRUCTURAL CHARACTERISTICS OF						
VEGETABLES COOKED UNDER SOUS-VIDE, COOK-VIDE AND						
CONVENTIONAL BOILING						
C. Iborra-Bernad ^a , P. García-Segovia ^a , J. Martínez-Monzó ^{a*} .						
^a Food Technology Department, Universitat Politècnica de València, Camino de Vera s/n., 46022 Valencia, Spain.						
*Corresponding author: Tel. 0034 963877361; fax 0034 963877369. E-mail address: xmartine@tal.upv.es						

ABSTRACT 47

48 In this paper, physico-chemical and structural properties of cut and cooked purple-flesh potato, green 49 bean pods and carrots have been studied. Three different cooking methods have been applied: traditional 50 cooking (boiling water at 100 °C), cook-vide (at 80 °C and 90 °C) and sous-vide (at 80 °C and 90 °C). Similar 51 firmness was obtained in potato applying the same cooking time using traditional cooking (100 °C), and 52 cook-vide and sous-vide at 90 °C, while in green beans and carrots the application of the sous-vide (90 °C) 53 required longer cooking times than cook-vide (90 °C) and traditional cooking (100 °C). Losses in 54 anthocyanins (for purple-flesh potatoes) and ascorbic acid (for green beans) were higher applying 55 traditional cooking. β-carotene extraction increased in carrots with traditional cooking and cook-vide 56 (p<0.05). Cryo-SEM micrographs suggested higher swelling pressure of starch in potatoes cells cooked in 57 contact with water, such as traditional cooking and cook-vide. Traditional cooking was the most aggressive 58 treatment in green beans because the secondary walls were reduced compared with sous-vide and cook-59 vide. Sous-vide preserved organelles in the carrot cells, which could explain the lower extraction of β carotene compared with cook-vide and traditional cooking. Sous-vide cooking of purple-flesh potato is 60 recommended to maintain its high anthocyanin content. Traditional boiling could be recommended for 61 62 carrots because increase β -carotenes availability. For green beans, cook-vide and sous-vide provided 4.04 63 products with higher ascorbic acid content.

KEYWORDS: 64

65 Firmness, color, antioxidants, microstructure, cooking treatment.

Practical Application 66

67 Knowledge of the effects of various culinary treatments on vegetables allows processors to provide 68 consumers with products with higher antioxidant contents and improved sensory properties, increasing 69 the consumer satisfaction and product loyalty.

70 **1. INTRODUCTION**

71 Vegetables play an important role in our diet due to elements such as fiber, water and phytochemical 72 components. Many vegetables can be consumed raw or cooked. Cooking provides softer products, it 73 gelatinizes the starch and the digestibility of the fiber is improved (Van Boekel and others 2010). Heat 74 treatments reduce the firmness, mainly by the β -elimination reaction of pectic substances. In addition to 75 depolymerization and solubilisation of pectic materials (Van Buggenhout and others 2009), the 76 phytochemical compounds could be destroyed or leached into the water media during cooking 77 treatments. The temperature reaches of about 100 °C and the presence of oxygen during the traditional 78 cooking reduces antioxidant content in vegetables (Leskova 2006).

79 Therefore, a possible strategy to increase the final quality is the use of temperatures below 100 °C 80 (reducing the damage of thermosensitive compounds) and reducing the oxidative process by diminishing 81 the oxygen (Hui and others 2003). Sous-vide and cook-vide are two cooking treatments with a reduced 82 access of the oxygen during cooking and the temperature applied is usually lower than 100 °C. There are 83 two main differences between both treatments. The first one is the presence of a pouch isolating the product of the cooking media in sous-vide, while in cook-vide products are in contact with the cooking 84 85 media (water). The other one is the way of atmospheric conditions are modified. In sous-vide, samples are 86 vacuum sealed in a pouch and the cooking media is maintained under atmospheric pressure (Baldwin 87 2012). Regarding cook-vide, products are cooked inside a cooker device with lower pressure causing the 88 water boiling below 100 °C (García-Segovia and others 2007). In addition, the surface heat transfer 89 coefficient could be higher in boiling water (cook-vide) than in liquid water (sous-vide) cooking at the 90 same temperature. Some studies comparing different methods suggest that sous-vide provide cooked 91 products with high sensory and nutritional values than others cooking methods, such as cooked potatoes 92 (Stea and others 2007), cooked red cabbage (Iborra-Bernad and others 2014b) and chicory stems (Renna 93 and others 2014). The presence of a pouch around the raw product before starting the cooking avoids the 94 leaching out of the nutrients in the cooking water compared to other types of cooking treatments, such as 95 boiling, steaming or microwaving (Charley and Weaver 1998). The use of sous-vide was largely used in the

96 90's and recently, Baldwin (2012) reviewed the sous-vide treatment. The other treatment, the cook-vide, 97 is a less studied treatment. Few reports using this low-pressure method have been conducted in meat, 98 such as beef muscle (García-Segovia and others 2008), and in vegetables, such as potatoes, carrots, green bean and kailan-hybrid broccoli (García-Segovia and others 2007; Iborra-Bernad and others 2013a; Iborra-99 100 Bernad and others 2013b; Martínez-Hernández and others 2013). Each cooking treatment can potentially 101 damage the cell walls and membranes of vegetables causing different amounts of degradation in the 102 antioxidant molecules contained in the cells. Some antioxidants are hydrophilic molecules, which could be 103 leach out in the cooking water, such as vitamin C, while others are hydrophobic molecules, such as β -104 carotene.

Regarding sensory quality, firmness and flavor are important properties to accept the intake of edible 105 106 substances (Szczesniak and Kahn 1971). The heat transfer method and temperature affect the physico-107 chemical properties of products such as firmness and antioxidant content. In the case of firmness, one of 108 the main factors of the softness process is the degradation of the pectic materials (Van Buggenhout and 109 others 2009). The knowledge of how each type of cooking treatment can affect each vegetable could be 110 interesting to increase the quality of the ready-to-eat products. Therefore, studies applying different 111 cooking treatments on the same product (Chiavaro and others 2006; Iborra-Bernad and others 2014a; 112 Lachman and others 2013) are relevant to select the right culinary factors and increase the nutritional and 113 sensorial quality offered to consumers.

114 The aim of the present work is to provide information about the most suitable cooking treatment for 115 different vegetables and understand better how the vegetable cells and tissues are modified using 116 different cooking treatments.

117 2. MATERIALS AND METHODS

118 2.1. MATERIALS

- Purple-flesh potatoes (*Solanum tuberosum* L. var. Vitelotte) provided by S.B.M. (Saveurs du Bout du
 Monde, Roscoff, France) were stored at 8 °C up to 5 days before carrying on the test. Potatoes were cut
 into cylinders (15 mm in height × 20 mm in diameter) using a metal clay hole cutter.
- 122 Green bean pods (Phaseolus vulgaris L. cv. Estefania) were purchased from a local producer (S.A.T.
- 123 Agricola Perichan, Valencia, Spain) one day before the experiments. The green beans were stored in the
- darkness at 5 °C until cooking process. The young pods of green beans cv. Estefania are very straight, long
- 125 (22-24 cm) and flattened. Before cooking, both ends of the pods were removed, and the green beans were
- 126 cut in 6-7 cm long pieces.
- 127 Carrots (*Daucus carota* L. cv. Nantesa) were purchased from a local company (Agrícola de Villena, Alicante, 128 Spain) one day before the experiments. The whole carrots were washed and cut into cylinders (15 mm in 129 height × 20 mm in diameter) using a metal clay hole cutter. The condition to accept samples was the 130 xylem tissue to be less than 10 mm diameter.

131 **2.2. COOKING METHODS**

Potato and carrot were cooked without blanching, while green beans were blanched for 1 min at 100 °C before the cooking treatment in order to reduce the cooking time to a less of an hour at 80 °C and provide product with a firmness well-done but firm to the bite. After blanching, samples were cooled down in water with ice for ten seconds.

Three methods were applied in the study: traditional cooking (boiling water at 100 °C) and two cooking treatments reducing the presence of the oxygen (sous-vide and cook-vide). Table 1 shows some characteristics of the cooking method used in this study. All treatments were carried out using distilled water for cooking to avoid interference of ions on the firmness and using the same device: Gastrovac[®] (International Cooking Concepts, Barcelona, Spain). This equipment consists of two elements: the main controller and the cooker. The controller contains a heating element and a vacuum pump. The

142 temperature is controlled and monitored through a digital system connected to a thermocouple 143 temperature sensor, which goes in the water bath (inside the cooker). To cook by cook-vide, products are 144 placed in the basket (Fig 1. panel 2) and it is hooked in a handle for lifting the cooking basket. The pot is 145 closed with a lid (3) that includes a handle. The basket is hung up avoiding contact with the heating media, 146 which is heated to a desired temperature. The vacuum pump is switched on (6) and the pressure is 147 reduced until the vapour-pressure-of-water at a selected temperature is reached. When the water is 148 boiling, the basket is taken down with the handle. At the end of the cooking time, the basket goes up and 149 the pressure is restored by opening the vacuum valve (7). After that, the lid is opened and the product can 150 be cooled to store refrigerated.

151 For sous-vide treatment, samples were placed avoiding the overlapping and they were vacuum-sealed 152 (98% vacuum) in heat-resistant polyethylene pouches (Cryovac® HT3050, Cryovac Sealed Air Corporation, 153 Barcelone, Spain) using a vacuum packaging machine (EV-25, Technotrip, Spain) applying a double sealing. 154 The permeability characteristics of pouches were: 10 g/24 h m^2 (ASTM F 1249) for the moisture vapor 155 transmission rate (MVTR); 10 cm³/m² 24 h (ASTM D3985) for O₂ transmission rate at 23 °C and 0% RH; 19 156 cm^3/m^2 24 h (ASTM D3985) for O₂ transmission rate at 23 °C and 80% RH; 35 cm^3/m^2 24 h (ASTM D1434) 157 for CO_2 transmission rate at 23 °C and 0% RH. The cooking treatment was performed with the previously mentioned device with the traditional lid for atmospheric cooking. 158

After cooking with traditional cooking and cook-vide treatments, samples were vacuum-sealed (98% vacuum) in heat-resistant polyethylene pouches (Cryovac®HT3050, Cryovac Sealed Air Corporation, Barcelone, Spain) using a vacuum packaging machine (EV-25, Technotrip, Spain).

All samples were stored at 3-4 °C for 24 h before the instrumental measurements to simulate the conditions in the catering industry that applies the sous-vide to minimize the workload during services.

164 **2.3 EXPERIMENTAL DESIGN**

165 For traditional cooking, temperature applied was 100 °C and cooking times are shown in Table 2. The

166 cooking times were different for sous-vide and cook-vide compared to traditional cooking in order to

167 achieve two criteria. The first one is to have one cooking time in common with traditional cooking to allow

a comparison; and the second one is that the cooked vegetables should be well-done but firm to the bite;

a sensorial test (data not showed) was applied to define the rest of cooking times.

170 For sous-vide and cook-vide the temperature applied was 80 °C and 90 °C. In the case of vegetables, the 171 temperature of the work is recommended to be above 80 °C to assure gelatinization of the starch, 172 softening of fibres and pectins (Sila and others 2009), and inactivation of enzymes; and below 100 °C to 173 avoid ballooning of the pouch applying sous-vide. De Baerdemaeker and Nicolaï (1995) explained the 174 phenomenon of ballooning based on the difference between inner and external pressure of the pouch. 175 The way to increase the pressure of the water vapour and air inside the pouch according to the 176 temperature are different. The air pressure increases linearly with the temperature according to the ideal 177 gas law; while the water vapour pressure increases exponentially following the Clausius-Clapeyron 178 equation. Therefore, the pressure becomes equal to atmospheric pressure at 90 °C. Above that packaging 179 pressure, ballooning may occur since the internal pressure becomes greater than the external pressure, 180 which is probably around 100 °C.

For cook-vide is necessary to reduce the water vapour pressure under the atmospheric pressure, for this reason the cooking temperature is less than 100 °C. To facilitate the comparison between sous-vide and cook-vide, the studied temperatures were 80 °C and 90 °C and each one was related to a specific time (Table 2). According to the temperature, pressure inside the device varied from 47.3 to 69.7 kPa (absolute pressure) because a vacuum pump low the pressure until the vapour pressure of water of the heating media is reached during the cook-vide.

187 2.4. INSTRUMENTAL TEXTURE ANALYSIS

The firmness of samples was measured at room temperature (25 °C) by a puncture test using a Texture Analyser TA-XT2 (Texture Technologies Corp., Scarsdale, NY, USA) according with the methodology followed by Garcia-Segovia and others (2008) in potatoes, Iborra-Bernad and others (2013a) in green beans and Iborra-Bernad and others (2013b) in carrots.

192 In potatoes and carrots cylinders, the firmness test was conducted with a 2 mm-diameter stainless-steel

193 flat-head probe (TA P/2). The probe completely penetrated perpendicular into the surface of the

194 cylinders. The penetration speed was 1 mm·s⁻¹, and post-speed was 10 mm·s⁻¹. Firmness was considered

195 as the maximum-recorded force during the puncture test. In carrots, one measurement for each tissue,

196 xylem and phloem, was carried out for each cylinder. In potatoes just one measurement was conducted in

197 each cylinder. Six cylinders were analyzed for each treatment.

In green beans the texturometer was equipped with a 2mm diameter stainless-steel needle probe (TA P/2N) and measurements were taken perpendicular to the surface of the pods and seeds were avoided. Three measurements were carried out for each pod and six pods were analyzed for each treatment. The vertical displacement of the needle probe was held constant in order to ensure the full penetration all along the thickness of the pod. The speed of penetration was 2 mm·s⁻¹, and pre- and post-speeds were both 5 mm·s⁻¹.

Data were collected and analyzed using Texture Exponent software (Stable MicroSystems, Godalming,
England).

206 2.5. COLOR MEASUREMENT

207 Color was recorded using a Minolta CM3600d colorimeter (Minolta Corp., Ramsey, NY, USA). The 208 instrument was calibrated with a ceramic reference, illuminant C, prior to use. CIE-L*a*b* coordinates 209 were obtained using D65 illuminant and 10° observer as reference system. Registered parameters were L* 210 (brightness: L* = 0 [black], L* = 100 [white]), a* (-a* = greenness, +a* = redness), b* (-b* = blueness, +b* 211 = yellowness) and total color differences (Δ E*ab) were calculated following the equation (Eq. 1):

212
$$\Delta E * ab = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$
 (Eq. 1)

In potatoes and carrots, the surface color in the top and in the bottom of each cylinder was measured in ten samples per treatment. For each treatment in green beans, ten samples of green beans were used to measure the skin color and the measure was repeated two times on each individual pod.

216 **2.6. DETERMINATION OF ANTIOXIDANTS**

217 2.6.1 DETERMINATION OF TOTAL MONOMERIC ANTHOCYANINS

218 The determination of total monomeric anthocyanins was based on the pH differential method (Lee and 219 others 2005). For the test, potatoes were removed from the pouches, they were placed on a paper for 1 220 min, and then the samples were slightly dried with a paper. For sample preparation 40 g of cooked potato 221 were chopped. After, 2 g of the chopped product was homogenized for 30 seconds with 20 mL of 222 methanol (Panreac, Barcelona, Spain) and 0.1 mL of hydrochloride acid (37% HCl, Panreac, Barcelona, 223 Spain). The homogenate was stored during 24 h at 4 °C in dark conditions, and after, it was centrifuged 224 (10.000 rpm, 10 min, 4 °C) to obtain a supernatant. Aliquots of 0.4 mL were added to 3.6 mL of pH 1.0 225 buffer and pH 4.5 buffer, prepared as suggested by Lee (2005). After waiting between 20 min and 50 min, samples were evaluated at λ = 700 and 530 nm in a spectrometer (Helios Zeta UV-VIS, Thermo Fisher 226 227 Scientific, UK). The anthocyanin pigment concentration was expressed as cyanidin-3-glucoside equivalents 228 per 100 g of cooked samples (molecular weight = 449.2 g/mol for cyanidin-3-glucoside (cyd-3glu); ε =26900 229 molar extinction coefficient, in L mol⁻¹ cm⁻¹). Four repetitions were done for each cooking treatment.

230 2.6.2 DETERMINATION OF ASCORBIC ACID

Ascorbic acid content was determined with a Titrino 702 SM (Metrohm, Ltd., Herisau, Switzerland) by bivoltammetric method using a Metrohm 6.0308.100 (Switzerland) double platinum electrode following the Metrohm method 42-J2 (Manual Methohm of analysis of Fruits and vegetables, Metrohm, Ltd., Herisau, Switzerland).The method is based on the oxidation of ascorbic acid to dehydroascorbic acid through the use of iodine. The results are independent of inherent coloration of the sample due to the bivoltametric indication.

For the test, green beans were removed from the pouches, they were placed on a paper for 1 min, and then the samples were slightly dried with a paper. Then, samples were liquefied, after 20 mL of the liquefied were placed into the titration beaker with 30 mL of oxalic acid solution (1 g/L, Panreac, Barcelona, Spain), treated with 2 mL glyoxal solution (40%, Panreac, Barcelona, Spain), briefly stirred and stood settle for 5 min. After the addition of 5 mL sulfuric acid (25% v/v, Panreac, Barcelona, Spain), it was

- titrated with iodine (0.01 M, Panreac, Barcelona, Spain) up to the endpoint, which was considered the
- 243 greatest loss of mV.
- The concentration was expressed as g of ascorbic acid per 100 g of product. Four repetitions were done for each cooking treatment.

246 2.6.3 DETERMINATION OF β-CAROTENE

247 The methodology of Olives and others (2006) was used to extract the carotenoids present in carrots. For 248 the test, carrots were removed from the pouches, they were placed on a paper for 1 min, and then the 249 samples were slightly dried with a paper. After, 5 g of sample were placed in a beaker, protecting them 250 from light, and then they were mixed with 100 mL of acetone/ethanol/hexane (25:25:50, v/v/v) extraction 251 solvent and magnetically stirred for 30 min. Then, 15 mL of distilled water were added and an upper 252 aliquot layer of 0.6 mL was dried under a stream of liquid nitrogen. The residue was dissolved with a 253 methanol/tetrahydrofuran/acetonitrile solution (55:15:30, v/v/v) to a final volume of 4 mL. The 254 spectrophotometric reference method of AOAC (2000) was used for quantification. Sample absorbance 255 was measured at 446 nm (Helios Zeta UV-VIS, Thermo Fisher Scientific, UK). The total carotenoid content 256 was expressed in mg of β -carotene per 100 g of cooked carrots. Standard β -carotene was provided by 257 Fluka-Biochemika (USA). Three repetitions were conducted per each cooking treatment.

258 2.7. MICROSTRUCTURE OF CELL WALL IN THE COOKED VEGETABLES

259 The sample microstructure was observed with a secondary electrons image using cryo-scanning electron 260 microscopy (cryo-SEM) with a JEOL JSM-5410 microscope (Jeol, Tokyo, Japan). A cryo-workstation Gatan 261 was used in this work. Samples were cut into rectangular pieces 4 x 1.5 x 5 mm. The samples, previously 262 frozen by immersion in slush nitrogen (-210 °C), fractured, etched and gold coated, were viewed in the cryo-SEM. The sublimation conditions were: 5 kV, at -90 °C, 10⁻⁵ Torr vacuum, for 15 min. The observation 263 264 conditions were 15 kV at 10 mm wd (working distance) and the liquid nitrogen temperature was -190 °C. 265 Using this technique, the fractured surface of the frozen sample was viewed directly at -150 °C or lower. 266 Micrographs of the preparation of purple-flesh potato, green bean pods and carrots were analyzed after a 267 day of storage at 4 °C. The micrographs were taken at 750 magnifications to observe changes in the cell 268 walls. Samples observed were raw ones, others cooked with traditional treatment (100 °C) and using sous-

vide and cook-vide.

270 **2.8. DATA ANALYSIS**

271 Variability in texture parameter, color coordinates and antioxidant content among conditions were

analyzed with one-way ANOVA. All ANOVA were followed by a LSD posthoc test to find out significant

differences (p≤0.05). The software employed was Statgraphics Centurion (STSC, Rockville, MD).

274 3. RESULTS AND DISCUSSION

3.1. EFFECTS OF COOKING TREATMENTS ON FIRMNESS

276 Firmness that consumers like for each vegetable was taken as target to compare different cooking 277 methods. Fig. 2a shows the results of firmness of purple-flesh potato cooked with traditional cooking, 278 cook-vide and sous-vide. Firmness was 12.5 (1.7) N (standard deviation showed in brackets) in raw 279 samples and ranged from 4.25 (0.98) N to 0.53 (0.06) N in cooked ones considering all cooking treatments. 280 No significant differences were found in firmness between samples cooked for 25 min with traditional 281 cooking and with the other treatments (cook-vide and sous-vide) at 90 °C. This means that a constant level 282 in firmness was reached during cooking as observed previously by Tijskens and Schijvens (1987). However, 283 cook-vide and sous-vide treatments applied at 80 °C provided firmer samples (p≤0.05) than traditional 284 cooked ones. The β -elimination reaction in pectic substances, main components of the lamella media, 285 increases substantially starting at 80 °C (Sila and others 2009). This observation explain that samples 286 cooked (cook-vide and sous-vide) at 80 °C were firmer than ones cooked at 90 °C with the same cooking 287 time.

Concerning green beans (Fig. 2b), raw samples had a firmness of 5.9 (0.6) N, while cooked samples displayed firmness between 0.64 (0.09) N and 2.66 (0.16) N. Comparing traditional cooking, cook-vide at 90 °C and sous-vide at 90 °C, differences were found between firmness of samples cooked for 20 min (p≤0.05). Traditional cooking (100 °C) provided the softer samples, the sous-vide at 90 °C maintained more of the firmness and the samples cooked with cook-vide had an intermediate degree of firmness. To 12

provide similar firmness (p>0.05) applying sous-vide and cook-vide with the same temperature (80 °C or 90 °C) 20 min more of cooking time were required in sous-vide treatment. The contact with the external water in traditionally and cook-vide cooked samples could increase the hydration of the secondary and primary walls, which characterize its hypodermis cells (Sterling and Shimazu 1961). The solubilisation of branched regions (rhamnogalacturonan) of the cell wall could increase, reducing the resistance to external strength, and then, the firmness (Stolle-Smits and others 1995)(Fig. 2b). This difference between heat treatments could be the origin of the differences detected in the puncture test.

300 Regarding carrots (Fig. 2c and Fig. 2d), the firmness of phloem (external) and xylem (internal) tissues were 301 studied. The firmness values measured in raw samples were 10.6 (0.9) N in phloem tissue and 12.2 (0.5) N 302 in xylem tissue. In phloem tissue, samples cooked with sous-vide and cook-vide at 80 °C were firmer than 303 samples cooked with shorter treatments at higher temperature (100 °C -traditional cooking- and 90°C -304 cook-vide and sous-vide-). As observed by Iborra-Bernad and others (2013b) the effect of temperature in 305 the softening process is greater than the cooking time. In xylem tissues, sous-vide samples cooked at 80 °C 306 were also the firmest ones. Similar firmness of cooked samples (sous-vide and cook-vide) (p>0.05) at this 307 temperature (80 °C) was achieved after cooking with sous-vide for 70 min and with cook-vide for 40 min. 308 Traditional cooked samples were the softer samples probably due to its high temperature which could 309 increase the degradation of pectic substances (Van Buggenhout and others 2009). Comparing samples 310 cooked with sous-vide and cook-vide at 90 °C, it was found that sous-vide kept samples firmer than cook-311 vide treatment in both tissues (phloem and xylem). Loss of firmness was associated with substantial 312 dissolution, depolymerization, and, apparently, destruction of cell wall pectins in carrots (Greve and 313 others 1994). Therefore, as commented with respect to green beans, external water available in the cook-314 vide treatment may have the effect of increasing the dissolution of pectic material as compared to the 315 sous-vide treatment. In addition, the heat transfer coefficient of surfaces is higher in boiling water (cook-316 vide) than in liquid water (sous-vide).

The obtained results highlight the different effect of each treatment according to the different compositions and histology of the vegetables.

319 **3.2. EFFECT OF COOKING TREATMENT ON COLOR**

Fig. 3a shows total color difference (ΔE^*ab) of cooked purple-flesh potato, the lower values were observed applying sous-vide at 80 °C and 90 °C (p≤0.05), meaning that the color of products is more similar to the raw samples. Larger ΔE^*ab values were observed in treatments where samples were in contact with boiling water (traditional cooking, cook-vide at 80 °C and 90 °C). Differences in ΔE^*ab between treatments could be explained by the leakage of anthocyanins (hydrophilic and choromophore compounds) in cooking water using cook-vide and traditional cooking, while sous-vide treatment isolated the product from the external cooking media.

327 Concerning green bean pods, raw samples were greener ($-a^* = -8.7$ (0.5)) than cooked ones (from -1.5 (1.0) 328 to -5.0 (0.4)). These differences could be related with chlorophyll degradation, which converts the bright 329 green color to olive-color (Van Boekel 1999). Total color difference (ΔE^*ab) (Fig. 3b) in samples cooked 330 with traditional cooking ranged from 12 to 14, similar to the majority of the other cooked samples 331 (p>0.05). It was observed a difference between cook-vide and sous-vide samples at 80 °C, mainly related 332 to coordinate a* as observed in previous studies (Iborra-Bernad and others 2013a). The isolation of 333 samples inside a pouch in sous-vide could retain the organic acids, which probably increased the degradation of the chlorophyll by a slight decrease of pH (Koca and others 2006). 334

In carrots (Fig. 3c), total color difference (ΔE^*ab) ranged from 12.2 (1.2) to 17 (3). The lowest differences belonged to samples cooked with traditional cooking for 10 min, the shortest cooking time. This treatment had lower values ($p \le 0.05$) than sous-vide at 90 °C and cook-vide at 80 °C for all cooking times. Higher temperatures maybe destabilize a little more the homeostasis of cells, facilitating the destruction of carotenoid–protein-complexes increasing the β -carotene extraction (Ryan and others 2008; Van het Hof and others 2000). However, this possible modification does not affect significantly the global color of the cooked carrots.

As observed in the previous section about firmness, color has been affected in different ways according to the cooking treatment and the nature of the main chromophore in each vegetable. In this sense, the

344 purple-flesh potato seemed to be more affected by the cooking treatment due to the hydrophilic nature

of the anthocyanins (easily leached).

346 3.3. EFFECT OF COOKING TREATMENTS ON ANTHOCYANINS, ASCORBIC ACID

347 AND β-CAROTENE

348 In purple-flesh potato, the anthocyanin content of raw samples was around 49 (10) cyanidin-3-glucoside 349 equivalents/100 g of cooked products (Fig. 4a). In cooked samples contents ranged between 22.3 (13) to 350 52.7 (8) cyanidin-3-glucoside equivalents/100 g of cooked products. Traditional cooking, sous-vide at 80 °C 351 and cook-vide at 80 °C for 25 min treatments showed lower anthocyanin values compared to raw samples 352 (p≤0.05). Traditional cooked samples had the lowest anthocyanin content probably due to the leakage 353 into the cooking water as a main effect, and a higher cooking temperature (100 °C) could destroy part of 354 the anthocyanins content as a second effect. However, their firmness are similar to potatoes cooked 25 355 min at 90 °C in sous-vide and cook-vide (Fig.1), highlighting the importance of the cooking treatment. In 356 cook-vide treatments, no differences were found between treatments at 80 °C and 90 °C, while the 357 extraction of the anthocyanins of samples cooked with sous-vide at 90 °C were higher than the one carried 358 out in sous-vide samples at 80 °C after 30 min cooking. Longer cooking times could increase the extraction 359 of the anthocyanins from the potato matrix by higher destruction of their cell walls (Van Boekel and others 360 2010). However, a higher diffusion of anthocyanins into the aqueous media in cook-vide treatments could 361 decrease the measured content, while the anthocyanin of the sous-vide samples could be retained in the 362 pouches (avoiding the contact with the cooking media). In other studies with purple onions and red 363 cabbage, lower losses of anthocyanin were also described in cooking treatments without cooking media 364 contact (Rodrigues and others 2009; Volden and others 2008). Further studies are required to understand 365 how these cooking treatments could affect the bioaccessibility and bioavailability of anthocyanins in 366 potatoes. Other studies in fruits and vegetables observe a low anthocyanin bioaccessibility. In the case of 367 raw figs, the bioaccessibility was quite low (0-5% of the initial values) in cyanidin-3-glucoside, whereas for 368 dried figs, anthocyanins were not observed (Kamiloglu and Capanoglu 2013). Studies conducted in 369 mulberry noticed that the bioaccessibility of anthocyanins were less than 5% after the intestinal digestion.

370 However, it seems that phenolics are generated from degradation of anthocyanins under intestinal 371 environment which explain the radical scavenging ability during the digest (Liang and others 2012). 372 Therefore, Bermúdez-Soto and others (2007) concluded that the study of bioaccessibility, bioavailability 373 and biological activity in dietary polyphenols are complex because they are transformed in the small 374 intestine into other unknown and/or undetected structural forms with different chemical properties due 375 to their highly sensitive to the mild alkaline conditions. Moreover, Yang and others (2011) suggests that 376 the bioavailability of anthocyanins varies markedly depending on food matrices, considering other 377 antioxidants and macronutrients in the same meal.

378 Fig. 4b shows the ascorbic acid content in green beans. The ascorbic acid content in raw green beans was 379 14.6 (1.0) mg of ascorbic acid/100 g of cooked product. The ascorbic acid content in cooked samples 380 ranged from 13.7 (0.7) to 18 (2) mg of ascorbic acid/100 g of cooked product. Samples cooked with 381 shorter treatments of sous-vide at 80 °C (40 and 50 min), cook-vide at 80 °C (40 min) and cook-vide at 90 382 C (20 and 30 min) had higher ascorbic contents (p<0.05) than raw samples. This increase of the ascorbic 383 content could be explained by a reduction of moisture in cooked samples due to the damage in cells for 384 heating (see next section). A reduction in moisture increases the current proportion of ascorbic content 385 despite losses of this thermosensitive molecule by heating effect (Barrett and Lloyd 2012).

386 In the case of carrots (Fig. 4c), β -carotene was selected as nutritional indicator because this compound is 387 chemically hydrophobic and sensitive to temperature and oxygen. β -carotene content in raw samples was 11 (2) mg of β -carotene/100 g of cooked product. This content was similar to the measured in sous-vide 388 389 samples (p>0.05) at 80 °C and 90 °C. Treatments in contact with boiling water (cook-vide at 80 °C and 90 390 °C, traditional cooking at 100 °C) resulted in higher β-carotene content than raw samples (p<0.05) 391 probably due to a larger denaturation of carotenoproteins and a higher solubilisation of pectic substances 392 of the cell wall, leading these cooked samples to a better extractability and higher concentrations 393 determined. This better extractability has been related with a higher bioaccessibility (Failla and others 394 2008; Hornero-Méndez and Mínguez-Mosquera, 2007). Lemmens and others (2009) observed that 395 modification in texture and the β -carotene *in vitro* bio-accessibility are inversely correlated, contrary to

396 our studies where carrots with similar texture are releasing in different amount of carotenes according to 397 the cooking treatment. In this regard, the bioaccessibility could also be different. Therefore, Aherne and 398 others (2010) noticed cooking not only enhances the bioaccessibility and bioavailability of all-trans β -399 carotenes but also its cis forms. Their study suggests food matrix and degree of processing play important 400 roles on carotenoid isomerisation and β -carotene isomer bioavailability. Micrographs (Fig. 5) suggest a 401 different damage in the carrots cells, which could explain the different releasing level of carotenoids.

402 As a conclusion, it has been observed that sous-vide treatment preserved better anthocyanins and higher 403 levels of β -carotene were extracted in carrots boiled directly in contact with water (cook-vide and 404 traditional cooking).

3.4. MICROSTRUCTURE OF COOKED VEGETABLES.

406 Fig. 5a shows potato micrographs of raw and cooked samples: traditional cooking (100 °C-15 min), cook-407 vide (90 °C-30 min) and sous-vide (90 °C-30 min). Raw cells showed cytoplasm organelles (the majority 408 containing starch granules) and lines (equivalent to the solute content) which were crystallized out in a 409 pure form after water sublimation process required during cryo-SEM preparation. Unlike raw samples with 410 lines of solutes in the cytoplasm, the starch gelatinized by high temperature completely filled the lumen of 411 the cytoplasm. During the gelatinization of starch, the molecule is hydrated with the available water, 412 creating the swelling pressure described by Jarvis (1992). In treatments with boiling water (traditional 413 cooking and cook-vide), the water media could diffuse through the damaged membranes and swells the 414 starch causing higher swelling pressure than sous-vide ones, which have only access to available water 415 from the internal water cells. Comparing sous-vide and cook-vide, starch gelatinized in cook-vide samples 416 was visually more homogeneous (probably due to a larger hydration) than that gelatinized in sous-vide 417 ones. Swelling pressure avoided the wrinkle of the cell wall observed in the micrographs of the sous-vide 418 cells. No differences were described in the firmness between those treatments (Fig. 2a), probably because 419 the gelatinization has a slight effect in the firmness loss as suggested by Verlinden (1995). Therefore, 420 Iborra-Bernad and others (2014b) observed that adhesiveness and cohesiveness was higher in purple-flesh

421 potato cooked with sous-vide compared to ones cooked with sous-vide, while firmness was similar

422 between samples cooked at the same temperature and cooking time.

423 Fig. 5b shows green beans micrographs of raw and cooked samples: traditional cooking (100 °C-10 min), 424 cook-vide (90 °C-20 min) and sous-vide (90 °C-20 min). In green beans, epidermal and hypodermal layer 425 cells were observed (Fig. 5b). As described by Reeve and Brown (1968), secondary walls were found in the 426 hypodermal tissue of the bean pods. In raw samples, lines drawn in cytoplasmic regions (equivalent of the 427 solute content) were more than in cooked ones. Moreover, traditional cooked cells (100 °C-10 min) had 428 fewer lines than cells of samples cooked by cook-vide or sous-vide. A higher temperature applied could 429 destabilize more intensely the cell homeostasis, which facilities the increment of the loss of the 430 intracellular content. Moreover, traditional cooked samples seem more damaged in secondary walls 431 because the walls reduced their thickness probably by the temperature applied (100 °C) which could 432 increase the solubilization and depolymerisation of pectic materials (Stolle-Smits 1995).

433 In carrot micrographs (Fig. 5c) phloem cells were examined in raw samples and cooked ones: traditional 434 cooking (100 °C-10 min), cook-vide (90 °C-30 min) and sous-vide (90 °C-30 min). Raw samples showed 435 mainly cells and areas full of lines (related to the solute content). Traditional cooked samples were poorly 436 filled compared with sous-vide and cook-vide samples pointing to more damaged membranes. Trejo-Araya 437 and others (2009) observed less tissue damage which resulted in smaller gaps in sous-vide carrots and 438 high pressure processing samples than in traditional cooked ones. The level of damaged cells seemed to 439 be inversely related with carotene content, because sous-vide samples had lower content than traditional 440 cooked and cook-vide samples (Fig. 4c). Furthermore, carotenoids are hydrophobic compounds present in 441 carrot root as large carotenoid crystals in chromoplasts (Schweiggert and others 2012), where they are 442 linked with proteins. Cooking treatment is able to break up the molecular linkages between carotenoids 443 and proteins increasing the extractability in cooked samples (Van het Hof and others 2000). Nevertheless, 444 sous-vide samples displayed some organelles which suggest a less aggressive treatment. It could explain 445 that β -carotene values of sous-vide samples were similar those measured in raw samples (Fig. 4c).

446 **4. CONCLUSIONS**

Changes in texture, color, nutritional indicators and structure provided by three different cooking
treatments (traditional cooking, cook vide and sous vide) in different vegetables were studied.

449 Our study shows why the suitability of the cooking treatment is different according to the characteristics 450 of nutritional compounds and the structural properties of each product. Purple-flesh potatoes should be 451 cooked with treatments isolating from the cooking media, such as sous-vide, in order to reduce the anthocyanin leakage in the cooking media. In addition, we noticed a different swelling of the starch in 452 453 potatoes cooked with different treatments despite similar firmness. The effect of temperature, the role of 454 the cooking media contact with vegetables during cooking and the impact of the low pressure in the cells 455 structures damages are the main factors which explain the different spreading of the starch in the cellular 456 lumen. Further studies could elucidate the role of each one of the factors in the modification of textural 457 and sensory properties in starchy vegetables. In the study with green beans it was noticed that the contact 458 with cooking media seems to have an important effect in the firmness at the same temperature and time 459 (cook-vide vs. sous-vide). The softening was higher when the cooking water was in contact with samples 460 and it was dependent on the temperature. Studies focused in the influence of the cooking media contact 461 and the pressure on the vegetable tissues should be conducted in order to model the relationship 462 between both factors. Another important result observed is that β -carotenes are more available in 463 traditional cooking (boiling water) and cook-vide compared to the sous-vide due to larger cell wall 464 damages in carrots, despite a similar firmness. Studies about the bioavailability and the bioaccessibility of 465 antioxidants could be recommended to better understand the impact of type of cooking in the nutrition 466 and modify the guidelines of public health according to these conclusions. Our results highlight that 467 individual studies of vegetables are required for comparing cooking treatments due to the complex 468 structure and the main antioxidant compounds characterizing each vegetable.

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473 **Author Contributions**

- 474 C. Iborra collected test data, interpreted results and drafted the manuscript J. Martinez designed the study
- 475 and interpreted results. P. Garcia prepared the samples to microstructure observation and interpreted the
- 476 microstructure data.

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591 Table 1. Comparison of three cooking methods: sous-vide, cook-vide and traditional cooking.

	Sous-vide	Cook-vide	Traditional cooking	
Temperature	Cooking temperature<100 °C	Cooking temperature<100 °C	Cooking temperature ≈100 °C	
Reduction of the presence of the oxygen	From the preparation of the pouch	During cooking process	Oxygen presence naturally in the atmosphere	
Material in contact with the	Samples are inside of a vacuum	Contact of samples and	Contact of samples and heating	
product	sealed pouch	heating media	media	
Cooking media state	Liquid water without boiling	Boiling water	Boiling water	

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594 Table 2. Experimental design for purple-flesh potato, green bean pods and carrots.

Treatment	Temperature	Vegetable	Cooking time (min)		
Traditional		Potatoes	20	25	30
	100 °C	Beans	10	15	20
cooking		Carrots	10	20	30
		Potatoes	25	30	35
	80 °C	Beans	40	50	60
Sous vido		Carrots	40	55	70
Sous-vice		Potatoes	25	30	35
	90 °C	Beans	20	30	40
		Carrots	30	45	60
		Potatoes	25	30	35
	80 °C	Beans	40	50	60
Cookvido		Carrots	40	55	70
COOK-VIDE		Potatoes	25	30	35
	90 °C	Beans	20	30	40
		Carrots	30	45	60

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- 598 Fig. 1. Vacuum cooking system: (1) heating element and temperature probe, (2) pan, (3) lid, (4)
- temperature selector, (5) manometer, (6) vacuum pump and (7) valve (source Iborra-Bernad and others
- 600 (2013)).



Fig 2. Firmness of purple-flesh potato (a), green bean pods (b) and carrots (in phloem tissue (c) and in

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xylem tissue (d)) at different treatment conditions. CV: cook-vide; SV: sous-vide.



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Fig. 4. Anthocyanin contents (a) in purple flesh potato (wet weight), ascorbic acid contents (b) in green

- 614 beans (wet weight) and β-carotene contents (c) in carrots (wet weight) of raw products and samples
- 615 cooked with traditional cooking (TC, at 100 °C), cook-vide (at 80 °C -CV80- and 90 °C -CV90-) and sous-vide
- 616 (at 80 °C-SV80- and 90 °C-SV90-).

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- Fig. 5. Cryo-scanning electron micrographs (magnification of x750) of tissues of purple flesh potato (a),
- 623 green beans (b) and carrots (c). O: Intracellular organelles; S: Separation between cell membranes and cell
- 624 wall; J: Intercellular space.





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