Effect of different cooking methods on structure and quality of industrially frozen carrots

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Abstract The effect of boiling, steaming and microwaving on microstructure, texture and colour of raw and industrially frozen carrots was investigated. The raw carrots, after cooking, showed dehydrated and separated cells with swollen walls. The carrots subjected to blanching, freezing and followed by frozen storage exhibited marked tissue damages indicating deep oriented fissures. Cooking caused cellular dehydration and separation in the tissue, with the same intensity between raw and frozen carrots and independently from the cooking treatment applied. Among different cooking methods, microwaving showed better retention of the initial texture and colour quality for both raw and frozen carrots. On the other hand, the steamed carrots revealed the highest degree of softening and colour differences from the control for both raw and frozen carrots, despite the worst tissue conditions were observed for the boiled carrots.

Highlights Industrial freezing and domestic cooking were evaluated on the main carrot quality attributes
Freezing process greatly affected carrot tissues and quality more than cooking step
Boiling significantly impacted on carrot tissue causing marked cell separation
Microwaving led to a good texture and colour retention due to the cell compactness
Steaming highly altered colour and texture despite tissue damages were similar to microwaving

Keywords Carrots · Industrial freezing · Cooking · Colour · Microstructure · Texture

Introduction

Vegetable consumption is well documented and recognized to beneficially affect the human health (Martin 2013). Vegetables are frequently consumed cooked (either industrially or at household level) to extend the shelf-life, to preserve the quality and to maintain the preference habits. Freezing is one of the most applied methods to process vegetables. Before the consumption, the raw material is subjected to several different steps (blanching, freezing, frozen storage, and final cooking) that greatly impact the final quality of the products (Canet et al. 2004).

From a structural point of view, blanching causes an alteration of organelles in the cytoplasm, a swelling of the cell wall (Prèstamo et al. 1998; Xu et al. 2015) and a gradual breakdown of the protoplasmic structure organization. This breakdown causes a subsequent loss of turgor pressure and a softening effect also related to changes in the pectic polymers of the cell wall and the middle lamella (Xu et al. 2015). Freezing leads to major modifications as compared to blanching because it induces the formation of ice crystals that mechanically stress and damage the tissue (Van Buggenhout et al. 2006). Frozen vegetables exhibited the weakening of the cell wall with the depolymerisation of the pectic materials, and a loss of turgor with softening effects (Prèstamo et al. 1998; Paciulli et al. 2015). Moreover, the storage of frozen products induced a further tissue softening (Neri et al. 2014). Finally, during industrial thermal processing and/or home cooking the high-temperature exposure caused the cell separation, which is related to the solubilisation of pectic components, often accompanied by the swelling of cell walls (Waldron et al. 2003).

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Carrots are one of the most widespread consumed vegetables. In the past, the impact of blanching and/or freezing and/or frozen storage on carrot microstructure has been extensively debated in literature (Gómez and Sjöholm 2004; Greve et al. 1994a, b; Préstamo et al. 1998; Xu et al. 2015). On the other hand, the relation among tissue changes after freezing and the texture mechanical properties has been investigated in few studies (Kidmose and Martens 1999; Neri et al. 2014; Roy et al. 2001; Van Buggenhout et al. 2006). In these studies, carrots were generally treated at laboratory scale and not industrially processed. In addition, the effect of cooking, the final process step before the consumption, on the main quality attributes (i.e. texture, colour), which in turn have a great impact on the final consumer acceptance, was scarcely considered (Kidmose and Martens 1999; Neri et al. 2014; Roy et al. 2001; Van Buggenhout et al. 2006). Thus, this topic needs to be more deeply investigated with the ultimate goal to offer to the consumers a high quality final cooked product with a great retention of the vegetable original features.

In this framework, the aim of this work was therefore to investigate the effect of three common cooking procedures (boiling, steaming and microwaving) on anatomical structure, texture and colour of carrots frozen in an industrial plant and to compare the results with those obtained from raw carrots provided by the same manufacturer and cooked with the same procedures.

Materials and methods

Samples and processing

Ten kilograms of carrots (*Daucus carota* L., Napoli variety) were obtained by a local manufacturer, having been harvested in the production site under the same season and agronomical conditions. Five kilograms of this sample were immediately transported to the laboratories under adequate refrigerated conditions (3 ± 1 °C) and processed within 48 h from harvesting as follows: washed with the tap water and drained, sorted for size and length, peeled and cut into slices of 8 mm of thickness. A portion of them was immediately analysed (R), while the other part underwent cooking treatments as described below. The other 5 kg were washed, peeled, sliced and processed in an industrial plant within 24 h from harvesting. Then, they were blanched by immersion in a hot water bath (100 °C) for 2 min 30 s to reach the peroxidase inactivation (Gonçalves et al. 2007) and frozen in an ammonia forced air cooling tunnel at -40 °C for 6 min. Frozen samples were then maintained for two months at -18 °C in a thermostat to mimic the common storage conditions prior to the commercialization. At the end of storage, frozen carrots were transported to the laboratories under

adequate temperature conditions (-18 °C). A portion of them was immediately analysed (F), the remaining part was cooked within 24 h from the arrival.

Cooking treatments

Boiling, steaming and microwaving were chosen as cooking procedures commonly applied on raw and frozen carrots. Cooking times were optimized for each treatment according to the common Italian habit of palatability and tenderness for this vegetable. Cooking times were obtained by the judgment of a large group of semi-trained panellists. Frozen carrots were not defrosted before cooking.

Boiling was performed by adding carrot slices to boiling tap water in a covered stainless steel pot (1:5, food/water) and cooking on a moderate flame. Cooking times, measured when the vegetable was put into boiled water, were 20 min for raw and 15 min for frozen carrots. Steaming 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 m³, an air speed of 0.5 m/s and a steam injection rate of 0.03 kg/min. Oven was pre-heated at the set temperature before inserting samples for each cooking trial. Cooking times were 45 and 30 min for raw and frozen carrots, respectively. Microwave treatments were carried out in a domestic microwave oven (De Longhi MW651, Treviso, Italy). Frozen carrot slices were placed in a plastic (PP) microwave steamer (1:2, food/water), not in direct contact with water, on the rotating turntable plate of the oven and exposed to a frequency of 2450 Hz at low power (450 W). Cooking time was 10 min for both raw and frozen vegetables. All cooking procedures were performed in triplicate. Abbreviations: R (raw), R_B (raw boiled), R_S (raw steamed), R_{MW} (raw microwaved), F (frozen); F_B (frozen boiled), F_S (frozen steamed), F_{MW} (frozen microwaved).

Histological analysis

Raw, frozen and cooked carrots were analysed. The samples were fixed in a formalin:acetic acid:60 % ethanol solution (2:1:17 v/v; FAA solution) and after at least 2 weeks they were dehydrated with gradual alcohol concentrations according to Ruzin (1999). The inclusion was made in a methacrylate resin (Technovit 7100 Heraeus Kulzer & Co., Wehrheim, DE), and the resulting blocks were sectioned at 4 µm thickness (transversal cuts) with a semithin Leitz 1512 microtome (Leitz, Wetzlar, DE). The sections were stained with Toluidine Blue (TBO) solution (Ruzin 1999), Periodic Acid Schiff (PAS) reagent and Amido Black (Ruzin 1999).

Six pieces of each vegetable were sampled for each treatment and stain. Sections were observed with a Leica DM 4000B optical microscope (Leica Imaging Systems Ltd., Wetzlar, DE) equipped with a Leica DC 100 digital camera (Leica Imaging Systems Ltd., Wetzlar, DE). The tissues were measured using an image analysis system (QWIN 5001 Leica Imaging Systems Ltd., Wetzlar, DE). The image analyses were carried out using a manual configuration of the image analysis system.

Texture analysis

Raw, frozen and cooked carrot slices were analysed using a TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) by means of a cut test. The test was performed using a 3 mm thick stainless steel knife blade driven through the entire diameter of the vegetable, positioned on a slot surface, at a speed of 3 mm/s. The following parameters were obtained from the force vs distance curves using the application software provided (Texture Expert for Windows, version 1.22): the maximum cutting force values ($F_{\max}$, N), the area under the cutting curve (Area, Nmm), the slope of the linear ascendant portion of the curve (Slope, Nmm^{-1}) (Canet et al. 2004). Ten slices were analysed for each sample.

Colour analysis

Colour determination was carried out using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a standard illuminant D₆₅ on raw, frozen and cooked carrots. L^* (lightness; black = 0, white = 100), a^* (redness > 0, greenness < 0) and b^* (yellowness > 0, blue < 0) were quantified using a 10° position of the standard observer. The colour differences (corresponding to the differences in L^* , a^* and b^* values) of products cooked in different way respected to the colour of the related raw and frozen samples were evaluated using the ΔE calculation (CIE 1978). The assessments were carried out on two pre-selected positions of each slice picking approximately the same points for the outer and the inner parenchyma of the specimens. Fifteen slices for frozen and cooked samples were analysed for a total of 30 determinations for each trial.

Statistical analysis

SPSS statistical software (Version 22.0, SPSS Inc., Chicago, IL, USA) was used to perform one-way analysis of variance (ANOVA) among samples. The least significant difference (LSD) at a 95 % confidence level ($p \leq 0.05$) was performed to further identify mean differences among groups. A t test was performed to compare raw and frozen samples cooked in the same way.

Results and discussion

Histological analysis

Figure 1 illustrates the inner and outer parenchyma of raw, and microwaved, steamed and boiled carrots. The cells presented large vacuoles and a slight plasmolysis due to the anatomical sample preparations (Fig. 1a). The wall appeared thin and few intercellular spaces were visible. The parenchyma tissues were represented by an outer parenchyma with tangentially oriented, elongated cells and an inner parenchyma with radially oriented, elongated cells. The inner parenchyma showed larger cells in comparison with the outer parenchyma (Fig. 1b). The general structure of carrot was similar as described earlier (Xu et al. 2015).

After boiling, the cells of R_B appeared plasmolysed, especially in the outer parenchyma (Fig. 1c). Moreover, some cells showed the evidence of the onset of separation in the middle lamella regions; the cell separation was due to the breakage of chemical bonds among the pectic components of middle lamellae of adjacent cells and/or to the hydrolysis of some other components of the cell wall (i.e., pectin, hemicelluloses, cellulose). Similar effect of thermal treatment was confirmed by several authors for different vegetable structures (Sila et al. 2005; Paciulli et al. 2015; Xu et al. 2015). In our study, the cells separation after cooking might be ascribed to a decrease in the strength of cell-cell interactions in the middle lamella adjacent to the intercellular spaces. In R_B, some cells showed the evidence of swollen cell walls (Fig. 1c) and this phenomenon was even more evident in the central cylinder. Préstamo et al. (1998) explained the swelling phenomenon as a gelation process of the cell wall components, represented by a network of cellulose microfibrils, embedded in a highly cross-linked matrix of polysaccharides (mainly pectins and hemicellulose) and glycoproteins. The pectin, particularly abundant in the middle lamella, was found to be mainly implicated interacting with the gel network. In R_{MW} carrots, the phenomenon of plasmolysis was evident; some cells showed swollen walls, while few of them exhibited broken walls. In any cases, the cell walls became less compact and less-densely stained due to the swollen cell wall. This swelling was more evident in the inner parenchyma (Fig. 1d). Moreover, the cell lyses was marked in the outer parenchyma near the epidermal tissue (Fig. 1e). In R_S samples, a strong swelling was observed on cell walls of inner and outer parenchyma, but less evident if compared with R_{MW} carrots (Fig. 1f). Plasmolysis was also detected in the inner parenchyma, but the structure was not affected.

Frozen carrots (F), blanched before freezing and subsequently frozen stored, were subjected to both the effects of heat and water crystals growth, which produced damages not detectable in the tissues of the raw carrots (Fig. 2a, b).

Fig. 1 Details of transverse sections of carrot samples stained with TBO or PAS – Amido Black: **a.** outer parenchyma of raw/uncooked carrots; **b.** inner parenchyma of raw/uncooked samples; **c.** outer parenchyma of boiled samples; **d.** inner parenchyma of microwaved samples; **e.** outer parenchyma of microwaved samples; **f.** outer parenchyma of steamed samples. Abbreviations: *p* plasmolysis, *cb* cell broken, *cs* cell separation, *sw* swollen cell wall

The freezing process apparently caused the disruption of the carrot parenchyma tissues; in some cases, the cells were clearly disrupted and the parenchyma appeared fissured (Fig. 2a, b), consistently with earlier report (Préstamo et al. 1998). In the outer and inner parenchyma, the direction of the fissures after freezing was variable, according to the different cell orientations. The fissures of the outer parenchyma showed a tangential orientation (Fig. 2a), while the ones of the inner parenchyma were oriented in a radial way (Fig. 2b). In F_B samples, the structure damages were increased, particularly in the inner parenchyma, near the vascular tissue (Fig. 2c). Moreover, it was visible a tendency to the cells to separation (Fig. 2c), and this could be due to a partial pectin solubilisation accompanied by depolymerisation, and/or to a partial damage of the cell wall in the middle lamella (Van Buggenhout et al. 2009). The anatomical structure of F_{MW} carrots appeared less damaged than the F_B ones (Fig. 2d); the structure, in spite of

fissures due to the freezing treatment, appeared compact and with no extra intercellular spaces. The most important structural changes were found in the cell wall thickening after cooking (Fig. 2e). Thus, it appeared that microwave heating (before and after freezing) caused few tissue plasmolysis, and that the rapid removal of water seemed to increase the tissue mechanical strength possibly by increasing the crystallinity of cellulose and hemicellulose in the cell wall (Kidmose and Martens 1999). An et al. (2008) found partially gelatinized starch after microwave cooking in carrots; conversely, in our study, the starch did not appear to undergo any gelatinization process. In F_S carrots (Fig. 2f), the general structure was preserved and comparable to that of F_{MW} carrots. It can be assumed that in the F_S and F_{MW} cooking treatments, the water present in the tissues was not expelled to the outside, but probably, due to dehydration, it moved in the matrix phase of the cell wall. This phenomenon did not happen in F_B , as

Fig. 2 Details of transverse sections of frozen carrots stained with TBO or PAS – Amido Black: **a.** outer parenchyma of frozen carrots, the fissures (*f*) were oriented in a tangential way; **b.** inner parenchyma of frozen carrots, the fissures (*f*) were oriented in a radial way; **c.** inner parenchyma of frozen boiled carrots; **d.** outer parenchyma of frozen microwaved carrots; **e.** detail of cell wall thickness of frozen microwaved carrots; **f.** inner parenchyma of frozen steamed carrots.

Abbreviations: *cb* cell broken, *cs* cell separation, *f* fissure, *p* plasmolysis, *rf* radial fissures, *sw* swollen cell wall, *tf* tangential fissures

in the boiling conditions the cooking takes place in a liquid medium and therefore a balance between external environment (cooking water) and cell sap was created.

Textural characteristics

Raw and cooked carrots showed different cut force vs deformation curves (Fig. 3a). In particular, the former exhibited a cutting profile characterised by a fast increase of the force, before the surface penetration of the probe, followed by a sudden fall. This behaviour probably indicated an hard and crunchy structure, as suggested by Neri et al. (2014), and as confirmed by the histological observations (Fig. 1a, b) in which the cells appeared turgid and strictly connected among them. After cooking, the force increased slowly (Fig. 3a). The decrease of the slope of the rising portion of the curve and the broaden curve profiles of the cooked carrots were probably in

relation with the softening and the increased deformability of the structure, due to heat, that caused plasmolysis and cell separations (Fig. 1c–f), as previously observed in other vegetables (Canet et al. 2004; Paciulli et al. 2015). Among cooking practices, R_B and R_S samples exhibited very similar force-distance curves, with narrower shapes than R_{MW} that presented instead a broader curve with a more complex profile (Fig. 3a insert a).

The parameters extrapolated from the curves were shown in Table 1. R carrots resulted significantly firmer than the corresponded cooked carrots, as expected. Focusing on the cooking practices, significant differences of cutting F_{max} values were observed among all the cooked carrots: the microwaved carrots were the hardest, followed by the boiled and steamed ones. It's possible to hypothesize that the water cooking media, in form of liquid or gas, had an effect on the middle lamella molecular bound hydrolysis, as confirmed by

Fig. 3 Force (N) vs deformation (mm) curves obtained for cutting test **a**. Raw group; **a**. Raw cooked group; **b**. Frozen group; **b** Frozen cooked group. Abbreviations: *R* raw, *R_B* raw boiled, *R_S* raw steamed, *R_{MW}* raw microwaved, *F* frozen; *F_B* frozen boiled, *F_S* frozen steamed, *F_{MW}* frozen microwaved

the more extended cell separation observed in the boiled carrots (Fig. 1c). On the other hand, the steamed carrots apparently revealed less cell separation, probably because hidden by the extended swelling of the cell walls (Fig. 1f) and this could justify the highest softness also in relation with the long cooking time.

All the cooking treatments showed a significant reduction of the Area in comparison to *R* (Table 1). *R_{MW}* carrots lost only 39.8 % of the initial energy required to cut *R* samples, while *R_B* and *R_S* exhibited higher percentage values (91.1 and 92.5 %, respectively), in accordance with the curve profiles (Fig. 3a and insert a). These results were supported by the histological observations as *R_{MW}* showed large tissue portions where no cell separation was present (Fig. 1d). The compactness of the *R_{MW}* tissue probably opposed a higher resistance to the probe penetration, resulting in a broadening of the curve and higher Area values than in other cooked raw carrots. The slope of the linear ascendant portion of the curve was also calculated as it provided information about the deformability of the sample; it decreased when an increase of tissue extensibility was recorded (Kidmose and Martens 1999). A significant reduction of the slope values in comparison to *R* was observed for all carrots in relation to the tissue thermal damages. This parameter was however not able to discriminate among the cooking methods, as no significant differences were observed.

F curve (Fig. 3b) showed a more complex shape in comparison to *R*, probably because of a more marked tissue damages due to the process steps, as also shown from the histological observations (Fig. 2a–f). A displacement of the *F_{max}* towards higher distances and a broadening of the curves were observed for the frozen group in comparison to raw, in particular for the *F* samples. Among cooked samples, *F_B* and *F_S* showed more elongated force/distance curves than *R_B* and *R_S*, respectively (Fig. 3, inserts a, b). *F* sample showed *F_{max}* reduction of 33.7 % in comparison to *R*. In addition, all the frozen cooked samples exhibited a marked decrease of *F_{max}* in comparison to *F* sample (Table 1). The more extended damages observed in the parenchyma of the frozen carrots (Fig. 2a) compared to raw group allowed an easy penetration of the probe and the reduction of the *F_{max}*. *F_{MW}* samples resulted as the firmest among the frozen cooked vegetables, being also *F_S* samples the softest. A consistent reduction of the cut force was previously reported for industrially frozen vegetables after cooking (Paciulli et al. 2015). Similarly, Kidmose and Martens (1999) observed highest values of maximum force (measured by Ottawa Texture Measuring System) for

Table 1 Texture parameters calculated from force-distance curves of raw, frozen and cooked carrot samples

	<i>R</i>	<i>R_B</i>	<i>R_S</i>	<i>R_{MW}</i>	<i>F</i>	<i>F_B</i>	<i>F_S</i>	<i>F_{MW}</i>
<i>F_{max}</i> (N)	71.8 ± 9.4**	6.7 ± 1.5 ^b **	4.4 ± 0.8 ^c **	9.7 ± 2.3 ^a	47.6 ± 5.6	4.4 ± 0.6 ^b	2.8 ± 0.6 ^b	7.5 ± 1.9 ^a
Area (Nmm)	163.0 ± 24.7	14.5 ± 3.5 ^b	12.2 ± 1.4 ^b	64.8 ± 9.8 ^a **	255.5 ± 39.8**	22.8 ± 2.0 ^b **	15.0 ± 1.9 ^c *	45.3 ± 8.2 ^a
Slope (Nmm ⁻¹)	31.5 ± 9.7**	2.9 ± 0.6 ^a **	2.1 ± 0.2 ^a **	2.7 ± 0.8 ^a *	16.9 ± 2.6	1.2 ± 0.2 ^b	1.0 ± 0.3 ^b	1.6 ± 0.5 ^a

R raw, *R_B* raw boiled, *R_S* raw steamed, *R_{MW}* raw microwaved, *F* frozen, *F_B* frozen boiled, *F_S* frozen steamed, *F_{MW}* frozen microwaved

n = 10, sample size = 30. Means in row followed by different letters differed significantly (*p* < 0.05). Statistical significance of raw carrots was not considered. Means in column followed by single (*p* < 0.05) or double (*p* < 0.01) asterisks differed significantly among raw and frozen samples cooked with the same treatment

frozen carrots slices stored for 1 month and pre-treated by microwave blanching. These authors hypothesized, on the basis of microscopical observations, that microwave heating caused a tissue dehydration, and that the rapid removal of water during cooking probably increased the mechanical strength of the tissue increasing the crystallinity of the cellulose and the hemicelluloses in the cell wall. Our tissue observations revealed the presence of swollen cell walls with amorphous material in F_{MW} carrots (Fig. 2d). It may be hypothesized that the water present in the tissues was not expelled to the outside, but rather, due to dehydration, moved in the matrix phase of the cell wall. F_S carrots resulted the softest among all the samples, even if their tissue appeared more similar to the one of the F_{MW} carrots, because of the high connections between cells (Fig. 2f). It's possible to assume that the presence of large swollen cell walls in the steamed carrots resulted in an apparent connection among the cells. The changes of the middle lamella after the treatments actually separated the cells allowing an easy penetration of the probe. The area values (Nmm) followed the same trend of the F_{max} (Table 1). F_{MW} required the highest energy to be cut, followed by F_B and F_S . F_B and F_S samples also showed significantly lower slope value than F_{MW} (Table 1), in accordance with Kidmose and Martens (1999). Thus, the latter sample appeared to be less deformable than the other two samples showing a softer and rubbery texture (Xu et al. 2015).

Comparing F_{max} of frozen and raw carrots, similar softening percentages after cooking were obtained in comparison to the corresponding controls. Results revealed that the cooking effect was independent from the original status of the vegetable. On the other hand, significant higher area values and lower F_{max} were observed for F , F_B and F_S carrots in comparison to the correspondent R and cooked samples. It's possible to hypothesize that the tissues assumed sticky traits and a higher attachment to the probe because of the greater damages of the frozen tissue in comparison to the raw one. Moreover, the consequent leakage of intracellular fluids in frozen carrots resulting in a greater friction during the probe penetration and a consequent extra energy amount to cut the samples. Conversely, F_{MW} carrots showed a significant lower area value with respect to R_{MW} . The extended plasmolysis observed in the microwaved samples, together with the fissures provoked by freezing (Fig. 2e), probably caused a significant drying effect, which in turn generated a less sticky sample and consequently a smaller area. Finally, the slope of the F group samples resulted significantly lower than R and cooked samples (Table 1), probably in relation with the extended tissue flexibility caused by the frozen fissuring.

Colour characteristics

The colour parameters of R and cooked carrots were reported in Table 2. Cooking significantly decreased all colour

Table 2 Colour parameters obtained on parenchyma tissue of raw and cooked carrot samples

Parenchyma	R	R_B	R_S	R_{MW}
Inner				
L^*	50.4 ± 2.4^b	42.4 ± 1.4^d	46.8 ± 1.7^c	53.4 ± 3.1^a
a^*	24.7 ± 3.8^a	14.6 ± 1.5^b	14.2 ± 1.9^b	15.2 ± 2.7^b
b^*	29.5 ± 3.7^a	21.6 ± 2.7^b	22.4 ± 3.1^b	21.0 ± 3.1^b
ΔE	–	15.3 ± 2.5^a	13.7 ± 2.6^a	14.0 ± 3.9^a
Outer				
L^*	55.9 ± 3.3^a	47.0 ± 1.0^c	49.1 ± 1.8^c	52.1 ± 1.9^b
a^*	35.7 ± 1.6^a	26.6 ± 1.6^b	20.6 ± 2.9^c	25.2 ± 2.3^b
b^*	46.2 ± 3.0^a	39.8 ± 2.8^b	32.8 ± 3.1^c	31.0 ± 3.1^c
ΔE	–	14.6 ± 2.5^b	21.9 ± 4.2^a	19.0 ± 3.6^a

R raw, R_B raw boiled, R_S raw steamed, R_{MW} raw microwaved

$n = 3$, sample size = 15. Means in row followed by different letters differed significantly ($p < 0.05$)

parameters with the exception of L^* in the inner parenchyma of R_{MW} . The decrease of L^* values indicated darkening, while the reduction of both a^* and b^* indicated a general colour loss mainly related to a decrease in α - and β -carotene and their isomerisation, as already reported for cooked carrots (Miglio et al. 2008) and carrot juice (Chen et al. 1995).

Different cooking processing did not affect the colour parameters in the same way. In the inner parenchyma, L^* resulted the only discriminating parameter: R_B exhibited the lowest L^* , while, as mentioned above, the lightness of R_{MW} was found to increase. In the outer parenchyma, where the presence of β -carotene was known to be higher than in the inner part and the distribution of α -carotene and lutein is similar to the inner one (Baranska et al. 2006), R_S resulted the worst affected in all the colour parameters, while R_B showed the lowest L^* values and R_{MW} the lowest b^* . Miglio et al. (2008) reported a slight increase of lutein and no variation in β -carotene during boiling, while all the analysed carotenoids suffer a decrement of their initial concentration during steaming. Czarniecka et al. (1996) found a carotenoid increase for “dry” cooking methods, such as microwaving, because of the increase of the dry matter due to the evaporation, and an opposite trend was found for the “wet” cooking methods. The overall colour changes induced by different cooking methods were evaluated with the ΔE parameter that, based on the numeric value, may indicate if the different colour is perceivable by the human eye; higher the value, higher the differences between sample and the reference sample (Limbo and Piergiovanni 2006). All carrots (both for inner and outer parenchyma) cooked with the different procedures were characterized by ΔE values indicating, according to Limbo and Piergiovanni (2006) scale, that the colour of the cooked samples was perceived as different by the human eye if compared with the raw sample. In particular, while in the

inner parenchyma the boiled sample appeared more changed, in the external portion, the colour changed more markedly in steamed carrots. In general, the colour of the outer parenchyma resulted more affected than that of inner one. It is well known that cooking can increase the extractability and therefore the bioaccessibility of β -carotene from the food matrix by softening or disruption of plant cell walls and the destruction of carotenoid–protein-complexes (Bernhardt and Schlich 2006; Miglio et al. 2008). This assumption, in agreement with our histological observations in which the outer parenchyma of all the treated carrots resulted more damaged than the inner one, can justify the more extended colour changes of the external portion of the samples after cooking, probably because of an extended pigments extraction and a further isomerisation or degradation. Similarly, Lemmens et al. (2009) found also an inverse correlation between the carrots hardness, measured by compression test, and the amount of β -carotene after thermal treatments. In agreement with this observation, the extended softening of the steamed samples could be related to an extended carotenoid extraction and a further degradation that allowed a higher ΔE values.

The effect of different cooking methods on the colour characteristics of frozen carrots was reported in Table 3. The freezing and storage significantly ($p < 0.05$) affected a^* of the inner parenchyma and both a^* and b^* values of the outer one. It could be associated to a first carotenoids loss during the blanching phase and/or their isomerisation (Gómez and Sjöholm 2004). The calculated ΔE of the F carrots in comparison to R resulted to be 7.5 and 7.8 for the inner and outer parenchyma, respectively, showing strong perceived colour differences (Limbo and Piergiovanni 2006). The cooking methods applied to frozen carrots affected the colour parameters in a very different way. Colour of the inner parenchyma

was not significantly affected when boiling treatment was used to process samples. a^* and b^* values of F_S carrots resulted instead decreased if compared with F. Otherwise, F_{MW} carrots showed only higher L^* than F. The outer parenchyma of the frozen samples was more affected by the heat treatments than the inner portion, as found for raw samples. Cooking induced a decrease of all colour parameters, with steaming being the most detrimental process. Mazzeo et al. (2011) found greater colour changes for frozen steamed carrots than boiled in relation with a higher carotenoid loss, probably because of the long cooking time, which led to a prolonged exposure to oxygen and light, rather than a temperature effect (Miglio et al. 2008). ΔE values of the inner parenchyma indicated that the colour of frozen boiled and microwave cooked carrots was significantly ($p < 0.05$) different from the steamed sample (Table 3). The same differences were found for the outer parenchyma, too. Thus, steaming was the cooking method that more drastically affected the colour of frozen carrots. Recently, Camorani et al. (2015) found that frozen carrots cooked under the same conditions used in this study exhibited carotenoids isomerisation with a significant increase in *cis*- β -carotene form. High correlations were found among all colour parameters and the ratio *cis*/all-*trans*- β -carotene, confirming the influence of the carotenoids isomerisation, as induced by heat treatment, on the final colour of the samples. Moreover, a pronounced carotenoids loss was observed, by the same authors, in steamed carrots in comparison to the other cooked samples. On the other hand, it was found that boiling and, to a lesser extent microwaving, caused an increase in carotenoid concentration, as better extracted from the damaged cells.

Comparing raw and frozen groups, it was clearly distinguishable that the freezing and storage process affected the colour more than cooking of frozen samples, as also shown by the histological observations. It appeared that, both in the inner and outer parenchyma, L^* value remained constant during the frozen storage period indicating that the blanching step avoided the darkening of the samples due to enzymatic browning, as suggested by Rawson et al. (2012). On the other hand, a^* and b^* values decreased significantly in the outer parenchyma ($p < 0.01$), suggesting detrimental effects on the carotenoid profile. Looking at the ΔE values of both the raw and frozen groups (Tables 2 and 3), lower colour changes were observed for the frozen cooked samples in comparison to those cooked from raw, especially for the outer parenchyma where β -carotene was more highly concentrated. In addition, ΔE values of frozen cooked carrots calculated in comparison to R (data not shown) for the outer parenchyma were lower than those obtained for the raw cooked carrots. The stabilizing effect of blanching prior to freezing and storage on β -carotene could be proposed as a possible explanation of this phenomenon, due to the decrease in chemical and enzyme-catalysed oxidation reactions, as already suggested (Mazzeo et al. 2011; Tansey et al. 2010).

Table 3 Colour parameters obtained on parenchyma tissue of frozen and cooked carrot samples

Parenchyma	F	F_B	F_S	F_{MW}
Inner				
L^*	49.8 ± 1.3^b	49.6 ± 1.6^b	49.8 ± 1.1^b	52.7 ± 2.8^a
a^*	19.2 ± 4.1^a	19.4 ± 2.8^a	14.5 ± 2.1^b	20.8 ± 4.3^a
b^*	28.7 ± 3.2^a	25.9 ± 4.0^a	18.1 ± 2.6^b	25.1 ± 4.0^a
ΔE	–	5.4 ± 1.6^b	11.6 ± 2.9^a	8.1 ± 2.5^b
Outer				
L^*	53.5 ± 0.7^a	51.9 ± 0.7^b	49.7 ± 1.5^c	52.3 ± 1.8^{ab}
a^*	30.7 ± 1.3^a	27.1 ± 1.5^b	23.4 ± 2.6^c	27.8 ± 2.1^b
b^*	40.9 ± 1.5^a	36.3 ± 2.6^b	31.3 ± 3.6^c	35.4 ± 2.3^b
ΔE	–	6.5 ± 1.9^b	12.8 ± 4.2^a	6.8 ± 2.5^b

F frozen, F_B frozen boiled, F_S frozen steamed, F_{MW} frozen microwaved, R raw

$n = 3$, sample size = 15. Means in row followed by different letters differed significantly ($p < 0.05$)

Conclusions

In conclusion, freezing (and the following storage commonly applied in an industrial plant) appears as more detrimental than cooking on carrot structure, as large oriented fissures in the parenchyma were observed. Cooking caused the cellular dehydration and the separation in the tissue, with the same intensity in raw and frozen groups and independently from the cooking treatment applied.

Among the cooking practices, steaming highly affected the texture and the colour of carrots under the conditions applied in this study, despite the worst structure conditions were found in the boiled samples. This aspect needs to be deeply investigated. On the other hand, microwaved carrot revealed a good compactness of the cells with a good retention of texture and colour and no significant differences on cooked raw and/or frozen samples.

Finally, the colour of frozen processed samples was better retained after cooking than that of raw cooked carrots, in view of the stabilizing effect of the blanching step. This was true especially for the outer parenchyma where β -carotene was notoriously found at high level, being this result of future interest for a nutritional evaluation of these products.

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