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

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

Introduction

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

In the literature, a device equipped with vacuum cooking and frying function called Gastrovac (International Cooking Concepts, Barcelona, Spain) has been already studied[4, 10, 11]. Unfortunately,

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

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

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

2. Materials and Methods

2.1 Plant material and samples preparation

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

2.2 Cooking trials

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

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

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

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

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

All the cooking conditions were defined by means of preliminary tests in order to achieve the same degree of cooking at the thermal centre expressed in terms of cook value C^{z}_{Tref}. The cook value was obtained from the integration of the heat penetration curve during preliminary tests:

$$C^{z}_{Tref} = \int_0^t 10^{(T-T_{ref})/z}\, dt$$

where:

t = time (min)

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

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

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

2.3 Histological analysis

The samples were fixed in FAA solution (formalin: acetic acid: 60% ethanol solution, 2:1:17 v/v).[18] After two weeks, they were dehydrated with gradual alcohol concentrations. The inclusion was made in a methacrylate resin (Technovit 7100, Heraeus Kulzer & Co., Wehrheim, Germany) and the 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[18] 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)[19] 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 mms^{-1} 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)[20]. 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° (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).[21] The samples were centrifuged at 12,000

178 g for 15 min at 4 °C. Then, the supernatant was collected for further analysis. 0.2 mL of 10-fold
179 diluted supernatant was mixed with 4.0 mL of a methanolic solution of DPPH (0.14 mmol/L).
180 Analyses were performed in triplicate and the absorbance of the solution was measured at 517 nm
181 after an incubation time of 70 min, in dark, at room temperature. All data were then expressed as
182 Trolox Equivalents (μmol/100 g pumpkin pulp) and antioxidant capacity referred to as Trolox
183 Equivalents Antioxidant Capacity (TEAC).[22]

184 *2.6 Analysis of phenolic compounds and of carotenoids*

185 Phenolic compounds were determined in the samples as previously described.[23] Briefly, lyophilized
186 sample (400 mg) was extracted with 2 mL of H_2O:CH_3OH 30:70 (v/v) for 10 min at room
187 temperature. After centrifugation, supernatant was analysed by HPLC with a diode array detector
188 (Shimadzu, Shimadzu, Kyoto, Japan) using a 40 min linear gradient from 20% to 80%. Phase A was
189 a mixture of H_2O:formic acid 99.8:0.2 (v/v) and phase B was a mixture of CH_3OH:CH_3CN 40:60
190 (v/v). A Prodigy column (5 μm ODS3 100A, 250 × 4.60 mm; Phenomenex, Torrance, CA, USA) was
191 used and the detector was set at 256 nm for flavonols and at 325 nm for phenolic acids. The
192 determination of carotenoids was carried out by HPLC analysis as previously described by Leonardi
193 et al. (2000).[24] Quantification was achieved extracting from the diode array data the chromatograms
194 recorded at 450 nm for α-, γ- and β-carotene, zeaxanthin and lutein, at 350 nm for phytofluene, and
195 at 290 nm for phytoene. β-, γ- and α-carotene, zeaxanthin, phytoene, and phytofluene were quantified
196 by calibration curves built with β-carotene pure standard. Lutein was quantified by a calibration curve
197 built with lutein pure standard.

198 *2.7 Statistical analysis*

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

202

3. Results and Discussion

3.1 Histological analysis

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

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

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

After the VC treatment, the parenchymal tissues appeared deeply damaged (Fig. 1D). The tissue resulted disorganized with loss of shape and turgidity of the cells due to breaking of the cell walls (Fig. 1E). The deformation of the cells after vacuum cooking can be due to the volume expansion of 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.[27] VC resulted the most aggressive treatment on pumpkin
230 cubes 'structure, among the all studied ones.
231 Iborra-Bernanrd et al. (2015)[7], 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 ± 1.19, 85.97 ± 1.55, 88.75 ± 1.74 and 85.17 ± 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 ± 0.51b, 8.95 ± 0.48b and 9.9 ± 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)[28] 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)[7] who observed firmer texture for SV green

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

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

3.3 Colour

Colorimetric parameters of cooked samples were all significantly different from RAW ones as a consequence of heat treatment (Table 2). *L** value of cooked samples decreased as expected and among cooked samples VC presented the significantly highest values followed by ST and successively by SV. The SV result is in disagreement with the general higher retention of colour reported after sous vide cooking and could be probably due to the wet appearance observed on the surface of these samples after the cooking, because of the plastic bag that prevented dehydration. VC results are in agreement with Iborra-Bernad et al. (2014a)[11] who reported that cook-vide potatoes were lighter (higher *L**) than sous vide ones. Redness values (*a**) decreased after the all cooking procedures in comparison to RAW. VC showed the highest *a** values, with the best retention of the pumpkin original colour. These results are in accordance with Okut et al. (2018)[12] that reported a 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 ΔE (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.[30] This result is directly related to the total carotenoid content (Table 3) and in agreement
285 with Dutta et al. (2006)[31] who reported a correlation between colorimetric parameters and total
286 carotenoids in pumpkin puree. **Similarly, de Almeida et al. (2019)[32] 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 β- carotene, lutein and α-carotene, in agreement with Bergantin et al. (2018)[33] 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[34,35], steaming was more effective
296 in releasing α- and β-carotene than other cooking methods, whereas lutein, zeaxanthin, and γ-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[36] 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.[37,38] Indeed, naringenin was the most representative flavonoid
310 in our pumpkin, but it was not present in 11 analysed pumpkin cultivars from Poland.[38] 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.[23] 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[39].
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.[22, 40] 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)

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

4. Conclusions

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

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References

1. Miller V, Mente A, Dehghan M, Rangarajan S, Zhang X, Swaminathan S, ... and Bangdiwala SI, Fruit, vegetable, and legume intake, and cardiovascular disease and deaths in 18 countries (PURE): a prospective cohort study. *Lancet*, **390**: 2037-2049 (2017).

2. Link LB and Potter JD, Raw versus cooked vegetables and cancer risk. *Cancer Epidem Biomar* **13**: 1422-1435 (2004).

3. Dewanto V, Wu X, Adom KK and Liu RH, Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J Agr Food Chem* **50**: 3010-3014 (2002).

4. Iborra-Bernad C, Tárrega A, García-Segovia P and Martínez-Monzó J, Comparison of vacuum treatments and traditional cooking using instrumental and sensory analysis. *Food Anal Methods* **7**: 400-408 (2014b).

5. García-Segovia P, Andrés-Bello A and Martínez-Monzó J, Effect of cooking method on mechanical properties, color and structure of beef muscle (*M. pectoralis*). *J Food Eng* **80**: 813-821 (2007).

6. Martínez-Hernández GB, Artés-Hernández F, Colares-Souza F, Gómez PA, García-Gómez P and Artés F, Innovative cooking techniques for improving the overall quality of a kailan-hybrid broccoli. *Food Bioprocess Tech* **6**: 2135-2149 (2013).

7. Iborra-Bernad C, García-Segovia P and Martínez-Monzó J, Physico-chemical and structural characteristics of vegetables cooked under sous‐vide, cook‐vide, and conventional boiling. *J Food Sci* **80**: E1725-E1734 (2015).

8. Mougin A, Mauroux O, Matthey-Doret W, Barcos EM, Beaud F, Bousbaine A, Viton F and Smarrito-Menozzi C, Impact of boiling conditions on the molecular and sensory profile of a vegetable broth. *J Agr Food Chem* **63**: 1393-1400 (2015).

9. Koç M, Baysan U, Devseren E, Okut D, Atak Z, Karataş H and Kaymak-Ertekin F, Effects of different cooking methods on the chemical and physical properties of carrots and green peas. *Innov Food Sci Emerg* **42**: 109-119 (2017).

10. Andrés-Bello A, García-Segovia P and Martínez-Monzó J, Effects of vacuum cooking (cook-vide) on the physical-chemical properties of sea bream fillets (*Sparus aurata*). *J Aquat Food Prod Technol* **18**: 79-89 (2009).

11. Iborra-Bernad C, García-Segovia P and Martínez-Monzó J, Effect of vacuum cooking treatment on physicochemical and structural characteristics of purple-flesh potato. *Int J Food Sci Tech* **49**: 943-951 (2014a).

12. Okut D, Devseren E, Koç M, Ocak ÖÖ, Karataş H and Kaymak-Ertekin F, Developing a vacuum cooking equipment prototype to produce strawberry jam and optimization of vacuum cooking conditions. *J Food Sci Tech* **55**: 90-100 (2018).

13. Schirò P, Insert for covers and/or containers for cooking foodstuff under vacuum. European Patent EP2671476A2 (2013)

14. Tomruk D, Devseren E, Koç M, Ocak ÖÖ, Karataş H and Kaymak-Ertekin F, Developing a household vacuum cooking equipment, testing its performance on strawberry jam production and its comparison with atmospheric cooking. *Agron Res*, **14**(Special Issue II): 1475-1487 (2016).

15. FAOSTAT. 2018. (Available at http://faostat.fao.org/site/339/default.aspx [verified 25 Sep. 2020]).

16. Silva MDFGD, Sousa PHMD, Figueiredo RW, Gouveia ST and Lima JSS, Cooking effects on bioactive compounds and sensory acceptability in pumpkin (Cucurbita moschata cv. Leite). Rev. Ciênc. Agron 50: 394-401 (2019).

17. Vittadini E, Rinaldi M, Chiavaro E, Barbanti D and Massini R, The effect of different convection cooking methods on the instrumental quality and yield of pork *Longissimus dorsi*. *Meat Sci* **69**: 749-756 (2005).

18. Ruzin S (ed). *Plant Microtechnique and Microscopy*. Oxford University Press, Oxford (1999).

19. AOAC, *Official methods for analysis of AOAC International*, 18th edn. AOAC International, Artington (2005).

20. Bourne MC, Texture profile analysis. *Food Technol* **32**: 62-66 (1978).

21. Paciulli M, Rinaldi M, Rodolfi M, Ganino T, Morbarigazzi M and Chiavaro E, Effects of high hydrostatic pressure on physico-chemical and structural properties of two pumpkin species. *Food Chem* **274**: 281-290 (2019).

22. Dini I, Tenore GC and Dini A, Effect of industrial and domestic processing on antioxidant properties of pumpkin pulp. *LWT — Food Sci Technol* **53**: 382–385 (2013).

23. Mazzeo T, Paciulli M, Chiavaro E, Visconti A, Fogliano V, Ganino T and Pellegrini N, The impact of the industrial freezing process on selected vegetables Part II. Colour and bioactive compounds. *Food Res Int* **75**: 89-97 (2015).

24. Leonardi C, Ambrosino P, Esposito F and Fogliano V, Antioxidative activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. *J Agr Food Chem* **48**: 4723-4727 (2000).

25. Paciulli M, Ganino T, Carini E, Pellegrini N, Pugliese A and Chiavaro E, Effect of different cooking methods on structure and quality of industrially frozen carrots. *J Food Sci Technol* **53**: 2443-2451 (2016).

26. Luza JG, Van Gorsel R, Polito VS and Kader AA, Chilling injury in peaches: a cytochemical and ultrastructural cell wall study. *J Am Soc Hortic Sci* **117**: 114-118 (1992).

27. Saurel R, The use of vacuum technology to improve processed fruit and vegetables. In: *Fruit and vegetable processing: Improving quality*, ed. by Jongen W. Woodhead Publishing Limited, Abington Hall, Abington Cambridge, pp 363-380 (2002).

28. Njintang NY, Scher J and Mbofung CM Texture, microstructure and physicochemical characteristics of taro (*Colocasia esculenta*) as influenced by cooking conditions. *J Food Eng* **91**: 373-379 (2009).

29. Belie ND, Laustsen AM, Martens M, Bro R and Baerdemaeker JD, Use of physico-chemical methods for assessment of sensory changes in carrot texture and sweetness during cooking. *J Texture Stud* 33: 367-388 (2002).

30. García-Segovia P, Garrido MD, Vercet A, Arboleya JC, Fiszman S, Martínez-Monzo J, Laguarda S, Palacios V and Ruiz J, Molecular gastronomy in Spain. *J Culin Sci Technol* **12**: 279-293 (2014).

31. Dutta D, Dutta A, Raychaudhuri U and Chakraborty R, Rheological characteristics and thermal degradation kinetics of beta-carotene in pumpkin puree. *J Food Eng* **76**: 538-546 (2006).

32. de Almeida AB, de Lima TM, de Oliveira Filho JG, Santana RV, Lima DS, Moreira EA and Egea MB, Relation between physicochemical characteristics and sensory profiles of cooked pumpkin varieties. Emir J Food Agr 31: 697-707 (2019).

33. Bergantin C, Maietti A, Tedeschi P, Font G, Manyes L and Marchetti N, HPLC-UV/Vis-APCI-MS/MS determination of major carotenoids and their bioaccessibility from "Delica" (*Cucurbita maxima*) and "Violina" (*Cucurbita moschata*) pumpkins as food traceability markers. *Molecules*, **23**: 2791 (2018).

34. de Carvalho LMJ, Smiderle LASM, de Carvalho JLV, Cardoso FSN and Koblitz MGB, Assessment of carotenoids in pumpkins after different home cooking conditions. *Food Sci Technol* **34**: 365-370 (2014).

35. Moreira LAS, de Carvalho JLV, Cardoso FSN, Ortiz GMD, Finco FDBA, José de Carvalho JLV, Different cooking styles enhance antioxidant properties and carotenoids of biofortified pumpkin (*Cucurbita moschata* Duch) genotypes. *Food Sci Technol*, **40**: 302-306 (2020).

36. Itle RA, Kabelka EA. Correlation between L* a* b* color space values and carotenoid content in pumpkins and squash (Cucurbita spp.). *Hort Science*, **44**: 633-637 (2009).

37. Kulczynski B and Gramza-Michałowska A, The profile of secondary metabolites and other bioactive compounds in *Cucurbita pepo* L. and *Cucurbita moschata* pumpkin cultivars. *Molecules*, **24**: 2945 (2019a).

457 38. Kulczynski B and Gramza-Michałowska A, The profile of carotenoids and other bioactive
458 molecules in various pumpkin fruits (*Cucurbita maxima* Duchesne) cultivars. *Molecules*, **24**: 3212
459 (2019b).
460 39. Renard CMGC, Watrelot AA and Le Bourvellec C, Interactions between polyphenols and
461 polysaccharides: Mechanisms and consequences in food processing and digestion. *Trends Food Sci*
462 *Tech* **60**: 43-51 (2017).
463 40. Azizah AH, Wee KC, Azizah O and Azizah M, Effect of boiling and stir frying on total phenolics,
464 carotenoids and radical scavenging activity of pumpkin (*Cucurbita moschata*). *Int Food Res J* **16**: 45-
465 51 (2009).
466 41. Lemmens L, Van Buggenhout S, Oey I, Van Loey A and Hendrickx M, Towards a better
467 understanding of the relationship between the β-carotene in vitro bio-accessibility and pectin
468 structural changes: a case study on carrots. *Food Res Int* **42**: 1323-1330 (2009).
469

Table 1. Textural parameters of raw and cooked pumpkin samples

	Hardness (N)	*Cohesiveness*	*Resilience (%)*	*Springiness (%)*	*Chewiness (N)*
RAW	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
ST	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
SV	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
VC	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

[a, b, c] Same letters within each column do not significantly differ ($n = 5$; $p < 0.05$); standard deviation given in parenthesis.

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

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

476

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

482

483 [a, b, c] Same letters within each column do not significantly differ ($n = 3$; $p < 0.05$); standard deviation

484 given in parenthesis.

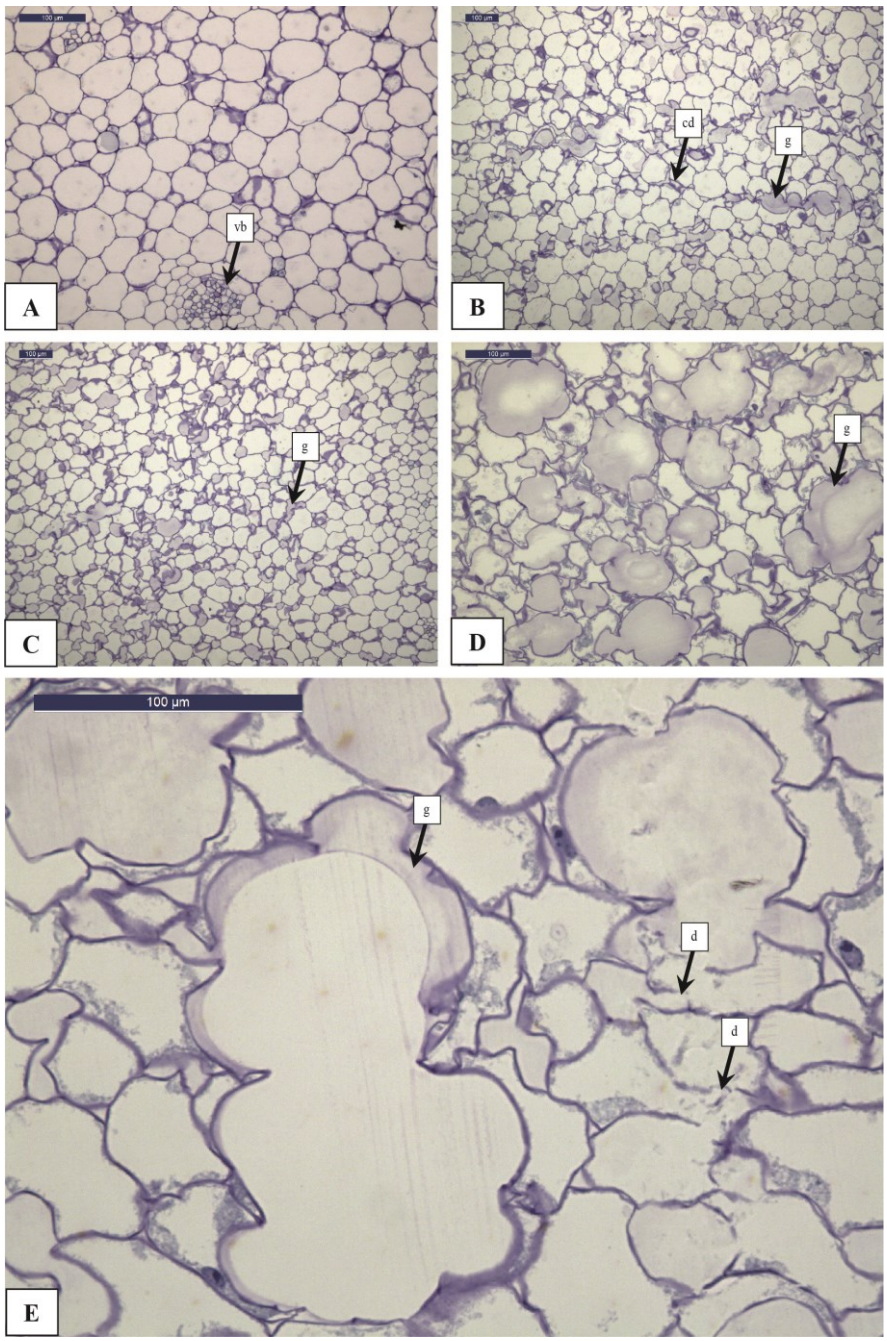
485

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

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

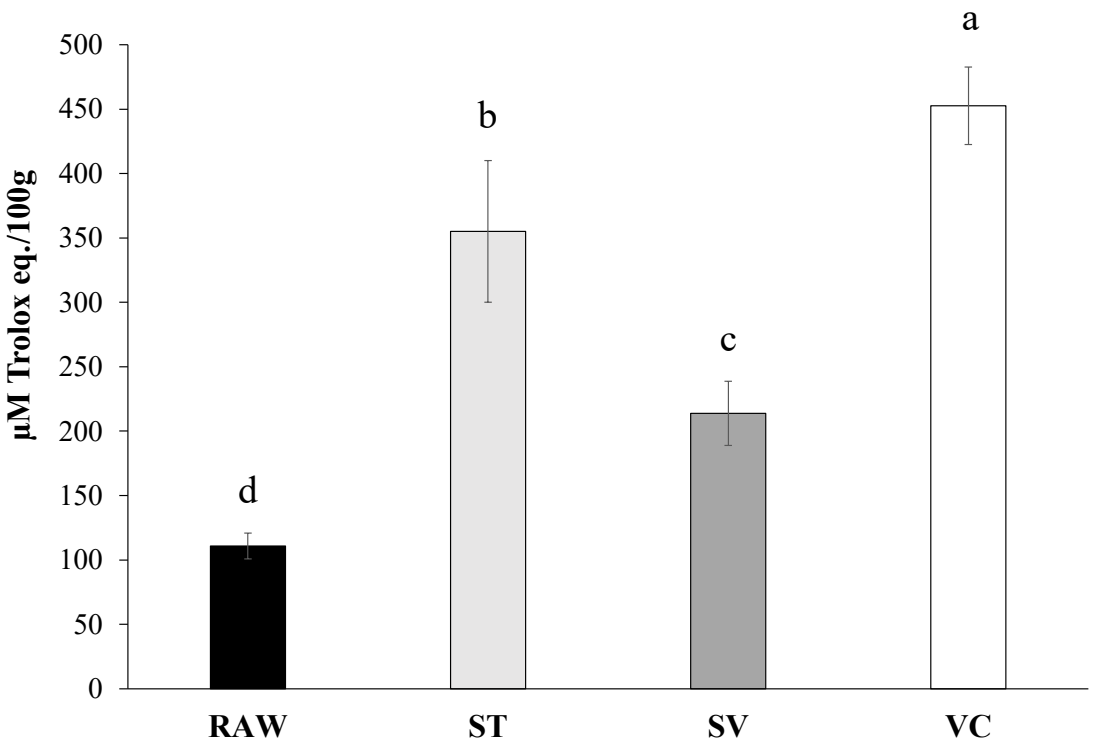
[a, b, c] Same letters within each column do not significantly differ ($n = 3$; $p < 0.05$); standard deviation is given in parenthesis.

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



Legend: vb=vascular bundles; cd=cell detachment; g = gaps; d=cell damage.

Figure 2. Total antioxidant capacity of pumpkin samples[a].



[a] n=3. Means followed by different letters significantly differ ($p < 0.05$).