

Effect of blanching on the quality of Brussels sprouts (*Brassica oleracea* L. *gemmifera* DC) after frozen storage

Daniela F. Olivera^a, Sonia Z. Viña^a, Claudio M. Marani^a, Ricardo M. Ferreyra^a,
Alicia Mugridge^a, Alicia R. Chaves^a, Rodolfo H. Mascheroni^{a,b,*}

^a CIDCA (Centro de Investigación y Desarrollo en Criotecnología de Alimentos), CONICET, Facultad de Ciencias Exactas Universidad Nacional de La Plata (UNLP), Calle 47 y 116 S/N°, La Plata 1900, Buenos Aires, Argentina

^b Facultad de Ingeniería UNLP, Calle 115 entre 48 y 49 S/N°, La Plata 1900, Buenos Aires, Argentina

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Abstract

Three blanching conditions previous to freezing and frozen storage of Brussels sprouts were compared in terms of their combined effects on texture, surface colour, total chlorophyll, radical scavenging activity (RSA), ascorbic acid and total flavonoids content. The blanching alternatives were: immersion in water (50 °C; 5 min) followed by blanching in water (100 °C; 3 min) (PB); microwave heating (700 W, 5 min) followed by blanching in water (100 °C; 2 min) (MW); and direct blanching in water (100 °C; 4 min) (DB). Fresh controls and treated samples were frozen then stored at –18 °C for 8 months. Texture of sprouts was significantly affected by blanching although all treatments had a similar effect. Freezing and frozen storage caused an additional loss of firmness, but the magnitude was lower than that induced by blanching. With respect to surface colour, the PB method gave the highest $-a^*$ values as well as a better Chroma retention than the controls. All treatments increased RSA, ascorbic acid and total flavonoids contents. The only significant differences between treatments was the slightly higher value for $-a^*$ and Chroma for the PB method and an increase in final RSA levels for the MW pre-treatment.

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1. Introduction

Looking back over the historical development of quality requirements for processed foods, freezing – when properly carried out – is undoubtedly the most satisfactory method for the long-term preservation of vegetable produce. The low temperatures commonly used for frozen foods (–18 °C) can maintain initial quality and nutritive value practically unchanged (Canet, 1989).

Commonly consumed cruciferous vegetables include broccoli, Brussels sprouts, cabbage, cauliflower, collard greens, kohlrabi, mustard, rutabaga, turnips and Chinese cabbage. Like other dark green vegetables, many cruciferous vegetables are rich in folate and chlorophyll. One of the unique things about cruciferous vegetables is that they are rich sources of glucosinolates, sulfur-containing compounds that give them their pungent aromas and spicy taste. The chemical composition of cruciferous vegetables might help to protect against cancer. Although many organizations, including the National Cancer Institute, recommend the consumption of 5–9 servings (2 1/2–4 1/2 cups) of fruits and vegetables daily, separate recommendations for cruciferous vegetables have not been established. Much remains to be learned regarding cruciferous vegetable consumption and cancer prevention, but the results of some

* Corresponding author. Address: CIDCA (Centro de Investigación y Desarrollo en Criotecnología de Alimentos), CONICET, Facultad de Ciencias Exactas Universidad Nacional de La Plata (UNLP), Calle 47 y 116 S/N°, La Plata 1900, Buenos Aires, Argentina. Tel.: +54 221 424 9287; fax: +54 221 425 4853.

E-mail address: rmasche@ing.unlp.edu.ar (R.H. Mascheroni).

epidemiological studies suggest that adults should aim for at least five servings (2 1/2 cups) per week of cruciferous vegetables (Higdon, 2006).

Brussels sprouts are mostly grown outdoors and harvested seasonally, which makes freezing a very important preservation process for extending their availability throughout the year. Many people have no opportunity to eat fresh vegetables every day and frequently use frozen vegetables, mainly for convenience, time-saving and practical reasons (Ninfali & Bacchiocca, 2003). Processing of frozen vegetables comprises a number of stages, i.e. preliminary operations to prepare the product such as selection, washing, peeling and cutting, blanching and/or other pre-freezing treatments, cooling, freezing and frozen storage. Nutritive quality of frozen vegetables is closely linked to the mildness of blanching. This operation is a thermal process designed to inactivate the enzymes responsible for generating off-flavours and odours and to achieve the stabilization of texture and nutritional quality and the destruction of microorganisms (Bahçeci, Serpen, Gökmen, & Acar, 2005). However, since blanching is a heat treatment, changes associated with thermal processing can be expected. These include loss of turgor in cells, due to thermal destruction of membrane integrity and partial degradation of cell wall polymers (Bahçeci et al., 2005). Additionally, freezing effects are notable in leafy vegetables that contain high moisture and thin cell walls (Fuchigami, Hyakumoto, & Miyazaki, 1995). Thus, blanching and freezing can be considered as critical steps since both might induce significant changes in the structure and integrity of plant tissues.

On the other hand, vegetables contain an impressive array of antioxidant compounds that includes carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols (Benzie, 2003; Hollman, 2001; Strain & Benzie, 1999). These are concentrated in the oxidation-prone sites of the plant. For example, up to 50 mM concentrations of ascorbic acid are found in chloroplasts (Benzie, 2003). Thus, fruits and vegetables are frequently mentioned as the main source of essential nutrients in the human diet and many studies have shown that a close relation exists between the intake of vegetables and the prevention of cancer and cardiovascular diseases (Jung et al., 2006). The dietary input of plant-based antioxidants, most notably the vitamins C and E, is strongly recommended (Gey, 1998).

Much research has been devoted to assess the way that different kinds of processing and frozen storage affect the antioxidants content of fruits and vegetables (Nicoli, Anese, & Parpinel, 1999; Ninfali & Bacchiocca, 2003; Prochaska, Nguyen, Donat, & Piekutowski, 2000; Zhang & Hamazu, 2004). Although the results about the degree of tissues damage reported by different authors do not agree, there is a general consensus that these operations should be optimized with the objective of reducing deterioration to a minimum (Ferreira, Canet, Alvarez, & Tortosa, 2006).

The objective of the present work was to study the effect of blanching method on sensory and nutritive quality changes of Brussels sprouts after freezing and frozen storage.

2. Materials and methods

2.1. Plant material

Brussels sprouts (*Brassica oleracea* L. *gemmifera* DC) cultivar Oliver were provided by a local grower (La Plata, Buenos Aires, Argentina). Plants were field-grown according to the usual practices in the region. Sprouts were harvested between June and August 2005. Harvest was carried out early in the morning and the sprouts were immediately carried to the laboratory and processed.

2.2. Pre-treatments, freezing process and frozen storage

Prior to the application of the different treatments, sprouts were selected to give a uniform size (25.1 ± 3.4 g weight; 35.8 ± 0.22 mm diameter; 49.2 ± 0.16 mm height) and the absence of physical and microbiological damage. Most external leaves and 2 mm of the lower ends were discarded. Blanching treatments were selected according to the results of previous work (Viña et al., 2007). Thus, two types of *combined treatments* were applied: immersion in water at 50 °C for 5 min followed by blanching in boiling water for 3 min (PB); microwave heating in an oven (BGH Quick Chef Sensor Infrared Model 17950, Argentina) at 700 W for 5 min, followed by blanching in boiling water for 2 min (MW). A direct blanching treatment was also applied: immersion of sprouts in water at 100 °C for 4 min (DB). In treatments PB and DB, approximately 250 g of the product were dipped in 5 L of water in order to minimize temperature reduction of the blanching bath. In treatment MW, approximately 250 g of sprouts were heated in the microwave oven and then dipped in 5 L of boiling water. After the complete application of the treatments, samples were immediately cooled by immersion in an ice-water bath for 3 min. Non-treated samples of fresh Brussels sprouts were maintained as controls (C). Samples were frozen in a commercial cabinet freezer by injection of liquid N₂ (temperature of the atmosphere equal to -35 °C) until -18 °C was reached at the thermal centre of the sprouts. Sample temperature was measured by Cu–Ct thermocouples connected to a Keithley KDAC Series 500 data acquisition and control system. The system allowed temperature to be measured or controlled within an accuracy of ± 0.5 °C. Freezing rate (from the initial freezing temperature to a final temperature of -18 °C in the centre of the sprouts) was approximately 2.3 °C/min. Control and treated samples were stored at -18 °C for 2, 4, 6 or 8 months. Samples were then thawed by immersion in boiling water for 7 min and immediately analyzed. All treatments were carried out at least twice.

2.3. Physical determinations

2.3.1. Texture

Firmness of Brussels sprouts was measured with a texturometer TA-XT2i (Stable Micro Systems Ltd, Godalming, Surrey, UK) and data were acquired and processed with the Texture Expert® Exceed software. The texturometer was operated in the compression mode, with a 25 kg load cell. The probe used was an aluminium compression plate (75 mm in diameter). Samples were laterally compressed at a constant rate of 1 mm s⁻¹ and curves of force (N) as a function of time (s) were automatically recorded. The force needed to compress 3 mm the sprouts heads was registered. Each reported value corresponds to the mean of at least ten determinations.

2.3.2. Surface colour

This determination was carried out using a Minolta colorimeter CR 300 Series (Osaka, Japan) with an 8 mm diameter measuring area. The instrument was calibrated with a standard white plate ($Y = 93.2$, $x = 0.3133$, $y = 0.3192$). The colour of Brussels sprouts was measured by L^* , a^* and b^* chromaticity co-ordinates of the CIELab scale (CIE, 1978). Also, Chroma [$C = (a^{*2} + b^{*2})^{1/2}$] was calculated. The absolute value of co-ordinate a^* ($-a^*$), which represents a measure of greenness of sprouts was considered for comparison of treatments and samples. Measurements were performed in three different positions on sprouts heads as well as in the cut zones (bases). Each reported value corresponds to the mean of at least 10 determinations.

2.4. Chemical determinations

2.4.1. Total chlorophyll content

Extractions were carried out in 80% v/v acetone at 0 °C from 0.6 g of sample. After centrifugation at 9000g for 10 min at 4 °C, aliquots of the extracts were taken to determine chlorophyll content by spectrophotometry. Absorbance readings were conducted at 646.8 and 663.2 nm and this data were used to calculate total chlorophyll content according to Lichtenthaler (1987). Extractions were performed at least in duplicate.

2.4.2. Radical scavenging activity (RSA)

Extractions were carried out in 5 mL of 96% w/w ethanol from 1 g of sample. RSA of the extracts was determined by reaction with the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]), according to Brand-Williams, Cuvelier, and Berset (1995), with slight modifications (Viña et al., 2007). Extractions and determinations were carried out at least in duplicate.

2.4.3. Ascorbic acid content

Samples were weighed accurately to 1 g each and extracted in 5 mL aqueous solution of citric acid 3% w/v. After microcentrifugation, aliquots (1 mL) of supernatants

were used to quantify ascorbic acid (Wimalasiri & Wills, 1983). A Waters Model 6000A (Milford, MA, USA) high-performance liquid chromatograph was used, fitted with an UV-vis detector. A C18 column Ultrasphere ODS (Beckman Instruments, Inc., San Ramón, CA, USA) was employed (particle diameter, 5 µm; internal diameter, 4.6 mm; length, 25 cm). The mobile phase was a 70:30 mixture of acetonitrile:water with 0.01 M NH₄H₂PO₄ and pH adjusted to 4.3. Flow rate was 2 mL min⁻¹. Detection was carried out at 254 nm. For calibration, a standard ascorbic acid solution was employed. Extractions and determinations were carried out at least in duplicate.

2.4.4. Total flavonoids content

It was determined by the technique described by Kim, Jeong, and Lee (2003), with slight modifications (Viña et al., 2007), using 5% NaNO₂; 10% AlCl₃ and 1 M NaOH as reagents. Absorbance at 510 nm was measured. A standard curve was constructed based on a range of catechin concentrations. Extractions and determinations were conducted at least in duplicate.

All results were expressed as relative values (%) and were calculated as the ratio between the parameter registered for each treatment (i) and the one corresponding to fresh Brussels sprouts (controls), both before (0 month) and after freezing and frozen storage.

2.5. Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was conducted with the Fisher's least significant difference (LSD) test, at a significance level $p = 0.05$.

3. Results and discussion

Results after 2, 4 and 6 months frozen storage were not significantly different to the 8 month results so only the later are reported.

3.1. Texture

Firmness variations registered for the different treatments and sampling points are shown in Fig. 1. The maximum force corresponded to 50.3 N on average for fresh Brussels sprouts. Blanching treatments caused a significant reduction in firmness by 86.0%, 83.5% and 81.3% of the value for fresh product, for DB, PB and MW methods, respectively. Differences between treatments were not significant ($p = 0.05$). Better pre-treatment performance have been reported for other vegetables. For example, in carrots Vu et al. (2004) have shown that pre-heating treatments can slow down texture degradation compared to the same final heating temperature but without pre-heating. These authors showed that pre-heating influenced the rate constant, the final texture value and the activation energy of

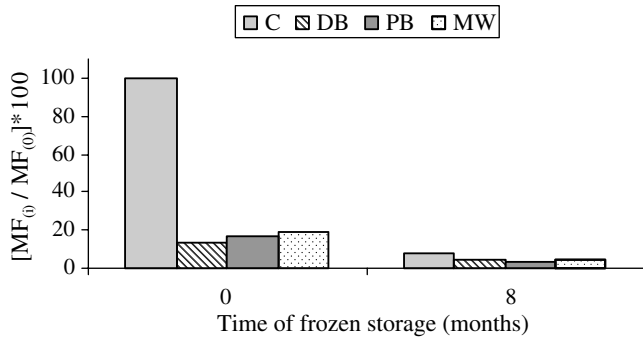


Fig. 1. Texture changes in blanched and frozen-stored Brussels sprouts, relative to fresh product (control) and expressed as percentage (%). MF: maximum force (N) for treated samples (i) or for controls (0). $LSD_{0.05} = 10.5\%$.

the texture degradation reaction, all in favour of texture improvement.

Fig. 1 shows that after freezing and 8 months of frozen storage, an additional reduction ($p = 0.05$) in firmness was registered for all treatments. No significant differences were found between control and treated samples after frozen storage, irrespective of the blanching method. Thus, after 8 months of frozen storage there were no outstanding advantages for the assayed blanching methods over the control (freezing without pre-treatments).

Loss of textural quality during blanching, freezing and thawing includes denaturation, dehydration damage, drip loss, tissue fractures and mechanical damage by ice crystals growth during freezing (Kidmose & Martens, 1999). Texture is definitely one of the quality indexes most affected by blanching, freezing and frozen storage of vegetables, and this effect could be clearly observed in our results. Similarly, some authors reported that freezing and frozen storage caused severe deterioration of the texture of tomatoes (Gradziel, 1988; Lisiewska & Kmiecik, 2000). Fuchigami, Miyazaki, and Hyakumoto (1995) observed worsened texture of carrots accompanied by a decrease in the content of pectic compounds. Ferreira et al. (2006), performed an in-depth study of freezing, thawing and cooking conditions on green beans quality indexes, mainly on texture and colour attributes, finding significant differences as a function of freezing and thawing rates. Also, Van Buggenhout et al. (2006) found significant influence of freezing rate on untreated frozen carrots texture. If pre-treatments were used (immersion in Ca^{2+} solution or mild heating) most of the advantages of rapid freezing on texture retention were lost.

3.2. Surface colour

Fresh Brussels sprouts heads were bright green as indicated by the CIE scale parameters: co-ordinate a^* was -19.5 ; Chroma was equal to 37.3 and the L^* co-ordinate, associated with lightness, was 61.5 . Fig. 2a shows variation in absolute values of co-ordinate a^* ($-a^*$) of Brussels sprouts heads as affected by different treatments and frozen

storage. As a general trend, treatments caused a significant ($p = 0.05$) increase in ($-a^*$) values relative to fresh product, denoting an improvement in greenness. The values obtained after the application of DB and MW were significantly higher than the ones corresponding to control samples, but there were no significant differences between these blanching methods. Highest absolute values of a^* were achieved by PB treatment application. After 8 months of frozen storage, ($-a^*$) values decreased both for control and treated samples suggesting loss in greenness of sprouts heads. Nevertheless, all treated samples showed significantly higher values of ($-a^*$) than the controls, with PB treatment giving the best scores. The PB treatment value did not differ significantly from fresh Brussels sprouts (control) prior to freezing.

The effects that heat treatments have on the colour of green vegetables have been studied extensively including use of mathematical models (Tijskens, Schijvens, & Biekman, 2001). Most of these studies only considered decrease in colour by heat treatments at constant and steady conditions. However, during the initial part of the heating, an increase in green colour is observed (Tijskens et al., 2001). Similarly, Herrmann (1993) reported that green beans blanched for 30 s showed higher scores for the green colour than non-blanched ones. Likewise, Lau, Tang, and Swanson (2000) noticed an initial increase in green colour of asparagus after heat treatments. In previous works (MacKinney & Weast, 1940; Meyer, 1960) this phenomenon was attributed to air removal around the fine hairs on the surface of plant tissues and to the expulsion of air between the cells, processes that are responsible for an alteration of the reflecting properties of the surfaces (Tijskens et al., 2001).

Fig. 2b shows variations of Chroma values of Brussels sprouts heads as affected by different treatments and frozen storage. Blanching treatments caused no significant variations in Chroma parameter. After 8 months of frozen storage, Chroma of blanched sprouts remained high, although it decreased significantly ($p = 0.05$) for the control, DB and MW treatments. The highest retention of Chroma relative to fresh Brussels sprouts was achieved by the PB treatment. Chroma values relate to the proportion of the grey component that characterizes colour. As Chroma decreases, colour becomes less intense (Lancaster, Lister, Reay, & Triggs, 1997) and this means that the colour of Brussels sprouts changes from a vivid to a dull green.

Fig. 2c shows variations of co-ordinate L^* of Brussels sprouts heads as affected by different treatments and frozen storage. All the treatments caused a significant decrease in L^* , that is related to loss of lightness, relative to the control. DB treatment induced the greatest reduction in L^* values, while there were no significant differences between PB and MW treatments. After 8 months of frozen storage, there was no additional reduction in L^* parameter for blanched samples. PB and MW alternatives seem to be the best for maintaining L^* parameter in higher levels relative to non-treated sprouts.

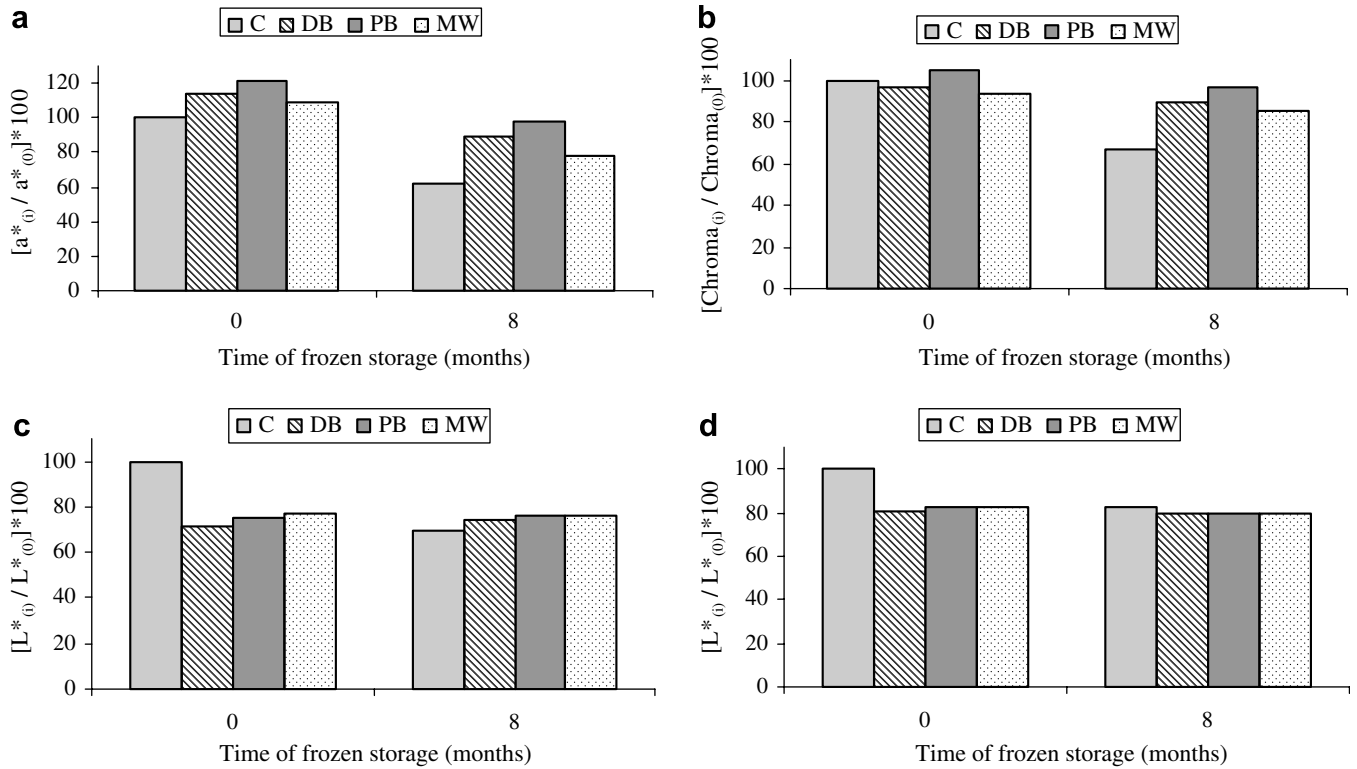


Fig. 2. Changes in colour of blanched (i) and frozen-stored Brussels sprouts heads, relative to fresh product (control, 0) and expressed as percentage (%). (a) Co-ordinate a^* (absolute values); $LSD_{0.05} = 6.1\%$. (b) Chroma; $LSD_{0.05} = 7.0\%$. (c) Co-ordinate L^* of heads; $LSD_{0.05} = 4.0\%$. (d) Co-ordinate L^* of cut zones; $LSD_{0.05} = 2.0\%$.

External colour was also measured in the cut zones of Brussels sprouts and the parameter selected to evaluate the main changes in colour was the L^* co-ordinate, whose initial value was 81.8. Fig. 2d shows changes in parameter L^* of Brussels sprouts bases. All treatments had no effect in controlling darkening (or browning) of cut zones. L^* co-ordinate reduced for all treatments, but after applying DB treatment, L^* values were slightly lower than those for MW method. However, this initial advantage of MW treatment was lost after 8 months of frozen storage.

3.3. Total chlorophyll (Chl) content

Fig. 3 gives the total Chl content of Brussels sprouts after treatment applications and frozen storage. Fresh sprouts had a total Chl content equal to $31.8 \mu\text{g/g}$ fresh tissue, with the Chl $a/\text{Chl } b$ ratio being 2.43. The analysis of variance showed that treatments had no significant effect on total Chl content ($p = 0.74$) of Brussels sprouts. After 8 months of frozen storage total Chl content decreased more significantly ($p = 0.05$) for the control than for treated samples. For blanched Brussels sprouts, frozen storage induced minor changes comparing with after blanching treatment alone. Bhohe and Pai (1986) pointed out that loss of chlorophyll during blanching of several green vegetables ranged from 3% to 7% but the duration of blanching was shorter (20–120 s, depending on the particular product) than in our treatments. Similarly, these authors found

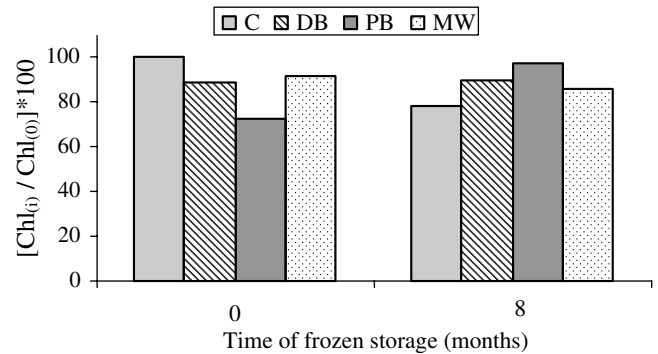


Fig. 3. Total chlorophyll content (Chl) changes in blanched (i) and frozen-stored Brussels sprouts, relative to fresh product (control, 0) and expressed as percentage (%). $LSD_{0.05} = 13\%$.

that about 17–24% of the chlorophyll was lost after 3 months of frozen storage. Total Chl content retention was higher in our experiments with Brussels sprouts, independently of the blanching method and even after 8 months of frozen storage.

3.4. Radical scavenging activity (RSA)

For control samples, mean RSA corresponded to $700 \mu\text{mol DPPH}/100 \text{ g}$ fresh tissue. Both pre-blanching methods (PB and MW) as well as the DB treatment gave a significant increase in RSA of Brussels sprouts

(Fig. 4a). These results agree with those of Turkmen, Sari, and Velioglu (2005) who have found that boiling (5 min), microwave cooking (1000 W for 1–1.5 min) and steaming over boiling water (7.5 min) induced significant increases in total antioxidant activity of selected green vegetables such as pepper, green beans, broccoli and spinach.

Frozen storage induced a significant ($p = 0.05$) further decrease in RSA for DB and PB-treated samples of Brussels sprouts. Conversely MW-treated samples showed an increase in RSA after 8 months at -18°C . Kidmose and Martens (1999) analyzed changes in microstructure of carrot slices during blanching and freezing. They found that in both water-blanching and steam-blanching samples, disruption of tissues was noted but no clear difference between the methods could be seen. The fractures took place mainly

intercellularly, meaning that individual cells remained intact. Instead, carrots exposed to microwave blanching had a significantly different appearance from those blanched by water or steam, with the tissue being composed of a patchwork of groups of well-preserved cells and layers of collapsed, sunken cells (Kidmose & Martens, 1999). They also found that cryogenic freezing at -30°C induced large cracks and cell collapse. This kind of study should be carried out for Brussels sprouts, to assess whether the blanching and freezing processes, as well as the frozen storage period, results in the release of radical species and antioxidant compounds from injured plant tissues and cells.

3.5. Ascorbic acid content

Recently harvested Brussels sprouts had an ascorbic acid content of 89 mg/100 g fresh tissue. PB and DB blanching methods significantly reduced ($p = 0.05$) initial ascorbic acid content (Fig. 4b), probably due to leaching. MW treatment induced an increase in ascorbic acid content. Some authors have explained similar phenomena by an increase in chemical extraction of certain organic compounds from the plant tissue, after microwave heating (Chun, Lee, Ye, Exler, & Eitenmiller, 2006; Oerlemans, Barrett, Suades, Verkerk, & Dekker, 2006; Verkerk & Dekker, 2004). Also, vegetable tissues suffer changes in the permeability of cells and vacuoles membranes upon heating and freezing, and this fact could modify the extraction of metabolites. Kidmose and Martens (1999) pointed out that microwave blanching of carrots resulted in higher nutritional quality compared to water blanching, while Bognár, Grünauer, and Doll (1987) found a higher content of dry matter, minerals, vitamin C and total sugars in carrots both immediately after blanching and after three months of frozen storage.

In our experiments, the analysis of variance showed that frozen storage had no significant effect on ascorbic acid content ($p = 0.31$) of Brussels sprouts.

3.6. Total flavonoids

The total flavonoids content (TFC) of fresh Brussels sprouts was 18.6 mg catechin/100 g fresh tissue. After blanching, TFC remained approximately constant ($p = 0.05$) although measurements showed a slight decrease due to blanching, regardless of the treatment. In all samples, a further rise in TFC was given by the combined effect of freezing and frozen storage. After 8 months at -18°C , control samples, DB, PB and MW-treated Brussels sprouts showed no significant differences in their TFC (Fig. 4c).

Flavonoids commonly accumulate in epidermal cells of plant organs, being found as glycosides and in non-glycosidic forms (aglycones) (Sakihama et al., 2002). Subcellular localization of the glycosides is mainly confined to hydrophilic regions such as vacuoles and apoplasts (McClure, 1975; Wollenweber & Dietz, 1981). Release of flavonoids

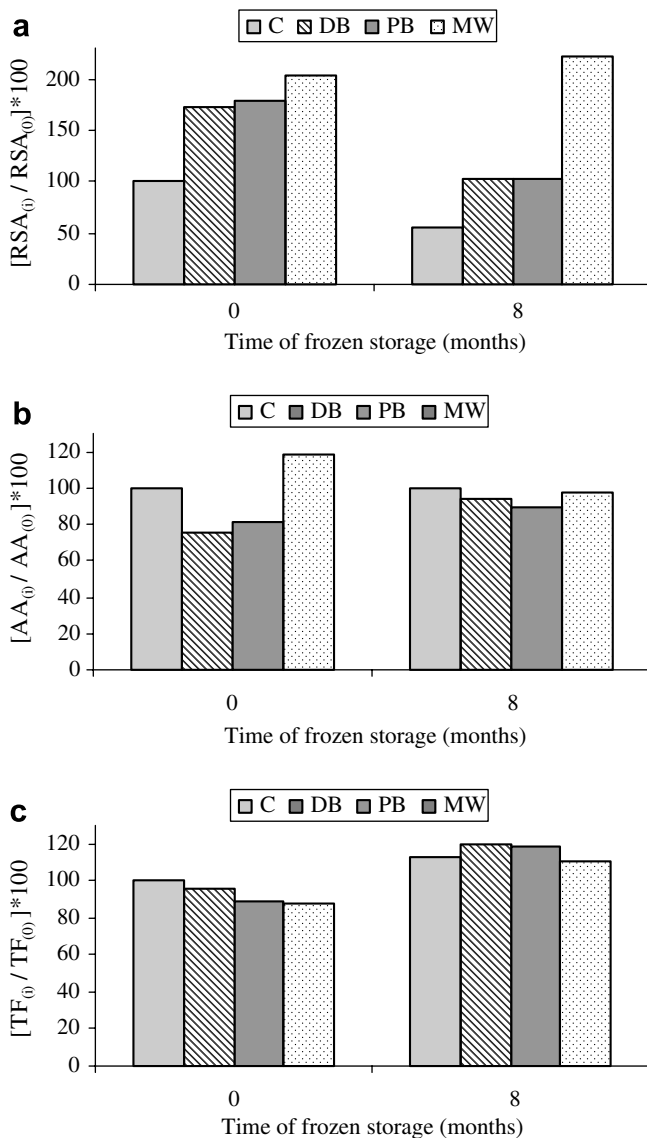


Fig. 4. Nutritive quality changes in blanched (i) and frozen-stored Brussels sprouts. Results were relative to fresh product (control, 0) and expressed as percentage (%). (a) Radical scavenging activity (RSA); $\text{LSD}_{0.05} = 17.4\%$. (b) Ascorbic acid content (AA); $\text{LSD}_{0.05} = 8\%$. (c) Total flavonoids content (TFC); $\text{LSD}_{0.05} = 16\%$.

and increased chemical extraction of these compounds could be induced by the combined effect of blanching, freezing and frozen storage similarly to the higher RSA for processed samples of Brussels sprouts.

4. Conclusions

Firmness of Brussels sprouts was mainly affected by the blanching operations and all the treatments had a similar effect. The combined action of freezing and long-term frozen storage caused an additional loss of firmness, but the effect was lower than that of blanching.

With respect to surface colour, all treatments caused a significant increase in ($-a^*$) values compared to fresh Brussels sprouts, increasing the greenness of the product. Highest absolute values of a^* were achieved after PB treatment but all treatments (specially PB) showed better Chroma retention than controls after 8 months of frozen storage.

Increases in measured RSA, content of ascorbic acid and total flavonoids might be related to loss of integrity of tissues, cells, membranes and organelles by the combined effect of blanching, freezing and frozen storage. Differences between treatments were not significant. Further research considering these aspects (mainly structural changes at membrane level) should be carried out.

The only significant difference determined among treatments was the slightly higher value for $-a^*$ and Chroma for the PB treatment and an important increase in final RSA levels for MW. Taking into account the trend towards more healthy foods, microwave pre-treatments seem to be the best industrial blanching methods for frozen Brussel sprouts.

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