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**Comparison of physical, microstructural and antioxidative properties of pumpkin cubes  
cooked by conventional, vacuum cooking and sous vide methods**

**Running title:** Impact of traditional and innovative cooking methods on pumpkin cubes

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## Abstract

**BACKGROUND:** The current dietary guidelines recommend five or more fruits, vegetables, and legumes servings per day. Often, these kinds of products are eaten cooked, resulting in organoleptic and nutritional changes. Vacuum cooking, both as cook vide and sous vide, is gaining attention as alternative cooking technique, thanks to its ability to preserve or even enhance sensory and healthy properties of food. The household application of these cooking method is poorly explored. In this work, the effect of domestic vacuum cooking, performed with a new patented system, was compared to sous vide and a traditional steam cooking on the quality of pumpkin cubes.

**RESULTS:** All the cooking treatments damaged pumpkin microstructure, leading to cells separation and plasmolysis; cook vide resulted the most aggressive method. The histological observations were related to the texture softening. Cook vide resulted the less impacting method on pumpkin colour, in relation to the largest extraction of some classes of carotenoids from the broken cells. A significant polyphenols extraction, especially gallic acid and naringenin, was instead observed for sous vide and steamed pumpkins. The total antioxidant activity, ascribable to the effect of both carotenoids and polyphenols, resulted thus enhanced after cooking, mainly for cook vide pumpkins, followed by the steamed and sous vide ones.

**CONCLUSIONS:** The use of vacuum cooking has often shown better performances than traditional steam cooking on pumpkin cubes. The implementation of cook-vide and sous-vide cooking at domestic or catering level would allow the consumption of vegetables with improved nutritional and sensorial characteristics.

**Keywords:** Cooking, sous vide, pumpkin, steaming, histological analysis, vacuum cooking

## 51    **Introduction**

52    Several guidelines recommend the consumption of five or more servings per day of fruits, vegetables,  
53    and legumes. Although fruits are mainly consumed fresh, vegetables are eaten raw or cooked (mainly  
54    in Europe and in USA), and mostly cooked in Asia and in other parts of the World.<sup>1</sup> Cooking process  
55    might alter the bioaccessibility and bioavailability of nutrients (such as phytochemicals, vitamins,  
56    minerals, and fibre). Some evidence **suggests** that cooking of vegetables can degrade nutrient and  
57    enzyme content and possibly also create harmful by-products.<sup>2</sup> However, for some phytochemicals,  
58    such as lycopene and  $\beta$  carotene, their bioaccessibility might be enhanced by cooking.<sup>3</sup> The most  
59    common way to cook vegetables is by immersing them in boiling water or exposing them to steam  
60    for several minutes; these treatments can generally lead to losses of nutritional compounds and  
61    molecules responsible for flavour.<sup>4</sup> For this reason, several alternative cooking techniques, such as  
62    microwaves, high pressure and vacuum treatments, are proposed to avoid some of these  
63    disadvantages. Among them, vacuum cooking has gained attention as an alternative cooking method  
64    in terms of (i) application at low temperatures in short processing time, (ii) non-oxygen environment,  
65    (iii) better protection of nutritional value and (iv) texture maintenance of food.<sup>5</sup> Generally, vacuum-  
66    based cooking treatments were reported to present a better microbial quality, colour, stem firmness  
67    and sensory acceptability.<sup>6</sup> For example, Iborra-Bernad et al. (2015)<sup>7</sup> reported that cook vide and sous  
68    vide cooking of green beans provided products with a higher ascorbic acid content than the  
69    conventional boiled ones. Mougin et al. (2015)<sup>8</sup> demonstrated that low-pressure cooking would allow  
70    preserving the most labile volatiles in vegetable broth due to the lower water boiling temperature and  
71    the reduced level of oxygen compared to traditional boiling. More recently, Koç et al. (2017)<sup>9</sup>  
72    reported that vacuum cooked green peas and carrots provided the highest general acceptance for the  
73    sensorial properties when compared to the sous vide and boiled ones.

74    In the literature, a device equipped with vacuum cooking and frying function called Gastrovac  
75    (International Cooking Concepts, Barcelona, Spain) has been already studied<sup>4, 10, 11</sup>. Unfortunately,

76 Gastrovac device was designed for more gastronomic cuisine and did not have the feature of  
77 household cooking appliance for its high cost.<sup>12</sup> Therefore, as there was no such household  
78 equipment, in 2013 an insert was designed and patented. This insert is applicable to a closing cover  
79 for a container body suitable for containing foodstuff under vacuum cooking also in house.<sup>13</sup> By  
80 means of the designed system, it is possible to obtain a vacuum cooking procedure also in domestic  
81 kitchens by preliminary generating the vacuum in the container without the need of a vacuum pump  
82 continuously connected to the appliance, as in Gastrovac system. The container could be then  
83 introduced in a domestic oven at the desired temperature and cooked. In the same way, Tomruk et al.  
84 (2016)<sup>14</sup> developed a kitchen appliance cooking equipment which can operate under vacuum and  
85 tested it on strawberry jam with very promising results: vacuum cooking reduced the 5-  
86 hydroxymethylfurfural HMF content of the strawberry jam but simultaneously gave also a higher  
87 sensorial quality in terms of colour, appearance, consistency, taste and overall acceptance comparing  
88 to the atmosphere processed jam.

89 **Among vegetables, pumpkin (*Cucurbita maxima* Duch.) is nutritionally and economically**  
90 **important species cultivated throughout the World with a global production of about 27 Mtons:**  
91 **in the European Union, Italy represents the second producer (0.6 Mtons) after Spain (0.7**  
92 **Mtons).<sup>15</sup> Regarding effects of different cooking techniques on pumpkin, Silva et al. (2019)<sup>16</sup>**  
93 **reported that sous vide cooked pumpkin had lower consumers acceptance for flavor, texture**  
94 **and overall acceptability compared to traditional cooking techniques due to the greater cooking**  
95 **time. However, to the Authors' best knowledge no information about effects of vacuum cooking**  
96 **on pumpkin is available.**

97 Thus, the aim of this **study** was thus the evaluation of cooking performances in terms of texture,  
98 colour, microstructural characteristics, antioxidant and carotenoids content, and organoleptic traits of  
99 pumpkin cubes by means of household vacuum cooking appliance compared to steaming and sous  
100 vide techniques.

## 102    **2. Materials and Methods**

### 103    *2.1 Plant material and samples preparation*

104    Twenty fresh pumpkins, *Cucurbita moschata* L. var. Violina, at commercial maturity (average weight  
105    3±0.5 kg), were kindly donated by Il Nuovo Fresco company (Montecchio Emilia, RE, Italy) and  
106    used in the experimental plan. The vegetables were brought to the laboratory within 24 h after  
107    harvesting and immediately stored at refrigerated temperatures (10 °C). The whole pumpkin was  
108    washed under running tap water to remove adhered dust. Pumpkins were then hand-peeled and cut,  
109    with a sharp knife, into small cubes of 1.0 cm side with a weight of about 1.1 g (RAW). In order to  
110    obtain homogeneous samples, only the equatorial part of the fruits was used.

### 111    *2.2 Cooking trials*

112    The pumpkin cubes were treated using three cooking trials: Sous vide (SV), Steaming (ST) and  
113    Vacuum cooking (VC). Three replicates were performed for each cooking method.

114    *Sous vide (SV)*: 120 cubes of pumpkin, divided into three vacuum bags (OPA/PP 15/65, Orved,  
115    Musile di Piave, Italy), were placed under vacuum using a packaging machine (Lavezzini Univac,  
116    Fiorenzuola d'Arda, PC, Italy). The samples were cooked in a stirred water bath at 90 °C (JULABO  
117    Labortechnik GmbH, Seelbach, Germany). Then, the bags were chilled in a rapid refrigerator  
118    (IRINOX Multifresh, IRINOX SpA, Corbanese di Tarzo, TV, Italy) and maintained under  
119    refrigerated storage at 4 °C until the time of analysis.

120    *Steaming treatments (ST)*: Twenty cubes for each replicate were used. The treatments were carried  
121    out at 100 °C under atmospheric pressure in a Combi-Steam SL oven (V-Zug, Zurich, Switzerland)  
122    that presented an internal volume of 0.032 m<sup>3</sup>, an air speed of 0.5 ms<sup>-1</sup> and a steam injection rate of  
123    0.03 kg min<sup>-1</sup>. Oven was pre-heated at the set temperature before inserting samples for each cooking  
124    trial. The cubes were equilibrated to room temperature before being placed in the oven for cooking.

125    *Vacuum cooking (VC)*: The samples were treated using the system described in the European Patent  
126    EP2671476A2. Thirty pumpkin cubes were placed in a closed container in which the pressure has

127 been brought at 0.8 bar using a vacuum pump (Tecla srl, Verona, Italy). Subsequently the samples  
128 were inserted in a preheated ventilated oven at 130 °C.

129 All the cooking conditions were defined by means of preliminary tests in order to achieve the same  
130 degree of cooking at the thermal centre expressed in terms of cook value  $C_{T_{ref}}^z$ . The cook value was  
131 obtained from the integration of the heat penetration curve during preliminary tests:

$$132 \quad C_{T_{ref}}^z = \int_0^t 10^{(T-T_{ref})/z} dt$$

133 where:

134 t = time (min)

135  $T_{ref}$  = reference temperature; set equal to 100 °C

136 z = temperature increase that induces a 10-fold increase of the reaction rate of the chemical reaction  
137 taken as reference; z was set at 33 °C, as previously reported.<sup>17</sup> All the cooking trials were designed  
138 to achieve a  $C_0$  at centre equal to 5.32 min equivalent corresponding to an acceptable cooking level  
139 expressed by a group of 20 untrained people which assessed samples cooked at different degrees  
140 during preliminary sensory experiments. Cooking times corresponded to 9 min for steaming, 18 min  
141 for sous vide and 29 min for vacuum cooking. Similarly,  $C_0$  values at samples' surfaces resulted very  
142 close each other (about 9 min).

143 The steam and ventilated oven air temperatures and water temperature in the stirred bath as well as  
144 those at the samples' centre and surface were monitored with 0.9 mm wire thermocouples (K-type;  
145 Ni/Al-Ni/Cr) with an acquisition rate of 5 s.

146

### 147 *2.3 Histological analysis*

148 The samples were fixed in FAA solution (formalin: acetic acid: 60% ethanol solution, 2:1:17 v/v).<sup>18</sup>  
149 After two weeks, they were dehydrated with gradual alcohol concentrations. The inclusion was made  
150 in a methacrylate resin (Technovit 7100, Heraeus Kulzer & Co., Wehrheim, Germany) and the  
151 resulting blocks were sectioned at 3 µm thickness (transversal cuts) with a semithin Leitz 1512

152 microtome (Leitz, Wetzlar, Germany). The sections were stained with Toluidine Blue (TBO)  
153 solution<sup>18</sup> for the evaluation of structure variation after each treatment. The section was observed by  
154 means of an optical microscopy Leica DM 4000 (Leica Imaging Systems Ltd., Wetzlar, Germany)  
155 equipped with a digital camera Leica DMC2900 (Leica Imaging Systems Ltd., Wetzlar, Germany).

156

## 157 *2.4 Physical analyses*

158 Moisture content (g/100 g) of pumpkin cubes was evaluated by means of gravimetric technique  
159 following the official method (AOAC, 1995)<sup>19</sup> both on raw and cooked samples.

160 Texture of the all treatments (RAW, VC, SV and ST) was analysed by Texture Profile Analysis (TPA)  
161 double compression test using a TA.XT2i Texture Analyzer equipped with a 35 mm diameter  
162 cylindrical aluminium with a pre-test, test and post-test speed of 1 mm s<sup>-1</sup> up to the 20% of the original  
163 sample height. The textural parameters considered were: hardness (maximum peak force of the first  
164 compression cycle, N), cohesiveness (ratio of positive force area during the second compression to  
165 that during the first compression area, dimensionless), resilience (area during the withdrawal of the  
166 penetration, divided by the area of the first penetration, dimensionless), and chewiness (product of  
167 hardness x cohesiveness x springiness, N)<sup>20</sup>. Ten samples of each cooking trial were analysed.

168 Colour determination was carried out using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka  
169 Japan) equipped with a standard illuminant D65. The assessments were carried out on four sides of  
170 four pumpkin cubes.  $L^*$  (lightness, black = 0, white = 100),  $a^*$  (redness >0, greenness <0),  $b^*$   
171 (yellowness,  $b^* > 0$ , blue <0), C (chroma, 0 at the centre of the colour sphere) and  $h^\circ$  (Hue angle, red  
172 =0°, yellow =90°, green=180°, blue=270°) were quantified on each sample using a 10-degree position  
173 of the standard observer. Ten samples of each cooking trial were analysed.

174

## 175 *2.5 DPPH free radical scavenging capacity test*

176 Antioxidant capacity was determined using DPPH assay (2,2-diphenyl-1-picrylhydrazyl free radical)  
177 following the procedure reported by Paciulli et al. (2019).<sup>21</sup> The samples were centrifuged at 12,000

g for 15 min at 4 °C. Then, the supernatant was collected for further analysis. 0.2 mL of 10-fold diluted supernatant was mixed with 4.0 mL of a methanolic solution of DPPH (0.14 mmol/L). Analyses were performed in triplicate and the absorbance of the solution was measured at 517 nm after an incubation time of 70 min, in dark, at room temperature. All data were then expressed as Trolox Equivalents ( $\mu\text{mol}/100\text{ g}$  pumpkin pulp) and antioxidant capacity referred to as Trolox Equivalents Antioxidant Capacity (TEAC).<sup>22</sup>

## 2.6 Analysis of phenolic compounds and of carotenoids

Phenolic compounds were determined in the samples as previously described.<sup>23</sup> Briefly, lyophilized sample (400 mg) was extracted with 2 mL of  $\text{H}_2\text{O}:\text{CH}_3\text{OH}$  30:70 (v/v) for 10 min at room temperature. After centrifugation, supernatant was analysed by HPLC with a diode array detector (Shimadzu, Shimadzu, Kyoto, Japan) using a 40 min linear gradient from 20% to 80%. Phase A was a mixture of  $\text{H}_2\text{O}:\text{formic acid}$  99.8:0.2 (v/v) and phase B was a mixture of  $\text{CH}_3\text{OH}:\text{CH}_3\text{CN}$  40:60 (v/v). A Prodigy column (5  $\mu\text{m}$  ODS3 100A,  $250 \times 4.60\text{ mm}$ ; Phenomenex, Torrance, CA, USA) was used and the detector was set at 256 nm for flavonols and at 325 nm for phenolic acids. The determination of carotenoids was carried out by HPLC analysis as previously described by Leonardi et al. (2000).<sup>24</sup> Quantification was achieved extracting from the diode array data the chromatograms recorded at 450 nm for  $\alpha$ -,  $\gamma$ - and  $\beta$ -carotene, zeaxanthin and lutein, at 350 nm for phytofluene, and at 290 nm for phytoene.  $\beta$ -,  $\gamma$ - and  $\alpha$ -carotene, zeaxanthin, phytoene, and phytofluene were quantified by calibration curves built with  $\beta$ -carotene pure standard. Lutein was quantified by a calibration curve built with lutein pure standard.

## 2.7 Statistical analysis

Means and standard deviations were calculated with SPSS (v. 25.0, SPSS Inc., Chicago, USA) and the same software was used to perform one-way analysis (ANOVA) with Tukey post-hoc test to evaluate the significant differences ( $p < 0.05$ ) between samples.

### 203 3. Results and Discussion

#### 204 3.1 Histological analysis

205 In RAW condition, the internal parenchyma (mesocarp) of *Cucurbita moschata* L. var. Violina fruits  
206 consisted of isodiametric, thickened cells with few and small intercellular spaces (Fig. 1A). The  
207 mesocarp cells was characterized, on average, by minor diameters, ranging from 12.6 to 77.8  $\mu\text{m}$  and  
208 by major diameters varying from 18.3 to 97.8  $\mu\text{m}$ . Immersed in the parenchyma tissue, vascular  
209 bundles surrounded by small parenchymatic cells are observed (Fig. 1A).

210 After SV, the parenchymatic tissue appeared damaged. The cells were detached (Fig. 1B) and this  
211 phenomenon seems to be related to the thermal treatment. Paciulli and collaborators (2016)<sup>25</sup>  
212 observed same damages in the parenchymatic tissues of different vegetables after cooking and they  
213 associated this event with the pectic bonds breaking at the level of the middle lamella between  
214 adjacent cells and/or with the hydrolysis of some components of the cell wall (pectin, hemicellulose,  
215 cellulose). SV treatment led to cell plasmolysis and, finally, to the formation of gaps in the  
216 parenchymatic tissue, due to cell separations. The gaps showed an intense colouration indicating the  
217 presence of inclusions (Fig. 1B). In this study, the content of the intercellular spaces could not be  
218 identified, but it seems that this phenomenon may be due to the separation of the mesocarp  
219 parenchymatic cells. This hypothesis is confirmed by the results obtained by Luza et al. (1992)<sup>26</sup>, who  
220 demonstrated that the damaged parenchymatic tissues, after a thermal treatment, accumulate pectic  
221 substances in the intercellular spaces.

222 After ST, the parenchymatic cells appeared with an irregular shape and intercellular cavities were  
223 visible in the tissue (Fig. 1C). In this case, the gaps were less evident and structure resulted less  
224 damaged than the previous treatment (SV), as consequence.

225 After the VC treatment, the parenchymal tissues appeared deeply damaged (Fig. 1D). The tissue  
226 resulted disorganized with loss of shape and turgidity of the cells due to breaking of the cell walls  
227 (Fig. 1E). The deformation of the cells after vacuum cooking can be due to the volume expansion of  
228 the air present in the tissues when it is vacuumized, followed by a partial collapse of the structure

229 when it returns to atmospheric pressure.<sup>27</sup> VC resulted the most aggressive treatment on pumpkin  
230 cubes 'structure, among the all studied ones.

231 Iborra-Bernanrd et al. (2015)<sup>7</sup>, studying the effect of boiling, cook-vide and sous-vide cooking on the  
232 structure of potatoes, green beans and carrots, found different scenarios, according to the type of  
233 vegetable. For potatoes and carrots, cook-vide resulted more aggressive than sous-vide, in accordance  
234 with the results of this study.

235

### 236 3.2 Textural analysis

237 Textural data of pumpkin samples are reported in Table 1. The differences observed between samples  
238 cannot be attributable to water changes, since the moisture content was not affected by the treatments.  
239 Moisture content resulted  $84.77 \pm 1.19$ ,  $85.97 \pm 1.55$ ,  $88.75 \pm 1.74$  and  $85.17 \pm 4.04$  g/100 g for RAW,  
240 SV, ST, and VC, respectively, with no significant differences among samples. Textural changes may  
241 be instead related to tissues modifications, as induced by treatments (Par. 3.1).

242 As expected, cooked samples resulted softer than the RAW ones. Among the cooked samples, VC  
243 showed the lowest hardness values, confirming the histological observations of a more damaged  
244 tissue structure. Even if cook values at centre were the same for all the cooked samples, (Par. 2.1),  
245 thermal degradation of VC samples resulted higher compared to the others showing a different  
246 consistency, probably due to the different temperature profiles in the whole product. Particularly, the  
247 surface cook effect resulted equal to  $9.01 \pm 0.51b$ ,  $8.95 \pm 0.48b$  and  $9.9 \pm 0.66a$  for ST, SV and VC,  
248 respectively; as a consequence, the mean cook value was higher in VC samples with reasonably  
249 higher heat damage to the cell structure.

250 Studies carried out on taro corms by Njintang et al. (2009)<sup>28</sup> suggest that multiple mechanisms are  
251 involved during cooking induced softening, including gelatinisation, starch hydrolysis, cells  
252 separations and proteins denaturation/leaching. The obtained results are in agreement with Iborra-  
253 Bernad, García-Segovia and Martínez-Monzó (2015)<sup>7</sup> who observed firmer texture for SV green

254 beans and carrots compared to VC, cooked with the same conditions. In the same way, Koç et al.  
255 (2017)<sup>9</sup> reported a higher softening percentage for VC cooked carrot and green pea compared to SV  
256 ones. Cohesiveness and resilience values (Table 1) significantly decreased after all cooking processes,  
257 as expected, with the greatest extent for VC samples with the same trend observed for the hardness  
258 values explained above. **Instrumental hardness measured with TPA method showed a high**  
259 **correlation with the sensory parameters on cooked carrots, providing a quick and cheap tool**  
260 **for determination of optimum cooking times as well as for cooked quality evaluation by the**  
261 **ready-to-use food products and catering industries.**<sup>29</sup>

262 Springiness values showed a different trend from the other textural data: all the cooked samples  
263 presented significantly higher values compared to the RAW ones probably because this latter showed  
264 a harder, more rigid and fragile structure that recovered less the original shape after the first  
265 compression. Finally, chewiness values were dramatically reduced after cooking and the obtained  
266 values were in agreement with the structural damages discussed above.

267

### 268 3.3 Colour

269 Colorimetric parameters of cooked samples were all significantly different from RAW ones as a  
270 consequence of heat treatment (Table 2).  $L^*$  value of cooked samples decreased as expected and  
271 among cooked samples VC presented the significantly highest values followed by ST and  
272 successively by SV. The SV result is in disagreement with the general higher retention of colour  
273 reported after sous vide cooking and could be probably due to the wet appearance observed on the  
274 surface of these samples after the cooking, because of the plastic bag that prevented dehydration. VC  
275 results are in agreement with Iborra-Bernad et al. (2014a)<sup>11</sup> who reported that cook-vide potatoes  
276 were lighter (higher  $L^*$ ) than sous vide ones. Redness values ( $a^*$ ) decreased after the all cooking  
277 procedures in comparison to RAW. VC showed the highest  $a^*$  values, with the best retention of the  
278 pumpkin original colour. These results are in accordance with Okut et al. (2018)<sup>12</sup> that reported a  
279 better retention of colour for a strawberry jam cooked by vacuum cooking compared to traditional

280 atmospheric cooking. On the contrary,  $b^*$  values of cooked samples were all significantly different  
281 from RAW but with no significant differences among the cooking conditions (Table 2). The lowest  
282 global colour difference expressed by  $\Delta E$  (Table 2) was observed for VC samples, confirming that  
283 vacuum cooking is able to preserve the colour of raw ingredients better than other cooking  
284 procedures.<sup>30</sup> This result is directly related to the total carotenoid content (Table 3) and in agreement  
285 with Dutta et al. (2006)<sup>31</sup> who reported a correlation between colorimetric parameters and total  
286 carotenoids in pumpkin puree. **Similarly, de Almeida et al. (2019)<sup>32</sup> reported high correlation**  
287 **between physicochemical and sensory characteristics of cooked pumpkin varieties with an**  
288 **important role of colour.**

289

#### 290 *3.4 Carotenoids, polyphenols and total antioxidant capacity*

291 Table 3 shows the content of carotenoids in raw and cooked pumpkins. This variety of pumpkin  
292 contained mainly  $\beta$ -carotene, lutein and  $\alpha$ -carotene, in agreement with Bergantin et al. (2018)<sup>33</sup> who,  
293 analysing two Italian varieties of pumpkin, found some other carotenoids in lower amount. As already  
294 shown in previous studies, the cooking procedures significantly increased almost all the carotenoid  
295 contents compared to RAW. In agreement with previous findings<sup>34,35</sup>, steaming was more effective  
296 in releasing  $\alpha$ - and  $\beta$ -carotene than other cooking methods, whereas lutein, zeaxanthin, and  $\gamma$ -carotene  
297 increased more upon vacuum cooking. During processing, carotenoids degradation, such as  
298 isomerisation and oxidation, can occur. In addition, processing can also break down food matrices  
299 and loosen carotene-binding fibres with an increase of carotenoids extractability. Among the studied  
300 treatments, vacuum cooking was the most aggressive on the tissues, leading to loss of cells turgidity  
301 due to the cell wall breaking. Such a strong breaking down of the structure can have led to the most  
302 effective release of total carotenoids compared to the other processes. Previous studies<sup>36</sup> have found  
303 in pumpkin samples strong correlations between the colour values  $a^*$  and  $b^*$  with the total carotenoid  
304 content and lutein, respectively; similar trends were also observed in our study.

305 The content of phenolics and flavonoids of raw and cooked pumpkins is reported in Table 4. *p*-  
306 hydroxybenzoic, coumaric and ferulic acids were the predominant phenolic acids, while naringenin  
307 the major flavonoid in this variety of pumpkin. There are a few publications on the profile of phenolic  
308 compounds in pumpkin that suggest a broad range of concentrations of single phenolic and flavonoid  
309 depending on the species and variety.<sup>37,38</sup> Indeed, naringenin was the most representative flavonoid  
310 in our pumpkin, but it was not present in 11 analysed pumpkin cultivars from Poland.<sup>38</sup> The effect of  
311 the thermal treatments on single phenolics and flavonoids was less clear than in the case of carotenoid  
312 values. Some of them increase after all the treatments (i.e., gallic and ferulic acids and naringenin),  
313 whereas others increased mainly after some treatments. On the other hand, caffeic acid consistently  
314 decreased after all the thermal treatments. Considering the sum of all phenolic and flavonoid  
315 compounds, ST and SV resulted the more effective treatments for release them and this result was  
316 mostly due to the increase of naringenin and gallic acid. Vacuum cooking also led to an increase in  
317 these compounds but in parallel some others decreased such as chlorogenic, coumaric and caffeic  
318 acids. The different effect of thermal treatments on single phenolic compounds has been already  
319 observed. This is the result of several, even opposite mechanisms. A partial hydrolysis of the ester  
320 bonds connecting phenolic acids to cell wall polysaccharides and the matrix softening favour the  
321 release of these compounds.<sup>23</sup> This might be for instance the case of gallic acid which was present in  
322 trace in raw pumpkin whereas it was determined in all the cooked pumpkins. However, when the  
323 phenolic compounds are released they can be oxidised by polyphenoloxidase and react again with  
324 cell-wall polysaccharides. The polyphenol-polysaccharides interaction modifies the extractability of  
325 these compounds, even though they can partially retain their antioxidant capacity<sup>39</sup>.

326 Total antioxidant capacity of pumpkin is ascribable to the effect of both carotenoids and polyphenols.  
327 The values, measured by means of DPPH method, are reported in Figure 2. All the cooked samples  
328 presented values significantly higher than RAW ones, in accordance to other studies on cooking of  
329 pumpkin.<sup>22, 40</sup> The increase in total antioxidant capacity after thermal processing is attributable to the  
330 release of phytochemicals from the cellular structures. Indeed, the highest DPPH values (Figure 2)

331 was measured for VC, in which the tissues were deeply damaged leading to the destruction of the cell  
332 wall and subcellular compartments and thus to the release of radical-scavenging antioxidants, such  
333 as carotenoids and p-hydroxybenzoic and ferulic acids (Tab. 3, 4). Similarly, Lemmens et al. (2009)<sup>41</sup>  
334 reported in carrot pieces an inverse relationship between structural characteristics, hardness and  $\beta$ -  
335 carotene *in vitro* bioaccessibility. Among cooked samples, the lowest values were observed in SV,  
336 probably due to the absence of a cooking medium able to soften the cell walls of the pumpkin tissues,  
337 accordingly to the lowest carotenoids extraction (Tab. 3). Similarly, Iborra-Bernad et al. (2015)<sup>7</sup>  
338 observed a higher content of  $\beta$ -carotene in purple flesh potato cooked by cook-vide method compared  
339 to sous vide one.

340

#### 341 4. Conclusions

342 The impact of vacuum cooking on pumpkin cubes is not largely debated in literature. The  
343 multidisciplinary approach of this study has highlighted how vacuum cooking can bring benefits both  
344 at **instrumental quality** and healthy level on cooked pumpkins, compared to a traditional steam  
345 cooking. The most interesting results were observed for cook vide; indeed, although it was the most  
346 impactful method on microstructure and texture, it showed the best colour retention and the highest  
347 enhancement of the antioxidant activity, mostly related to a better carotenoids extraction from the  
348 broken cells. Sous vide proved to be similar to steaming for most of the studied characteristics. The  
349 implementation of cook vide at domestic level, has great potential to increase the consumption of  
350 organoleptic and nutritional improved pumpkin cubes. **In further studies, for supporting the**  
351 **implementation of this cooking technique sensorial evaluation will be performed on cooked**  
352 **samples.**

353

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357

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- 469

470 **Table 1.** Textural parameters of raw and cooked pumpkin samples

	<i>Hardness (N)</i>	<i>Cohesiveness</i>	<i>Resilience (%)</i>	<i>Springiness (%)</i>	<i>Chewiness (N)</i>
<b>RAW</b>	71.24 (8.74) a	0.63 (0.05) a	35.41 (4.25) a	66.86 (4.79) b	30.15 (5.26) a
<b>ST</b>	13.32 (1.26) b	0.42 (0.07) b	21.64 (4.05) b	81.18 (5.71) a	4.59 (0.91) b
<b>SV</b>	10.00 (2.01) b	0.44 (0.12) b	22.31 (4.23) b	82.02 (4.37) a	3.68 (0.69) c
<b>VC</b>	7.25 (1.34) c	0.36 (0.10) c	15.48 (3.14) c	78.49 (5.71) a	2.10 (0.44) d

471

472 <sup>a, b, c</sup> Same letters within each column do not significantly differ (n = 5; p < 0.05); standard deviation  
473 given in parenthesis.

474

475 **Table 2.** Colourimetric parameters of raw and cooked pumpkin samples

	<i>L</i> <sup>*</sup>	<i>a</i> <sup>*</sup>	<i>b</i> <sup>*</sup>	$\Delta E$
<b>RAW</b>	63.27 (1.37) a	34.68 (1.19) a	51.24 (3.14) a	-
<b>ST</b>	49.15 (1.70) c	17.68 (4.27) c	35.17 (4.85) b	27.50 (4.70) a
<b>SV</b>	53.10 (3.46) d	20.19 (2.82) c	38.63 (5.63) b	21.69 (4.25) b
<b>VC</b>	57.18 (2.13) b	26.87 (3.80) b	40.34 (5.80) b	14.63 (3.87) c

476

477 <sup>a, b, c</sup> Same letters within each column do not significantly differ (**n = 10; p < 0.05**); standard deviation  
478 given in parenthesis.

479

480 **Table 3.** Carotenoids of raw and cooked pumpkin samples (µg/g dw)

481

	RAW	ST	SV	VC
<i>lutein</i>	1.04 (0.008) c	1.72 (0.02) b	1.80 (0.03) b	2.46 (0.004) a
<i>zeaxanthin</i>	0.015 (0.001) c	0.030 (0.001) b	0.027 (0.001) b	0.056 (0.001) a
<i>α-carotene</i>	0.838 (0.007) b	1.035 (0.008) a	0.723 (0.002) c	0.723 (0.001) c
<i>β-carotene</i>	2.21 (0.005) c	2.70 (0.005) a	2.52 (0.003) b	2.57 (0.002) b
<i>γ-carotene</i>	0.168 (0.002) d	0.47 (0.03) b	0.390 (0.005) c	0.607 (0.008) a
<i>phytoene</i>	0.044 (0.007) c	0.093 (0.002) a	0.071 (0.004) b	0.090 (0.006) a
<i>phytofluene</i>	0.046 (0.002) b	0.092 (0.002) a	0.108 (0.004) a	0.096 (0.003) a
<i>Total carotenoids</i>	4.37 (0.05) d	6.10 (0.11) b	5.64 (0.04) c	6.60 (0.02) a

482

483 <sup>a, b, c</sup> Same letters within each column do not significantly differ (n = 3; p < 0.05); standard deviation  
484 given in parenthesis.

485

486 **Table 4.** Principal and total polyphenols of raw and cooked pumpkin samples (µg/g dw)

	RAW	ST	SV	VC
<i>gallic acid</i>	0.0 (0.0) c	6.30 (0.19) a	5.86 (0.54) a	1.42 (0.15) b
<i>chlorogenic acid</i>	2.82 (0.04) a	2.28 (0.07) a	0.17 (0.03) b	0.69 (0.03) c
<i>p-hydroxybenzoic acid</i>	8.80 (0.06) b	8.75 (0.34) b	6.28 (0.13) c	9.81 (0.80) a
<i>caffeic acid</i>	0.86 (0.03) a	0.61 (0.01) b	0.42 (0.02) c	0.76 (0.01) b
<i>coumaric acid</i>	3.02 (0.12) b	2.79 (0.20) b	3.98 (0.07) a	1.71 (0.23) c
<i>ferulic acid</i>	1.40 (0.01) d	2.21 (0.03) b	1.85 (0.08) c	3.12 (0.10) a
<i>Total phenolic acids</i>	94.0 (0.3) b	107.3 (1.0) a	106.4 (0.7) a	93.1 (0.8) b
<i>quercetin</i>	0.70 (0.09) c	1.26 (0.01) b	1.51 (0.02) a	0.70 (0.09) c
<i>rutin</i>	9.70 (0.01) a	9.73 (0.01) a	9.72 (0.01) a	8.17 (0.53) b
<i>naringenin</i>	54.3 (0.2) d	64.5 (0.1) a	62.5 (0.2) b	55.8 (1.5) c
<i>Total flavonoids</i>	64.3 (0.4) c	74.9 (0.7) a	72.9 (0.7) b	64.7 (0.1) c
<i>Total polyphenols</i>	158.7 (0.7) b	182.6 (0.6) a	180.7 (0.5) a	157.4 (0.6) b

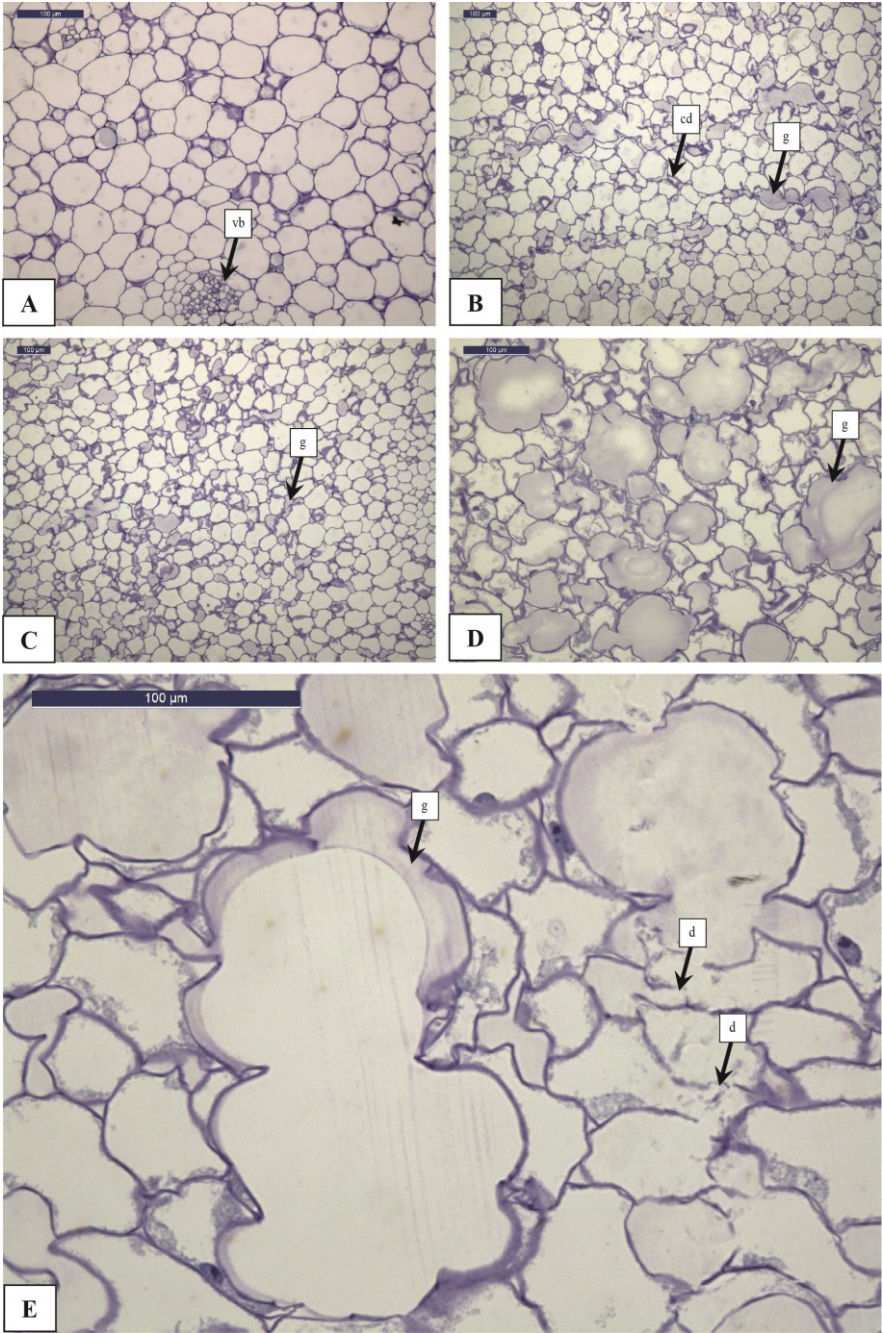
487

488 <sup>a, b, c</sup> Same letters within each column do not significantly differ (n = 3; p < 0.05); standard deviation

489 is given in parenthesis.

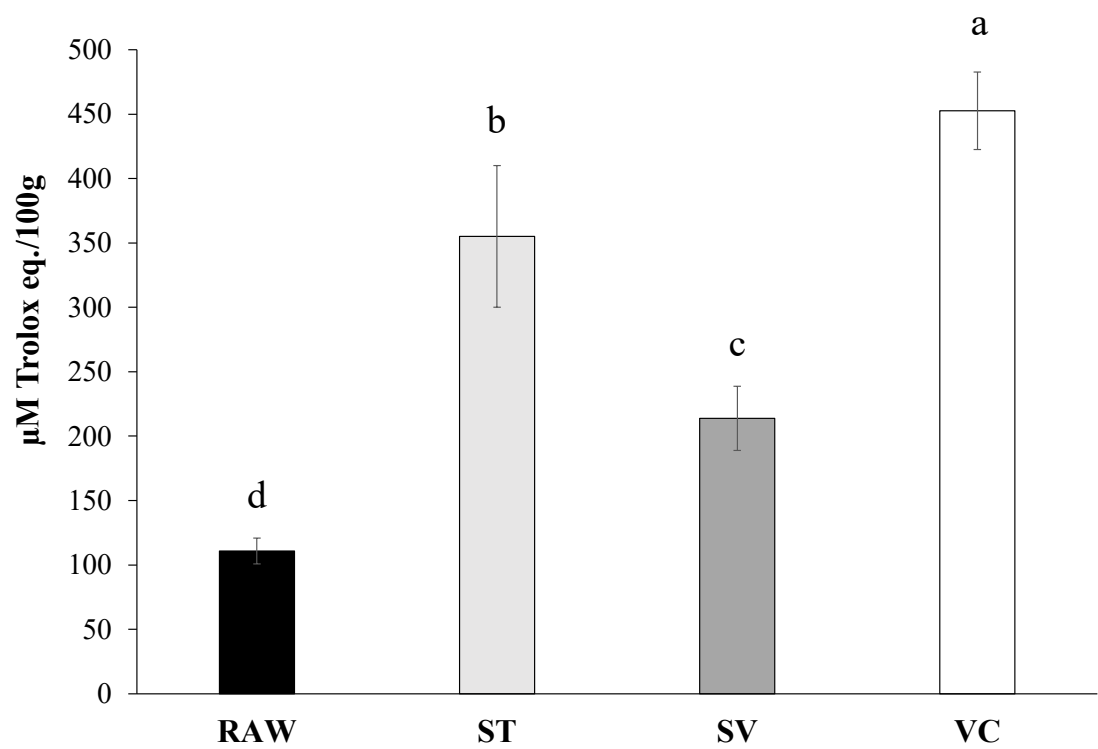
490

491 **Figure 1.** Transverse sections of *Cucurbita maxima* L. var. Violina samples stained with Toluidine  
492 Blue: RAW (A), ST (B), SV (C), VC (D. and E).  
493



494  
495 Legend: vb=vascular bundles; cd=cell detachment; g = gaps; d=cell damage.  
496  
497

498 **Figure 2.** Total antioxidant capacity of pumpkin samples<sup>a</sup>.



499

500 <sup>a</sup> n=3. Means followed by different letters significantly differ ( $p < 0.05$ ).