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Changes in Carotenoids, Antioxidant Properties and Quality of Purple Carrot (*Daucus carota* L.) during Cold Storage by Post-harvest Elicitation

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PONER EN LOS AGRADECIMIENTOS A LAURA CUANDO HAYA QUE REVISARLO: financiado por la Unión Europea – Next Generation EU

Abstract: Black-purple carrots possess, apart from carotenoids, high content of other bioactive pigments such as anthocyanins. However, both carotenoids and anthocyanins are modified over cold storage due to their lability. In this work, we investigated the effect of the postharvest elicitation on nutritional and organoleptic quality of purple carrots over storage at 5°C for 21 days. Based on our experience, we considered methyl jasmonate and abscisic acid as elicitors. The values obtained were compared with those provided by the fresh-untreated sample on the treatment day (ie, day 0) and after three-week storage (ie, on day 21), which were used as a reference. As a result, carotenoid content increased naturally in untreated samples over storage whereas anthocyanins maintained invariable. Also, physicochemical parameters reflected apparent organoleptic quality loss. However, the postharvest treatment of purple carrot samples with elicitors resulted in similar carotenoid content in elicited samples to fresh carrots on day 0 as well as lower concentration of anthocyanins and antioxidant activity. Besides, elicitation, particularly with abscisic acid, enabled purple carrot deterioration to be slowed down and, therefore, the organoleptic quality to be preserved. This suggests delaying effect of both elicitors on the spoilage rate of purple carrots over the storage time. These findings indicate that the postharvest application of elicitors may be an interesting conservation method, alternative to traditional procedures, to decelerate decomposition and extend shelf-life of purple carrots.

Keywords: elicitors; carotenoids; anthocyanins; antioxidant activity; purple carrot; spoilage; methyl jasmonate; abscisic acid

1. Introduction

Carrot (*Daucus carota* L.) is one of the most widely cultivated vegetables in the world. Its consumption has progressively increased in the last few years thanks to its pleasant flavor and health benefits related to its nutritional value. Carrot is an important source of carotenes, minerals, vitamins and dietary fiber (Alasalvar et al., 2001). Apart from the conventional orange-root carrots, there are materials with different colors owing to the accumulation of different carotenoids and anthocyanin pigments. In particular, black or purple carrots are rich in anthocyanins, which are known for their health promoting properties. In general, diets including both pigment types, carotenoids and anthocyanins, have been associated with the reduction of the risk of chronic pathologies such as cardiovascular, cancer, stroke and neurodegenerative diseases (Wrolstad, 2004).

Nevertheless, the chemical composition of foods is often altered over the storage period, which affects both their sensorial characteristics and nutritional value. Carotenoids are usually degraded in presence of oxygen, light, high temperatures and certain enzymes due to their highly unsaturated structure (Van den Berg et al., 2000). Also, anthocyanins are modified by pH, enzyme activity, oxygen and, in particular, high temperatures (de Pascual-Teresa and Sanchez-Ballesta, 2008). In relation to organoleptic properties of carrots, it is common to observe loss of water content and firmness, slimy texture, discoloration, bitterness and an oxidized odor (Yen et al., 2008). For these reasons, it is

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essential to select carefully the storage conditions with a view to assuring sensory and health-related quality.

Interestingly, cold storage has been reported as inconvenient to preserve orange carrot carotenoid during the post-harvest period. In fact, milder temperatures (ie, around 20 °C) have been demonstrated not only to avoid carotenoid loss but also to promote their increase (Hammaz et al., 2021). In addition, room temperature over storage has been recommended rather than colder values to minimize anthocyanin degradation in black carrot juice (Özen et al., 2011). On the other hand, it is evident that the temperature used to store carrots affects significantly sensory characteristics in such a way that the storage conditions applied must always guarantee the absence of rooting and decomposition. In this respect, raw carrot shelf-life without refrigeration varies from 5 to 7 days but the use of cold temperature can prolong this period until 3 or 4 weeks (Opoku et al., 2009). Therefore, cold temperatures are essential for consumer acceptance. We intended to propose a procedure that enables purple carrot shelf-life to be prolonged, preserving, in turn, bioactive compound content.

In this context, the pre-and post-harvest treatments with chemical elicitors have been already described to enrich plant-based foods in biologically active compounds such as carotenoids and anthocyanins (Moreno-Escamilla et al., 2020; Flores and Ruiz del Castillo, 2016; Blanch et al., 2020). Nonetheless, the effect of elicitors on dark carrots has been scarcely studied (Barba-Espín et al., 2021; Barba-Espín et al., 2019).

The aim of this research was to study the effect of the post-harvest treatment with elicitors on bioactive compounds (ie carotenoids and anthocyanins) in purple carrots over storage. Assessment of the antioxidant activity by two different assays and physicochemical quality parameters were also considered. Our final intention was to find an approach enabling nutritional and sensory quality of purple carrots over storage to be preserved.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade methanol (MeOH), acetone, ethyl acetate and acetonitrile were purchased by Macron Fine Chemicals (USA). Hexane and chloroform were obtained from LabScan (Bangkok, Thayland). Ultrapure water was obtained from a purification system (Macron Fine Chemicals, USA). 2,2-diphenyl-2-picrylhydrazil (DPPH), methyl jasmonate (MJ), abscisic acid (ABA), lanolin, B-carotene, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium chloride, sodium hydroxide (NaOH), sodium acetate anhydrous standards were all supplied by Sigma-Aldrich (Steinheim, Germany). Ethanol (EtOH), pentane, cyclohexane, acetic acid glacial and hydrochloric acid (HCl) were acquired from Scharlau Chemie S.A. (Barcelona, Spain) and lutein standard was obtained from Extrasynthese (Genay, France).

2.2. Samples

Fresh purple carrot roots (*Purple Haze* variety, Figure 1) were supplied by a local producer (Madrid, Spain). The variety, used for both fresh market and processing, was characterized by a dark purple color with an orange core and 3-5 cm in diameter. After reception, samples were split into three different groups. Carrots in the first group were immediately analyzed to be used as a reference (so-called fresh-untreated on day 0). Samples in the second group were stored at 5°C in the dark for 21 days to assess the natural progress of purple carrots without elicitation under the specific storage conditions used in this study (so-called fresh-untreated on day 21). Finally, carrot roots in the third group were first subjected to the treatments with MJ and ABA and subsequently stored as explained below (so-called MJ-treated and ABA-treated, respectively).

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Figure 1. Purple carrot (*Purple Haze* variety): (a) whole, (b) cut in half, (c) sliced.

2.3. Treatments

For the experiments, the same size carrots with no sign of sprouts or any other damage were selected. The application of MJ and ABA was carried out at room temperature. To consider root-to-root variation, every treatment was performed in duplicate by using different roots. Based on our previous results, MJ and ABA were chosen to carry out the treatments for their higher effectiveness (Flores et al., 2016; 2018; Ruiz del Castillo et al., 2010). To apply the elicitors on purple carrots, two different approaches were tested (ie, paste and spray).

2.3.1. Treatments with MJ an ABA paste

Approximately 25 mg of the elicitor (ie, 0.2 mg g^{-1}) was mixed with lanolin paste, which was prepared by merging lanolin with distillated water (1:2 w/w). This mixture was applied on one half of the root by using a spatula (treated), while the other half was treated with lanolin paste without elicitor (control). The four treated carrots (ie, two for each elicitor) were stored at 5°C in absence of light for 21 days. After this time, the paste was carefully removed from the carrots using a dry paper and control and treated samples were analyzed.

2.3.2. Treatments with MJ and ABA spray

To prepare the elicitor emulsion, 20 mg of MJ or ABA (ie, 0.2-mg g^{-1} sample) was dissolved in 0.05% Tween-20 (ie, 50 μL of Tween-20 in 100 mL of distilled water). Then, the mixture was sprayed over two roots for each elicitor. Control samples were prepared by running off extra carrots with Tween-20 solution alone. All purple carrot roots, both treated and controls, were stored at 5°C in the dark for 21 days. Subsequently, the remaining of the emulsion was removed by using a paper and all samples were analyzed.

2.4. Physicochemical parameters

The physicochemical properties of fresh-untreated (on day 0 and on day 21) and treated (with MJ or ABA) purple carrots were studied by determining juiciness, moisture content, total soluble solid (TSS) content, acidity (as both pH and total titratable acidity, TAA) and maturity index. Prior to the actual measurements, juice was obtained from purple carrots by following this procedure: carrots were cut and put in a blender. The resulting paste was then centrifuged at 20°C and 10000 rpm for 15 min. The juice obtained was separated from the paste remaining by vacuum filtration and the physicochemical parameters were immediately measured.

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2.4.1. Juiciness

The juiciness of the samples was estimated from weight ratio percentage between the juice obtained and the carrot root used in its preparation.

2.4.2. Moisture content

The moisture content of the purple carrots was calculated gravimetrically. Purple carrots were sliced and ground with a food processor. After that, the carrot powder obtained was dried in a conventional stove at 105°C until constant weight. The weight difference indicated the moisture content.

2.4.3. Total soluble solids (TSS)

An Atago digital refractometer, model dbx-30 (Labexchange, USA) was used to perform the measurements. Just a drop of carrot juice was placed onto the refractometer. The result was obtained from the mean value of three measurements for each sample. The results were expressed as percentage brix degrees and grams of sucrose per dry weight (DW).

2.4.4. Acidity

2.4.4.1. Active acidity (or Hydrogen concentration pH)

The active acidity was calculated by measuring the pH using a 913 pH-meter (Metrohm, Madrid, Spain). To that end, the corresponding electrode was just sunk into a 50 mL-volume of purple carrot juice.

2.4.4.2. Total titratable acidity (TTA)

The determination was accomplished by acid-base titration. A 50-mL volume of juice was neutralized with NaOH solution (0.1 N). A pH of 8.2 was fixed to consider the endpoint for titration. The amount of base used until neutralization showed the acid content. The results were expressed as percentage grams of major organic acid and gram of organic acid per DW.

2.3.5. Maturity index

The maturity index was estimated from the ratio between the TSS values and the TTA values. The numeric value of ratio expresses maturity index: higher numerical value, more advanced the root will be in the postharvest maturity stage.

2.5. Health-related quality

The content in carotenoids and anthocyanins and the antioxidant activity by the DPPH and FRAP assays were determined in fresh-untreated and treated purple carrot samples as detailed below.

2.5.1. Determination of carotenoids

2.5.1.1. Extraction

Purple carrots were ground in a food processor. A 20-mL volume of acetone was added to 2 g of ground carrot. Then, the mixture was homogenized by using an Ultra-Turrax (T18 Digital, IKA) for 5 min. After that, it was passed through a filter paper. Additional acetone was used to sweep the remaining of the extract until the sample became colorless. Subsequently, a 25-mL volume of hexane was added and the mixture was shaken. Finally, the acetone phase was discarded and the hexane layer was up to 50 mL.

Total carotenoid content (TCC) and individual carotenoids were determined in the extracts obtained as explained below.

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2.5.1.2. Total carotenoid content (TCC)

A spectrophotometer (Beckman Coulter DU-800 spectrophotometer, Barcelona, Spain) was used to accomplish the measurements. The absorbance of the extracts was registered at 485 nm and all the measurements were performed twice. TCC was determined by applying the extinction coefficient ($E_{1\%}$ = 2500) (Britton 1991). Results were expressed as μg of β-carotene equivalents (EβC) per g of DW.

2.5.1.3. Individual carotenoids by HPLC

The content of individual carotenoids in purple carrot extracts was determined by HPLC (Alliance Separation Module 2695, Waters, Mildford, CT, USA) equipped with an automatic injector and a photodiode array detector 996 (DAD, Waters, Mildford CT, USA). The separation was carried out on a 3.9 mm × 150 mm reversed-phase Nova-Pak C_{18} column (particle size 4 μm, Waters, Milford, USA). The mobile phase consisted of acetonitrile:methanol:ethyl acetate (73:20:7, v/v/v) and isocratic mode was used. Based on the literature (Zelenkova et al., 2015) and on the test of different flow rate values, the analysis were perform at 0.3 mL min^{-1}. The chromatographic signals were registered at 450 nm by using the software (Empower2 software, Waters, Mildford, CT, USA). Carotenoids (ie, lutein and β-carotene) were identified by comparison with the retention time of the corresponding standards. In addition, the idenficiation was verified with bibliographic information (Zelenkova et al., 2015). Quantitative determination of carotenoids was accomplished by using calibration curves of β-carotene. All analyses were performed in duplicate.The results were expressed as μg EβC per g of DW.

2.5.2. Anthocyanins

2.5.2.1. Extraction

Anthocyanins were extracted from fresh-untreated and treated purple carrots by adding 50 mL EtOH:H_2O (1:1, v/v) containing 0.01% HCl (37% v/v) to 25 g of ground sample. Then, the mixture was stirred for 2 h at room temperature. The extract was purified by adding chloroform (2 × 25 mL), pentane (2 × 25 mL) and cyclohexane (2 × 25 mL). The aqueous fraction was properly separated and the solvent was removed at 30°C until a final volume of 25 mL. The extract obtained was stored in the dark at -20 °C. Each extraction was carried out in duplicate. Total anthocyanin content (TAC) and antioxidant activity by DPPH and FRAP assays was determined in the extracts obtained, as described below.

2.5.2.2. Total anthocyanin content (TAC)

TAC was determined by applying the pH differential method (Giusti and Wrolstad, 2001). In brief, carrot extracts were diluted with two different buffer solutions: 0.025 M potassium chloride at pH 1 and 0.4 M sodium acetate at pH 4.5. The measurements were performed by using the same equipment as that used for TCC and at two different wavelengths (ie, 520 and 700 nm). The results were expressed as μg of cyanidin-3-*O*-glucoside equivalents (EC3G) per g of DW. The absorbance was calculated by applying the equation (1), for which the molar extinction coefficient (ie, 26900 L cm^{-1}) and the molecular weight of C3G (ie, 449.4 g/L) were used.

$$(1)\ Abs_t = (Abs_{520nm} - Abs_{700\ nm})_{\ pH=1} - (Abs_{520\ nm} - Abs_{700\ nm})_{\ pH=4.5}$$

2.6. Antioxidant activity

The antioxidant activity of the samples was evaluated in terms of the free radical scavenging activity by using the DPPH assay and in terms of the capacity to reduce ferric ion (Fe^{3+}) to ferrous iron (Fe^{2+}) by the FRAP assay. A BioTek Synergy HT multi-mode microplate reader with BioTek´s Gen 5TM software (BioTek Instruments Inc., Winooski, VT, USA) and 96-well microplates was used for the measurements. The working DPPH• reagent was prepared by dissolving DPPH• standard in methanol (1000 μM) whereas the working FRAP reagent was made by mixing acetate buffer (0.3 M)

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at pH 3.6 with 2,4,6-tripyridyl-s-triazine (TPTZ) in HCl (40 mM) and $FeCl_3 \cdot 6H_2O$ (20 mM) at a ratio of 10:1:1. The same procedure was applied for both assays. A 290-µL volume of the reagent (i.e. DPPH• or FRAP) was added to a 10 µL of the sample in each well. Subsequently, the mixtures were incubated at 37°C for 30 and 20 min in the dark, respectively. The absorbance was measured at 515 nm for the DPPH assay and at 593 nm for the FRAP assay. The value of absorbance provided by the DPPH• or FRAP reagent solutions were used as a blank in each case. A Trolox standard curve was used to obtain the data and all analyses were performed in duplicate. When necessary, extracts were diluted to be adapted to the linear range of the curve. Results were expressed as mg of Trolox equivalents per g of DW.

2.7. Statistical study

An analysis of variance for TCC, TAC, DPPH and FRAP data of was performed using one-way analysis of variance (ANOVA) method. The results are presented as the average of all values obtained and standard deviation (SD). Data obtained from fresh-untreated and treated samples, were statistically compared. Comparisons of means were made by using the Fishers´protected LSD. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Physicochemical quality

3.1.1. Visual observation

By comparing fresh-untreated on day 0 purple carrots (Figure 2a) with that fresh-untreated stored for 21 days (Figure 2b), whitening was apparent as a result of the natural evolution of the samples over storage. The reason of the color change is the formation of lignin as a healing process of wounded tissues (Cisneros-Zavallos et al., 1995). Besides, visible softness and moisture loss was also apparent in untreated roots as compared with fresh carrots on day 0. However, treated samples, whatever the elicitor used, maintained very similar color and apparent firmness to that of fresh-untreated sample on day 0 (Figures 2c and 2d). A comparison between MJ- and ABA-treated carrots reflected brighter color and visually higher water content for the carrots exposed to ABA.

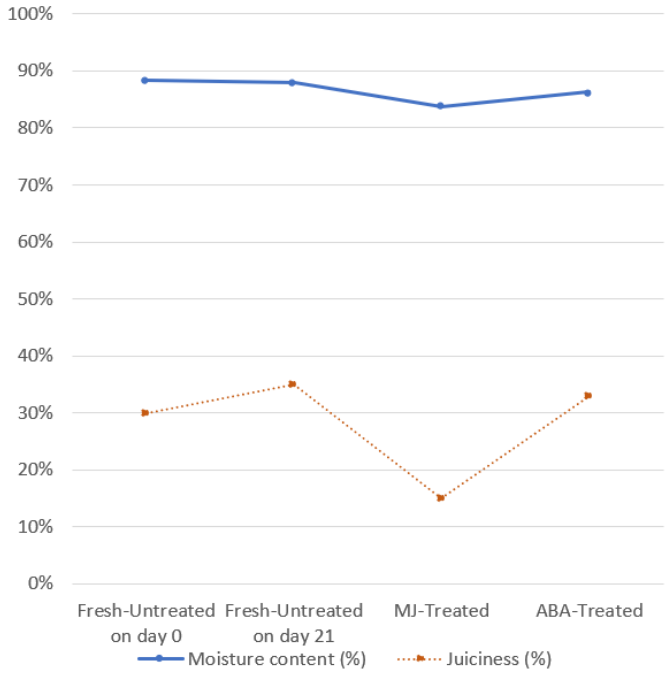

Figure 2. Purple carrot (*Purple Haze* variety): Fresh on the reception day (a), Untreated after storage (b), MJ-treated after storage (c), ABA-treated after storage (d).

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3.1.2. Physicochemical parameters

Figure 3 depicts moisture content and juiciness of purple carrot samples. As observed, both parameters maintained reasonably steady in most samples. Specifically, moisture content always ranged between 86.3 and 88.4% and juiciness between 30 and 35%. No significant ($p > 0.05$) influence of storage or of elicitation was found. An exception was found in MJ-treated samples, which displayed significantly ($p < 0.05$) lower moisture content (ie, 83.8%) and, in particular, juiciness (ie, 15 %).

Figure 4 represents TSS (% °Brix) (a) and acidity, expressed as TTA(%) and pH (b). As seen, TSS increased significantly ($p < 0.05$) in fresh-untreated purple carrots from day 0 (ie, °Brix 5.0%) to day 21 (ie, °Brix 5.65%). This reflects an increment of sugar content in carrot roots during the 21-day cold storage. The enhancement of sugars during ripening and storage has already been described in tubers (Senkumba et al., 2017; Hashem et al., 2014) and other vegetables (Al-Dairi et al., 2021). It is attributed to hydrolysis of polysaccharides into oligosaccharides and monosaccharides. It can also be due to the degradation of pectin substances into simple sugars occurring over ripening (Munhuewyi, 2012). The increment of TSS with storage diminished with elicitation, regardless the elicitor used (ie, 5.25% for MJ and 5.45% for ABA). No significant ($p > 0.05$) differences were found between both elicitors. Since TSS increment is related with ripening, the reduction in the TSS increment with elicitation indicates that the treatment with MJ or ABA is effective in delaying spoilage and extending, hence, purple carrot shelf-life.

From Figure 4 (b), TTA, measured as % malic acid, increased significantly ($p < 0.05$) in fresh-untreated purple carrots with storage (ie, from 0.08% on day 0 to 0.12% on day 21). The increase of TTA with storage here observed is in agreement with the findings earlier published by other researchers (Senkumba et al., 2017; Hashem et al., 2014). It is most like due to the formation of acids by oxidation of reducing sugars or by breakdown of pectic substance, which can be in turn converted into organic acids or simple sugars, as previously commented. Regarding treatment effect, the effect of both elicitors was similar. They both reduced TTA increase during the post-harvest period (ie, 0.10% for MJ-treated carrots and 0.07% for ABA-treated carrots). Although it is interesting to point out the higher impact of ABA than that of MJ. In fact, ABA-treated carrots provided TTA values similar to those measured in fresh-untreated carrots on day 0 (ie, 0.07% and 0.08%, respectively). The slowdown of TTA increase over storage as a consequence of elicitation supports the hypothesis above mentioned about the delaying effect of MJ and ABA on the decay rate.

It is also important to mention that the changes in TTA had a positive correlation with changes in pH values, which increased from 5.9 in fresh-untreated carrots on day 0 to 6.12 on day 21 whereas the treatment with MJ and ABA resulted in values of 6.01 and 6.04, respectively. These results are in accordance with those reported by Senkumba et al., 2017. Since acidity and pH are inversely proportion to each other, it is believed that protons are released from organic acid hydrolysis in such a way that the lower acid content in the sample, the higher hydrogen concentration.

Figure 5 depicts maturity index of purple carrot samples. Since maturity index is a sugar:acid (TSS:TTA) ratio, it decreased significantly ($p < 0.05$) in fresh-untreated samples over cold storage (ie, 62% on day 0 *vs* 49% on day 21). This patter is a direct result of the marked increase of TTA measured in fresh-untreated samples throughout the storage period. It is also interesting that the decrease in maturity index was not affected by MJ elicitation. In fact, no significant ($p > 0.05$) differences between MJ-treated samples and fresh-untreated carrots on day 21 were measured (ie, 49% *vs* 51%, respectively). On the contrary, the exposition of purple carrots to ABA resulted in a preservation of their maturity index over storage as compared with that of fresh-untreated carrots on day 0 (ie, 75% *vs* 62%, respectively).

In view of these results, the post-harvest elicitation of purple carrots by using ABA as an elicitor was effective in preserving moisture content, juiciness, sugar content and organic acid content throughout the cold storage for 21 days. Considering that sugar and organic acid contents determine the tasting quality of a product, elicitation with ABA seems a recommendable approach to preserve physicochemical quality of purple carrots during 21-day cold storage. Additionally, ABA treatment also provided satisfactory results in terms of visual appearance.

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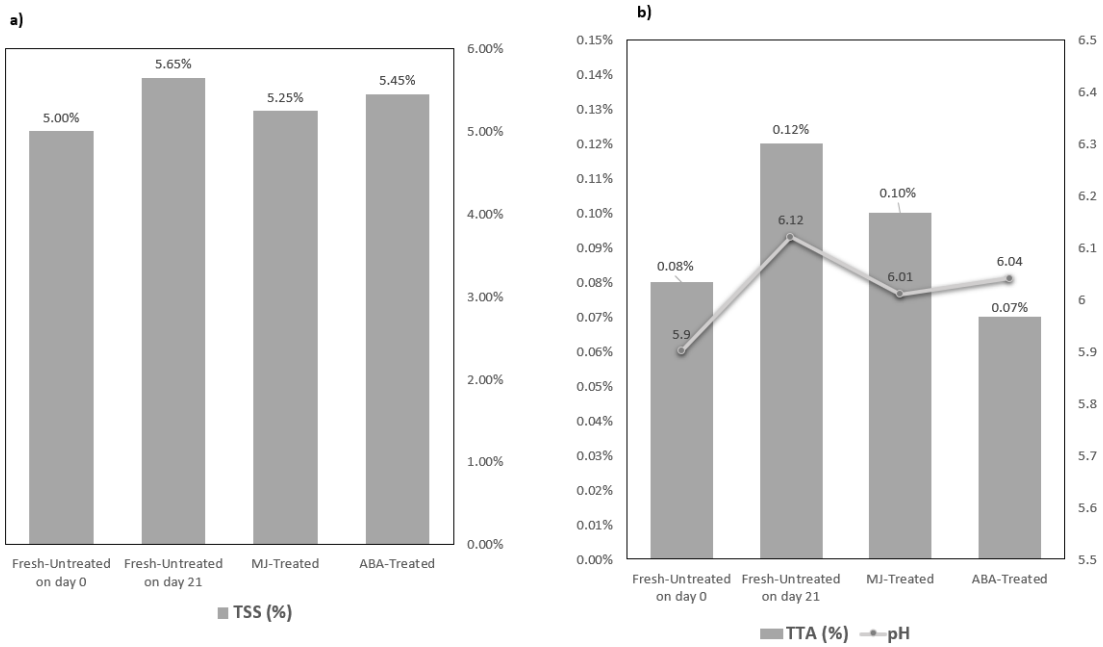

Figure 3. Moisture content (%) and juiciness (%) of fresh-untreated and treated purple carrot samples.

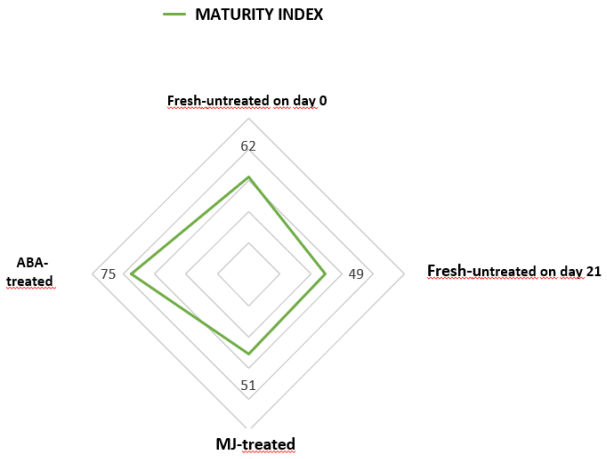

Figure 4. TSS (%) (a) and TTA (°Brix) and pH (b) of fresh-untreated and treated purple carrot samples.

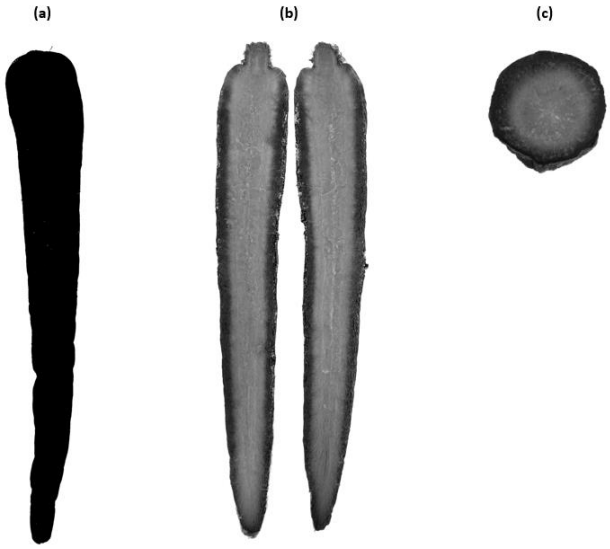

Figure 5. Maturity index of fresh-untreated and treated purple carrot samples.

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3.2. Health-related quality

Table 1 indicates TCC, expressed as mg EβC g^{-1} DW, and TAC, expressed as mg EC3G g^{-1} DW in fresh-untreated purple carrots on day 0 and day 21 and in MJ- and ABA treated samples, including their corresponding controls. Data are expressed as mean values (n = 2) ± SD. Different letters between purple carrot samples indicate differences at $p < 0.05$. From the table, it is interesting that TCC values in fresh-untreated purple carrots on day 0 (ie, 1.41 mg EβC g^{-1} DW) were similar to those reported in the literature for the typical orange-rooted carrots (ie, 1.29 mg g^{-1} DW) (Blando et al., 2021). As also observed, TCC increased significantly ($p < 0.05$) in fresh-untreated carrots after cold storage (from 1.41 mg EβC g-1 DW on day 0 to 3.79 mg EβC g-1 DW on day 21). Other authors have also stated an increase in typical orange carrot β-carotene during the first two or 21 days of refrigerated storage (Howard et al. 1999; Lee, 1986; Berger et al., 2008; Imsic et al., 2010). However, the reason why this increase occurs is not well known. It is speculated that either biosynthesis of carotenoids continues over storage or extractability of β-carotene improves because of the slow degradation of carrot matrix. In fact, it is possible that both mechanisms are playing in conjunction. On the one hand, carotenoid biosynthesis after harvest has been suggested in many climacteric fruits and tuberous vegetables other than carrots, e.g. tomatoes or sweet potatoes. On the other, it is known that carrot matrix is disaggregated with time and it is reasonable to believe that this disaggregation enables carotenoids to be released. In any case, it is obvious that the degradation of carotenoids is always slower than any of the two mechanisms above described. From a nutritional standpoint the results are interesting regardless the mechanism involved. There are several factors contributing to the accumulation of carotenoids in fresh-untreated purple carrots during the three-week cold storage such as relative humidity or chilling stress. In this respect, various pre-and postharvest factors (ie., high light, salinity, drought, cultivation practice, etc) have been reported to positively modulate the enhanced biosynthesis of carotenoids (Saini et al., 2018). However, all these studies also describe decrease in carotenoid content in longer storage periods and highlight the extreme importance of the storage conditions.

Table 1. TOTAL CONTENT OF BIOACTIVE COMPOUNDS.

	FRESH-UNTREATED		MJ TREATMENT		AA TREATMENT	
	On day 0	**On day 21**	**Control**	**Treated**	**Control**	**Treated**
TCC (mg EβC g^{-1}DW)	1.41±0.03a	3.79±0.05b	1.59±0.02a	1.61±0.03a	2.06±0.04c	2.15±0.03c
TAC (mg ECGg^{-1}DW)	2.23±0.05a	2.18±0.04a	0.43±0.02b	0.37±0.01b	0.26±0.01b	0.30±0.02b

Different letters between samples in the same row indicate differences at $p < 0.05$.

As also seen in Table 1, TAC in fresh-untreated purple carrots maintained steady over storage (from 2.23 mg EC3G g^{-1} DW on day 0 to 2.18 mg EC3G g^{-1} DW on day 21). As no bibliographic studies about the storage effect on anthocyanin-rich carrots have been, to our knowledge, carried out on purple carrot, no comparison with the literature could be properly accomplished. However, works on black carrot juice have revealed degradation of anthocyanins in cold environment in such a way that storage at 20°C is recommendable for longer shelf-life (Özen et al., 2009). Supporting this study, the decrease of anthocyanin content as a result of the refrigeration has also been observed by other researchers in plant-based foods other than purple carrots (Galani et al., 2017).

Regarding elicitation effect, the same trend was observed for TCC and TAC. In all cases, for both MJ and ABA, the comparison between the treated with their corresponding controls showed no significant differences ($p > 0.05$). This reflects the transference of the elicitor at some level from the treated half to the control half of the root. However, the statistical comparison of treated purple carrots by using any elicitor with fresh-untreated on day 21 exhibited a significant ($p < 0.05$) drop in both TCC and TAC. This confirms, once more, the hypothesis on the slowdown of the decay process as a result of the elicitation. Although, in principle, this is undesirable from a nutritional perspective, the sensory quality is essential for commercial purposes.

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Also, it is interesting to point out the results on TCC obtained from ABA treatment. From Table 1, although ABA treatment values decreased significantly ($p < 0.05$) with respect to those measured in fresh-untreated samples on day 21, the contents were still significantly ($p < 0.05$) higher than those on day 0 (2.15 mg EβC g-1 DW in ABA-treated *vs* 1.41 mg EβC g-1 DW in carrots on day 0). The potential of ABA as an elicitor is also supported by the fact that the organoleptic characteristics of ABA-treated carrots after storage were still adequate from a commercial standpoint.

Table 2 represents the HPLC-DAD analysis of carotenoids in fresh-untreated (on day 0 and day 21) and treated (with MJ and ABA) purple carrot samples. Data are expressed as mg EβC g^{-1} DW (n = 3) ± SD. Different letters between purple carrot samples indicate differences at $p < 0.05$. Quantification was carried out by interpolating the results of the samples in a calibration curve of β-carotene whose concentrations ranged from 10 μg mL^{-1} to 300 μg mL^{-1} ($r^2 = 0.9897$). As can be seen in Table 2, lutein and β-carotene were the carotenoids identified in the extracts, being the latter major. Some authors have also reported minor concentrations of α-carotene in certain dark-colored carrot cultivars (Macura et al., 2019; Arscott et al., 2010, Blando et al., 2021). However, its presence depends on the specific cultivar and part of the carrot-root used in the analysis (ie, core, cortex or a mixture). In general, the darker the cultivar is, the lesser amount of α-carotene. Similarly, the closer to the core, the lesser amount of α-carotene (Blando et al., 2021). For this reason, the absence of α-carotene is not surprising. In fact, reports in the literature have determined lutein and β-carotene as the main carotenoids present in black-purple carrots (Pérez et al., 2023). From Table 2, the concentrations of lutein and β-carotene estimated in fresh-untreated purple carrots on day 0 (ie, 0.23 mg EβC g^{-1} DW and 4.87 mg EβC g^{-1} DW, respectively), are in accordance with data described for dark carrot cultivars (Pérez et al., 2023; Macura et al., 2019). A comparison among the samples revealed, in general, similar trend for individual carotenoids as that of TCC. Fresh-untreated purple carrots on day 21 exhibited an increase of lutein and β-carotene with respect to fresh samples on day 0 (ie, 0.68 and 28.16 mg EβC g^{-1} DW on day 21 *vs* 0.23 and 4.87 mg EβC g^{-1} DW on day 0). Also, MJ-treated carrots did not show significant ($p < 0.05$) differences in the content of lutein and β-carotene with respect to fresh-untreated samples on day 0 (ie, 0.18 and 2.06 mg EβC g^{-1} DW in MJ treated *vs* 0.23 and 4.87 mg EβC g^{-1} DW in fresh samples on day 0). In contrast to the results on TCC, ABA treated purple carrots showed unexpectedly lutein and β-carotene contents similar to those estimated in fresh-untreated samples on day 0 (ie, and 0.16 and 4.93 mg EβC g^{-1} DW in ABA treated *vs* 0.23 and 4.87 mg EβC g^{-1} DW in fresh samples on day 0). This is probably due to the determination of additional analytes other than carotenoids together with lutein and β-carotene in the TCC assay.

Table 2. CAROTENOIDS BY HPLC-DAD.

	FRESH-UNTREATED		MJ TREATMENT	ABA TREATMENT
	On day 0	On day 21		
LUTEIN (mg EβC g^{-1}DW)	0.23±0.01a	0.68±0.03b	0.18±0.01a	0.16±0.02a
β-CAROTENE (mg EβC g^{-1}DW)	4.87±0.07a	28.16±0.10b	2.06±0.05c	4.93±0.09a

Different letters between samples in the same row indicate differences at $p < 0.05$.

Table 3 represents the antioxidant activity in terms of free radical scavenging activity, measured by DPPH assay, and the capacity to reduce ferric ion (Fe^{3+}) to ferrous iron (Fe^{2+}), measured by the FRAP assay. Data are expressed as mean values (n = 2) ± SD. Different lowercase letters between purple carrot samples indicate differences at $p < 0.05$. By comparing fresh-untreated samples on day 0 with day 21, the free radical scavenging activity did not vary significantly ($p > 0.05$) throughout the whole storage period (12.24 mg Trolox g^{-1} DW *vs* 12.71 mg Trolox g^{-1} DW) whereas the ferric reducing power showed a significant ($p < 0.05$) increase of the values obtained (17.57 mg Trolox g^{-1} DW on day 0 vs 30.03 mg Trolox g^{-1} DW on day 21).

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Table 3. ANTIOXIDANT ACTIVITY.

ASSAY	FRESH-UNTREATED		MJ TREATMENT		AA TREATMENT	
	On day 0	On day 21	Control	Treated	Control	Treated
DPPH (mg TROLOX g^{-1}DW)	12.24±0.06a	12.71±0.05a	4.73±0.02b	4.09±0.03b	4.25±0.02b	4.89±0.02b
FRAP (mg TROLOX g^{-1}DW)	17.57±0.08a	30.03±0.07a	7.38±0.02b	6.02±0.02b	6.37±0.04b	7.02±0.03b

Different letters between samples in the same row indicate differences at $p < 0.05$.

DPPH values were directly related with TAC results. This involves that anthocyanins were the main contributors to the free radical scavenging activity. From the results on fresh-untreated samples, it can be stated that the antioxidant activity of purple carrot root was not affected by the 21-day cold storage. Concerning the treated samples, no significant ($p > 0.05$) differences were stablished between the treated samples, whatever the elicitor, and their corresponding controls. Besides, the application of both MJ and ABA, resulted in a significant ($p < 0.05$) decrease of the antioxidant activity as compared with fresh-untreated samples by using either assay.

4. Conclusion

Storage of purple carrots at 5°C for 21 days resulted in an increase of carotenoid content as well as in the stability of anthocyanin content and antioxidant activity. However, sensorial quality in terms of sugar and organic acid contents and visual appearance reflected evident decay of the roots after the storage time. The elicitation of purple carrots with ABA enabled spoilage rate during the postharvest period to be reduced. As a consequence, organoleptic properties were preserved over the whole storage. However, the natural increase of carotenoids and invariability of anthocyanins initially observed in unelicited carrots were partially lost. In short, elicitation with ABA allowed to extent shelf-life of purple carrots over cold storage in exchange of the partial loss of health-promoting benefits. Since taste and firmness are imperative to ensure the commercial value of purple carrots, the aim now is to minimize anthocyanin loss in ABA treated purple carrots with a view to obtaining a long-lasting functional food. For that purpose, studies on purple carrots including combination of elicitors with additional storage conditions are scheduled.

Credit authorship contribution statement: Gracia Patricia Blanch and Maria Luisa Ruiz del Castillo designed the experiments. Laura Saéz-Escudero performed the experiments and analyzed the data. Gracia Patricia Blanch also contributed to the experimental work. Maria Luisa Ruiz del Castillo wrote the manuscript. Laura Saéz-Escudero, Gracia Patricia Blanch and Maria Luisa Ruiz del Castillo interpreted the data.

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Data Availability: The authors are unable or have chosen not to specify which data has been used.

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