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# Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots

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#### Abstract

Ready-to-eat shredded orange and purple carrots, packed in air (control), or in modified atmosphere packaging [MAP;  $(90\%N_2 + 5\%O_2 + 5\%CO_2$  and  $95\%O_2 + 5\%CO_2$ ], and stored chilled for up to 13 days, were examined for their antioxidant activity and contents of anthocyanins, carotenoids and phenolics, as well as sensory quality. Total antioxidant activity, carotenoids and phenolics of purple carrots were initially 2.8-, 2.3- and 2.9-fold higher than orange carrots, respectively. Total antioxidant activity remained relatively constant in orange carrots during storage under all treatment conditions, whereas a highly significant decrease  $(P < 0.01)$  in (95%O<sub>2</sub> + 5%CO<sub>2</sub>)-treated purple carrots was observed. The content of anthocyanin, only found in purple carrots, was decreased slightly during the storage period, and this was particularly significant in the  $95\%O_2 + 5\%CO_2$  treatment. In both orange and purple carrots, loss of total carotenoids occurred in the  $95\%O_2 + 5\%CO_2$  treatment. Total phenolic content of purple carrots increased at a much higher rate during storage than orange carrots. The MAP treatment (90%N<sub>2</sub> + 5%O<sub>2</sub> + 5%CO<sub>2</sub>) gave better sensory quality and extended shelf-life for purple carrots ( $\sim$ 2–3 days longer shelf-life than other treatments,) but, no difference was observed for orange carrots. Shredded purple carrot can be stored under  $90\%N_2 + 5\%CO_2 + 5\%O_2$  treatment for up to 10 days and high nitrogen treatment may be used in maintaining the storage quality of shredded purple carrots. Thus, purple carrots may be used in place of orange carrots to take advantage of their nutraceutical components.

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Keywords: Orange and purple carrots; MAP; Antioxidant activity; Anthocyanins; Carotenoids; Phenolics; Sensory assessment

#### 1. Introduction

Epidemiological and clinical investigations have associated diets rich in fruits and vegetables with reduced risk of heart, cardiovascular, neurological and chronic diseases, and various forms of cancer (Ames, Shigenaga, & Hagen, 1993; Block, Patterson, & Subar, 1992; Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Joseph et al., 1999; Steinmetz & Potter, 1991; Surh, 2003). A major benefit from such diets may be increased consumption of antioxidants (Ames et al., 1993), including carotenoids, ascorbate, tocopherols and phenolics. Among phenolics, flavonoids are potent in vitro antioxidants (Cao, Sofic, & Prior, 1997; Rice-Evans, Miller, & Paganga, 1996; Wang, Cao, & Prior, 1997). Flavonoids include different groups of flavones, isoflavones, flavonols, flavononones, catechins, and the pink, red, purple and blue pigments known as anthocyanins (Mazza & Miniati, 1993; Shahidi & Naczk, 2004). Interest in anthocyanins has increased because they are potential natural alternatives to artificial colorants in the food and pharmaceutical industries (Bridle & Timberlake, 1997; Francis, 1989; Giusti, Rodrıguez-Saona, Baggett, Reed, Durst, & Wrolstad, 1998). In

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addition, apart from their food colorant properties, anthocyanins have many positive health benefits, such as reduction of risk of coronary heart disease, improved visual acuity, antioxidant activities and anticancer activities (Kamei et al., 1995; Tamura & Yamagami, 1994; Timberlake & Henry, 1988; Waterhouse, 1995).

Fruits and vegetables are rich sources of different phytonutrients, many of which have antioxidant properties (Prior et al., 1998). Research has shown that fruits and vegetables contain other phytonutrients, in addition to well-known antioxidants, such as vitamins C and E, or  $\beta$ carotene, which significantly contribute to their total antioxidant activity (Cao, Sofic, & Prior, 1996; Wang, Cao, & Prior, 1996). Among vegetables, carrots are now increasingly consumed, mainly due to their pleasant flavour and perceived health benefits related to their vitamins, minerals and dietary fibre (Alasalvar, Grigor, Zhang, Quantick, & Shahidi, 2001; Holland, Welch, Unwin, Buss, Paul, & Southgate, 1995). Carrots have been ranked tenth in terms of their nutritional value among 38 other fruits and vegetables, and seventh for their contribution to nutrition (US Agricultural Statistics, 1971). However, little is known about the antioxidant activity and phytonutrients in either orange or purple carrots.

Interest in the role of antioxidants in human health has prompted research in the fields of horticulture and food science to assess fruit and vegetable antioxidants and to determine how their content and activity can be maintained or even improved through cultivar development, maturity, harvesting methods, post-harvest procedures, processing technologies and storage conditions. In the past few years, ready-to-eat shredded carrots (usually mixed with other vegetables) have become more popular in the UK's supermarkets. Whenever the storage temperature and packaging film of ready-to-eat carrots is not strictly maintained, some deterioration occurs before their expiry date and spoilage of readyto-eat shredded carrots has been reported (Carlin, Nguyen-The, Cudennec, & Reich, 1989). In order to extend the shelf-life and maintain the quality of fresh cut carrots, modified atmospheres with controlled concentrations of  $CO<sub>2</sub>$  and  $O<sub>2</sub>$  have been used (Amanatidou, Slump, Gorris, & Smid, 2000; Babic, Amiot, Nguyen-The, & Aubert, 1993; Barry-Ryan, Pacussi, & O'Beirne, 2000; Carlin, Nguyen-The, Hilbert, & Chambroy, 1990). However, the effect of modified atmosphere packaging (MAP) and storage on antioxidant phytonutrients of ready-to-eat shredded orange and purple carrots has never been quantified. Purple carrots, which are not yet in the market, contain higher amounts of antioxidant vitamins, carotenoids and phenolics (Alasalvar et al., 2001) than other coloured carrot varieties (orange, yellow and white). More detailed research on comparison of nutraceutical components of ready-to-eat shredded orange and purple carrots stored under MAP will enhance our knowledge and appreciation for the use of purple carrots and their products in a variety of food and speciality products.

The purpose of this study was to compare the effect of chill storage and MAP on antioxidant activity, contents of anthocyanins, carotenoids, and phenolics and sensory quality of ready-to-eat shredded orange and purple carrots.

### 2. Materials and methods

## 2.1. Materials

Orange and purple carrots were sown in the same location (Elsoms Seeds Ltd.) in sandy, silt soil in May and were lifted for analysis in November of the same year. All chemicals used for the analyses were obtained from Sigma-Aldrich-Fluka Company Ltd. (Dorset, UK), unless otherwise specified.

#### 2.2. Sample preparation

Both orange and purple carrots (uniform size and without blemish) were delivered on the day of harvest (Elsoms Seeds Ltd.). Carrots of each colour  $(\sim 5 \text{ kg})$  were '' topped'' and ''tailed'' using a sharp knife, thoroughly washed with tap water, trimmed and peeled using a hand peeler, and subsequently disinfected for 5 min (100 ppm free chlorine solution, pH 6.9) and then rinsed under tap water. They were then left to drip-dry for 15 min in a perforated cage. Afterwards, carrots were shredded (2  $mm \times 2$  cm), using a food processing machine (Kenwood Ltd., Hatford, UK) equipped with a grating disk. Shredded carrots were then packaged in 150 g lots in 30  $cm \times 35$  cm polyethylene bags (Dynopack, Hampshire, UK). Finally, the bags were flushed with pure  $N_2$ ,  $O_2$  and  $CO<sub>2</sub>$ , at desired ratios using a controller (WITT KM100-3M, Witten, Germany), and sealed automatically using a Multivac model A300 packaging machine (Multivac Ltd., Wolfertschwenden, Germany). The following combinations of gases were used: (a) air (control); (b)  $95\%O_2$  + 5%CO<sub>2</sub>; and (c)  $90\%N_2 + 5\%O_2 + 5\%CO_2$ . Polyethylene bags with the carrots were placed in a temperature-controlled fridge ( $\sim$ 5 $\pm$ 2 °C) for 13 days. Samples were analysed in triplicate (three bags per treatment) on days 1, 3, 6, 8, 10 and 13.

#### 2.3. Gas analysis of bags

The gas composition in the polyethylene bags was monitored daily through a septum using a 1 ml airtight syringe. The  $N_2$ ,  $O_2$  and  $CO_2$  concentrations were determined by injection of 0.5 ml of a gas sample into a Star 3400 CX gas chromatograph (Varian Associates Inc., Palo Alto, CA) equipped with a thermal conductivity detector (TCD).

#### 2.4. Measurement of antioxidant activity

An improved oxygen radical absorbance capacity (ORAC) assay was carried out according to the method of Ou, Hampsch-Woodill, and Prior (2001) with a slight modification. Unlike other popular antioxidant activity methods, the improved ORAC assay directly measures the antioxidant activities of chain-breaking antioxidants against peroxyl radicals. The ORAC assay measures the ability of antioxidative compounds in test materials to inhibit the decline in fluorescence (FL) that is induced by peroxyl radical AAPH [2,2'-azobis (2-amidinopropane) dihydrochloride]. The sequence of the reaction mixture was as follows:  $150 \mu l$  of sample or blank or standard, 150 ll FL, 2.55 ml of 75 mM phosphate buffer (pH 7.4) and 150 µl AAPH. FL was measured and recorded every 5 min at the emission wavelength of 515 nm and excitation of 493 nm using a RF-540 Shimadzu spectrofluorophotometer (Shimadzu, Kyoto, Japan) until the FL of the final reading declined by 95% from the first reading. ORAC values were expressed as umol Trolox equivalents (TE) per g of fresh carrots. Trolox is a watersoluble tocopherol analogue, devoid of phytyl side chain, used as a reference compound for antioxidant activity.

#### 2.5. Measurement of total anthocyanins

Total anthocyanins were determined by a pH differential method (Cheng & Breen, 1991). All manipulations were carried out under yellow fluorescent lighting (Thorn, UK) because anthocyanins are highly sensitive to light, heat and air. Absorbance was measured in a UV-1601 Shimadzu spectrophotometer (Shimadzu) at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using  $A = [(A_{510} - A_{700})pH_{1,0} - (A_{510} - A_{700})pH_{4,5}]$  with a molar extinction coefficient of cyanidin 3-glucoside of 26900. Results were expressed as mg cyanidin 3-glucoside equivalents per 100 g of fresh carrots.

## 2.6. Measurement of total carotenoids

Total carotenoids were measured according to the method of Cyanotech Corporation (2001) with slight modifications. Grated carrots (0.5 g) were homogenised in 25 ml of acetone containing dimethyl sulphoxide (10%) in an ice bath. All manipulations were carried out under a yellow fluorescent lighting (Thorn) because carotenoids are highly sensitive to light, heat and air. The homogenate was filtered through a Whatman No. 4 filter paper and washed until the residue was colourless. Finally, the filtrate was brought to 100 ml with the extraction solvent, to afford the extract, and the absorbances at 471 and 477 nm were measured against an acetone blank using a UV-1601 Shimadzu spectrophotometer (Shimadzu). Total carotenoids were calculated according to the following equation.

Total carotenoids (
$$
\%
$$
) =  $\frac{\text{Abs}_{\text{max}}}{250}$   
  $\times \frac{25 \text{ml acetone} \times \text{dilution}}{\text{sample weight}} \times 100.$  (1)

#### 2.7. Measurement of total phenolics

Total soluble phenolics in the methanol extracts were determined colorimetrically using Folin–Ciocalteu reagent, as described by Slinkard and Singleton (1977), using ferulic acid as a standard. Results were expressed as mg ferulic acid equivalents per 100 g of fresh carrots.

#### 2.8. Sensory assessment

Sensory evaluation was used to discriminate between the appearance, smell/odour and browning of shredded orange and purple carrots packed under different MAP conditions over 13 days of storage. A panel of 6 judges (all research students at the Food Research Centre, University of Lincoln), with sensory evaluation experience, were trained in discriminative evaluation of shredded orange and purple carrots. The carrots used during the training sessions had been subjected to various conditions for shelf-life studies. Fresh orange and purple carrots were used as the control (high quality). The training panel was familiarised with the effects of storage over 2 weeks in air in contrast to modified atmospheres. Panellists relied on their training experience to evaluate products.

#### 2.9. Statistical analysis

SigmaStat was used to normalise the data and differences in mean values were determined using Tukey's procedure of analysis (SAS, 1990).

## 3. Results and discussion

## 3.1. Antioxidant activity

Antioxidant activities of fresh orange and purple carrots, measured by ORAC fluorescein assay on day 1, were  $76.7 \pm 1.6$  and  $217 \pm 2.9$  µmol TE/g, respectively (Fig. 1). Purple carrot contained, initially, a 2.8-fold higher ORAC value than its orange counterpart. Antioxidant activity of orange carrots remained very stable in both air (control) and under MAP and no significant differences ( $P > 0.05$ ) existed between any treatments over the storage period, whereas a highly significant decrease  $(P < 0.01)$  was observed under the MAP condition for purple carrots  $(95\%O_2 + 5\%CO_2)$ .



Fig. 1. Comparison of antioxidant activity of shredded orange and purple carrots stored chilled ( $\sim 5 \pm 2$  °C) under air or MAP. Error bars show the variations of three determinations in terms of SD. Percentage average RSD: 2.8%.

Antioxidant activities of 13 freeze-dried vegetables, from various locations, at different harvest seasons, based on ORAC and ferric reducing antioxidant power (FRAP) results have previously been reported by Ou, Huang, Hampsch-Woodill, Flanagan, and Deemer (2002). The reports show that the ORAC and FRAP values of vegetables are not only dependent on species, but also highly dependent on geographical origin and harvest time. On the basis of ORAC results, antioxidant activity varied between  $19 \pm 3$  and  $154 \pm 60$  µmol TE/g, dry weight, being lowest in pea and highest in green pepper. Freeze-dried orange carrots were quoted as having  $60 \pm 15$  µmol TE/g, dry weight. Our value for fresh orange carrots was much higher than that reported for freeze-dried samples, although the same method was employed for their determination. This large variability can apparently be explained by the influences of different varieties, processing, location, weather conditions, and harvest season, among others, which would affect the level of antioxidants present in carrots. In addition, antioxidant activity of carrots might be lost during freeze-drying.

#### 3.2. Total anthocyanins

Anthocyanins, which were only found in purple carrots, showed no significant decrease  $(P > 0.05)$  for all treatments over the entire storage period, except under MAP treatment  $(95\%O_2 + 5\%CO_2)$ , which significantly  $(P < 0.05)$  decreased them on day 13 (Fig. 2). The initial concentration of total anthocyanins was  $5.1 \pm 0.2$  mg/ 100 g, which decreased to  $4.8 \pm 0.1$ ,  $4.6 \pm 0.2$  and  $4.9 \pm 0.1$  mg/100 g on day 13 in air,  $95\%O_2 + 5\%CO_2$ and  $90\%$ N<sub>2</sub> + 5O<sub>2</sub> + 5%CO<sub>2</sub>, respectively.

Blueberry (87 Highbush blueberry cultivars) anthocyanins range from 89 to 331 mg/100 g (Ehlenfeldt  $\&$ Prior, 2001). High amounts of anthocyanins have also been reported in different berries (Moyer, Hummer, Finn, Frei, & Wrolstad, 2002; Prior et al., 1998). Purple carrots contained less anthocyanins than berries.

## 3.3. Total carotenoids

Fig. 3 shows the effect of MAP on total carotenoid content of chilled stored shredded orange and purple carrots. Total carotenoid content of purple carrots was initially 2.3-fold higher than orange carrots  $(19.5 \pm 0.5)$ mg/100 g in purple and  $8.6 \pm 0.2$  mg/100 g in orange), in agreement with values reported by Alasalvar et al. (2001). They found that purple carrots contained 2.3 fold more  $\alpha$ - and  $\beta$ -carotenes than orange carrots. The orange and purple carrot varieties used in this trial were different from varieties used in the above study. Although gradual decreases in both air- and MAP-treated orange samples were found, these changes in the total



Fig. 2. Comparison of total anthocyanins of shredded purple carrot stored chilled ( $\sim 5 \pm 2$  °C) under air or MAP. Error bars show the variations of three determinations in terms of SD. Percentage average RSD: 2.9%.



Fig. 3. Comparison of total carotenoids of shredded orange and purple carrots stored chilled ( $\sim$ 5 $\pm$ 2 °C) under air or MAP. Error bars show the variations of three determinations in terms of SD. Percentage average RSD: 1.9%.

carotenoid contents of samples stored in air on MAPtreated were highly significant  $(P < 0.01)$ . In both orange and purple carrots, the main loss of total carotenoids was in the  $95\%O_2 + 5\%CO_2$  treatment.

#### 3.4. Total phenolics

Total phenolic contents of fresh orange and purple carrots (on day 1) were  $34.8 \pm 1.9$  and  $102 \pm 3.8$  mg/100 g, respectively (Fig. 4). Total phenolics content of purple carrots was initially 2.9-fold higher than that of orange carrots. Alasalvar et al. (2001) found sizable varietal differences in the content of total phenolics among different coloured carrot varieties (orange, purple, yellow and white). The total amount of phenolics in purple carrots was 74.6 mg/100 g, whereas the corresponding values in orange, yellow and white varieties ranged from 7.72 to 16.2 mg/100 g (Alasalvar et al., 2001). The purple and orange carrot varieties used in this study were different from those used in the earlier study.

The total phenolics content of purple carrots increased at a much higher rate during storage than that of the orange counterpart (Fig. 4). This increase could be related to the developmental changes and wound-like response. Dixon and Paiva (1995) reported that plants respond to wounding with increases of phenolic compounds involved in the repair of wound damage and in defence against microbial invasion. According to Sarkar and Phan (1979), the total phenolics of carrots stored at  $3 \pm 1$  °C and at  $\sim$ 90% relative humidity increased steadily with storage time. Chubey and Nylund (1969) suggested that carrots richer in phenolics are more susceptible to browning, but their contributions (in fruits and vegetables) for resisting parasitic attack could be of benefit to minimally processed carrots stored under MAP.

As shown in Fig. 4, storage under  $90\%$ N<sub>2</sub> +  $5O_2$  +  $5\%CO<sub>2</sub>$  significantly reduced the accumulation of total phenols compared to those stored under air and  $95\%O_2 + 5\%CO_2$ . Although no significant difference  $(P > 0.05)$  was observed between air and  $95\%O_2$  +  $5\%CO<sub>2</sub>$  over 13 days of storage in orange carrots, a highly significant difference ( $P < 0.01$ ) existed between air and MAP treatments in purple carrot. Amanatidou et al. (2000) studied the impact of high  $O_2$  and high  $CO_2$ modified atmospheres for shelf-life extension of minimally processed carrots. They found that the quality of carrots stored under  $20\%N_2 + 50\%O_2 + 30\%CO_2$  was similar to or better than their quality when stored under  $89\%N_2 + 1\%O_2 + 10\%CO_2$  after 8–12 days at 8 °C. In addition, oxygen levels above 70% resulted in poor quality of product when combined with  $10-30\%$  CO<sub>2</sub>. Although we used a different gas ratio, our results were in good agreement with those of Amanatidou et al. (2000), thus confirming that high  $N_2$ , and low  $O_2$  and  $CO<sub>2</sub>$ , prevents the accumulation of phenolics in stored



Fig. 4. Comparison of total phenolics of shredded orange and purple carrots stored chilled ( $\sim$ 5 $\pm$ 2 °C) under air or MAP. Error bars show the variations of three determinations in terms of SD. Percentage average RSD: 3.3%.

Table 1

Descriptive terms obtained from sensory assessment of shredded orange and purple carrots packed in both air and under MAP, and stored chilled (~5  $\pm$  2 °C)

Days	Treatments	Orange			Purple		
		Appearance	Smell/odour	Browning	Appearance	Smell/odour	Browning
1	Air (control)	Very good	Fresh carrot	Absent	Very good	Fresh carrot	Absent
	$95\%O_2 + 5\%CO_2$	Very good	Fresh carrot	Absent	Very good	Fresh carrot	Absent
	$90\%N_2 + 5\%O_2 + 5\%CO_2$	Very good	Fresh carrot	Absent	Very good	Fresh carrot	Absent
3	Air (control)	Very good	Fresh carrot	Absent	Good	Fresh carrot	Absent
	$95\%O_2 + 5\%CO_2$	Very good	Fresh carrot	Absent	Good	Fresh carrot	Absent
	$90\%N_2 + 5\%O_2 + 5\%CO_2$	Very good	Fresh carrot	Absent	Good	Fresh carrot	Absent
6	Air (control)	Good	Fresh carrot	Absent	Good	Fresh carrot	Slight
	$95\%O_2 + 5\%CO_2$	Good	Fresh carrot	Absent	Good	Fresh carrot	Slight
	$90\%$ N <sub>2</sub> + $5\%$ O <sub>2</sub> + $5\%$ CO <sub>2</sub>	Good	Fresh carrot	Absent	Good	Fresh carrot	Slight
8	Air (control)	Good	Slightly lost	Absent	Acceptable	Slightly lost	Moderate
	$95\%O_2 + 5\%CO_2$	Good	Slightly lost	Absent	Acceptable	Slightly lost	Moderate
	$90\%N_2 + 5\%O_2 + 5\%CO_2$	Good	Slightly lost	Absent	Acceptable	Slightly lost	Slight
10	Air (control)	Good	Slightly lost	Absent	Acceptable	Slightly off	Very moderate
	$95\%O_2 + 5\%CO_2$	Good	Slightly lost	Absent	Acceptable	Slightly off	Very moderate
	$90\%N_2 + 5\%O_2 + 5\%CO_2$	Good	Slightly lost	Absent	Acceptable	Slightly lost	Moderate
13	Air (control)	Acceptable	Slightly off	Absent	Poor	Off	Very moderate
	$95\%O_2 + 5\%CO_2$	Acceptable	Slightly off	Absent	Poor	Off	Very moderate
	$90\%N_2 + 5\%O_2 + 5\%CO_2$	Acceptable	Slightly off	Absent	Poor	Slightly off	Moderate

Data are mean of results from 6 trained panellists.

shredded carrots. Increased phenolic content in purple carrots during storage agreed well with sensory scores (Fig. 4 and Table 1).

#### 3.5. Sensory assessment

A list of descriptive terms obtained from sensory assessment of shredded orange and purple carrots stored under air or MAP treatments is shown in Table 1. No differences between the air and MAP treatments for orange carrots were observed over the 13 days of chill storage. All orange carrots products were acceptable, and absence of browning and slight off smell/odour were only evident on day 13. Compared to orange carrots, significant browning was observed in all treatments of purple carrots during the 13 days of storage. These increases agree well with the total phenolics content of purple carrots (Fig. 4). All purple carrot products were unacceptable by day 13. Despite that,  $(90\%N_2 + 5O_2 +$  $5\%CO<sub>2</sub>$ )-treated purple carrots gave better storage quality compared to those kept under air and  $95\%O_2 + 5\%CO_2$  in terms of their smell/odour and formation of browning products.

## 4. Conclusions

Total antioxidant activity, carotenoids and phenolics of purple carrots were initially 2.8-, 2.3- and 2.9-fold higher than those of orange carrots, respectively. Anthocyanins were found only in purple carrots. Thus,

purple carrots may be used in place of orange carrots in order to take advantage of their nutraceutical components. Accumulation of phenolics in purple carrots showed a trend similar to sensory assessment (browning). High browning product formation in purple carrots over the entire storage period had a negative effect on their overall appearance. MAP treatment did not extend the shelf life of orange carrots, whereas  $90\%N_2 + 5O_2 + 5\%CO_2$  treatment delayed the browning and off-smell/odour development in purple carrots. Shredded purple carrots could be stored under  $90\%$ N<sub>2</sub> + 5O<sub>2</sub> + 5%CO<sub>2</sub> treatment for up to 10 days. Therefore, high nitrogen treatment may be used in maintaining the storage quality and nutraceutical properties of shredded purple carrots.

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