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Effect of Cooking Methods on Bioactive Pigments in Purple Carrots (*Daucus carota* L.)

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Abstract: Carotenoids and anthocyanins are plant-based molecules that have shown potent antioxidant and anti-inflammatory activities contributing to human health. Purple carrots contain not only the carotenoids occurring in the typical orange carrots, but also a high content of anthocyanins, which makes them nutritionally more interesting. However, most of these bioactive compounds are partially lost during cooking. This study aimed to determine total carotenoid content (TCC), total anthocyanin content (TAC), total phenol content (TPC) and antioxidant activity (AA) in terms of DPPH and ABTS radical scavenging activity in cooked purple carrots. The identification of the main anthocyanins was also performed by HPLC-DAD. With a view to evaluating the effect of cooking, purple carrots were subjected to freeze-drying, boiling, steaming and roasting. To our knowledge, this is the first extensive study about the effect of various cooking methods on different parameters related to the beneficial health properties of purple carrots. As a result, freeze-drying brought about significant loss of carotenoids (TCCs 554.90 $\mu g \cdot g^{-1}$ vs. 1136.66 $\mu g \cdot g^{-1}$ in fresh carrots) whereas boiling resulted in a significant decrease of polyphenols (TPCs 15.71 $\mu g \cdot g^{-1}$ vs. 66.71 $\mu g \cdot g^{-1}$ in fresh carrots) and anthocyanins (TACs 1.41 $\mu g \cdot g^{-1}$ vs. 4.83 $\mu g \cdot g^{-1}$ in fresh carrots). The results in this paper can be used by the food industry to elaborate more nutritious purple-carrot-based products.

Keywords: purple carrot; anthocyanins; carotenoids; bioactive compounds; antioxidant activity; cooking methods

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1. Introduction

Carrots are one of most valuable vegetables in terms of commercial interest. Their nutritional value is widely known and is in part due to high content of carotenoids [1]. Conventional orange carrots contain α- and β-carotene and a small amount of lutein [2]. Carotenoids not only are responsible for the colors of red- and orange-colored fruits and vegetables, but also play an important role in human nutrition. They possess provitamin A activity and they are regarded as potent antioxidants. In addition, they are widely used as food colorants and precursors of aroma compounds by the food industry [3]. In addition to carotenoids, carrots are also rich in phenolic compounds, including anthocyanins, which also contribute to a majority of their in vitro antioxidant capacity [4].

In the last few years, awareness of the health benefits of anthocyanins has increased, resulting in the development of new vegetables enriched in anthocyanins, including purple carrots [5]. Among other benefits, anthocyanins have been linked to reduced risk of cardiovascular diseases [6] and cancer preventive properties [7]. The color of carrots indicates the different levels of carotenoids and anthocyanins occurring in them. Orange and yellow are usually related to the presence of β-carotene and lutein, respectively, whereas blue, purple and black are associated with anthocyanins. The more commonly known anthocyanins are based on six anthocyanidins: cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin. However, more than 500 different anthocyanins have been isolated from plants [8]. In the case of purple carrots, the main anthocyanins are all derived from cyanidin being essentially acylated [9].

Although carrots can be consumed fresh, they are frequently consumed cooked to extend their shelf-life and preserve their quality. Moreover, the high content of terpenes in carrots causes them to have a strong, harsh smell when consumed fresh [10], which is usually unacceptable, especially by children. Among the most common cooking procedures applied to carrots, the following can be highlighted" freeze-drying, boiling, steaming and roasting. Bibliographic reports indicate that all these procedures lead to relevant improvements in the sensory quality since they reduce the brightness, redness or yellowness color saturation and taste, which has a direct influence on carrots' acceptability to consumers. However, these methods can in turn result in the loss and/or alteration of the most unstable constituents. In this regard, it is known anthocyanins are particularly temperature labile, and they are easily affected by exposure to heat and light. Several studies have demonstrated that anthocyanins occurring in pigmented foods other than purple carrots decrease by about 80% after cooking [11].

In the present research, we evaluated the impact of cooking on pigmented bioactive constituents as well as on the AA in purple carrots (*Daucus carota* L.). To that end, we studied the effects of freeze-drying, boiling, steaming and roasting on the content of carotenoids, phenolics and, particularly, anthocyanins. The impact on free radical scavenging activity as measured by DPPH and ABTS assays was also considered. Although the influence of different cooking methods on bioactive compounds including anthocyanins has been reported in other purple-pigmented foods [12–14], a very limited study has been carried out on carrots [15]. In this work, an extensive study considering various cooking methods as well as various parameters related to the health-promoting properties of purple carrots is, to our knowledge, addressed for the first time.

2. Materials and Methods

2.1. Materials and Chemicals

HPLC-grade methanol (MeOH), acetone and acetonitrile (ACN) were purchased from Macron Fine Chemicals, Gliwice, Poland). Hexane and chloroform were obtained from LabScan (Bangkok, Thailand). Ultrapure water was obtained from a purification system (Macron Fine Chemicals, Gliwice, Poland). Further, 2,2-diphenyl-2-picrylhydrazil (DPPH) and 2,2′-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) reagents and gallic acid (GA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sodium carbonate, potassium chloride, potassium persulfate and sodium acetate anhydrous standards were supplied by Sigma-Aldrich (Steinheim, Germany). Sodium sulphate anhydrous was obtained from Quality Chemicals S.L. (Barcelona, Spain); as well, ethanol (EtOH), pentane, cyclohexane, formic acid, acetic acid glacial and hydrochloric acid (HCl) were acquired from Scharlau Chemie S.A. (Barcelona, Spain). Folin-Ciocalteu reagent was acquired from Merck (Darmstadt, Germany), and kuromanin chloride standard (cyanidin-3-*O*-glucoside, C3G) was obtained from Extrasynthese (Genay, France).

Fresh purple carrots (*Purple Haze* variety) with 3–5 cm diameters and no scars or split roots were supplied by the local supermarket (Madrid, Spain). Immediately after acquisition, water content on a mass basis was calculated gravimetrically, and then the carrots were frozen at −20 °C until their use.

2.2. Cooking Methods

Freeze-drying, boiling, steaming and roasting were chosen as cooking procedures because of their usual application on raw carrots. Before cooking, frozen carrots were, in all cases, sliced into pieces that were 0.5 cm thick and then immediately cooked without previous defrosting. Freeze-drying treatment was accomplished using a Beta 2-8LD plus freeze-dryer (Christ, Staufen, Germany) at room temperature for 5 days. Boiling was performed by adding carrot slices (200 g) to 1 L of boiling tap water in a covered glass container and home cooking for 10 min. The remaining water after boiling was collected, lyophilized and analyzed to evaluate the possible loss of phenolics. Steaming was carried out at 100 °C by placed a 200 g weight of carrot slices in a steamer (i.e., a pot with a lid

with holes in it that fits inside another pot) for 10 min. Roasting was carried out using a conventional air oven (Combi Master CM6, Rational, Ibérica, Barcelona, Spain). Prior to the actual roasting, the oven was preheated at the set temperature before inserting the carrots. Different roasting times were tested (i.e., 10, 20 and 30 min). Then, purple carrot slices (250–350 g, depending on the baking time) were put on baking paper and inserted into the oven at 100 °C for the selected time. All cooking procedures were performed in duplicate. Then, the carrot slices were all vacuum-packed and frozen at −20 °C without exposure to light until their analyses. Extraction of carotenoids, polyphenols and anthocyanins from the carrot samples was accomplished by following the same procedure regardless of the cooking method used.

2.3. Extraction and Analysis of Carotenoids

A 20 mL volume of acetone was added to 1 g of uncooked and cooked purple carrots. The mixture was then homogenized using an Ultra-Turrax (T18 Digital, IKA, Staufen, Germany) for 5 min and subsequently passed through a filter paper. The remaining extract was swept with an extra 20 mL of acetone until the orange/yellow colors were completely removed and the sample became colorless. Then, a 25 mL volume of hexane was added and shaken into the mixture. For phase separation, water (approx. 200 mL) was added without splashing to reducing emulsion formation. After letting it settle down, the aqueous phase was discarded and the hexane layer containing carotenoids was up to 50 mL, removing possible amounts of surfactant-like compounds with sodium sulphate anhydrous. The absorbance was immediately measured at 485 nm. The quantification of TCC was carried out using the extinction coefficient ($E_{1\%}$ = 2500) [2]. Data were expressed as micrograms of β-carotene equivalents per g of dry weight (DW). All the analyses were carried out in duplicate.

2.4. Extraction of Total Polyphenols and Anthocyanins

A 50 g weight of fresh and cooked purple carrots was subjected to extraction with 50 mL EtOH:H_2O (1:1, *v*/*v*), containing 0.01% HCl (37% *v*/*v*). The mixture was stirred for 2 h at room temperature, with previous homogenization by using an Ultra-Turrax. It was then vacuum-filtered with a Büchner funnel. To remove interferences, the filtered extract was purified with chloroform (4 × 25 mL), pentane (4 × 25 mL) and cyclohexane (4 × 25 mL). The aqueous fraction was passed through a 0.45 µm filter and concentrated in a rotary evaporator at 30 °C until reaching a final volume of 25 mL. The resulting extract was stored at −20 °C in the absence of light. Each sample was extracted in duplicate. The extracts obtained were used for the determination of TPC, TAC and individual anthocyanins by HPLC-DAD and AA assays, as explained below.

2.5. Total Phenol Content (TPC)

TPC measurements in the extracts were performed by following the Folin–Ciocalteu method [16]. A BioTek Synergy Mx multi-mode microplate reader with BioTek's Gen 5TM software (BioTek Instruments Inc., Winooski, VT, USA) and 96-well plates (Thermo Fisher Scientific, Madrid, Spain) were used. In vials, 200 µL of the suitable dilution of the extracts, 1600 µL of distilled water and 400 µL of Folin-Ciocalteu reagent were added. The mixture was stirred in a vortex for 15 s and left in the dark for 5 min. Then, 800 µL of a sodium carbonate solution (75 $g \cdot L^{-1}$) was added and the mixture was stirred again for 15 s and left in the dark for 1 h. Finally, the absorbance was measured at 750 nm. The same mixture without extract (using 200 µL of distilled water instead) was used as a blank, and the total phenolic concentration was calculated from a calibration curve using GA as a standard. The results were expressed as micrograms of GA equivalents per g of DW. All analyses were carried out in quadruplicate.

2.6. Anthocyanins

2.6.1. Total Anthocyanin Content (TAC)

The TAC was determined by applying the pH differential method [17]. In brief, the uncooked and cooked purple carrot extracts were diluted with 0.025 M potassium chloride buffer solutions at pH 1 and with 0.4 M sodium acetate buffer at pH 4.5. A spectrophotometer (Beckman Coulter DU-800 spectrophotometer, Barcelona, Spain) was used to carry out the measurements and a 520 and 700 nm sweep was carried out. All the analyses were carried out in duplicate. The TAC values obtained were expressed as micrograms of C3G equivalents per g of DW. A molecular weight of 449.4 $g \cdot mol^{-1}$ and a molar extinction coefficient of 26,900 $L \cdot mol^{-1} \cdot cm^{-1}$ were used to estimate the TAC values, and the following equation was applied to calculate the total absorbance:

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

2.6.2. Individual Anthocyanins by HPLC-DAD

The content of individual anthocyanins in uncooked and cooked 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 of anthocyanins was performed on a 250 mm × 4.6 mm ID reversed-phase C_{18} column (particle size 5 µm, ACE, Madrid, Spain) at a flow rate of 1 $mL \cdot min^{-1}$. An Altima 5-µm C_{18} pre-column and column operated at 25 °C. The solvents were (A) water/formic acid (9:1) and (B) water/acetonitrile/formic acid (6:3:1). The gradient changed from 80/20 (A/B) at the beginning of the analysis to 15/85 (A/B) for 70 min up to the end. Then, it was stabilized in the initial condition for another 20 min. The detection was carried out at 520 nm using a diode array detector (DAD). The chromatographic signals were registered using Empower2 software (Waters, Mildford, CT, USA). Identification of the anthocyanins was carried out tentatively by comparison with bibliographic reports [18]. The quantification was performed using calibration curves of C3G, which was run under the experimental conditions indicated by the supplier. In brief, the solvents were water with 0.1% formic acid (A) and methanol (B). A linear gradient was applied from 95/5 (A/B) to 40/60 (A/B) for 20 min and then from 40/60 (A/B) to 100% B for another 5 min. The results were expressed as microgram C3G equivalents per g of DW. The range of the linear calibration curves was from 0.5 $mg \cdot L^{-1}$ to 1000 $mg \cdot L^{-1}$. All analyses were accomplished in duplicate.

2.7. Antioxidant Activity (AA)

The AA in terms of free radical scavenging activity was determined in the extracts by the DPPH• [19] and ABTS• [20] assays with slight modifications. In both cases, a BioTek Synergy Mx multi-mode microplate reader with BioTek's Gen 5TM software (BioTek Instruments Inc., Winooski, VT, USA) and 96-well microplates (Thermo Fisher Scientific, Madrid, Spain) were used for the analyses. The AA data were always calculated by interpolating the results into a Trolox standard curve ranging between 0 and 1000 $\mu mol \cdot L^{-1}$. When necessary, the concentration of the extracts was diluted to be adapted to this linear range. For both assays, the results were expressed as milligrams of Trolox equivalents per g of DW. All analyses were performed in quadruplicate. The specific experimental details for each assay were as follows:

2.7.1. DPPH• Assay

For these determinations, 200 µL of DPPH• reagent (60 µM) was added to 10 µL of the sample in each well. The mixtures were then incubated for 60 min in absence of light, and the absorbance was measured at 515 nm. The value of absorbance obtained from the DPPH• reagent solution was used as a reference.

2.7.2. ABTS Radical Scavenging Activity

The ABTS radical cation ($ABTS^{\bullet+}$) was created with a stock solution (7 mM) in 2.45 mM potassium persulfate and placing the mixture under stirring in the dark at room temperature for 12 h before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. A 10 µL-volume of each extract was mixed with 200 µL of the ABTS radical in each well. After incubation at 30 °C for 20 min, the absorbance at 734 nm was measured.

2.8. Statistical Analysis

An analysis of variance for TCC, TPC, TAC, DPPH and ABTS data was carried out using the 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 purple carrots subjected to different cooking methods were statistically compared with fresh carrot data, which were used as a reference. The Fisher's protected LSD method was used for comparison. Differences were considered significant at $p < 0.05$.

3. Results and Discussion

Figure 1 depicts purple carrots subjected to different cooking methods. Table 1 represents the water content of purple carrots estimated gravimetrically after cooking.

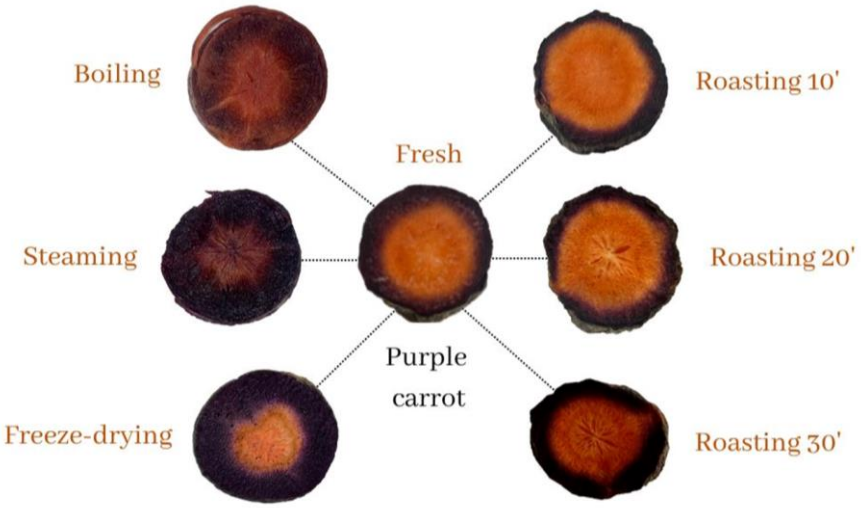

Figure 1. Purple carrots (*Purple Haze* variety) cooked by different methods.

Table 1. Water content (%) of purple carrots cooked by different methods.

COOKING METHOD	WATER CONTENT (%)
Fresh	85.3
Freeze-drying	14.1
Boiling	93.0
Steaming	86.4
Roasting (10 min)	81.2
Roasting (20 min)	73.0
Roasting (30 min)	58.5

Table 2 summarizes TCC (µg β-carotene equivalent g^{-1} DW), TPC (µg Trolox equivalent g^{-1} DW) and TAC (µg C3G equivalent g^{-1} DW) in purple carrots cooked by different procedures. Data are expressed as mean values ($n = 2$) ± SD. Different lowercase letters between the sample subjected to each cooking method and fresh carrots (used as a reference) indicate differences at $p < 0.05$. First, it is interesting to highlight the contents of bioactive

pigments in fresh purple carrots. As seen in the table, particularly high TCC was measured in uncooked carrots (i.e., 1136.66 μg·g^{-1} DW). Contradictory results on TCC in purple carrots have been reported in the literature. Whereas some authors have found considerably lower TCC in purple carrots than in typical orange varieties [4], others have described twice the α- and β-carotene content in purple carrots as compared with orange carrots [21]. In any case, carotenes in carrots have been reported to vary within a wide range depending on various factors such as genotype, climatic factors, degree of ripening, etc. [22]. From Table 2, TPC and TAC values measured in the fresh material were slightly lower than those reported in the literature for purple carrots [18,23]. In fact, TPC data were closer to those published for the typical orange carrots than for purple carrots [18].

Table 2. Effect of cooking on the antioxidant pigments of purple carrots. Different lowercase letters between the sample subjected to each cooking method and fresh carrots (used as a reference) indicate differences at $p < 0.05$.

	TOTAL CONTENT OF BIOACTIVE PIGMENTS		
UNCOOKED	**TCC (μg β-carotene g^{-1} DW)**	**TPC (μg GA g^{-1} DW)**	**TAC (μg C3G g^{-1} DW)**
Fresh	**1136.66 ± 0.08 a**	**66.71 ± 0.08 a**	**4.83 ± 0.16 a**
COOKING METHOD			
Freeze-drying	554.90 ± 0.02 b	2919.02 ± 0.03 b	364.78 ± 0.11 b
Boiling	3245.61 ± 0.07 b	15.71 ± 0.11 b	1.41 ± 0.06 b
Steaming	2061.95 ± 0.01 b	82.49 ± 0.05 a	7.79 ± 0.14 b
Roasting (10 min)	1596.76 ± 0.04 b	99.65 ± 0.01 b	4.73 ± 0.12 a
Roasting (20 min)	1292.47 ± 0.05 a	199.40 ± 0.01 b	10.99 ± 0.18 b
Roasting (30 min)	1247.22 ± 0.03 a	317.82 ± 0.01 b	18.72 ± 0.11 b

Regarding the influence of cooking, it was seen that different effects were observed according to the method used. Freeze-drying resulted in significant ($p < 0.05$) losses of TCC (51.2% off) when compared with fresh carrots. It is possible that the accessibility of carotenoids decreases as a consequence of the extremely low water content in freeze-dried samples. Although freeze-drying is a well-known technique to preserve food quality, optimization of variables is regarded as essential to avoid losses of bioactive phytochemicals [24]. However, TPC and TAC values increased significantly ($p < 0.05$) in freeze-dried carrots with respect to the fresh material (from 66.71 to 2919 μg·g^{-1} DW and from 4.83 to 364.78 μg·g^{-1} DW, respectively). The promoting effect of freeze-drying on phenolics has already been observed in other vegetables [25]. It is believed that freeze-drying brings about structural changes during the freezing in such a way that different metabolic pathways may lead to the conversion of other compounds into phenolic compounds and their accumulation. Further, large molecular weight phytochemicals might break down into smaller compounds, which can increase the measurements. In contrast to freeze-drying, boiling resulted in the highest values of TCC (3245.61 μg·g^{-1} DW) but the lowest values of TPC (15.71 μg·g^{-1} DW) and TAC (1.41 μg·g^{-1} DW) out of all the cooking methods. In fact, the drop of TPC (from 66.71 to 15.71 μg·g^{-1} DW) and TAC (from 4.83 to 1.41 μg·g^{-1} DW) measured in boiled purple carrots was particularly significant ($p < 0.05$) as compared with uncooked samples. These results are directly associated with the different polarity of the pigments studied. Whereas polyphenols including anthocyanins are basically polar compounds and, therefore, removable by water, carotenoids are non-polar and, therefore, stable in an aqueous environment. In order to evaluate the loss of polyphenols in general and anthocyanins in particular, the water resulting from the cooking was analyzed for TPC and TAC. As a result, values of TPC and TAC of 10.30 ± 0.14 mg GA g^{-1} DW and 1.60 ± 0.15 mg C3G g^{-1} DW, respectively, were obtained in the cooking water. This con-

firms the major transference of polyphenols including anthocyanins from the samples to the aqueous medium. Both the increase of carotenoids and the reduction of polyphenols after boiling have been described in both orange [26] and purple [15,27] carrots. Actually, maximum concentrations of α- and β-carotene have been found after 20–30 min boiling as compared with fresh vegetables. Although there are also a few studies that describe opposite results (i.e., decrease of carotenoids [28] and increase of TPC values after boiling [29]), the values shown here support the substantial loss of water-soluble bioactive compounds as a consequence of boiling. It is interesting that steaming, despite also resulting in an increase of TCC (from 1136.66 $\mu g \cdot g^{-1}$ to 2061.95 $\mu g \cdot g^{-1}$), did not bring about the loss of either TPC (from 66.71 $\mu g \cdot g^{-1}$ to 82.49 $\mu g \cdot g^{-1}$) or TAC (from 4.83 $\mu g \cdot g^{-1}$ to 7.79 $\mu g \cdot g^{-1}$). This suggests that steaming is a less aggressive cooking method than boiling.

As far as roasting is concerned, it not only preserved naturally occurring carotenoids in uncooked purple carrots, but also led to a significant ($p < 0.05$) increase of all bioactive pigments. This increase was higher for carotenoids when the roasting time was as short as 10 min. However, TPC and TAC exhibited even higher values when the roasting times were longer than 10 min. It is known that the heat treatment of vegetables can cause two opposite effects. On the one hand, it can induce the thermal degradation of phytochemicals, and on the other hand, it can increase the extractability of the compounds as a result of matrix softening [30]. Our results evidenced that, under the roasting conditions here applied, all studied pigments, including carotenoids and anthocyanins, not only were not degraded, but their formation was slightly stimulated. This phenomenon has also been observed in previous studies on purple-corn-derived products [31]. Although the heating temperature applied was 100 °C, it is believed that the slight formation of non-enzymatic browning compounds might occur during the roasting. These compounds are able to stabilize anthocyanins by interaction with the resulting aglycone during thermal degradation.

All in all, boiling was the cooking method with the highest ability to release carotenoids from purple carrots, whereas freeze-drying would be recommendable in terms of TPCs and TACs. That said, it is also important to bear in mind the relatively high contents in bioactive pigments, in general, in roasted purple carrots. This, together with the palatability attributed to roasting, makes this cooking method an attractive procedure, from both sensorial and nutritional standpoints.

Table 3 represents the individual anthocyanins identified and quantified in fresh purple carrots (i.e., uncooked) and cooked by different methods by HPLC-DAD. Data are expressed as mean values ($n = 2$) ± SD. The HPLC chromatograms corresponding to the anthocyanin profile of purple carrots cooked by different methods are depicted in Figure 2. From Table 3 and Figure 2, it is observed that purple carrots contain basically acylated anthocyanins. The major anthocyanins were cyanidin-based, containing different sugar moieties non-acylated (numbered as 1 and 2), or acylated with sinapic acid (number 3), ferulic acid (number 4) and coumaric acid (number 5). The predominant anthocyanin was cyanidin-3-xylosyl-glucosyl-galactosyl acylated with ferulic acid (numbered as 4), which is typical of purple carrots. Its content was 9.76 $\mu g \cdot g^{-1}$ DW in fresh purple carrots and represented more than 50% of the total anthocyanins in all cases. Both the anthocyanin qualitative profile and the total content measured in fresh purple carrots agreed well with the literature [18,32]. It is worth mentioning that the total amount of anthocyanins measured was in general higher than the TAC values previously observed (see Table 2). This is most likely due to interferences in the TAC method, which might prevent anthocyanins from exhibiting. As expected, the effect of the different cooking methods on individual anthocyanins was the same as that previously observed in TAC (see Table 2). As seen in both Table 3 and Figure 2, boiling resulted in major losses of anthocyanins (5.72 $\mu g \cdot g^{-1}$ vs. 12.56 $\mu g \cdot g^{-1}$ in fresh carrots) while freeze-drying was the method that enabled them to be preserved the most. In fact, total anthocyanins in freeze-dried carrots increased considerably with respect to the reference value measured in the fresh material (1937.39 $\mu g \cdot g^{-1}$ vs. 12.56 $\mu g \cdot g^{-1}$). Similar to TAC, steaming did not lead to the loss of anthocyanins. This reflects that steaming is a procedure gentle enough for anthocyanins not to be degraded. Once more, roasting also

represented a more moderate option, particularly when applied for 20 and 30 min. As seen, anthocyanins did not increase as much as they did after freeze-drying (i.e., 1937.39 $\mu g \cdot g^{-1}$), but their improvement was in any case noteworthy (i.e., 103.75 and 198.21 $\mu g \cdot g^{-1}$ after roasting at 20 and 30 min, respectively, vs. 12.56 $\mu g \cdot g^{-1}$ in uncooked samples). These results are also in accordance with those reported elsewhere [18].

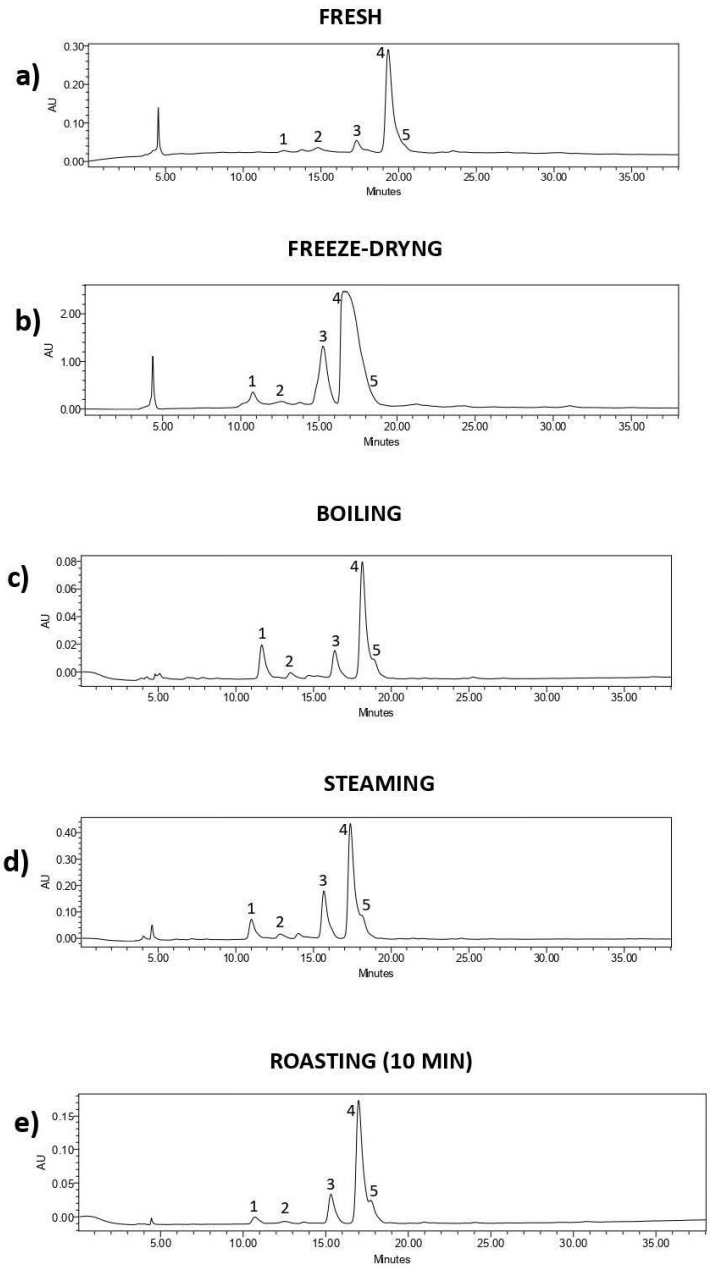

Figure 2. HPLC chromatogram of the purple carrots cooked by different methods recorded at 520 nm (**a**–**e**). Anthocyanin identification: 1. Cyanidin 3-xylosylglucosylgalactoside, 2. Cyanidin 3-xylosylgalactoside, 3. Sinapic acid derivative of cyanidin 3-xylosylglucosylgalactoside, 4. Ferulic acid derivative of cyanidin 3-xylosylglucosylgalactoside, 5. Coumaric acid derivative of cyanidin 3-xylosylglucosylgalactoside.

Table 4 indicates the AA in terms of free radical scavenging activity measured by DPPH (mg Trolox equivalent g^{-1} DW) and ABTS (mg Trolox equivalent g^{-1} DW) assays. Data are expressed as mean values (n = 2) ± SD. Different lowercase letters between the sample subjected to each cooking method and fresh carrots (used as a reference) indicate

differences at $p < 0.05$. In view of Table 4, the results obtained from both DPPH and ABTS assays were in good agreement. Freeze-drying resulted in a significant ($p < 0.05$) increase of the AA with respect to raw purple carrots (6.44 and 5.26 mg·g^{-1} DW vs. 0.16 and 0.13 mg·g^{-1} DW). Actually, freeze-dried carrots were by far the samples with the highest radical scavenging activity, followed by the roasted carrots. Regarding roasting, it is worth mentioning that the longer the roasting time, the higher the AA. Steaming did not have any effect on the AA, whereas boiling, as expected, produced a significant ($p < 0.05$) decrease of the radical scavenging activity (0.04 and 0.03 mg·g^{-1} DW vs. 0.16 and 0.13 mg·g^{-1} DW in fresh carrots). AA losses after boiling have been reported in purple foods other than carrots [12,13] and pigmented carrots [15]. A possible correlation between the content of bioactive pigments (Table 2) and the AA as measured by both assays (Table 4) was assessed by linear regression. The results obtained are shown in Table 5. As observed, TPC and TAC were directly correlated ($r^2 > 0.99$ in all cases) with the free radical scavenging activities of the carrots cooked by different methods. In contrast, no correlation was established between TCC values and those provided by DPPH and ABTS assays. This reflects that polyphenols, and particularly anthocyanins, are mainly responsible for the AA measured in purple carrots regardless of the cooking method used. In this respect, although carotenoids are efficient antioxidants scavenging singlet molecular oxygen radicals, anthocyanins act on a regular basis as an H-atom donator or in a single electron transfer. Both of them are part of the antioxidant defense system in the human organism, but anthocyanins may prevent inflammation and protect against type 2 diabetes, cancer and heart disease. All in all, a balanced diet including carotenoid- and anthocyanin-rich foods contributes to benefiting human health.

Table 3. Effect of the cooking on anthocyanins identified and quantified by HPLC-DAD.

Peak	Fresh (μg·g^{-1} DW)	Freeze-Drying (μg·g^{-1} DW)	Boiling (μg·g^{-1} DW)	Steaming (μg·g^{-1} DW)	Roasting (10 min) (μg·g^{-1} DW)	Roasting (20 min) (μg·g^{-1} DW)	Roasting (30 min) (μg·g^{-1} DW)
1	0.22 ± 0.09	83.06 ± 0.03	0.89 ± 0.02	3.35 ± 0.01	0.67 ± 0.01	8.62 ± 0.21	16.93 ± 0.11
2	0.66 ± 0.11	51.60 ± 0.20	0.16 ± 0.04	1.06 ± 0.16	0.36 ± 0.01	2.45 ± 0.47	3.95 ± 0.19
3	0.96 ± 0.20	380.39 ± 0.03	0.78 ± 0.06	7.03 ± 0.25	2.40 ± 0.01	26.36 ± 0.14	48.13 ± 0.03
4	9.76 ± 0.13	1017.23 ± 0.01	3.26 ± 0.14	20.32 ± 0.05	10.19 ± 0.01	52.23 ± 0.01	115.66 ± 0.18
5	0.96 ± 0.03	405.11 ± 0.10	0.64 ± 0.18	3.63 ± 0.21	1.89 ± 0.01	14.09 ± 0.35	13.55 ± 0.07
Total	12.56	1937.39	5.72	35.40	15.51	103.75	198.21

Table 4. Effect of cooking on antioxidant activity. Different lowercase letters between the sample subjected to each cooking method and fresh carrots (used as a reference) indicate differences at $p < 0.05$.

	ANTIOXIDANT ACTIVITY	
UNCOOKED	**DPPH (mg Trolox g^{-1} DW)**	**ABTS (mg Trolox g^{-1} DW)**
Fresh	0.16 ± 0.01 a	0.13 ± 0.01a
COOKING METHOD		
Freeze-drying	6.44 ± 0.08 b	5.26 ± 0.07 b
Boiling	0.04 ± 0.02 b	0.03 ± 0.02 b
Steaming	0.20 ± 0.02 a	0.16 ± 0.02 a
Roasting (10 min)	0.31 ± 0.08 b	0.25 ± 0.07 b
Roasting (20 min)	0.54 ± 0.10 b	0.43 ± 0.09 b
Roasting (30 min)	0.86 ± 0.07 b	0.69 ± 0.07 b

Table 5. Correlation by linear regression between total pigment contents and AA.

Total Pigment Content/AA Assay	Linear Regression Equation	Coefficient (r^2)
TCC/DPPH	y = −218.41x +1857.6	0.3457
TCC/ABTS	y = −266.86x + 1855.8	0.3449
TPC/DPPH	y = 456.76x − 29.47	0.9994
TPC/ABTS	y = 558.78x − 26.36	0.9995
TAC/DPPH	y = 58.089x − 11.85	0.9936
TAC/ABTS	y = 71.012x − 11.47	0.9940

4. Conclusions

The procedure used during cooking had a deep impact on the content of the beneficial-to-health pigments and free radical scavenging activity of purple carrots. Freeze-drying resulted in the increase of polyphenols including anthocyanins, but it led to the loss of carotenoids. On the contrary, both boiling and steaming caused an increase of carotenoids, although only boiling resulted in significant losses of polyphenols. Roasted carrots, however, exhibited a more balanced result. On the one hand, roasting did not result in particularly high contents of either carotenoids or polyphenols, but on the other hand, no relevant losses of any of the studied pigments were observed. This fact has also been reported in other purple-pigmented foods [31]. The content of polyphenols correlated directly with the free radical scavenging activity of purple carrots, while a direct correlation between carotenoids and the antioxidant capacity was not observed. This reveals that polyphenols, mainly anthocyanins, are the main contributors to the AA of purple carrots, whatever the cooking method used. Overall, freeze-dried and roasted purple carrots exhibited higher AA than carrots subjected to boiling and/or steaming. However, each cooking method possesses advantages and drawbacks in such a way that the selection of one or another will depend, to a great extent, on the sought objective.

In view of the results of this work, the purpose now is to study in depth the effect of roasting on purple carrots. For that purpose, new variables such as the influence of moisture and type of oven, together with heating time and temperature, will be considered. In addition, vitamins, aroma compounds and sensorial evaluation will also be included. Future studies estimating the antioxidant and anti-inflammatory properties of the digested fractions after cooking are also scheduled.

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