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Influence of blanching and low temperature preservation strategies on antioxidant activity and phytochemical content of carrots, green beans and broccoli

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Work dedicated to my grand parents

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ABSTRACT

The objective of this study was to investigate the effect of blast freezing and blanching in combination followed by chilling, on the antioxidant activity (ARP), phenols, ascorbic acid and colour of broccoli, carrots and green beans. No significant changes ($p > 0.05$) in ARP of blanched frozen (BLFR) broccoli, carrot and green beans were observed. In contrast, UBFR (unblanched frozen) treatments caused a significant decrease ($p < 0.05$) in ARP and ascorbic acid content of vegetable samples. BLFR treated samples had better retention of antioxidant activity and ascorbic acid as compared to UBLR counterparts at chill storage (4 °C) for 7 days. However, no significant changes were observed in phenol content for all vegetables. Ascorbic acid decreased exponentially for both blanched and unblanched samples. The reaction rate constant (k) increased from 1.06×10^{-1} day^{-1} to 1.17×10^{-1} day^{-1} for blanched and unblanched broccoli florets and from 4.6×10^{-3} day^{-1} to 1.98×10^{-1} day^{-1} for blanched and unblanched carrots during 7 days storage. Result presented here indicates greater stability of nutritional parameters for BLFR samples compared to UBFR samples during 7 days storage at 4 °C for all vegetables.

1. Introduction

Fruits and vegetables contain range of phytochemicals, in addition to well-known antioxidants, such as vitamins C and E, or polyphenols, which significantly contribute to their total antioxidant activity (Cao, Sofic, & Prior, 1996; Prior et al., 1998). 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). Due to the seasonal and perishable nature, raw vegetables are subjected to some form of preservation in order to make them available for later consumption (Lin & Brewer, 2005). Freezing of fruits and vegetables is generally regarded as superior to other food preservation techniques such as canning and dehydration, with respect to retention in sensory attributes and nutritive properties. Freezing is often employed to maintain fresh-like characteristics with minimal loss of nutrients such as vitamins, and antioxidant content over long periods (Prochaska, Nguyen, Donat, & Piekutowski, 2000). However, while freezing on its own helps to preserve food through the slowing of enzymatic reactions, senescence and microbial growth; it does not fully stop these processes (Bahceci, Serpen, Gokmen, & Acar, 2005). The result can be the development of off-odours, off-colours, off-flavours, changes in texture and nutrient loss. Fruits and vegetables are blanched prior to freezing mainly to inactivate enzymes, reduce microbial load, remove gases from the plant tissue, cause shrinkage of the product to facilitate packaging, fix texture, colour, and clean the surface of the vegetable (Bahceci et al. 2005; Barrett & Theerakulkait, 1995).

Typically, blanching is carried out by treating the vegetables with steam or hot water for 1–10 min at 75–95 °C, the time/temperature combination depending on the type of vegetable (Cano, 1996). Blanching of foods involves mild heating in water and serves. Blanching can have negative effect on nutrients such, as vitamins and phenolic compounds which are relatively unstable when subjected to heat treatments (Prochaska et al., 2000). Apart from processing, storage conditions and domestic cooking and preparation have significant effect on phytochemicals such as ascorbic acid and phenols (Patras, Brunton , Tiwari, & Butler.,2009a; Rawson, Koidis, Patras, Tuohy,& Brunton, 2010; Vallejo, Tomás-Barberán, García-Viguera, 2002; Verkerk & Dekker, 2004).

Consumers are now becoming aware of the need to consume a variety of fresh vegetables to maximise their intake of beneficial

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antioxidants (Prochaska et al., 2000). Several studies show the effects of freezing on green peas and spinach, carrots, broccoli, green beans (Howard, Wong, Perry, & Klein, 1999; Korus, Lisiewska, & Kmiecik, 2002; Murcia, López-Ayerra, Martinez-Tomé, Vera, & García-Carmona, 2000; Saccani et al., 2001). In addition, freezing has, in some studies, been shown to influence the quality and nutritive value of foods (Srinivasan, Xiong, & Blanchard, 1997). There is very little information on how these processes in combination influence the quality and nutritive value of foods despite the fact that they are commonly used together to prolong shelf life.

Although freezing is an effective method of preserving foods some deterioration in frozen food quality occurs during storage. Therefore the objective of the current study was to examine the effect of blast freezing and blanching in combination followed by chilling, on the ARP, phenols, ascorbic acid and colour of broccoli, carrots and green beans with a view to their use as components of ready-meals.

2. Materials and methods

2.1. Vegetable material and sample preparation

Fresh carrots (*Daucus carota* L. cv. Nairobi), green beans (*Phaseolus vulgaris* L cv. Emerit) and broccoli (*Brassica oleracea* L cv. Monaco) were purchased from a local supplier (Donnelly's Ltd., Dublin, Ireland). The carrots were washed, peeled (using a hand held domestic peeler) and sliced into discs of 5 mm thickness using a mechanical slicer (Berkel Ltd, Ontario, Canada). The green beans were washed, topped and tailed. Broccoli was separated into florets and leaves and inedible stems were removed.

2.2. Blanching and blast freezing experiment

Two different pre-treatments were used in this trial. Carrot, green beans and broccoli were equally divided (200 g each) into two batches [Batch A and Batch B]. Batch A was subjected to blanching (BL), whereas no blanching treatments (UB) were given to the batch B. Blanching was carried out by placing samples in water at 95 °C for 3 min. The proportion of water to the raw material used was 5:1 by weight. Following this, samples were cooled in distilled in water for 2–3 min. Prior to blast freezing, samples (200 ± 1 g) were vacuum-sealed in 20 cm × 30 cm in Polypropylene pouches (thickness- 75 μm, gas permeability- 2.7 g m^{-2} d, sealing temperature- 100–180 °C, Packex Industries Ltd., Wicklow, Ireland using Vac-star S220 vacuum sealer (Vicquip Ltd., Dublin, Ireland)). The blanched and unblanched vegetables were blast frozen at −30 °C for 2.5 h (Nu-Avon, Wiltshire, England; air speed 3.8 m s^{-1}) (Fig. 1).

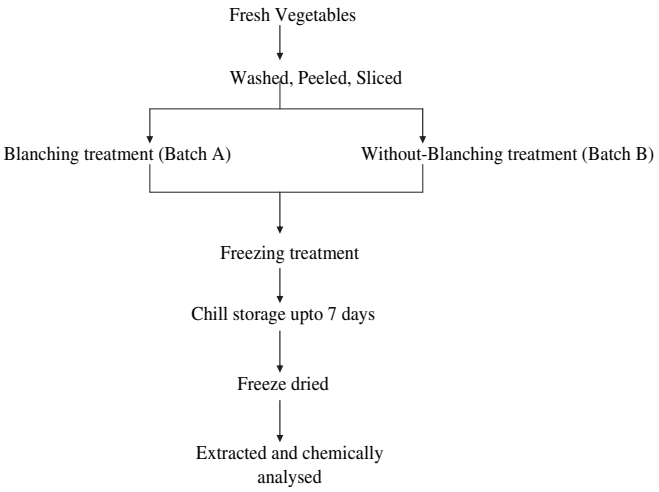

Fig. 1. Overview of the steps involved in blanching, freezing and chill storage treatments.

2.3. Chill storage

Blanched frozen [BLFR] and unblanched frozen [UBFR] samples were placed in different propylene pouches and stored in air at 4 °C for 0, 3, 5, 7 days. Each treatment was replicated three times. At each sampling point, samples were removed; freeze dried (Frozen in Time Ltd., York, UK) at a temperature and pressure of −50 °C and 0.03 mbar respectively for more than two days and tested for antioxidant indices and instrumental colour.

2.4. Measurement of total antioxidant activity

Methanolic extracts were prepared by adding 25 mL of HPLC grade methanol to 1.25 g of freeze dried powder. Samples were then homogenised for 1 min at 24,000 rpm using an Ultra-Turrax T-25 Tissue homogenizer (Janke & Kunkel, IKA®-Labortechnik, Saufen, Germany). DT20 tube with a rotor stator element with a dispersing element was utilized. The stator diameter was 19 mm and rotor diameter of 12.7 mm with gap between rotor and stator of 0.3 mm with shaft length of 19.2 cm was used. The samples were then vortexed with a V400 Multitude Vortexer (Alpha laboratories, North York, Canada) for 20 min at 800 g and centrifuged for 15 min at 2000 g (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, UK). 10 mL of the sample was filtered through PVDF Acrodisc syringe filters (pore size 0.45 μm, Sigma, Ireland,) and stored at −20 °C for subsequent analysis. Total antioxidant activity was measured using the DPPH assay as described by Goupy, Hugues, Boivin, and Amiol (1999). Briefly 500 μL of diluted sample and 500 μL of the DPPH (0.238 mg/mL^{-1}) working solution were mixed in a microcentrifuge tube. After vortexing, the tubes were left in the dark for 30 min at room temperature after which the absorbance was measured against methanol at 515 nm using a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Milton Keynes). The decrease in absorbance of a sample was calculated in comparison to a blank sample (500 μL methanol and 500 μL DPPH). The relative decrease in absorbance (PI) was then calculated as follows: PI (%) = 1 − (Ae/Ab), with Ae = absorbance of sample extract and Ab = absorbance of blank. The PIs used to calculate the related antioxidant activity were superior (PI1) and inferior (PI2) to the value estimated at 50%. Antioxidant activities were expressed as the IC50 i.e. the concentration of antioxidant required to cause a 50% reduction in the original concentration of DPPH. For ease of interpretation, antiradical powers were also calculated and defined as the inverse of the IC50 value Eq. (1) and (2). Finally, the antioxidant activity of the extracts was compared to that of a synthetic antioxidant (Trolox) and expressed as Trolox equivalent antioxidant activity (TEAC) values. The analyses were done in triplicate and all quantitative results are reported on a dry weight basis.

$$\Delta C = \frac{(C_1 - C_2) \times (PI_1 - 50)}{PI_1 - PI_2} \quad (1)$$

$$IC_{50} = C_1 - \Delta C \quad (2)$$

$$ARP = \frac{1}{IC_{50}} \quad (3)$$

2.5. Measurement of phenolic content

Total phenols (TP) in vegetable samples were determined using the Folin–Ciocalteu reagent according to the method of Singelton, Orthofer, and Lamuela-Raventos (1999). Briefly 100 μL of methanolic extract, 100 μL of MeOH, 100 μL Folin–Ciocalteau reagent (FC) and 700 μL of Na_2CO_3 was added to 1.5 mL microcentrifuge tubes and the samples were vortexed briefly. The tubes were then left in the dark for 20 min at room temperature. Following this, the samples were centrifuged (Eppendorf, Centrifuge 5417R., Germany) at 10,000 g for 3 min. The absorbance of the sample was read at 735 nm using aqueous Gallic acid (10–400 mg L^{-1}) as a standard. Results were expressed as mg of Gallic acid equivalent per 100 g of dry weight of sample.

2.6. L-Ascorbic acid analysis (AA)

Extraction of L-ascorbic acid was carried out using metaphosphoric acid (6 g/100 mL) and 1.25 g of freeze-dried powder as described for antioxidant extractions above. Ascorbic acid determination was carried out by reverse phase high performance liquid chromatography (HPLC) according to the method of Lee and Coates (1999) with slight modifications (Patras, Brunton, Da Pieve, Butler, & Downey 2009b; Patras, Brunton, Da Pieve, & Butler, 2009c). A 20 μl of aliquot was injected into the chromatographic column. The chromatographic system (Shimadzu Model no SPD- M10A VP, Mason Technology, Dublin 8, Ireland) consisted of a pump, a vacuum degasser, a Diode–Array Detector and it was controlled through EZ Start 7.3 software (Shimadzu) at 40 °C. A hypersil ODS column (150 mm × 4.6 mm, 5 μm, Supelco., US) fitted with hypersil ODS guard column(Gemini C18 [4 mm L × 3.0 mm ID], Phenomenex., UK) was utilised with a mobile phase (isocratic) of 25 mMol L^{-1} monobasic potassium phosphate adjusted to pH 3 at a flow rate of 1 mL min^{-1} at 40 °C. The detector was set at 245 nm. For quantification external calibration curves for ascorbic acid in metaphosphoric acid (6 g/100 mL) were prepared at concentrations from 25 μg mL^{-1} to 500 μg mL^{-1}. The total run time was 4.0 min.

2.7. Colour measurements

The colour of the samples was measured using a Hunter Lab colour meter (Hunter Lab DP-9000 colour difference meter, Hunter Associates Laboratory, Virginia, USA) fitted with a 2.5 cm diameter aperture. The instrument was calibrated using the black and white tiles provided. Colour was expressed in Hunter Lab units L^*, a^* and b^*. Three replicate measurements were performed and results were averaged. In addition, Chroma and total colour difference (TCD) were calculated using the following equations, where L_0, a_0, b_0 are the control values (untreated) for vegetables. TCD indicates the magnitude of colour difference between stored and control samples. Differences in perceivable colour can be analytically classified as very distinct (TCD > 3), distinct (1.5 < TCD < 3) and small difference (TCD < 1.5) (DrLange, 1999; Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008). It should be noted that combinations of colour parameters may be more effective to evaluate the overall colour changes of processed vegetables than the individual L^*, a^*, b^* parameters.

$$Chroma = \sqrt{a^2 + b^2} \tag{4}$$

$$\Delta E = \left[(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2\right]^{1/2} \tag{5}$$

3. Statistical analyses

A two-way analysis of variance (ANOVA) was performed using the GenStat Release 10.1 software. Values were considered significant at $p < 0.05$. Three replications were carried and mean values were reported. The degradation of ascorbic acid loss in blanched and unblanched samples was calculated by using the standard equation for first-order reactions and degradation rate constants were determined by fitting (Eq. (6)) to experimental data.

$$C = C_0 e^{kt} \tag{6}$$

where, C is the studied parameter (AA) at any given reaction time, C_0 are initial values of treated samples (day 0) and k are rate constants. Data fitting was considered significant at a probability level of 95%.

4. Results and discussion

4.1. Effect of pre-treatment (BL/UB) and subsequent blast freezing on antioxidant activity, phytochemical content of broccoli, carrots and green beans

ARP of fresh, BLFR and UBFR broccoli, carrots and green beans are presented in Table 1. ARP values of all BLFR vegetables were not significantly lower than fresh samples indicating that blanching with subsequent blast freezing did not affect the antioxidant activity of any of the vegetables. These results agree with the findings of Hunter and Fletcher (2002), they demonstrated that frozen vegetables have similar antioxidant activities to their fresh counterparts. Myojin et al. (2008) suggested that blanching of bitter gourd improves the retention of antioxidant activity and total phenolics during subsequent frozen storage. Puupponen-Pimia et al. (2003) found that total phenolics and DPPH index of cauliflower decreased slightly during blanching, but no further changes were observed during storage. Similarly, Cooper, Chen, and King (1983) studied the effects of laboratory-scale blanching and freezing on folate levels of spinach. They found that blanching in water at 100 °C for 3 min caused a 33% loss compared with fresh spinach. Gębczyński and Lisiewska (2006) studied the process of freezing (−20, −30 °C) in broccoli florets and found significant decrease in the antioxidant activity. In the present study, non-inclusion of blanching step with blast freezing did significantly affect ARP values for all vegetables investigated. It is important to note that the retention of antioxidant activity (ARP) is significantly improved by blanching, indicating possible involvement of enzymatic reactions (PPO) persisting significantly in unblanched samples and in a less extent still in blanched samples (Table 1).

Conversely levels of phenols in all vegetables were unaffected by BLFR and UBFR treatments. Gębczyński and Lisiewska (2006) studied the process of freezing (−20, −30 °C) in broccoli florets and found significant decrease in the level of polyphenol content. Myojin et al. (2008) found that at subsequent frozen storage at −18 °C, radical scavenging activity and total phenolic content of unblanched and blanched bitter gourd underwent little change for 90 days and then gradually declined. The authors also reported that radical scavenging activity and total phenolic levels, practically remained unchanged throughout the entire storage period at −40 °C.

Levels of ascorbic acid were unaffected by BLFR for broccoli and carrot samples but decreased significantly ($p < 0.05$) by UBFR treatments compared to fresh samples. This was particularly evident for broccoli florets with an 83% reduction in AA levels being detected. Davey et al. (2000) compared processing techniques most relevant to vegetables, i.e. canning, freezing and dehydration, they

Table 1
Influence of pre-treatment (BLFR/UBFR) and subsequent blast freezing [day 0] on antioxidant activity, ascorbic acid, phenols and colour of broccoli, carrots and green beans.

Samples	ARP[d] $(g/L)^{-1}$	Phenols (mgGAE[e]/100g)	AA[g] (mg/100g)	L^*	TCD[f]	Chroma
Broccoli						
Fresh	0.53 ± 0.01	446.0 ± 12.21	374.1 ± 2.12	29.5 ± 3.78	0.00	12.8 ± 1.21
Blanched Frozen [BLFR][a]	0.52 ± 0.02	448.0 ± 0.98	373.2 ± 5.67	28.1 ± 5.89	0.2 ± 1.21	11.2 ± 0.04
Unblanched Frozen [UBFR][b]	0.45 ± 0.04	521.0 ± 2.32	62.7 ± 2.34	18.3 ± 0.01	10.9 ± 0.76	8.8 ± 0.56
LSD[c]	0.06	87	12.57	2.68	0.04	2.24
Carrots						
Fresh	0.12 ± 0.45	88.4 ± 1.78	43.9 ± 0.05	56.3 ± 6.89	0.00	36.2 ± 1.78
Blanched Frozen	0.12 ± 0.01	89.0 ± 1.56	40.6 ± 4.89	56.2 ± 1.78	0.9 ± 2.78	36.2 ± 1.56
Unblanched Frozen	0.09 ± 0.03	92.5 ± 3.78	20.7 ± 3.90	54.2 ± 2.13	1.8 ± 6.78	36.1 ± 4.21
LSD	0.02	15.79	12.37	3.81	3.32	3.78
Green beans						
Fresh	0.24 ± 0.01	225.6 ± 1.89	ND	43.6 ± 2.67	0.00	18.7 ± 0.01
Blanched Frozen	0.22 ± 0.56	200.5 ± 3.21	ND	40.2 ± 2.78	5.1 ± 2.78	15.1 ± 0.98
Unblanched Frozen	0.11 ± 0.34	186.6 ± 4.32	ND	23.4 ± 3.01	19.3 ± 5.01	13.1 ± 0.21
LSD	0.06	40.27		4.45	0.19	2.16

All values are means of three replicates of three batches and expressed in dry weight basis.
[a] Blanched Blast frozen.
[b] Unblanched Blast frozen.
[c] least significant difference ($p = 5\%$).
[d] Total antioxidant activity.
[e] Gallic acid equivalent.
[f] Total colour difference.
[g] L- Ascorbic acid.

found that L-ascorbic acid losses were greatest during dehydration and lowest during freezing. Similarly, Favell (1998) reported negligible losses in ascorbic acid of carrots during freezing. It is envisaged that ascorbic acid losses can occur, particularly during blanching (Lathrop & Leung, 1980) but slight change may be expected thereafter during deep frozen storage. Several studies demonstrated that effects of freezing on the product quality are minimal (Howard et al., 1999; Murcia et al., 2000).

4.2. Effect of pre-treatment (BL/UB) and subsequent blast freezing on colour parameters of carrots and green beans and broccoli

Table 1 shows the colour attributes of fresh, BLFR, UBFR carrots, green beans and broccoli. In general, TCD values for this study were found to be very distinct after UBFR treatment but not following BLFR. For both broccoli florets and green beans TCD values were significantly different for UBFR samples compared to fresh samples. The lightness values (L^*) for fresh broccoli, carrots and green beans were 29.5, 56.3, and 43.6 respectively. UBFR treatment had more pronounced affect on lightness of vegetable samples whereas no significant reduction in L^* was observed for BLFR samples. Chroma, one of the important colour attribute decreased significantly for broccoli and green beans with the exception of carrots. BLFR treatment maintained colour intensity of the samples better than UBFR treated samples. A study conducted by Lin and Brewer (2005) demonstrated that blanched peas were visually lighter green than unblanched peas immediately after blanching and after 12 weeks of frozen storage whereas they did not observe any significant difference for a^* and b^* value of blanched frozen green peas.

4.3. Effect of treatment (BLFR/UBFR) and short term chill storage on antioxidant activity, phytochemical content of broccoli, carrots and green beans

ARP values for broccoli, carrots and green beans with or without blanching and followed by chill storage are presented in Table 2. Significant effect of treatment and storage days on ARP levels for all vegetable samples was observed (Tables 2 and 4). In all cases ARP values for unblanched samples were significantly lower than for blanched samples. However, it is well known that processing of any kind induces physical, structural, chemical and nutritional modifications in different vegetables (Di Scala & Crapiste, 2008). For example Ismail and Lee (2004) blanched five species of cruciferous vegetables and observed a 9–40% decrease in the total antioxidant activity. In contrast, Nicoli, Anese, Parpinel, Franceschi, and Lerici (1997) reported that inspite of a reduction in antioxidant phytochemicals brought about by thermal process treatments, the overall antioxidative activity of food products can be preserved or even increased owing to the possibility of other antioxidants arising, particularly as a result of the Maillard reaction. Similar results were reported by (Patras et al., 2009a). This was not the case for water blanching in the present study and this may be related to the fact that blanching is a mild process and preserves antioxidant components whilst also protecting them from enzyme mediated degradation by inactivating enzymes such as polyphenol oxidase.

ARP values for UBFR samples were significantly lower than for than for BLFR for all vegetables types after 7 days of storage. This indicates that initial effect of blanching in retaining more antioxidants at the beginning of the storage period was maintained even after 7 days chill storage. In the present study, heat treatment in blanching may induce some losses, but inactivation of oxidative enzymes prevents further losses during chill storage. Subsequent chill storage for up to 7 days had significant effect on ARP values of broccoli, carrot disks and green beans with significantly low values being present in samples stored for 7 days as compared to fresh samples for all vegetable types and treatments. Puupponen-Pimia et al. (2003) also observed a pronounced decrease in the DPPH index during the refrigerated storage of broccoli. It is also very important to note that decrease in ARP is due to the loss of antioxidant components such as ascorbic acid, polyphenols and glucosinolates etc in green vegetables. In the present study, it is difficult to make direct statements on the relationship between ascorbic acid and antioxidant activity in methanolic extract as ascorbic acid is not sparingly soluble and therefore would not be present in the extract. Linear correlations demonstrate that the antioxidant activity determined by DPPH assay, was well correlated to total phenolic content ($r = 0.76$ $p < 0.001$).

Table 2
Influence of treatment (BLFR/UBFR) and subsequent chill storage on antioxidant activity, ascorbic acid, phenols of broccoli, carrots and green beans.

Days of storage	ARP[b] $(g/L)^{-1}$		Phenols mgGAE[c]/100g		AA[d] mg/100g	
	BL[e]	UB[f]	BL	UB	BL	UB
Broccoli						
0	0.51 ± 0.02	0.45 ± 0.04	422.0 ± 3.35	521.0 ± 5.06	211.8 ± 9.81	62.7 ± 1.38
3	0.53 ± 0.02	0.40 ± 0.02	404.0 ± 11.25	671.0 ± 0.89	153.6 ± 15.21	41.1 ± 0.51
5	0.52 ± 0.03	0.46 ± 0.05	403.1 ± 4.56	679.1 ± 2.26	110.3 ± 0.37	31.7 ± 0.51
7	0.29 ± 0.02	0.17 ± 0.02	387.0 ± 9.56	636.0 ± 12.61	96.7 ± 0.64	30.5 ± 0.40
LSD[a]	0.25		29.4		16.14	
Carrots						
0	0.11 ± 0.01	0.09 ± 0.80	92.5 ± 5.90	92.5 ± 2.45	39.0 ± 2.67	20.6 ± 1.89
3	0.09 ± 0.01	0.07 ± 1.23	110.1 ± 6.90	110.1 ± 7.89	38.5 ± 3.67	7.5 ± 1.78
5	0.08 ± 0.02	0.04 ± 0.90	104.1 ± 1.32	104.1 ± 0.03	38.6 ± 7.21	7.2 ± 0.04
7	0.06 ± 0.01	0.03 ± 0.05	90.0 ± 8.90	90.0 ± 0.06	38.6 ± 0.02	6.4 ± 4.89
LSD	0.01		19.16		0.70	
Green beans						
0	0.24 ± 0.89	0.11 ± 0.21	224.2 ± 6.01	186.6 ± 2.89	nd	nd
3	0.25 ± 0.78	0.07 ± 2.67	226.5 ± 8.21	187.9 ± 6.69	nd	nd
5	0.11 ± 0.12	0.07 ± 0.08	220.5 ± 0.34	219.4 ± 2.78	nd	nd
7	0.09 ± 4.78	0.06 ± 0.01	198.3 ± 0.81	188.4 ± 1.32	nd	nd
LSD	0.07		40.94			

All values are means of three replicates of three batches and expressed in dry weight basis.
nd = non-detectable.
[a] least significant difference ($p = 5\%$).
[b] Total antioxidant activity.
[c] Gallic acid equivalent.
[d] L- Ascorbic acid.
[e] Blanched.
[f] Unblanched.

Ascorbic acid levels ranged from 30.5 to 211.8 mg/100 g DW and 6.40–39.0 mg/100 g DW for UBFR and BLFR broccoli and carrots samples respectively as illustrated in Table 2. Observed values were within the range of those reported elsewhere for these vegetables (Howard et al., 1999). Ascorbic acid levels were below the limit of detection in green beans. Following chill storage, ascorbic acid decreased significantly for unblanched samples, whereas better retention of ascorbic acid was observed for blanched broccoli samples. In contrast no changes in ascorbic acid content was observed for BLFR carrots whereas 30% ascorbic acid was lost for UBFR samples ($p < 0.05$). The retention of ascorbic acid is often used as an estimate for the overall nutrient retention of food products (Murcia et al., 2000). Ascorbic acid is by far the least stable nutrient during processing; it is highly sensitive to oxidation and leaching into water-soluble media during processing, storage and cooking of fresh, frozen and canned fruits and vegetables (Franke, Custer, Arakaki, & Murphy, 2004). Higher losses of vitamin C in vegetables due to blanching in water are reported in the literature (Murcia et al., 2000; Sikora, Cieślik, Leszczyńska, Filipiak-Florkiewicz, & Paweł, 2008). Some authors also suggested that steam blanching (96 °C for 3 min) prior to freezing storage decreased vitamin C concentration by about 32% and these results are similar to those of Howard et al. (1999). The authors reported a vitamin C loss of about 30% in steam-blanched broccoli (92 °C for 1.8 min).

In a study carried out by Albrecht, Schafer, and Zottola (1991) reported vitamin C losses ranging from 2% to 48% for 6 different broccoli cultivars stored at 2 °C for 21 days, similarly, Howard et al. (1999), observed vitamin C losses of 13% and 48%, respectively, after 3 wk of storage of broccoli at 4 °C. The author also suggested that AA retention during prolonged storage was better in vegetables stored at −20 °C than during refrigerated storage at 4 °C. The obtained results underline the important role of several pre-processing factors on the qualitative and nutritional characteristics of carrot and broccoli samples.

In our results, non-inclusion of a blanching step, decreased ascorbic acid significantly over 7 days of storage period. AA content was found decrease exponentially with $R^2 > 0.74$ (Fig. 2) with degradation rate constant of 1.1×10^{-1} day^{-1} and 1.2×10^{-1} day^{-1} for blanched and unblanched broccoli samples respectively. Similarly, higher degradation rate constant was observed for unblanched carrot samples (2.0×10^{-1} day^{-1}) as compared to blanched samples ($0.05 \times 10^{-1} day^{-1}$).

Phenolic content (TP) of broccoli, carrots, green beans ranged from 387 to 679 mg GAE/100 g; 90.0–110.1 mg GAE/100 g; 186.6–226.5 mg GAE/100 g, respectively among all the samples analysed (Table 2). The observed values were within the range for these vegetables by other authors (Leja, Mareczek, Starzyńska, & Rozék, 2001; Puupponen-Pimia et al., 2003; Zhang & Hamauzu, 2004). Phenolic content of blanched broccoli samples were fairly consistent throughout the chill storage. Results also demonstrated that phenolic content of unblanched broccoli samples increased significantly from day 0 to day 5 and than decreased thereafter.

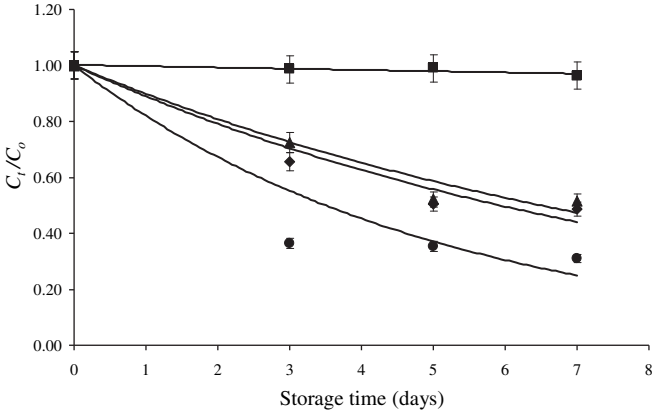

Fig. 2. First- order plot of ascorbic acid degradation in blanched and unblanched vegetable samples. [Blanched broccoli (▲),Unblanched broccoli (◆); Blanched carrot (■), Unblanched carrot (●)].(values are average of $n = 3$).

Table 3
Influence of treatment (BLFR/UBFR) and subsequent chill storage on colour parameters of blanched and unblanched (95 °C, 3 min) broccoli florets, carrots and green beans during storage for 7 days at 4 °C.

Days of storage	L^*		TCD[b]		Chroma	
	BL[c]	UB[d]	BL	UB	BL	UB
Broccoli						
0	28.8 ± 1.34	18.3 ± 0.08	1.2 ± 1.21	10.9 ± 0.04	12.1 ± 4.45	8.8 ± 2.10
3	27.5 ± 1.64	18.1 ± 1.01	1.8 ± 3.01	11.8 ± 2.67	11.9 ± 3.90	6.9 ± 3.90
5	27.4 ± 1.55	18.1 ± 0.66	2.2 ± 0.05	11.2 ± 3.32	12.0 ± 3.54	8.7 ± 0.67
7	28.0 ± 1.04	14.0 ± 0.06	1.2 ± 2.45	7.4 ± 1.45	11.7 ± 2.78	9.1 ± 0.89
LSD[a]	3.67		0.92		1.35	
Carrots						
0	56.1 ± 1.32	56.3 ± 2.21	1.4 ± 1.56	1.8 ± 2.78	35.3 ± 2.78	36.1 ± 2.78
3	56.9 ± 1.67	42.4 ± 0.80	1.6 ± 1.89	16.2 ± 4.78	34.5 ± 0.06	42.0 ± 3.78
5	56.1 ± 2.56	44.5 ± 3.67	1.9 ± 2.67	13.3 ± 2.01	34.6 ± 0.04	38.7 ± 0.01
7	55.1 ± 4.31	43.1 ± 0.45	4.1 ± 3.01	14.4 ± 0.01	33.3 ± 2.43	35.8 ± 0.56
LSD	0.52		0.98		1.39	
Green beans						
0	41.3 ± 5.90	23.4 ± 0.34	2.9 ± 6.61	19.3 ± 5.89	12.9 ± 2.78	8.8 ± 2.90
3	41.6 ± 0.01	28.8 ± 4.78	4.3 ± 5.89	13.4 ± 0.09	11.9 ± 4.78	6.9 ± 0.01
5	41.0 ± 0.45	27.0 ± 2.45	2.5 ± 0.91	15.6 ± 0.20	12.0 ± 4.17	8.7 ± 0.02
7	41.7 ± 0.78	26.1 ± 2.90	2.3 ± 5.89	16.5 ± 1.67	11.7 ± 3.89	9.1 ± 2.71
LSD	3.04		1.08		1.35	

All values are means of three replicates of three batches and expressed in dry weight basis.
[a] least significant difference ($p = 5\%$).
[b] Total colour difference.
[c] Blanch.
[d] Unblanch.

A similar trend was observed for carrots and green beans. Studies in vegetables confirm that processing significantly alters the physical and bio-chemical composition and functionality (Patras, Brunton, Tiwari, & Butler, 2008; Zhang & Hamauzu, 2004) and may play a vital role in non-uniform behaviour of phenols towards different processing treatments. The total phenolic content of unblanched broccoli increased at a much higher rate during storage than that of blanched samples (Table 2). This increase could be related to the developmental changes and wound-like response due to freezing. 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 ± 1 °C and at ≈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.

4.4. Effect of treatment (BLFR/UBFR) and chill storage on instrumental colour parameters of broccoli, carrots and green beans

Colour is often the first parameter by which a consumer judges a food product before purchase (Gormley, 1978). Colour is used to judge the quality, maturity and age after harvest of many foods. During chilled storage, the colour of vegetables can become grey or brownish, or in some cases paler (Zacharias, 1980). In our study, total colour difference and the colour intensity calculated from colour parameters L^*, a^*, and b^* varied significantly among storage time and treatments (Table 3). Analysis of variance indicated that this variation was in most cases due to differences between vegetable types. Mean L^* values decreased from 28.85 to 28.07 for BLFR broccoli samples, whereas low values (14.04–18.3) of lightness were observed for UBFR samples at the end of storage. (Table 3). Similar results were reported by (Cruz, Vieira, & Silva, 2007) for watercress (*Nasturtium officinale*). In the case of carrots and green beans, L^* value was fairly stable in BLFR samples, whereas UBFR samples had lower values (Table 3). A decrease in L^* value is related to product lightness loss. These results demonstrate the significant effect of UBFR and BLFR treated samples on the colour degradation of the vegetable samples studied. The major colour changes in vegetable samples were largely due to the non-inclusion of blanching step.

Mean colour intensity (chroma) values were higher for blanched samples than unblanched counterparts. In the case of carrots, not much variation was observed in colour intensity for blanched or unblanched samples as shown in Table 3. Interestingly, Chroma

Table 4
Effect of treatment (BL/UB), storage days and interactions on quality parameters of broccoli, carrots and green bean.

Sample	Treatment	Storage days	Interaction (Treatment × Storage days)
Broccoli			
ARP[a]	S	S	NS
Phenols	S	NS	NS
AA[b]	S	S	S
L^*	S	S	S
TCD[c]	S	S	S
Chroma	S	S	S
Carrots			
ARP	S	S	NS
Phenols	NS	NS	S
AA	S	S	S
L^*	S	S	S
TCD	S	S	S
Chroma	S	S	S
Green beans			
ARP	S	S	S
Phenols	NS	NS	NS
L^*	S	S	S
TCD	S	S	S
Chroma	NS	NS	S

S = significant at 5% level; NS = not-significant.
[a] ARP.
[b] L- Ascorbic acid.
[c] Total colour difference.

values of green beans decreased significantly as a combined effect of storage and treatment as illustrated in Table 4. Proportion of grey component characterizing colour is given by values of Chroma. As Chroma decreases, colour becomes less intense (Lancaster, Lister, Reay, & Triggs, 1997) changing from a vivid to a dull green in case of broccoli and green beans in the present study.

Total colour difference (TCD) is a function of the three CIE L^* a^* b^* coordinates (Eq. (2)). TCD values increased from 1.2 to 2.2, 1.4 to 4.1, 2.3 to 4.3 for BLFR broccoli, carrots and green beans respectively, whereas greater differences were observed for UBFR samples. Colour change was highest in unblanched samples as compared to blanched samples for all three vegetables (Table 3). This may be due to lower L^* values in unblanched samples as level of enzyme activity (PPO) would be high in unblanched samples. A study conducted by (Cruz et al., 2007) demonstrated that blanching by heat and a combined treatment of heat/ultrasound (thermosonication) resulted in higher TCD values of 6 and 12 respectively in for watercress (*N. officinale*) as compared to control. Higher TCD values were reported by (Patras et al., 2009a) in heated treated carrot samples as compared to fresh sample. Hue angle provides more information about the spatial distribution of colours than direct values of tristimulus measurements (Sigge, Hansmanw, & Joubert, 2001) but an inconsistent affect of this colour parameter was observed in this study (data not shown).

5. Conclusions

The retention of total antioxidant activity, total phenols and ascorbic acid in broccoli, carrots and green beans was strongly influenced by the different pre-treatment and preservation methods investigated. BLFR was an effective technique for preserving ascorbic acid and antioxidant activity in all vegetables. The retention of these moieties being negatively influenced by non-inclusion of a blanching pre-treatment step. Vegetables subjected to blanching treatments had better retention of ARP and ascorbic acid and instrumental parameters as compared to unblanched counterparts. Total colour change was found to be substantially higher (TCD $>$ 3) for unblanched samples. Future directions for research might include investigation of methods to prevent losses during blanching that would be feasible in a home or industrial environment.

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