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ORIGINAL RESEARCH

Combined Use of Blanching and Vacuum Impregnation with Trehalose and Green Tea Extract as Pre-treatment to Improve the Quality and Stability of Frozen Carrots

Veronica Santarelli[1] · Lilia Neri[1] · Roberto Moscetti[2] · Carla Daniela Di Mattia[1] · Giampiero Sacchetti[1] · Riccardo Massantini[2] · Paola Pittia[1]

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Abstract

The use of green tea extract (GTE) and trehalose for the production of frozen carrots fortified with bioactive compounds was investigated. Carrot enrichment was performed by blanching and vacuum impregnation (VI). Functional properties and quality attributes of carrots were evaluated after pre-treatments, freezing and frozen storage (FS). Blanching effectively inactivated peroxidase and pectinmethylesterase enzymes. Blanching in trehalose and VI in trehalose and/or GTE solutions limited the soluble solid and firmness losses through pre-treatments, while effect on colour was similar to other pre-treatments ($\Delta h° \approx +6$; $\Delta L^* \approx -6$). Pre-treatments did not affect the carotenoid content while increased the total polyphenol content (TPC) and antioxidant activity (AOA), which in samples enriched with GTE resulted respectively three and two times higher than the fresh vegetable. After FS, carrots showed a drastic firmness loss, and colour change. Trehalose showed a cryoprotective effect on carrots' firmness and colour only when its penetration in plant cells was promoted by blanching. All samples highlighted a significant carotenoid loss (−40%), whilst a TPC decrease was observed only on GTE polyphenol fortified carrots. Despite this loss, AOA of carrots enriched with GTE after 60 days of frozen storage was two times higher than AOA of fresh carrots.

Keywords Carrots · Green tea · Freezing · Vacuum impregnation · Bioactives · Quality

Introduction

Green tea (GT), from *Camelia sinensis* L., is a worldwide consumed beverage recognised as valuable source of polyphenols and, in particular, of catechins such as epigallocatechin gallate (ECGC), epigallocatechin, epicatechin gallate and epicatechin. Besides their antioxidant properties, these polyphenols show pharmacological activities, such as lowering effects on glucose, lipid and uric acid, and their consumption has been correlated with low incidence of chronic pathologies in which oxidative stress is deeply involved (e.g. cardiovascular and neurodegenerative diseases, cancer) (Del Rio et al., 2013; Peluso & Serafini, 2017).

The amount of GT that needs to be consumed daily to obtain health benefits is rather large and difficult to reach (Vuong et al., 2011). In this context, the development of food products enriched with GT could help consumers to reach the amount of GT catechins capable to exert beneficial effects on human health.

Fortification with GTE for functional purposes has been studied in bakery products, such as bread (Bajerska et al., 2010), biscuits (Sharma et al., 2011), noodles (Li et al., 2012), probiotic yogurt (Muniandy et al., 2016) and frozen surimi gel (Pérez-Mateos et al., 2006), as well as in dried (Lavelli et al., 2010) and minimally processed apples (Tappi et al., 2017).

However, the addition of GTE to food products can affect their physio-chemical and sensory characteristics such as colour, flavour and taste (Ananingsih et al., 2013). Polyphenols, in fact, contribute to define the sensory characteristics of

✉ Lilia Neri
lneri@unite.it

✉ Paola Pittia
ppittia@unite.it

1 Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Via Renato Balzarini 1, 64100 Teramo, Italy

2 Department for Innovation in Biological, Agro-food and Forest System, University of Tuscia, Via San Camillo de Lellis snc, 01100 Viterbo, Italy

foods, affect the rate of enzymatic browning, influence the emulsification processes and the physical and chemical stability of the dispersed systems, and interact with proteins (Bora et al., 2018; Giacintucci et al., 2016; Scroccarello et al., 2019). Moreover, concerning GT catechins, and EGCG in particular, there is evidence from interventional clinical trials that doses equal or above 800 mg EGCG/day taken as a food supplement have potential hepatotoxicity effects (Younes et al., 2018). Thus, the enrichment of a food product with GT must be optimised basing on the abovementioned nutritional recommendations and to minimise the side effects exerted by these compounds on the quality attributes of the food product.

Vacuum impregnation (VI) is recognised as a powerful technology to directly introduce in a controlled way dissolved or suspended substances into products with porous structure, thus allowing a fast modification of nutritional, functional and/or structural properties as well as improving the processability of fruits and vegetables (Gómez Galindo & Yusof, 2014). A mechanistic description of the VI treatment in solid porous food systems, in which hydrodynamic mechanism (HDM) and deformation-relaxation phenomena (DRP) models are coupled, was given by Fito et al. (1996).

During vacuum impregnation (VI), the plant porous fraction composed by intercellular spaces is filled by an external solution of defined composition and/or concentration to a degree that depends on various factors, including (i) the process conditions (i.e. vacuum level, vacuum time, equilibration time at atmospheric pressure and temperature), (ii) the osmotic pressure and viscosity of the impregnation fluid, (iii) the size and shape of the samples and its effective porosity (pore size and distribution); this last parameter affects the capillary pressure of fluids within the vegetable tissue and its response to mechanical stress (Neri et al., 2016). Recent investigations (Demir et al., 2018; Dymek et al., 2016) have also shown that VI of plant tissues is not only about mass transfer but dependently on type of solutes in the impregnation liquid and the optimization of the oxygen amount left in the residual tissue porosity; it can stimulate the cell metabolic activity within short time scales after the treatment (Tylewicz et al., 2013; Yusof et al., 2017) and promote the metabolization of the solutes and their transfer within the cells.

By VI, various compounds of various nature and functionality (e.g. firming, antimicrobial and antioxidant agents, flavours, cryoprotectants, vitamins, minerals, probiotics, organic acids, etc.) can be introduced inside a plant food matrix, as a single component, or in mix (Comandini et al., 2010; Demir et al., 2018; Derossi et al., 2013; Neri et al., 2014) . Depending on the type of component chosen, the final product after VI can be characterised by modified/improved sensorial quality, extended shelf-life and/or varied composition so as to increase the daily intake of nutrients and meet nutritional recommendations or health benefits. With this last purpose, recent studies (Batista de Medeiros et al., 2019; Betoret et al., 2012; Castagnini et al., 2015; Mejía-Águila et al., 2021) exploited the use of VI for fruit fortification with fruit juices or plant extract with a high content in bioactive compounds such as green tea (Tappi et al., 2017). However, beside its application for minimally processed fruit production, VI can be exploited in several industrial processes as an innovative pre-treatment inserted into a more complex processing with several applications (Betoret et al., 2015; Derossi et al., 2012; Zhao & Xie, 2004), e.g. in combination with other unit operations such as pulsed electric fields (Phoon et al., 2008) or blanching (Leunda et al., 2000; Del Valle et al., 1998; Bellary & Rastogi, 2014; Moreno et al., 2000) for the production of products with extended shelf life, e.g. dried or frozen products.

Freezing is one of the most important preservation technologies available for fruits and vegetables. Despite its stabilising effects, however, it may cause chemical and physical changes in the plant tissues that may compromise the quality characteristics of the products such as colour and texture (Bonat Celli et al., 2015; Neri et al., 2014), and decrease their acceptability.

Quality and stability of frozen vegetables is enhanced when freezing is coupled with other processing operations such as blanching. Applied as pre-treatment, blanching inactivates endogenous enzymes responsible for producing off-flavours and changing colour, texture and nutritional attributes. However, despite this positive effect, this heat treatment can negatively affect the overall quality of the processed vegetables and acceptance by consumers, as well as their content in bioactive compounds (Xin et al., 2015). The use of sugar solutions as heat carriers in blanching treatments of vegetables is an interesting tool since it allows the preservation of tissue microstructure by preserving the cell wall and membrane integrity. Mono- and disaccharides have been shown, in fact, to stabilise membranes against stress conditions by preserving their fluid state and also to protect proteins by preventing denaturation phenomena (Neri, Hernando, et al., 2011). Moreover, with trehalose (α-D-glucopyranosyl α-D-glucopyranoside) being among the most effective, at low temperatures these sugars act as anti-freezing molecules. This is due to their effect on lowering the freezing point and chemical potential of water, and destructuring the tetrahedral hydrogen bond network of water. Thanks to these properties, their addition to plant foods was shown to preserve cell structures upon freezing (Phoon et al., 2008) and frozen storage (Neri et al., 2014).

The aim of this study was to investigate the use of blanching in combination with vacuum impregnation to produce frozen carrots fortified with green tea extract. Trehalose was added in both VI and blanching solutions as stress protectant for carrot slices in order to limit quality loss induced by both processing and frozen storage. The mass transfer phenomena, the quality attributes of the final products, and their functional properties were evaluated after blanching and VI pre-treatments, as well as after freezing and frozen storage.

Materials and Methods

Materials

Organic carrots (*Daucus carota* L., cv Romance) grown in the Abruzzo region (Italy), and harvested in June 2018 were purchased in a local farm. A single batch (40 kg) was supplied and used for all the experiments. Upon arrival, carrots were selected for size (i.e. length: 18–20 cm and diameter: 1.5–2 cm), stored in a chilled chamber at 3 °C at 90% RH and processed within 10 days. Before processing, carrots were washed, peeled and cut in 0.5-cm thick slices. Raw material had the following characteristics: moisture = 90.8 ± 0.1%; soluble solids = 9.6 ± 0.6 Brix; pH = 5.82 ± 0.03; porosity = 0.031% (methods of analyses are depicted in the following sections).

Trehalose was purchased from Adea s.r.l. (Italy). All other chemicals and standards were purchased from Sigma (Steinheim, DE).

Green tea extract (GTE) in powder (*Camelia sinensis* L., PromoPharma, Republic of San Marino) with a standard content of polyphenols (95%, of which 65% were catechins, of which the 40% was epigallocatechin gallate) was purchased in a local drug store. Before use, GTE was diluted 1:200 in water, agitated for 30 min to favour dissolving, and filtered under vacuum with paper. Immediately after, this GTE stock solution was used for the preparation of the vacuum impregnation agents.

Sample Preparations

Carrot slices (200 g) were blanched for 108 s in water (BL_w) or trehalose 4% w/v (BL_{4T}) solution pre-heated at 90 °C by using a pot and a magnetic hotplate stirrer with temperature control (AREX, VELP Scientifica, Italy). The product:blanching medium ratio was equal to 1:15 w/w. After blanching, samples were quickly drained, and the surplus liquid was removed from surfaces by dabbing with absorbent paper. After packaging in single layer into high barrier polyethylene bags, the carrot slices were immediately cooled in ice bath for 3 min to minimise over-processing. In order to collect about 2 kg of both BL_W and BL_{4T} sliced carrots; each treatment was repeated 10 times.

Vacuum impregnation was carried out on BL_w or BL_{4T} samples by using as impregnant agents water (VI_w), trehalose 10% w/v (VI_{10T}), green tea extract 0.25% w/v (VI_E), or trehalose 10% w/v in combination with green tea extract 0.25% w/v ($VI_{10T\text{-}E}$). The solutions VI_E and $VI_{10T\text{-}E}$ were prepared starting from the GT stock solution ("Materials").

The blanching parameters (time, temperature, product:blanching agent ratio), as well as the trehalose and GTE concentrations were set up basing on preliminary tests (*data not shown*) and results of previous investigations (Neri et al., 2014; Neri, Di Mattia, et al., 2011; Neri, Hernando, et al., 2011).

The vacuum impregnation parameters (vacuum and post-vacuum times, sub-pressure level, temperature) and solutes concentrations were selected after preliminary tests (*data not shown*) aimed to maximise the impregnation efficiency, to preserve the sensory properties of the product and to comply with nutritional recommendation indicated by Younes et al., 2018 as regards the ECGC maximum daily intake.

The VI treatments were performed by applying a pressure of 50 mbar for 10 min and a post-vacuum time of 10 min by using vacuum laboratory equipment composed of (i) a glass cylindrical chamber (10 L volume) including a stainless steel perforated holder connected to a vacuum pump (SC 920, KNF ITALIA, Milan, Italy) and (ii) an external water flow chamber for the process temperature control connected to a thermostated water bath set at 20 ± 1 °C. Carrot slices (200 g) were added to the VI solution in a 1:15 (w/w) product:impregnant agent ratio. Thereafter, samples were drained, and excess liquid was removed by lightly dabbing carrot surfaces with absorbent paper. In order to produce about 1.2 kg of each sample, the VI treatment was repeated 6 times.

Fresh (non pre-treated, F) and pre-treated sliced carrots, were portioned, packed in air immediately after their preparation as a single layer in bi-oriented polypropylene (BOPP) bags and frozen at −40 °C (±0.5 °C) using a direct-freeze deep freezer (NUVE, Ankara, Turkey). During freezing, the carrot temperature was recorded at the product core by a thermocouple connected to a digital thermometer (DT68A Cocoda, Italy). The average freezing rate was of 1.47 ± 0.03 °C/min. After 24 h, the freezer temperature was increased up to –18 °C. During freezing and storage the freezer temperature was monitored and recorded through the system's temperature controller and the temperature fluctuations were at maximum of ±0.5 °C.

Moisture and Mass Transfer Parameters

Moisture content was determined according to the AOAC gravimetric method (Ref 925.10, AOAC, 1990) by drying ~5 g of vegetable tissues at 70 °C until constant weight.

To evaluate the total mass variation of samples subjected to blanching and VI, the weight of the vegetable was measured before and after pre-treatments (blanching or blanching and vacuum impregnation). Changes in total mass ($\Delta M^0{}_t$) at time *t* (g/g), water mass ($\Delta M^w{}_t$) at time *t* (g/g) and soluble solids ($\Delta M^{ss}{}_t$) at time *t* (g/g) of each sample were calculated as described by Neri et al. (2019).

Soluble Sold and pH

Soluble solids content (°Brix) and pH were measured with a refractometer (Optech, Optical Technology, Vigo, Spain), previously calibrated with distilled water (°Brix = 0), and a pH-meter MP220 (Mettler Toledo International, Polaris Parkway, USA), respectively. All the analyses were performed in duplicate on the aqueous phase obtained from the carrot samples after homogenisation with Ultra-Turrax for 1 min at 13,000 rpm and centrifugation at 3606×*g* for 10 min.

Density and Porosity

Carrot apparent density (ρ_v) and real solid-liquid density (ρ_{SL}) were determined by using a pycnometer. The apparent density (ρ_v) was measured on a reference liquid consisting of an isotonic solution whilst the real solid-liquid density (ρ_{SL}) on sample purée after its degasifying at vacuum. These parameters were both expressed as kg m^{-3}. Due to the difficulty in air removal of some samples and corresponding inconsistent ρ_{SL} values, the real solid-liquid density was estimated from water mass fraction X_w (Gras et al., 2002) using the following equation:

$$\rho_{SL} = 1590(1 + 0.590 \times Xw)-1 \quad (1)$$

Sample porosity (ε) was calculated from density values as:

$$\varepsilon = 1-(\rho_v/\rho_{SL}) \quad (2)$$

Sugar Identification and Quantification

Content of mono- and disaccharides has been determined by HPLC analysis using an ICS 3000 Ionic Chromatograph (Dionex, San Donato Milanese, Italy) equipped with ICS 3000 SP pump and ICS 3000 ED detector. Sugar extraction was carried out in duplicate and according to the method used by Neri, Hernando, et al. (2011). The chromatographic analysis was carried out using a carbohydrates separation column (CarboPac PA20, 3 mm × 150 mm, Dionex) with a pre-guard column (CarboPac PA20, 3 mm × 30 mm, Dionex). NaOH 150 mM was used as mobile phase. Sugar detection was carried out using the time/potential waveform A as indicated by Dionex (Technical note 21).

Operating conditions were the following: flow rate 0.5 mL min^{-1}, a 35-min run with a column temperature of 30 °C and a volume injection of 10 μL. For sugar identification and quantification, standard solutions containing from 0.1 to 100 g mL^{-1} of trehalose, sucrose, fructose and glucose were used. All the analyses were carried out in duplicate.

Enzyme Extraction from Carrot Tissue

Peroxidase (POD) extraction was carried out by homogenising for 2 min (ultraturrax Yellow line Di 25 basic, IKA Germany), 25 g of sample with 50 mL of 0.1 M phosphate buffer (pH 6,5); the solution was then filtered through filter paper and centrifuged for 10 min at 4 °C and 3606×*g*. The supernatant was used as POD extract.

Pectinmethylesterase (PME) extraction was carried out by homogenising 50 g of carrots with 100 mL of NaCl 8.8% (w/v) for 2 min. The homogenised solution was stirred for 15 min, filtered through filter paper and centrifuged for 30 min at 4 °C and 2346×g. The pH of each enzymatic extract was adjusted to pH 7.5 by adding a few drops of 0.1 N NaOH.

POD and PME Assay

POD and PME activity were tested according to the method used by Neri, Hernando, et al. (2011). One unit (U) of POD was defined as the quantity of enzyme necessary to obtain an increase in absorbance of 0.001 in 1 min while one unit (U) of PME was defined as the quantity of enzyme necessary to obtain a decrease in absorbance of 0.01 in 1 min, under the assay conditions.

POD and PME residual activity after treatments was determined as A/A_0, where A is the enzymatic activity of the blanched samples and A_0 is the enzymatic activity of the fresh carrots.

Colour Analysis

Colour analysis was performed using a spectrophotocolorimeter Konica Minolta Chroma Meter CR-5 (Konica Minolta, Osaka, Japan) equipped with a D65 illuminant. The measures were carried out on the carrot phloem using a 10° standard observer and a target mask with a measure area of 3 mm (Moscetti et al., 2017). Colour was assessed in CIELab coordinates and the hue angle (h°) was calculated as h° = $\tan^{-1}$ (b^* a^{*-1}). For each sample, 10 slices were analysed.

Mechanical Properties

To determine the mechanical properties of the samples, a compression test was performed using an Instron dynamometer (mod 5542, Instron Universal Testing Machine, Wycombe, UK) equipped with a 3.5 cm diameter descending plunger. A speed of 50 mm min^{-1} has been set to produce a deformation on the sample equal to 15% of its height. For each sample, analysis was carried out on 20 different carrot slices with a diameter between 2 ± 0.5 cm. Maximum force in compression was used as firmness index.

Determination of Total Carotenoids

Total carotenoids were determined using the modified method described by Koca et al. (2007). Dehydrated carrot powder (0.125 g) was extracted in duplicate in a 25 mL 7:3 hexane:acetone mixture using a shaker. The extract was filtered, and the residue was re-extracted until it became colourless. The filtrates were combined in a separatory funnel and washed with a 50-mL distilled water. The water phase was discarded and Na_2SO_4 (10 g) was added as desiccant. The hexane phase was transferred to a 50-mL volumetric flask and brought to volume with hexane. The total carotenoid concentration in the solution was determined by its absorbance at 450 nm with a UV/Vis spectrophotometer and determined as β-carotene equivalent (β-CE) from the standard curve prepared in the β-CE range from 0.5 to 30 mg/L. Results were reported as mg of β-CE g^{-1} d.w.

Polyphenol Extraction

Polyphenol extraction was carried out as described by Ma et al. (2013) with slight modifications. Freeze-dried carrots (2.5 g) were homogenised with 10 mL of 70% v/v methanol for 10 min using a homogeniser (Yellowline DI25basic, IKA, France); the solution was stirred for 20 min and then centrifuged for 20 min at 6000 rpm at 15 °C. The supernatant obtained was filtered with a 0.45-μm nylon filter and stored at −40 °C until analysis. For each sample, the polyphenol extraction was performed in duplicate.

Determination of Total Phenolic Content and Antioxidant Activity

Total polyphenol content (TPC) and the antioxidant activity were determined on the polyphenolic extracts by using the Folin-Ciocalteau reagent according to Singleton and Rossi (1965) and the ABTS radical cation decolorization assay according to Santarelli et al. (2020). On each extract, the TPC analysis was performed in duplicate and the AOA analysis in triplicate.

Viscosity and Surface tension Measurements

The air/water surface tension of the VI solutions was measured at 25 °C with an Attension Sigma 700/701 tensiometer (Biolin Scientific Oy, Espoo, Finland) equipped with a Wilhelmy platinum plate (length, width, and thickness 19.6, 10 and 0.1 mm, respectively). Surface tension measurements were carried out after 60 min of equilibration time.

Viscosity data were obtained from flow curves (shear stress versus shear rate) obtained with a rotational rheometer (MRC 302, Anton Paar, Graz, Austria) equipped with a concentric cylinder configuration (CC27). Flow curves were collected in the shear rate range from 1 to 1000 s^{-1}. Samples were loaded into the cylindrical cup and allowed to equilibrate at a set-point temperature of 25 °C for 2 min before analysis.

Statistical Analyses

Data were expressed as mean and standard deviation and additionally analysed by one-way ANOVA analysis. Significant differences between means were computed by LSD test at a significance level of 0.05.

Firmness and colour data collected on samples after blanching in combination with vacuum impregnation and during the frozen storage were further processed by three-way ANOVA to highlight single effects of the solvent/solution used respectively for the blanching (S_{BL}) and vacuum impregnation (S_{VI}) pre-treatment, and the effect of freezing and frozen storage. Effective hypothesis decomposition was computed for the significant factors ($p < 0.05$). The sigma-restricted coding of effects was used, and for each effect its sum of squares is the difference of the model sums of squares for all other effects from the whole model sums of squares. As such, the effective hypothesis decomposition sums of squares provide an unambiguous estimate of the variability of predicted values for the outcome uniquely attributable to each effect.

Data were processed using STATISTICA for Windows (StatSoftTM, Tulsa, OK, USA) software.

Results

Effect of pre-treatments on enzymatic activity and carrot composition

The activity of peroxidase (POD) and pectinmethylesterase (PME) was determined on fresh and blanched carrots in order to assess the effectiveness of the blanching pre-treatment in inactivating endogenous enzymes otherwise responsible for quality loss during vacuum impregnation and frozen storage. Fresh carrots showed a POD and PME activity respectively of 311 ± 61 U g^{-1} dw and 519 ± 46 U g dw^{-1} while, after blanching, independently from the blanching agent used, no PME activity was detected and a POD residual activity lower than 10% was achieved in accordance with industrial requirements (Moscetti et al., 2017).

Mass transfer data and trehalose uptake of blanched and VI carrots are reported in Fig. 1. In order to better describe the effect of pre-treatments on carrots' composition, the content of native sugars and trehalose was also reported (Fig. 2).

After water blanching (BL_w), carrots showed a weight loss due to the loss of both water and soluble solids, as an effect of leaching phenomena promoted by thermal damages induced on cell structures by the heat treatment. In particular, the sugar content drastically decreased with a loss of glucose, fructose and sucrose ranging between 43 and 51%.

Carrots blanched in trehalose solution (BL_{4T}) highlighted a lower weight decrease than the water blanched ones thanks to the sugar uptake and lower loss of water and soluble solids,

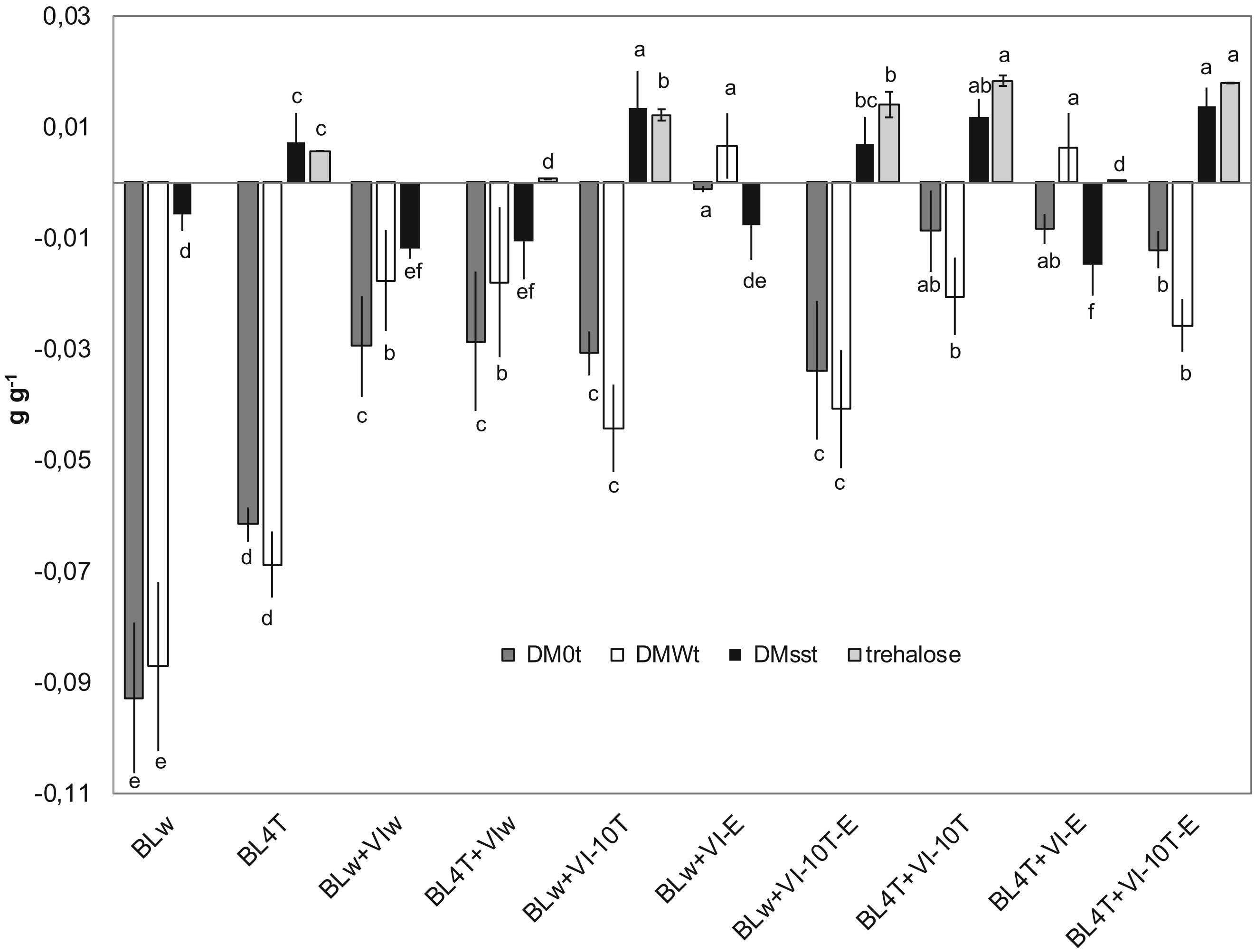

Fig. 1 Mass transfer parameters and trehalose uptake of pre-treated carrots. Total mass change ($\Delta M^0{}_t$); water mass change ($\Delta M^w{}_t$); soluble solids change ($\Delta M^{ss}{}_t$). Water blanched: BL_W; blanched in trehalose solution: BL_{4T}; vacuum impregnated with water: VIw; VI in trehalose 10% w/v : VI_{10T}; VI with green tea extract 0.25% w/v :VI_E; VI with trehalose 10% w/v and green tea extract 0.25% w/v :$VI_{10T\text{-}E}$. [a-e]: different letters stand for statistically significant differences at $p < 0.05$ within each independent variable

including native sugars (Fig. 2). This result can be attributable both to the thermo-protective effect exerted by trehalose on cell structures (Neri, Hernando, et al., 2011) and also to the reduction of diffusion phenomena as a result of the lower osmolarity difference between the product and the blanching agent.

VI affected the composition of blanched carrots by the enrichment with water and solutes of the impregnant medium (GTE and/or trehalose), while causing the depletion of trehalose in samples previously blanched in trehalose solution and, in general, a loss of endogenous soluble solids. This loss, in samples pre-treated by blanching in trehalose solution was due to the drop of both glucose, fructose and sucrose (Fig. 2); conversely, samples pre-treated by water blanching, after the sugar loss due to water blanching, in general did not highlight any further variation due to VI. The results are ascribed to gas-liquid and to liquid-liquid substitution occurring at extracellular level in carrot tissue (Gras et al., 2002) as an effect of hydrodynamic mechanisms and deformation and relaxation phenomena induced by the pressure variation, and due to diffusion phenomena provoked by the difference in solute concentration between the vegetal matrix and the VI medium.

Samples blanched and VI in trehalose solutions (BL_{4T} + VI_{10T} and BL_{4T} + $VI_{10T\text{-}E}$) showed the highest uptake of trehalose, and thus, a total sugar content slightly lower than that measured in fresh carrots (Fig. 2).

The addition of tea extract to the VI trehalose solution did not influence the sugar penetration (Figs. 1 and 2) into the carrot tissue, in fact, both samples BL_w + VI_{10T} and BL_w + $VI_{10T\text{-}E}$, and samples BL_{4T} + VI_{10T} and BL_{4T} + $VI_{10T\text{-}E}$ showed a similar ($p > 0.05$) trehalose uptake. It is generally assumed that solute penetration during VI process is affected by the impregnation solution viscosity and by the liquid interfacial tension (Gómez Galindo & Yusof, 2014). Both the solutions used for VI showed similar ($p > 0.05$) viscosity ($1.4 \pm 0.1 \times 10^{-3}$ Pa•s). The addition of GTE to the trehalose solution

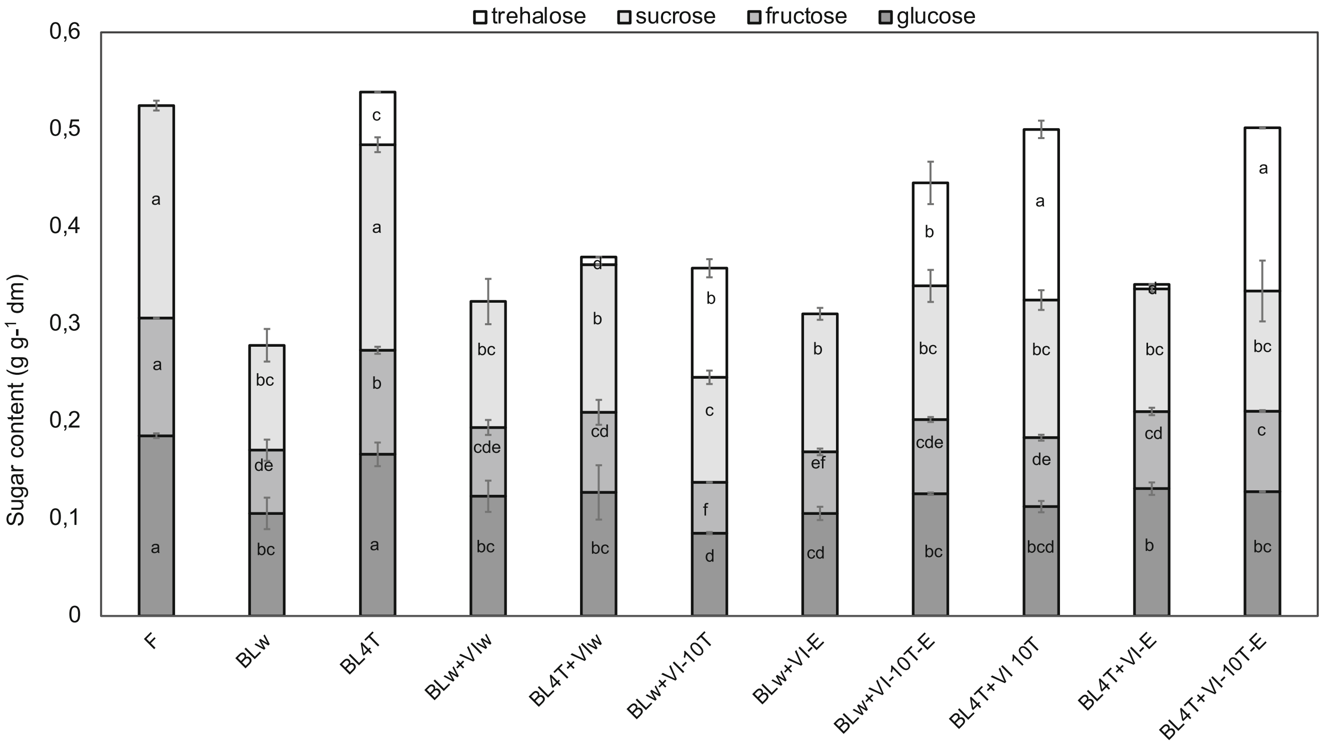

Fig. 2 Content of endogenous sugars (glucose, fructose and sucrose) and trehalose in carrots blanched and/or vacuum impregnated in trehalose solutions. Water blanched: BL_W; blanched in trehalose solution: BL_{4T}; vacuum impregnated with water: VIw; VI in trehalose 10% w/v: VI_{10T}; VI with green tea extract 0.25% w/v :VI_E; VI with trehalose 10% w/v and green tea extract 0.25% w/v :$VI_{10T\text{-}E}$. For each sugar, different letters in the bars stand for statistically significant differences at $p < 0.05$

lowered the surface tension from 71.6 ± 2.5 to 49.4 ± 0.1 mN m^{-1} due to the ability of catechins to modify the interfacial properties of the impregnating agent (Di Mattia et al., 2010). In the light of the observed results, it is possible to assume that the viscosity of the solution is the main driving factor of trehalose penetration in the experimental conditions of this study.

Effect of Pre-treatments, Freeing and Frozen Storage on Physical Properties

In Fig. 3, firmness of fresh (non pre-treated) and pre-treated carrots before and after freezing and frozen storage for 7 and 60 days is shown.

Blanching treatments significantly impaired the carrots' firmness irrespective of the blanching agent used (i.e. water or 4% trehalose solution). This effect is attributable to the gelification, solubilisation and degradation of pectins located in the cell wall occurring during heat treatment as an effect of the temperature increase. In general, VI treatment did not affect carrot firmness. However, samples blanched in trehalose solution, subsequently vacuum impregnated with tea extract or tea extract in combination with trehalose (BL_{4T} + VI_E and BL_{4T} + $VI_{10T\text{-}E}$) highlighted a significant (p < 0.05) higher firmness compared to the other pre-treated samples. This result could be partly ascribed to trehalose, whose thermo-protective effect during blanching of carrots was already highlighted in a previous study by microstructural analysis, but not by mechanical test (Neri, Hernando, et al., 2011), and to polyphenols adsorption to cell walls. Phan, Flanagan et al. (2017) showed, in fact, that polyphenols, when added to cell wall extracts, selectively and extensively interact with different types of cell wall polysaccharides with reversible binding (hydrogen bonding and hydrophobic interactions) in very short times (<1 min). Thus, these interactions could have increased the cell wall strength contributing to the firmness increase observed on the processed vegetable tissues.

After freezing and frozen storage, all samples showed a drastic firmness decrease (Fig. 3). Mechanical damages provoked by ice formation and crystal growth, as well as cell turgor loss due to the alteration of local osmotic pressure caused by migration of unfrozen water to the frozen area, could be responsible for this effect. The alteration of osmotic pressure, in particular, provokes cell dehydration and shrinkage, denaturation of cell components such as proteins and loss of pectin mediated cell connection (Li et al., 2018). This phenomenon could not be completely inhibited under the storage conditions used (−18 °C) as the vegetable products were kept at a temperature above the glass transition temperature of the maximally concentrated solution (T'g) of carrots (ca. −40 °C) (Chirife et al., 1996), allowing non-convective diffusion processes of water in the matrix (Van der Sman, 2020).

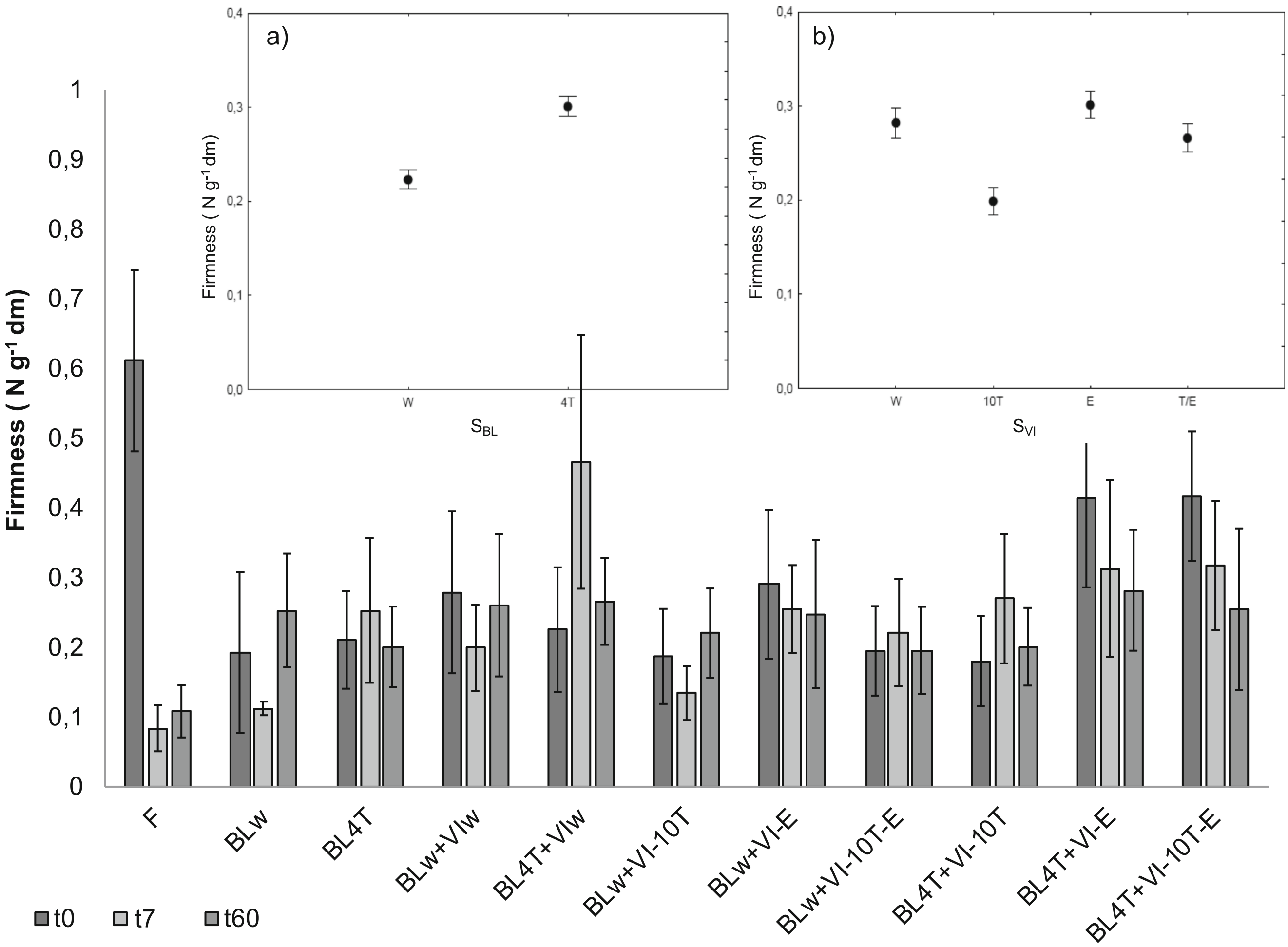

Fig. 3 Firmness of fresh and pre-treated carrots before (t_0) and after freezing and frozen storage for 7 (t_7) and 60 (t_{60}) days. Water blanched: BL_W; blanched in trehalose solution: BL_{4T}; vacuum impregnated with water: VIw; VI in trehalose 10% w/v : VI_{10T}; VI with green tea extract 0.25% w/v :VI_E; VI with trehalose 10% w/v and green tea extract 0.25% w/v :$VI_{10T\text{-}E}$. Insert: effective hypothesis decomposition of factorial ANOVA for the individual effect of the solvent/solution used respectively for blanching (S_{BL}) and vacuum impregnation (S_{VI}) pre-treatments on firmness

Non pre-treated (F) carrots showed a higher firmness loss compared to the pre-treated ones due to their PME activity. After 7 days of frozen storage, a higher firmness in the carrots blanched in trehalose solution than the water-blanched ones was observed; however, after 60 days, no differences were found between these samples. This result is in agreement with Neri et al. (2014) who observed a positive effect of trehalose on firmness of blanched and frozen carrots only for short times of frozen storage.

Firmness data collected on all the samples pre-treated by blanching in combination with vacuum impregnation were further processed by ANOVA analysis (*data not shown*) and a significant effect ($p < 0.05$) was observed for both the factors S_{BL} and S_{VI}, i.e. the solvent/solution used respectively for the blanching and vacuum impregnation pre-treatment. The effective hypothesis decompositions of these effects are reported in Fig. 3 (insert a and b, respectively).

S_{BL} and S_{VI} significantly ($p < 0.05$) affected the samples' firmness with samples blanched in trehalose being harder than the water-blanched ones, and samples VI in water, GTE solution and GTE in combination with trehalose solution being harder than those impregnated with trehalose solution. These results indicate that trehalose is able to exert a cryoprotective effect on frozen carrots only when its addition is carried out by blanching. In fact, when the product is exposed to high temperatures, its penetration in plant cells is promoted by the increase of membrane permeability. This hypothesis is also supported by recent studies carried out on spinach leaves by Phoon et al. (2008) and Demir et al. (2018), who highlighted a cryoprotective effect of trehalose only when its penetration was induced at intracellular level by coupling pulsed electric field treatments (PEF) with VI or, vice versa, combining the VI treatment with PEF. PEF and VI, depending on process conditions, can influence the solutes penetration into the cells respectively by changing the permeability of the cell membranes and by activating cell metabolic processes (Dymek et al., 2016; Gómez Galindo and Yusof, 2014). In carrots, it is excluded that this latter action mechanism contributed to the penetration of trehalose at intracellular level as cells were inactivated by the blanching treatment.

The sugar addition only at extracellular level could determine, on the contrary, a negative effect on cell turgidity probably due to the promotion of osmotic phenomena.

Figures 4 and 5 respectively report the lightness and hue angle of fresh and pre-treated carrots before and after freezing and frozen storage.

Fresh carrots showed a lightness (L*) and hue angle (h°) respectively lower and higher than those reported by Moscetti et al. (2017) on the same variety. Blanching and VI pre-treatments affected the colour of the fresh carrots by determining a significant ($p < 0.05$) decrease in lightness (Fig. 4) and a slight increase ($p < 0.05$) in the hue angle (Fig. 5). The shift of colour from orange to yellow could be related to pigment degradation, whilst sample darkening is related to the replacement of the gases and native liquids present in the vegetable matrix with the impregnant solutions as an effect of the blanching and VI pre-treatment (Neri et al., 2016).

Overall, no significant differences were found in terms of L* and h° among the differently pre-treated carrot samples and this indicates that the solutes used in blanching and VI treatments, as well as the vacuum impregnation itself, did not affect the carrot colour.

After freezing and frozen storage carrots showed, in general, a slight but significant ($p < 0.05$) L* decrease, with sample F (i.e. non pre-treated) being the most affected. When ANOVA analysis was performed on data collected on samples pre-treated by blanching in combination with VI (*data not shown*), a significant effect ($p < 0.05$) was noted for the individual S_{BL} and S_{VI} factors; the effective hypothesis decompositions for these effects are reported in Fig. 4 (insert a

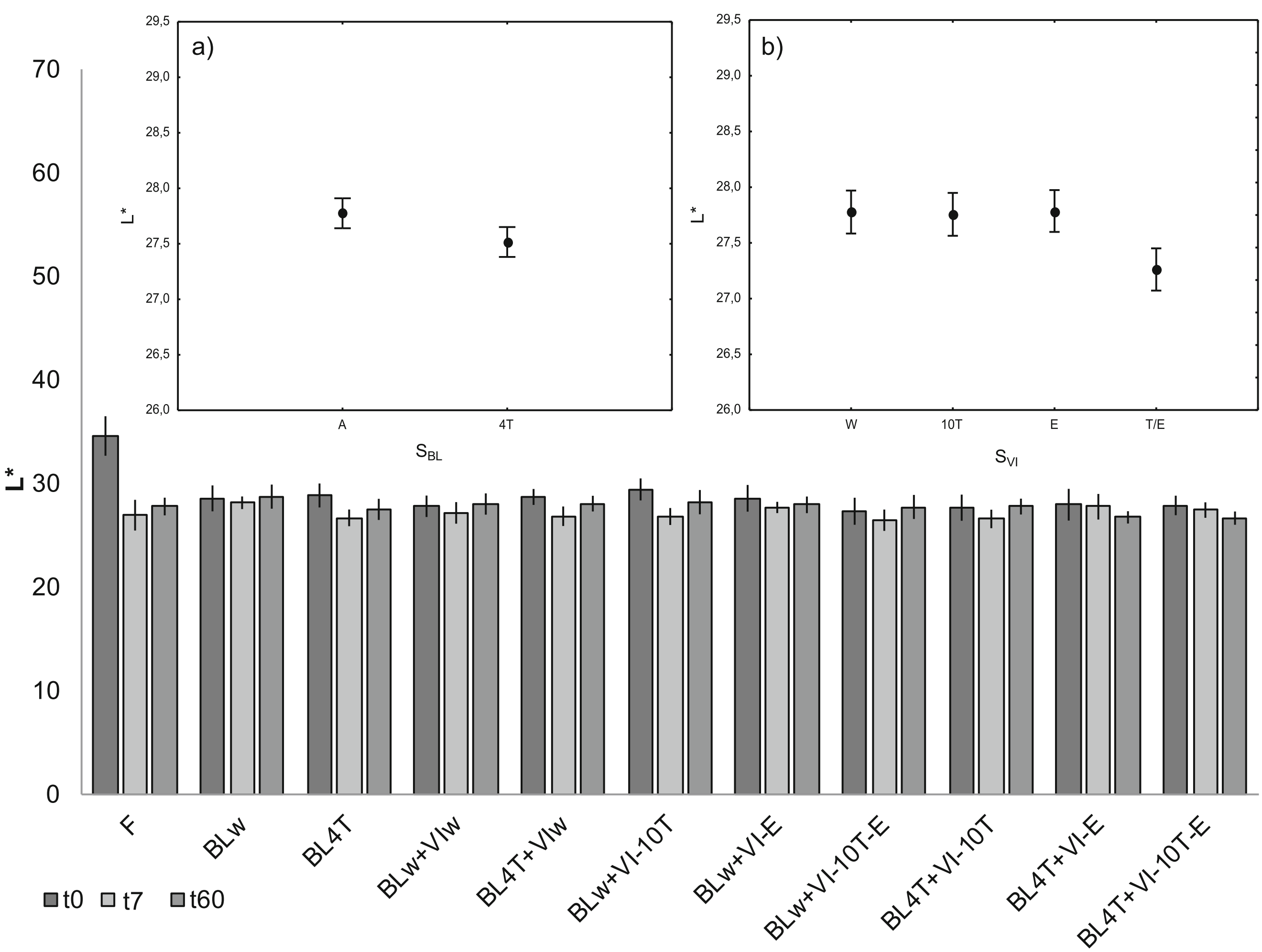

Fig. 4 Lightness (L*) of fresh and pre-treated carrots before (t_0) and after freezing and frozen storage for 7 (t_7) and 60 (t_{60}) days. Water blanched: BL_W; blanched in trehalose solution: BL_{4T}; vacuum impregnated with water: VIw; VI in trehalose 10% w/v : VI_{10T}; VI with green tea extract 0.25% w/v :VI_E; VI with trehalose 10% w/v and green tea extract 0.25% w/v :$VI_{10T\text{-}E}$. Insert: effective hypothesis decomposition of factorial ANOVA for the individual effect of the solvent/solution used respectively for blanching (S_{BL}) and vacuum impregnation (S_{VI}) pre-treatments on L*

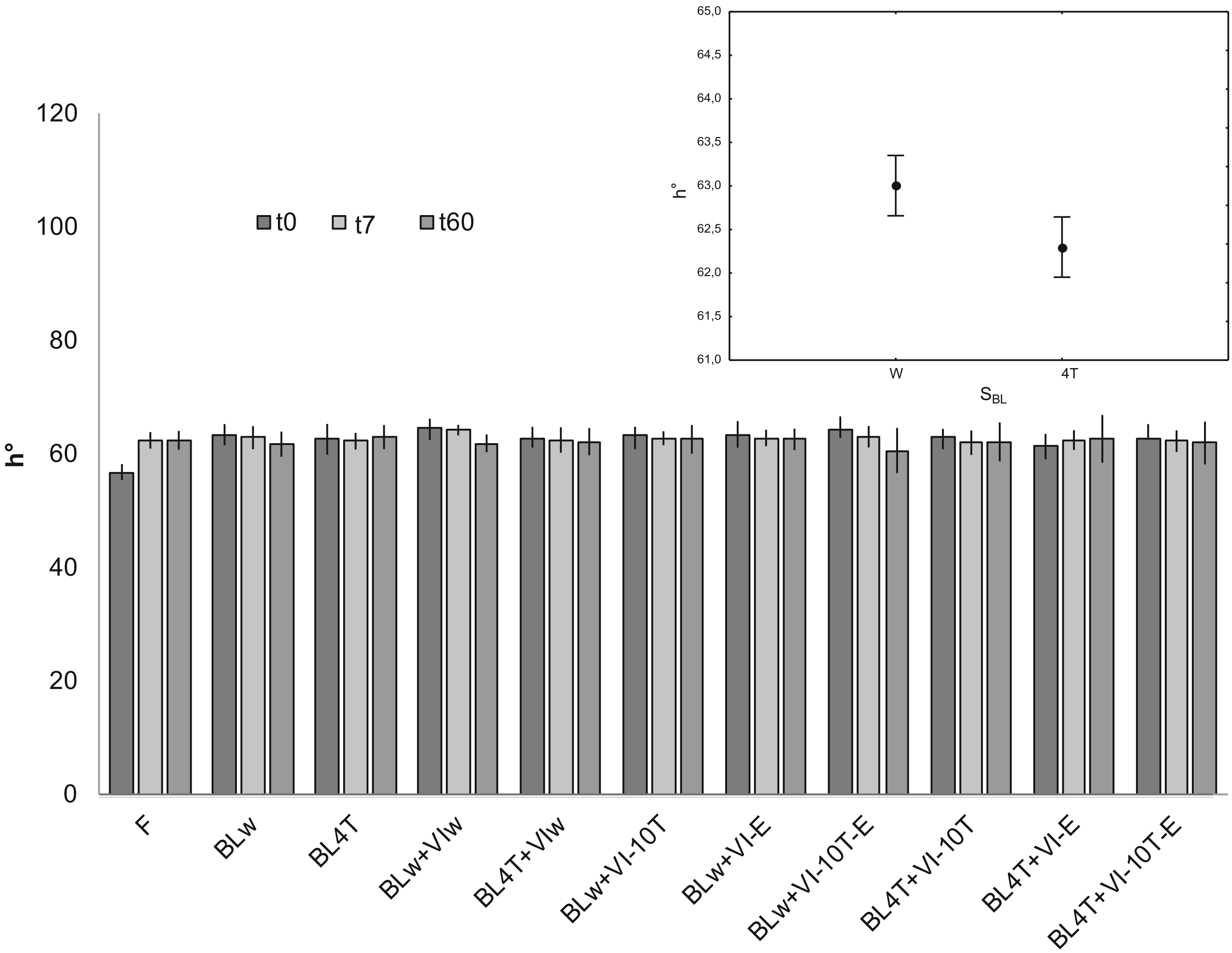

Fig. 5 Hue angle (h°) of fresh and pre-treated carrots before (t_0) and after freezing and frozen storage for 7 (t_7) and 60 (t_{60}) days. Water blanched: BL_W; blanched in trehalose solution: BL_{4T}; vacuum impregnated with water: VIw; VI in trehalose 10% w/v : VI_{10T}; VI with green tea extract 0.25% w/v :VI_E; VI with trehalose 10% w/v and green tea extract 0.25% w/v :$VI_{10T\text{-}E}$. Insert: effective hypothesis decomposition of factorial ANOVA for the individual effect of the solvent/solution used respectively for blanching (S_{BL}) and vacuum impregnation (S_{VI}) pre-treatments on h°

and b respectively). Lightness was negatively influenced by both blanching in trehalose solution and vacuum impregnation with GTE + trehalose solution. As far as h° is concerned (Fig. 5), freezing did not determine any significant ($p > 0.05$) variation on sample BL_w and BL_{4T} whilst sample F showed an h° increase ($p < 0.05$), or rather a yellowish colour. ANOVA analysis of samples pre-treated by blanching and VI highlighted a slight but significant effect ($p < 0.05$) for the individual S_{BL} factor; by observing the effective hypothesis decompositions for this effect (insert in Fig. 5), it is possible to observe that samples blanched in trehalose had a lower h° than the water blanched ones, corresponding to a more orange colour.

The different effect exerted by freezing on the colour of the F sample and of the pre-treated ones is due to the presence in the former of oxidative enzymes which were not completely inhibited by the low storage temperatures (−18 °C), and still exhibit activity, being at a temperature above the products' T′g. The lightness and hue angle of the frozen samples pre-treated by blanching in trehalose in combination with VI could be due to the protective effect of the sugar on tissue pigments (Forni et al., 1997).

Effect of Pre-treatments, Freezing and Frozen Storage on Functional Properties

In Table 1, the data of the total polyphenol content (TPC), total carotenoid content and antioxidant activity (AOA) of fresh and differently pre-treated samples before and after freezing and 60 days of frozen storage, are reported.

Fresh carrots showed a TPC similar to that reported by other authors (Gonçalves et al., 2010; Patras et al., 2011). Blanched samples depicted a total polyphenol content significantly higher than the fresh vegetable ($p < 0.05$). This result is in agreement with what observed by Ma et al., (2013) and by

Table 1 Mean and standard deviation of carotenoids content, total polyphenol content (TPC), and Trolox equivalent antioxidant capacity (TEAC) of fresh/not pre-treated (F) and pre-treated carrots before (t_0) and after freezing and 60 days of frozen storage (t_{60}).

		Carotenoids (mg g^{-1} dw)	sd	TPC (mg GAE g^{-1} dw)	sd	TEAC (μmol g^{-1} dw)	sd
t_0	F	0.98^{bcd}	0.12	7.55^{l}	0.27	9.09^{hij}	0.02
	BL_W	1.07^{ab}	0.01	8.81^{hi}	0.68	10.6^{f}	0.23
	BL_{4T}	0.95^{cd}	0.03	9.46^{gh}	0.96	11.7^{e}	0.07
	BL_W+VI_W	1.05^{abc}	0.03	9.36^{gh}	0.16	9.16^{hij}	0.49
	BL_{4T}+VI_W	1.14^{a}	0.04	8.76^{hi}	0.46	8.80^{ijk}	0.05
	BL_W+VI_{10T}	1.14^{a}	0.15	5.39^{m}	0.22	9.20^{hij}	0.52
	BL_W+VI_E	1.10^{ab}	0.02	23.7^{a}	0.16	20.43^{c}	4.65
	BL_W+$VI_{10T\text{-}E}$	1.01^{bcd}	0.10	11.0^{f}	0.61	22.4^{b}	8.78
	BL_{4T}+VI_{10T}	0.89^{d}	0.04	8.64^{i}	0.10	10.5^{fg}	0.55
	BL_{4T}+VI_E	1.10^{ab}	0.02	22.6^{b}	1.58	20.2^{c}	8.98
	BL_{4T}+$VI_{10T\text{-}E}$	0.99^{bcd}	0.13	14.9^{d}	0.73	23.9^{a}	0.36
t_{60}	F	0.56^{efg}	0.08	7.62^{l}	0.52	8.49^{jk}	0.42
	BL_W	0.58^{efg}	0.01	8.99^{hi}	0.68	9.64^{ghi}	0.55
	BL_{4T}	0.51^{g}	0.00	8.87^{hi}	0.05	6.60^{m}	0.13
	BL_W+VI_W	0.67^{e}	0.03	9.18^{ghi}	0.61	8.15^{jk}	0.52
	BL_{4T}+VI_W	0.66^{e}	0.01	9.81^{g}	0.66	9.87^{fgh}	0.29
	BL_W+VI_{10T}	0.56^{efg}	0.03	5.21^{m}	0.32	6.94^{lm}	0.37
	BL_W+VI_E	0.65^{e}	0.06	21.6^{c}	0.06	18.91^{d}	3.14
	BL_W+$VI_{10T\text{-}E}$	0.57^{efg}	0.01	12.6^{e}	0.11	20.2^{c}	0.82
	BL_{4T}+VI_{10T}	0.52^{fg}	0.02	4.97^{m}	0.32	7.93^{k}	0.55
	BL_{4T}+VI_E	0.64^{ef}	0.01	20.9^{c}	0.95	20.4^{c}	1.24
	BL_{4T}+$VI_{10T\text{-}E}$	0.46^{g}	0.03	12.2^{e}	0.23	20.0^{c}	2.12

Water blanched: BL_W; blanched in trehalose solution: BL_{4T}; vacuum impregnated with water: VI_w; VI in trehalose 10% w/v: VI_{10T}; VI with green tea extract 0.25% w/v: VI_E; VI with trehalose 10% w/v and green tea extract 0.25% w/v: $VI_{10T\text{-}E}$. sd: standard deviation

$^{a\text{-}m}$: Data with different letters stand for statistically significant differences at $p<0.05$.

Santana-Gálvez et al. (2019), and confirms that short thermal treatment does not impair the functional properties of the plant, while it promotes the extraction of bioactives. When the blanched samples were further VI treated in water, no variation of the phenolic content was observed, indicating that the experimental conditions used for the vacuum impregnation treatment allowed to preserve the functional properties of the vegetable. VI treatment in green tea solutions determined a significant increase ($p < 0.05$) in polyphenols, which resulted three times higher than that in the fresh vegetable. The addition of trehalose to the GTE solution negatively influenced the TPC of the VI samples and this result could be due to (i) the higher viscosity of the 10 T-E solution compared to the GTE solution (1.4 ± 0.1 mPa·s vs. 1.1 ± 0.05 mPa·s), which could have hindered the penetration of the impregnant solution into the vegetable tissue; (ii) the protective action of trehalose on the plant tissue and, in turn, the lower extractability of the phenolic compound; (iii) the lower capacity of the extracts to reduce the Folin-Ciocalteu's reagent as a consequence of phenol–sugar interactions. In fact, as highlighted by Kopjar et al. (2016) and by Lončarić et al. (2018), trehalose can affect the TPC of plant extracts to a very different extent depending on the phenolic profile and thought mechanisms, which still need to be explained.

After freezing and 60 days of frozen storage, no effect was observed on the TPC of unblanched and blanched carrots ($p > 0.05$) in accordance with Patras et al. (2011), as well as on samples blanched in water and trehalose solution. In contrast, samples blanched and VI with GTE solutions were negatively affected by freezing and frozen storage. These results could be explained by a different sensitivity of endogenous and exogenous GTE polyphenols towards oxidation reactions as an effect of their different localization and binding to cell wall constituents.

Despite the highest TPC decrease upon frozen storage, samples impregnated with GTE solutions after freezing and frozen storage confirmed their highest polyphenol content, which was up to 2.9 times higher than the fresh product.

Fresh carrots showed a carotenoid content slightly higher than that reported by de Jesus Junqueira et al. (2018) and Moscetti et al. (2017). In general, the carotenoid content was not influenced by pre-treatment despite what observed for the h° parameter (Fig. 4) which suggests a pigment degradation. This result could be due to the different extractability of carotenoids from the plant tissue. In the blanched samples, in fact, as observed for polyphenols, it could have been positively influenced by the heat treatment which, in turn, could have led to a modification in cell membrane permeability. The enhancement of the carotenoid extraction efficiency after blanching was observed on carrots and other fruit and vegetable products also by other authors (Behsnilian & Mayer-Miebach, 2017; Palermo et al., 2014; Santana-Gálvez et al., 2019).

Freezing and frozen storage negatively affected ($p < 0.05$) the carotenoid content (Table 1) in agreement with what observed by Bouzari et al. (2015). Sample F, despite what observed with respect to the h° and L* colour parameters, showed a carotenoid content similar ($p > 0.05$) to all the other pre-treated samples, which were stabilised by the blanching treatments through the inactivation of endogenous enzymes. No differences ($p > 0.05$) in the carotenoid content were observed also among the other pre-treated samples, regardless of their process and formulation.

The antioxidant activity (AOA) (Table 1) increased in carrots blanched in trehalose solution and VI with GTE solution; in particular, AOA was higher in samples vacuum impregnated with GTE and trehalose, despite their lower TPC values. As reported by Lončarić et al. (2018), interactions occurring in complex food matrices between phenols and trehalose can explain this result. Trehalose, in fact, differently affected the reactivity of single or mixed polyphenols depending on the mechanisms of action exploited by the method used to evaluate the polyphenols' antioxidant activity, and this could thus explain the discrepancy between the ABTS and TPC results. Although TPC and ABTS results are generally correlated, TPC exclusively measures the reducing power of extracts (Sánchez-Rangel et al., 2013) whilst, in the TEAC assay, the ABTS radical could undergo reduction by both single electron transfer (SET) and hydrogen atom transfer (HAT), therefore presenting a mixed mechanism of action towards the antioxidants present in the extracts (Apak et al., 2016; Prior et al., 2005).

After freezing and frozen storage, a significant AOA reduction ($p < 0.05$) was observed as an effect of both the carotenoid and polyphenol content decrease (Table 1). Despite these variations, the samples blanched and VI in GTE solutions after freezing and frozen storage showed AOA values doubled respect to fresh carrots.

Conclusions

The results presented in this study show that blanching in combination with vacuum impregnation can be effectively used as freezing pre-treatments for the production of innovative frozen plant products allowing for both the enzymatic stabilisation and direct formulation with functional ingredients.

In particular, the enrichment of carrots with trehalose by blanching and of green tea extract by VI showed to slightly affect the quality properties of carrots, while allowing for a considerable increase in the carrots' functional properties and firmness preservation of plant tissue.

Freezing and frozen storage caused a significant loss of carotenoids while the total polyphenol content decreased only in carrots previously enriched with green tea polyphenols. Despite these variations, blanching and vacuum impregnation in green tea extract solution permitted to obtain after 60 days of frozen storage, carrots with an antioxidant activity doubled compared to that of the fresh vegetable.

Trehalose showed a cryoprotective effect on colour and on the mechanical properties of the frozen carrots only when its transfer into the vegetable matrix occurred by blanching, and this result could be related to its penetration at intracellular level promoted by changes of the membrane permeability due to the thermal treatment.

Further investigations and microstructural analysis are necessary to better understand the role of trehalose and polyphenols on preserving and/or enhancing the firmness of processed plants. In addition, the effect of both BL and/or VI treatments on quality and storability of carrots slices subjected to different processing methods (e.g. drying) should be tested.

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