Food Chemistry 112 (2009) 967–976

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Glucosinolates, L-ascorbic acid, total phenols, anthocyanins, antioxidant capacities and colour in cauliflower (*Brassica oleracea* L. ssp. *botrytis*); effects of long-term freezer storage

Jon Volden [a,b,*], Gunnar B. Bengtsson [b], Trude Wicklund [a]

[a] Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Aas, Norway
[b] Nofima Food, Matforsk AS, Osloveien 1, NO-1430 Aas, Norway

ARTICLE INFO

Article history:
Received 15 January 2008
Received in revised form 15 May 2008
Accepted 9 July 2008

Keywords:
Cauliflower
Blanching
Freezing
Glucosinolates
Polyphenols
Anthocyanins
L-ascorbic acid
FRAP
ORAC
CIELAB

ABSTRACT

Blanching and one year freezer storage were performed on one purple, two white and two green varieties of cauliflower (*Brassica oleracea* L. ssp. *botrytis*) to assess the effects on several health-related phytochemicals. Blanching, prior to freezing, reduced total aliphatic and indole glucosinolates (GLS) by 31% and 37%, respectively. L-ascorbic acid (L-AA), total phenols (TP), anthocyanins, FRAP and ORAC were on average reduced by 19, 15, 38, 16 and 28%, respectively. The colour measured as CIELAB parameters was partly affected by blanching: lightness was significantly reduced in the white cultivars and in a romanesco type cultivar. Chromaticity was reduced in the coloured cultivars with a twice-as-large reduction in the purple cultivars. Hue increased for all samples, except for the purple cultivar where a decrease was found. Long-term freezer storage did not affect total aliphatic and indole GLS in cauliflower in a major way. Freezer storage did result in an average L-AA decrease of 24% for all but the purple cultivar. Some reductions in the TP levels were also found but not to the extent found for L-AA. When an effect was found in the FRAP and ORAC values, the reductions occurred towards the end of the storage period and were 15% and 37%, on average, respectively. Long-term freezer storage did not affect the anthocyanin content and only minor effects were found for the colour parameters.

1. Introduction

The compositions of ingested foods have relationships to positive but also adverse health effects, such as cardiovascular disorders, cancers, diabetes, hypertension and obesity (World Cancer Research Fund/American Institute for Cancer Research, 2007; World Health Organization, 2003). Vegetables are important components of the diet, supplying a multitude of health-related phytochemicals. In this era, with its fast pace and requirements to quickly prepare pre- or half-fabricated meals, the use of frozen vegetables is increasing (Barbosa-Cánovas, Altunakar, & Mejía-Lorío, 2005). In addition, many parts of the world have limited access to fresh vegetables and prolonged frozen storage is a requirement. Short-term thermal treatment, i.e. blanching, is necessary as a pre-treatment to freezing procedure, mainly in order to inactivate degradative enzymes (Cano, 1996). After blanching, the temperature is rapidly brought down by cooling, the vegetable is frozen and maintained at constant temperature, typically around −20 to −30 °C. Storage takes place at the producer, retailer or consumer stage, up to 18 months prior to consumption, depending on the product. As health awareness is increasing and the evidence supporting the notion that a diet rich in vegetables may lead to significant health benefits are mounting, the need for accurate and reliable data concerning the effects of long-term freezer storage on commonly used vegetables is clear.

In recent decades, flavonoids and glucosinolates (GLS) have been the focus of much research, due to their potential as health-promoting phytochemicals (Rice-Evans, Miller, & Paganga, 1996; Verhoeven, Verhagen, Goldbohm, van den Brandt, & van Poppel, 1997). Flavonoids are polyphenols, with a C6–C3–C6 flavan structure, present in many plants. GLS are thioglucosides, distinctive to the plant order *Capparales*, especially the family *Brassicaceae*, to which the cabbage-type vegetables belong. Flavonoids exhibit antioxidant and antimicrobial properties and have been investigated extensively regarding their ability to lower the risk of cardiovascular diseases (Hertog et al., 1995; Knekt, Jarvinen, Reunanen, & Maatela, 1996). GLS will upon degradation by either enzymes within the plant or by decomposition within the alimentary tract,

* Corresponding author. Address: Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Aas, Norway. Tel.: +47 64 96 58 70; fax: +47 64 96 59 01.
E-mail address: jon.volden@umb.no (J. Volden).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.07.018

yield secondary compounds that have been linked to a reduced risk of specific forms of cancer (van Poppel, Verhoeven, Verhagen, & Goldbohm, 1999; Verhoeven et al., 1997). L-ascorbic acid (L-AA, vitamin C) is closely linked to human health and, as it is not formed within the human organism, has to be administered on a regular basis (Davey et al., 2000). Colour is one of the most important consumer-related quality attributes and the visual appearance of many vegetables and fruits is determined by the presence of a distinct group of flavonoids, the anthocyanins, conferring blue, purple, red and orange colours (Andersen & Jordheim, 2006).

The most investigated phytochemical related to processing and storage of fruits and vegetables is L-ascorbic acid and, as it is both labile and highly water-soluble, losses during processing can occur due to ascorbic acid oxidase, thermal breakdown and leaching (Davey et al., 2000). Naturally, heat denaturation prevents further action by degradative enzymes. This is one of the reasons that long-term freezer storage of blanched vegetables is generally believed to impose minimal loss of health-related phytochemical properties, thus leading to superior preservative attributes (Cano, 1996). Lisiewska and Kmiecik (1996) showed that blanching reduced the vitamin C content in broccoli and cauliflower by 41–42% and 28–32%, respectively. The blanched vegetables were then freezer-stored for up to one year with 15–18% and 6–13% losses for broccoli and cauliflower, respectively. Similar results were obtained by Puupponen-Pimiä et al. (2003) for a range of different vegetables. Cieślik, Leszczyńska, Filipiak-Florkiewicz, Sikora, and Pisulewski (2007) investigated the effects of blanching and freezing for 48 h at −22 °C in several different vegetables, finding that blanching reduced the total GLS levels by 2–30%, followed by inconsistent effects of freezing.

There have been relatively few studies related to processing, and long-term freezer storage in particular, of vegetables even though they constitute a significant part of the ingested diet in many parts of the world. The majority of investigations conducted have focused on single constituents (especially ascorbate) or groups of similar compounds. It is becoming clearer that a significant beneficiary health gain is linked to the action of multiple fundamentally different compounds present (World Cancer Research Fund/American Institute for Cancer Research, 2007; World Health Organization, 2003). For these reasons, the objectives of this study were to investigate the effects of blanching and long-term frozen storage of cauliflower (*Brassica oleracea* L. ssp. *botrytis*) in five different varieties (two white, one green, one purple and one green pyramidal/romanesco), including one industrially processed cauliflower. Cauliflower was chosen as it is rich in numerous health beneficial chemicals and is one of the major frozen vegetable commodities. The growing interest in brassicas is related to the GLS and polyphenolics present and these are thus important vegetables, beyond their palatable qualities. The presented study includes determinations of the following parameters in untreated, blanched and freezer-stored (3, 6 and 12 months) cauliflower: glucosinolates (GLS), L-ascorbic acid (L-AA), total phenols (TP) and total monomeric anthocyanins (TMA), the CIELAB colour coordinates, as well as antioxidant capacity-related parameters, as measured by ferric reducing ability power (FRAP) and oxygen radical absorbance radical assay (ORAC).

2. Materials and methods

2.1. Chemicals

Methanol, acetonitrile, acetic acid, hydrochloric acid, $FeCl_3 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, anhydrous sodium carbonate, sodium acetate, potassium chloride, potassium hydrogen phosphate (K_2HPO_4), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) and L(+)-ascorbic acid were obtained from Merck KGaA (Darmstadt, Germany). Sinigrin (prop-2-enylglucosinolate), glucoraphanin (4-methylsulfinylbutylglucosinolate), glucoiberin (3-methylsulfinylpropylglucosinolate) and glucotropaeolin (benzylglucosinolate) were purchased from C_2 Bioengineering (Karlslunde, Denmark). Oxalic acid was purchased from BDH Chemicals Ltd. (Poole, England). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), 2,4,6-tri(2pyridyl)-*s*-triazine (TPTZ) and trifluoroacetic acid (TFA, 1 ml ampoules) were obtained from Fluka Chemie GmbH (Buchs, Switzerland). Methanol with 0.1% TFA was supplied by Riedel-de Haën/Honeywell Specialty Chemicals Seelze GmbH, Hanover, Germany. Folin–Ciocalteu phenol reagent (2.0 N), 3,4,5-trihydroxybenzoic acid (gallic acid), 2,2′-azobis(2-methylpropionamidine)dihydrochloride (AAPH) and fluorescein (3′,6′-dihydroxyspiro[isobenzofuran-1[3H],9′[9H]-xanthen]-3-one) disodium salt were from Sigma–Aldrich (St. Louis, MO, USA). Liquid nitrogen and helium gas were supplied by Yara International ASA (Oslo, Norway). All chemicals and gases implemented were of analytical grade.

2.2. Plant material

Cauliflower (*Brassica oleracea* L. ssp. *botrytis*) cv. 'Aviso' (white), 'Flamenco' (white), 'Celio' (green pyramidal/romanesco), 'Emeraude' (green) and 'Grafitti' (purple) were grown, cultivated and harvested at the Norwegian University of Life Sciences, 30 km south of Oslo. The median weight and range in grammes of the cauliflower heads were: 'Aviso' 1283 (916–1712), 'Flamenco' 1111 (785–1559), 'Celio' 740 (497–1004), 'Emeraude' 890 (637–1389) and 'Grafitti' 847 (631–1447). All material was processed on the day of harvesting. Frozen industrial samples of cv. 'Aviso' (denoted 'Aviso'IND in the tables) were obtained from a major Norwegian vegetable-processing company.

2.3. Blanching and long-term freezer storage

Blanching was conducted by immersing 1000 g of fresh cauliflower florets, pooled from 7 × 500 g heads, in 10,000 g of tap water (96–98 °C) for 3 min. Afterwards, the blanched material was drained for 1 min, cooled in 10,000 g of ice-water for 2 min and drained for 1 min. For each cultivar, blanching was performed three times. Blanched cauliflower florets were divided into three equally sized portions, put into polyethylene-coated paper bags (Frantschach Industrial Packaging Deutschland GmbH, Hammelburg, Germany) and placed in a freezer at −24 °C. Cv. 'Aviso' samples, from the industrial supplier, were blanched at 90 ± 2 °C for 8.5 ± 0.2 min, cooled to <10 °C, single-freezed and packed in polyethylene-coated paper bags. Three 25 kg bags were obtained from the industrial supplier.

When sampled, cauliflower florets were immersed in liquid nitrogen, crushed to a coarse powder in a porcelain mortar and further ground using a Braun CombiMax K 700 grinder (Braun GmbH, Kronberg, Germany) at speed 10 for 1 min. Great care was taken to omit thawing of material and all equipment in contact with the frozen material was at subzero temperatures.

2.4. Glucosinolates

Analyses of native GLS were performed as described elsewhere (Volden et al., 2008). Briefly, samples were lyophilised and milled and 200 mg were extracted in 4.5 ml of 70% (v/v) 73 °C methanol and held at that temperature for 3 min. Two hundred microliter of 2 mM glucotropaeolin were added as control standard. The sample was then homogenised for 1 min (Ultra-Turrax T25 Basic from IKA-Werke GmbH & CO, Staufen, Germany) at 21,500 rpm fitted with a S25N–8G dispersion tool, and centrifuged (4300*g* for 15 min). The supernatant was collected, the pellet re-suspended

with 3 ml of 70% methanol and centrifuged. Aliquots (1.5 ml) of the combined supernatants were evaporated to dryness at 45 °C (Savant SPD131DDA Speed Vac Concentrator, Thermo Electron Corp., Vantaa, Finland) and re-dissolved with water. Prior to LC–MS analysis, all samples were filtered using a 0.45 μm PVDF filter (Millex-HV, Millipore). An Agilent 1100 Series LC/MSD Trap XCT system (Agilent Technologies, Waldbronn, Germany) with a photodiode array detector using a Betasil C18 column (250 × 2.1 mm, 5 μm) with a guard column (40 mm × 2.1 mm, 5 μm) from Thermo (Thermo Fisher Scientific Inc., Waltham, MA), was used. Separation and detection conditions were as described earlier (Volden et al., 2008). Detection was carried out at 227 nm and quantification was according to Tian, Rosselot, and Schwartz (2005). Identification was facilitated by using the ion trap in a similar manner to Bennett, Mellon, and Kroon (2004). All samples were analyzed simultaneously and the results reported as μmol GLS per 100 g fresh weight (FW).

2.5. L-ascorbic acid

Determination of L-AA was performed as described earlier (Volden et al., 2008). Briefly, 25 g of frozen cauliflower material were added to 50 ml of 1.0% (w/v) oxalic acid, homogenised for 1 min using a Braun MR 400 hand processor, filtered and the filtrate applied onto an activated (5 ml methanol + 5 ml water) Sep-Pak C18 from Waters Corp. (Milford, MA, USA) in order to remove unwanted constituents. Prior to injection, the sample was filtered using a 0.45 μm Millex-HA (Millipore Corp., Bedford, MA, USA). Separation and detection were performed using an Agilent 1100 Series LC system fitted with a Zorbax SB-C18 (250 × 4.6 mm, 5 μm) column with Zorbax XDB C18 (4 × 4 mm, 5 μm) guard column (Agilent Technologies) at 254 nm. L-AA was quantified by external calibration and results are reported as mg L-AA acid per 100 g FW.

2.6. Methanolic extraction for total monomeric anthocyanin, total phenol and antioxidant capacity analysis

Frozen ground 5 g samples were weighed into pre-cooled 50 ml centrifuge tubes prior to addition of 15 ml of cold acidified (10 mM HCl) methanol and homogenisation at 22,000 rpm for 45 s using a Polytron PT 3000 (Kinematica AG, Littau-Lucerne, Switzerland). The homogenate was centrifuged at 31,000*g* for 10 min at 4 °C using a Beckman J2-21 M/E centrifuge (GMI Inc., Ramsey, MI, USA). The supernatant was decanted, the pellet resuspended with 10 ml of acidified methanol and centrifuged. The combined supernatants were placed in a –40 °C freezer in fully filled tightly capped storage tubes prior to analysis of antioxidant capacities, total phenols and total monomeric anthocyanins. All samples were extracted in duplicate and analyzed in triplicate.

2.7. Total phenols

The contents of TP were determined using the Folin–Ciocalteu reagent (FCR), reading the resultant reduced analytes at absorbance maximum at 765 nm. The method used was based on Singleton, Orthofer, and Lamuela-Raventós (1999) with modifications (Volden et al., 2008). TP was determined using a Konelab 30i clinical chemical analyser (Thermo Electron Corp.). In brief, 20 μl samples were added to 100 μl of FCR (diluted 1:10 with dist. water), mixed and incubated at 37 °C for 60 s prior to addition of 80 μl of 7.5% (w/v) sodium bicarbonate solution. The reaction mixture was incubated at 37 °C for 15 min prior to absorbance reading. TP were assessed against a calibration curve of gallic acid, and the results presented as mg gallic acid equivalents (GAE) per 100 g FW.

2.8. Ferric reducing ability power

FRAP was analyzed according to Benzie and Strain (1996), detecting the absorbance change of the reduction of $Fe(TPTZ)^{3+}$ to $Fe(TPTZ)^{2+}$ (intense blue) at 595 nm. The method was carried out as described earlier using a Konelab 30i (Volden et al., 2008). Briefly, 200 μl of the FRAP reagents (3.0 mM acetate buffer, 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$, ratio 10:1:1) were automatically pipetted separately and mixed in the cuvettes; 8 μl of sample were added, mixed and left to incubate at 37 °C for 10 min prior to absorbance measurement. Trolox (Vit. E analogue) was used as control. Results are expressed as mmol Fe^{2+} per 100 g FW.

2.9. Oxygen radical absorbance capacity

ORAC, developed and validated by Ou, Hampsch-Woodill, and Prior (2001), was performed as described by Dávalos, Gómez-Cordovés, and Bartolomé (2004) with minor adjustments (Volden et al., 2008). Measurements were carried out on a FLUOStar Optima from BMG Labtech GmbH (Offenburg, Germany) equipped with a fluorescent filter (exitation, 485 nm; emission, 520 nm) using a black 96-F MicroWell (Nunc A/S, Roskilde, Denmark). Reaction was carried out in 75 mM phosphate buffer (pH 7.4). Twenty micro liter diluted samples and 120 μl fluorescein were pipetted into the wells of the microplate and left to pre-warm for 10 min at 37 °C inside the instrument prior to addition of 60 μl fresh made 37 °C AAPH, using the instrument pump. Concentrations of fluorescein and AAPH in the wells were 70 nM and 24 mM, respectively. Trolox calibrations were performed in triplicate and samples in duplicate. The areas under the curve of blank were subtracted and the results were expressed as mmol Trolox equivalents (TE) per 100 g FW.

2.10. Total monomeric anthocyanins

TMA were determined using the pH-dependent absorption characteristics of anthocyanins, allowing assessment of levels by using different buffers (Giusti & Wrolstad, 2001). TMA concentration was assessed using a Konelab 30i photometer (Thermo Electron Corp.) by the comparing the 520 nm absorbance maximum at pH 1.0 to no absorption at pH 4.5 (Volden et al., 2008). Bichromatic measurements were made with both buffer/sample solutions at 520 nm and 700 nm (side-wavelength) after adding 20 μl of sample to 200 μl of pH 1.0 or pH 4.5 buffer solution (0.025 M potassium chloride or 0.4 M sodium acetate, respectively), mixing and incubation at 37 °C for 5 min. TMA contents are expressed as mg cyanidin-3-glucoside equivalents (cy-3-gluE) per 100 g FW.

2.11. Colour

A HunterLab LabScan XE (Hunter Assoc. Lab. Inc., Reston, VA, USA) was used to measure the spectral reflectance as CIE (CIE, Colorimetry, & pub. 015:, 2004) chromatic coordinates L^* (lightness, 0–100; black–white), a^* (red, + or green, −) and b^* (yellow, + or blue, −) values. The illuminant D_{65}, the 10° observer condition and Universal ver. 3.3 software were used. Frozen samples were thawed in a refrigerator. Triplicate measurements were performed on different floret areas. Results are reported as L^*, hue angle (h_{ab}, Eq. (1)), which is related to the red/blueness (green/yellowness) and chroma (C^*, Eq. (2)), which is related to the quantitative qualities of colour.

$$h_{ab} = \tan^{-1}\left[\frac{b*}{a*}\right] \quad (1)$$

$$C* = \sqrt{a*^2 + b*^2} \quad (2)$$

2.12. Statistical analysis

In all experiments one-way analysis of variance, in combination with Tukey's test for individual comparisons, was used to evaluate the effects of blanching, freezer storage and cultivar. The significance level used was $p < 0.05$. MINITAB® Release 14.20 Statistical Software (Minitab Inc., State College, PA, USA) was used for all statistical assessments.

3. Results and discussion

3.1. Glucosinolates

The levels of total aliphatic GLS in the untreated, blanched and long-term frozen cauliflower are shown in Fig. 1A. The highest total levels of aliphatics were found in 'Aviso' and the lowest, at about half the amount, were found in the other white cauliflower, 'Flamenco'. Of the total indole GLS (Fig. 1B) the highest levels were found for the green cv. 'Emeraude', followed by the green pyramidal cv. 'Celio'. The total GLS levels were, from high to low, 'Emeraude' > 'Celio' > 'Aviso' > 'Grafitti' > 'Flamenco'. Individual aliphatic GLS are shown in Table 1. Glucoiberin and progoitrin were found in all cultivars. The inherent levels varied between the cultivars and the most abundant aliphatic was progoitrin, except for 'Emeraude', wherein glucoraphanin was the most plentiful. Glucoerucin was only detected in the green cultivars ('Emeraude', 'Celio'). Glucoraphanin and sinigrin were absent in 'Flamenco' and 'Emeraude', respectively. The investigated cultivars all contained the four indole GLS shown in Table 2. Glucobrassicin was the most abundant indole GLS. The GLS composition and levels determined are in accordance with previous investigations (Cieślik et al., 2007; Schonhof, Krumbein, & Brückner, 2004; Tian et al., 2005).

Blanching reduced the total aliphatic and indole GLS levels significantly ($p < 0.05$) in all cauliflowers bar total indoles in 'Flamenco' (Fig. 1A). The reductions ranged from 23% to 38% and 23 to 53% for total aliphatic and total indole GLS, respectively. Cieślik et al. (2007) blanched cauliflower at 80 °C for 3 min, finding losses in total GLS of 2.7% and 13.0% in green and white, respectively. The amount of water used was not given and the reported losses were lower than those determined in our study for the different cultivars. Tables 1 and 2 show how the individual GLS were affected and, for most of the cultivars, the reductions were statistically significant. The aliphatic GLS, glucoiberin, progotrin, sinigrin, glucoerucin and glucoraphanin, were reduced by 29, 31, 36, 32 and 37%, respectively. The reductions of indole GLS, 4-hydroxyglucobrassicin, glucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin levels were 35, 44, 31 and 37%, respectively. The individual levels reported by Cieślik et al. (2007) in blanched cauliflower are puzzling, e.g. 4-hydroxyglucobrassicin was reduced by blanching in white, but increased in green cauliflower. The reasons for these discrepancies are unknown. Goodrich, Anderson, and Stoewsand (1989) blanched broccoli and Brussels sprouts (4 min, 99 °C, unknown vegetable-water ratio), finding significant losses in GLS in the former and none in the latter, contributing this to the differences in the physical configurations.

The effect of long-term freezer storage on GLS was less severe than that caused by blanching (Fig. 1, Tables 1 and 2). The total aliphatic and indole GLS levels were, overall, unaffected. However,

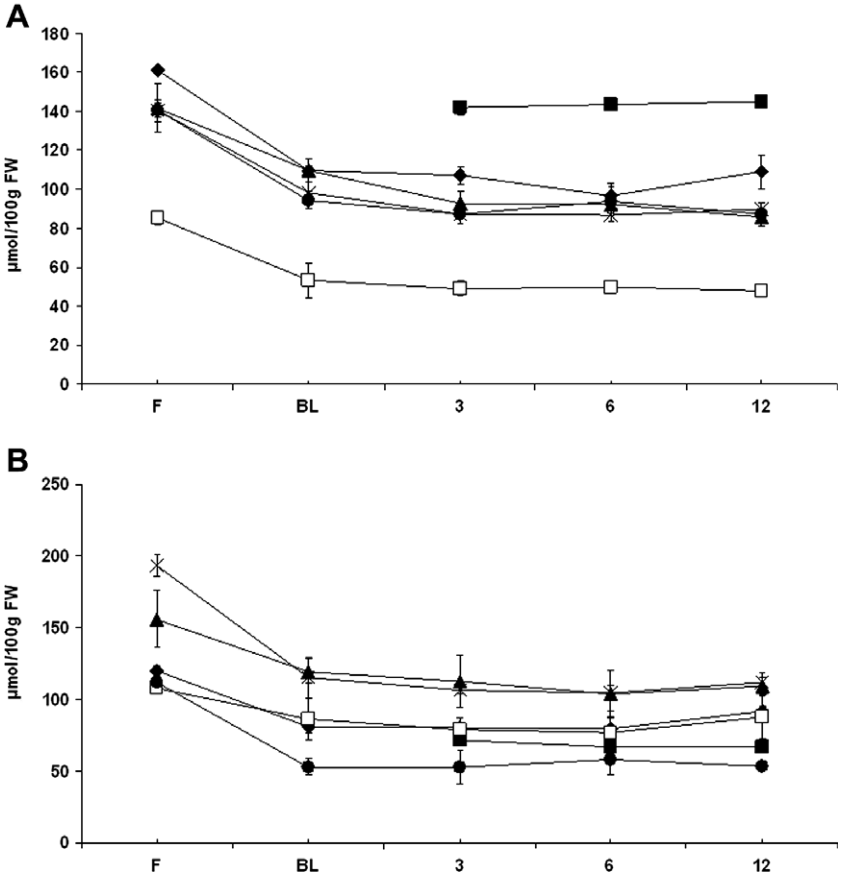

Fig. 1. Effects of blanching and long-term freezer storage on total aliphatic (A) and indole (B) GLS in cauliflower. F = fresh, BL = blanched cauliflower. 3, 6 and 12 denote the number of months of frozen storage. Cultivars: ◆ = 'Aviso', ■ = industrial 'Aviso', □ = 'Flamenco', ▲ = 'Celio', x = 'Emeraude' and ● = 'Grafitti'. Vertical bars indicate standard deviation, $n = 3$.

Table 1
Effects of blanching and long-term freezer storage on aliphatic glucosinolates in cauliflower

	Cv.	Fresh	Blanched	Freezer storage (months)		
				3	6	12
Glucoiberin	Aviso	^{a}34.3 ± 0.6	^{*a}23.5 ± 2.8	^{a}19.7 ± 1.1	^{a}18.2 ± 0.7	^{a}19.6 ± 2.5
	AvisoIND‡	–	–	^{ab}24.6 ± 0.7	^{c,B}22.3 ± 0.3	^{abd,C}23.6 ± 0.1
	Flamenco	12.7 ± 0.7	*9.9 ± 0.8	6.4 ± 1.8	6.6 ± 2.2	^{A}5.5 ± 0.4
	Celio	47.8 ± 3.4	*33.3 ± 3.3	^{b}28.4 ± 2.0	^{b,A}26.3 ± 0.5	^{b,A}26.5 ± 0.7
	Emeraude	^{ab}30.5 ± 2.1	^{*ab}21.8 ± 2.1	^{ac}16.3 ± 3.0	^{a}15.7 ± 2.1	^{ac}17.2 ± 2.6
	Grafitti	^{ab}35.6 ± 2.4	^{*ab}23.6 ± 2.4	^{ac,A}18.7 ± 1.8	^{bc,B}23.9 ± 0.3	^{acd,AC}19.6 ± 0.6
Progoitrin	Aviso	103.5 ± 1.6	^{*a}68.7 ± 1.6	72.0 ± 3.3	^{a}65.2 ± 6.5	74.2 ± 4.8
	AvisoIND‡	–	–	88.3 ± 4.4	93.5 ± 0.9	94.0 ± 2.8
	Flamenco	^{a}58.6 ± 2.7	*33.6 ± 9.2	32.3 ± 2.4	^{b}33.2 ± 4.5	32.5 ± 3.3
	Celio	80.4 ± 7.9	^{*a}66.1 ± 5.0	61.4 ± 6.1	^{a}62.9 ± 9.0	56.9 ± 4.3
	Emeraude	27.1 ± 2.4	*18.1 ± 2.3	16.4 ± 0.7	17.0 ± 0.4	16.6 ± 0.5
	Grafitti	^{a}66.7 ± 2.5	*49.0 ± 5.9	44.8 ± 3.3	^{b}46.9 ± 3.3	45.5 ± 0.5
Sinigrin	Aviso	16.7 ± 1.2	13.3 ± 1.6	12.1 ± 0.3	^{a}10.7 ± 0.5	^{a}11.9 ± 1.1
	AvisoIND‡	–	–	26.7 ± 0.5	^{b}25.4 ± 0.4	^{B}24.8 ± 0.8
	Flamenco	13.7 ± 0.1	*9.6 ± 0.6	10.5 ± 0.2	^{a}9.9 ± 2.0	^{a}10.0 ± 0.3
	Celio	8.8 ± 1.4	7.2 ± 0.4	nd	nd	nd
	Emeraude	nd	nd	nd	nd	nd
	Grafitti	32.5 ± 0.6	*18.6 ± 0.6	20.9 ± 0.6	^{b}19.9 ± 4.8	19.2 ± 0.5
Glucoerucin	Aviso	nd	nd	nd	nd	nd
	AvisoIND‡	–	–	nd	nd	nd
	Flamenco	nd	nd	nd	nd	nd
	Celio	^{a}4.7 ± 0.5	^{*a}3.2 ± 0.4	^{a}3.1 ± 0.4	^{a}3.2 ± 0.2	2.6 ± 0.1
	Emeraude	^{a}3.4 ± 1.0	^{a}2.2 ± 0.4	^{a}4.0 ± 1.1	^{a}3.8 ± 0.6	^{A}4.3 ± 0.7
	Grafitti	nd	nd	nd	nd	nd
Glucoraphanin	Aviso	^{a}6.4 ± 0.1	^{*a}4.1 ± 0.1	^{a,A}3.3 ± 0.1	^{a,AB}2.6 ± 0.1	^{a,AC}3.4 ± 0.4
	AvisoIND‡	–	–	^{ab}2.4 ± 0.0	^{ab}2.3 ± 0.1	^{ab,BC}2.7 ± 0.0
	Flamenco	nd	nd	nd	nd	nd
	Celio	20.4 ± 2.3	*13.3 ± 1.7	12.8 ± 1.5	12.9 ± 0.7	12.4 ± 0.8
	Emeraude	79.4 ± 4.0	*56.0 ± 4.8	50.6 ± 1.4	50.4 ± 2.0	51.4 ± 0.5
	Grafitti	^{a}5.8 ± 0.6	^{*a}3.1 ± 0.3	^{ab}2.6 ± 0.1	^{ab}3.0 ± 0.5	^{ab}2.8 ± 0.4

All values are in μmol/100 g FW ± SD, $n = 3$.
*Significantly different from fresh.
Superscripts of capital letters A, B and C denote significant difference from blanched, 3 and 6 months samples, respectively.
Superscript of lower case letters: same letters indicate no significant differences between cultivars.
‡Industrial sample of cv. 'Aviso'.

significant declines were found for total aliphatics in the 3 and 6 month samples in the romanesco cultivar ('Celio'). Also, for the indole GLS, the 6 and 12 month samples of the industrially processed white cultivar ('Aviso'IND) were significantly lower than the 3 month sample. No effects were found on the total GLS content during the freeze-storage. Cieślik et al. (2007) kept blanched frozen vegetables at −22 °C for 48 h prior to analysis, finding inconsistent changes in the total GLS levels, losses in Brussels sprouts and broccoli, and increases in green cauliflower. The authors did not discuss why this was the case. In our study, the individual levels were not severely altered throughout the long-term freeze-storage, neither for the aliphatic nor the indole GLS. Regarding the aliphatic GLS (Table 1), glucoiberin was reduced ($p < 0.05$) towards the end of the storage period for 'Flamenco', 'Celio' and 'Grafitti'. No effects were detected for progoitrin and only the 12 month sample of 'Aviso'IND was lower than the 3 month sample. An increase was found for glucoerucin in 'Emeraude'; however, this may be related to the low level quantitation. Glucoraphanin was unaffected by storage in all cultivars, except for the white 'Aviso' in which the content was reduced. The individual indole GLS (Table 2) were also not severely affected. However, some modest effects were found. At month 12, all indole GLS in the industrial cultivar ('Aviso'IND) were lower than at month 3; 4-hydroxyglucobrassicin, glucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin were reduced by 13, 3, 15 and 11%, respectively. The levels of 4-methoxyglucobrassicin in cv. 'Aviso' were higher at 3, 6 and 12 months than in freshly blanched samples. We have no explanation for this. On the whole, the findings indicate that freezing is an excellent way to preserve the contents of GLS in cauliflower. Rodrigues and Rosa (1999) came to the same conclusion in a study of broccoli.

3.2. L-ascorbic acid

L-AA levels were highest in the green pyramidal cv. 'Celio' and lowest in white cv. 'Flamenco' (Table 3). The range corresponded well with previously reports of values (Davey et al., 2000). L-AA levels were significantly reduced (13–23%, Table 3) by blanching for all samples, which matches findings by Puupponen-Pimiä et al. (2003) for vitamin C. Lisiewska and Kmiecik (1996) found slightly larger losses of 28–32%, which can probably be explained by the fact that they used a 1 min longer blanching time and no subsequent cooling, consequently leading to a higher degree of loss through leaching and thermal breakdown.

The freezer storage negatively affected the levels of L-AA ($p < 0.05$), especially towards the end of the 12 month period (Table 3). Compared to blanched, the 12 month samples were reduced by 26% for the white cultivars 'Aviso' and 'Flamenco'. The 7% reduction found in the white industrial sample ('Aviso'IND) might be linked to the applied blanching conditions. The L-AA content was reduced in the green cultivars by 14% and 18% for 'Emeraude' and 'Celio', respectively. The lowest loss was found in the purple cultivar 'Grafitti', by 8% in the 12 month sample. Lisiewska and Kmiecik (1996) also investigated the effect of freezer storage over a one year period, finding reductions in vitamin C of 6–13% and 15–18% for cauliflower and broccoli, respectively. In our study only L-AA was investigated and the lower losses reported by Lisiewska

Table 2
Effects of blanching and long-term freezer storage on indole glucosinolates in cauliflower

	Cv.	Fresh	Blanched	Freezer storage (months)		
				3	6	12
4-hydroxyglucobrassicin	Aviso	^{a}15.3 ± 0.5	^{*a}9.5 ± 0.9	^{a}11.1 ± 0.5	^{a}10.2 ± 1.5	^{a,A}12.1 ± 0.1
	AvisoIND‡	–	–	^{ac}11.9 ± 0.5	^{ac,B}9.9 ± 0.2	^{ac,B}10.3 ± 0.1
	Flamenco	^{b}4.7 ± 0.9	^{*b}2.5 ± 0.6	^{b,A}0.9 ± 0.1	^{b,A}1.5 ± 0.3	^{b,B}2.1 ± 0.1
	Celio	^{a}17.1 ± 1.1	^{*a}14.2 ± 0.7	^{ac}13.6 ± 1.6	^{ac,AB}9.7 ± 0.6	^{ac,AB}11.0 ± 0.4
	Emeraude	49.8 ± 4.4	*31.2 ± 3.6	32.7 ± 1.7	28.9 ± 0.8	^{c}35.7 ± 2.8
	Grafitti	^{b}2.4 ± 0.9	^{b}2.7 ± 1.0	^{b,A}0.9 ± 0.1	^{b,A}0.7 ± 0.0	^{b}2.0 ± 0.3
Glucobrassicin	Aviso	^{a}81.8 ± 1.7	^{*a}56.1 ± 4.5	^{a}49.6 ± 1.3	^{a}50.5 ± 7.4	^{a}59.3 ± 4.6
	AvisoIND‡	–	–	^{abd}44.9 ± 0.4	^{abde,B}42.2 ± 0.9	^{acd,B}43.4 ± 0.4
	Flamenco	^{ab}81.4 ± 0.8	^{ab}68.1 ± 11.8	^{ab}60.9 ± 7.3	^{ab}60.5 ± 12.5	^{ab}69.4 ± 14.5
	Celio	^{c}110 ± 17	^{b}82.8 ± 8.1	^{b}74.2 ± 12.5	^{abc}70.0 ± 12.6	^{ab}71.4 ± 4.3
	Emeraude	^{abcd}98.2 ± 7.2	^{*abc}53.8 ± 8.2	^{abc}48.9 ± 4.1	^{abcd}50.4 ± 4.9	^{ac}50.4 ± 3.4
	Grafitti	^{abd}83.5 ± 6.2	^{*ac}36.4 ± 3.6	^{acd}35.2 ± 12.9	^{abde}42.8 ± 9.0	^{cd}37.5 ± 1.9
4-methoxyglucobrassicin	Aviso	^{a}15.0 ± 1.8	^{*a}10.3 ± 1.0	^{a,A}13.2 ± 0.7	^{a,A}12.9 ± 1.3	^{a,A}12.9 ± 0.6
	AvisoIND‡	–	–	^{bd}8.8 ± 0.1	^{bc,B}8.2 ± 0.2	^{b,BC}7.5 ± 0.1
	Flamenco	7.1 ± 1.0	^{b}6.3 ± 0.7	^{b}5.7 ± 0.2	^{b}6.2 ± 0.6	^{b}6.2 ± 0.3
	Celio	^{b}23.5 ± 1.3	^{*c}18.8 ± 1.4	21.1 ± 3.4	20.7 ± 2.6	22.6 ± 1.1
	Emeraude	^{b}22.4 ± 2.3	^{*c}16.3 ± 2.3	^{ac}14.2 ± 0.5	^{a}13.8 ± 0.6	^{a}14.6 ± 1.0
	Grafitti	^{a}14.5 ± 1.0	^{*ab}8.1 ± 0.7	^{abcd,A}10.6 ± 1.1	^{abc}9.5 ± 0.8	10.0 ± 0.6
Neoglucobrassicin	Aviso	^{a}7.5 ± 0.1	^{*a}5.1 ± 0.5	^{a}6.1 ± 1.4	^{a}6.0 ± 0.4	^{a}7.3 ± 1.4
	AvisoIND‡	–	–	^{ad}6.2 ± 0.2	^{abcd}6.3 ± 0.2	^{acd,BC}5.5 ± 0.2
	Flamenco	14.6 ± 0.5	*9.0 ± 1.4	^{b}11.2 ± 0.8	^{ab}8.9 ± 1.7	^{b}9.7 ± 0.1
	Celio	^{a}5.1 ± 0.3	^{*ab}3.7 ± 0.1	^{c}3.5 ± 0.9	^{ac}3.4 ± 0.9	^{c}4.2 ± 0.4
	Emeraude	23.1 ± 3.0	*13.8 ± 2.0	^{b,A}10.7 ± 0.7	^{b}11.4 ± 0.7	^{b}11.1 ± 0.5
	Grafitti	^{a}10.1 ± 0.9	^{*b}5.6 ± 0.9	^{acd}5.9 ± 0.7	^{acd}5.3 ± 1.8	^{cd}4.1 ± 1.1

All values are in μmol/100 g FW ± SD, n = 3.
*Significantly different from fresh.
Superscripts of capital letters A, B and C denote significant difference from blanched, 3 and 6 months samples, respectively.
Superscript of lower case letters: same letters indicate no significant differences between cultivars.
‡Industrial sample of cv. 'Aviso'.

Table 3
Effects of blanching and long-term freezer storage on L-ascorbic acid, total phenols, FRAP and ORAC in cauliflower

	Cv.	Fresh	Blanched	Freezer storage (months)		
				3	6	12
L-AA[1]	Aviso	^{a}65.4 ± 2.0	^{*a}53.3 ± 2.3	^{a}54.3 ± 2.4	^{a,AB}45.0 ± 0.8	^{a,ABC}39.2 ± 1.5
	AvisoIND‡	–	–	^{acd}58.4 ± 0.6	^{b}56.1 ± 0.7	^{B}54.6 ± 1.2
	Flamenco	54.0 ± 0.7	*47.0 ± 0.3	^{b}46.6 ± 2.0	^{a,A}42.5 ± 2.1	^{ab,ABC}34.8 ± 1.7
	Celio	77.1 ± 2.5	*59.1 ± 1.2	^{ac}58.5 ± 1.2	^{b}54.8 ± 1.0	^{c,ABC}47.9 ± 2.9
	Emeraude	^{ab}68.1 ± 1.5	^{*a}55.4 ± 0.5	^{acd}54.1 ± 2.0	^{c,A}49.3 ± 2.2	^{ab,ABC}40.5 ± 3.5
	Grafitti	^{ab}64.2 ± 3.1	^{*a}50.6 ± 1.5	^{abd}49.6 ± 2.2	^{c}49.5 ± 1.2	^{c,A}46.6 ± 0.4
TP[2]	Aviso	^{a}66.6 ± 3.1	*58.4 ± 2.9	^{a}61.7 ± 4.1	^{a,B}52.8 ± 2.2	^{a,B}53.7 ± 1.6
	AvisoIND‡	–	–	^{abcd}68.3 ± 8.9	^{bc}71.9 ± 1.0	^{c}70.4 ± 0.8
	Flamenco	^{a}63.5 ± 0.6	*53.4 ± 0.3	^{ab}55.7 ± 1.0	^{a}53.5 ± 1.5	^{a,B}52.0 ± 1.5
	Celio	^{b}74.2 ± 1.6	^{*a}66.8 ± 0.9	^{abc}67.2 ± 0.9	^{b}66.3 ± 2.2	^{b,AB}62.6 ± 1.6
	Emeraude	^{ab}71.8 ± 2.0	^{a}69.7 ± 0.5	^{abcd}70.2 ± 2.6	^{bc}68.4 ± 2.2	^{bc}65.6 ± 3.6
	Grafitti	146 ± 2	*116 ± 1	121 ± 10	124 ± 3	122 ± 3
FRAP[3]	Aviso	^{a}0.86 ± 0.04	^{*a}0.69 ± 0.04	^{a}0.72 ± 0.04	^{a}0.70 ± 0.03	^{a,ABC}0.59 ± 0.02
	AvisoIND‡	–	–	^{acd}0.84 ± 0.02	^{b,B}0.89 ± 0.01	^{bc,C}0.81 ± 0.01
	Flamenco	^{ab}0.81 ± 0.01	^{*a}0.64 ± 0.01	^{ab}0.65 ± 0.01	0.60 ± 0.03	^{a,ABC}0.54 ± 0.02
	Celio	^{ac}0.93 ± 0.01	^{*b}0.80 ± 0.02	^{ac}0.86 ± 0.01	^{b}0.83 ± 0.03	^{b}0.81 ± 0.05
	Emeraude	^{abc}0.89 ± 0.02	^{*b}0.80 ± 0.00	^{abcd}0.79 ± 0.02	^{ab}0.77 ± 0.06	^{abc}0.70 ± 0.05
	Grafitti	1.79 ± 0.04	*1.50 ± 0.03	1.57 ± 0.12	1.50 ± 0.02	1.64 ± 0.12
ORAC[4]	Aviso	^{a}0.99 ± 0.06	^{a}0.77 ± 0.15	^{a}0.89 ± 0.06	^{a}0.88 ± 0.06	^{a,B}0.64 ± 0.07
	AvisoIND‡	–	–	^{a}1.11 ± 0.08	^{abc}1.13 ± 0.18	^{b}1.08 ± 0.04
	Flamenco	^{a}0.75 ± 0.01	^{*a}0.56 ± 0.00	0.57 ± 0.09	0.40 ± 0.02	^{AB}0.35 ± 0.05
	Celio	2.03 ± 0.06	*1.46 ± 0.09	1.49 ± 0.09	^{b}1.28 ± 0.12	^{b,ABC}0.95 ± 0.04
	Emeraude	1.50 ± 0.16	^{*a}1.04 ± 0.11	^{a}0.83 ± 0.10	^{ac}0.84 ± 0.06	^{a,A}0.66 ± 0.16
	Grafitti	2.57 ± 0.08	*1.85 ± 0.18	1.79 ± 0.05	1.67 ± 0.16	^{AB}1.33 ± 0.12

All values are per 100 g FW ± SD, n = 3.
*Significantly different from fresh.
Superscripts of capital letters A, B and C denote significant difference from blanched, 3 and 6 months samples, respectively.
Superscript of lower case letters: same letters indicate no significant differences between cultivars.
‡Industrial sample of cv. 'Avisó.
[1] L-ascorbic acid in mg.
[2] Total phenols in mg GAE.
[3] Ferric reducing ability power in mmol Fe^{2+}.
[4] Oxygen radical absorbance capacity in mmol TE.

and Kmiecik (1996) might be caused by their observation that the content of dehydroascorbic acid increased as a result of the freezing procedure. It is possible that the anthocyanin pigments present in the purple cauliflower ('Grafitti') could have influenced the stability of L-AA, leading to a somewhat smaller loss in comparison to the other cultivars.

3.3. Total phenols

The contents of TP found in untreated cauliflower (Table 3) are in agreement with what others have found (Gębczyński & Kmiecik, 2007; Lo Scalzo, Bianchi, Genna, & Summa, 2007; Puupponen-Pimiä et al., 2003). The level determined in the purple variety ('Grafitti') was approximately twice as high as for the other cultivars, likely attributed to the occurrence of anthocyanins not present in the other cultivars. Blanching significantly reduced the TP levels by 10–21% for all cultivars, except for 'Emeraude' which was unaffected. The losses observed are of the same magnitude as reported by Puupponen-Pimiä et al. (2003) and Gębczyński and Kmiecik (2007).

Overall, the TP levels did not change extensively over the course of the freezer storage. Compared to blanched, only romanesco ('Celio') was significantly reduced (by 6% after 12 months). Comparing the 12 with the 3 month sample, significant reductions of 13% and 7% were found in the white cultivars 'Aviso' and 'Flamenco', respectively. In line with our findings, Puupponen-Pimiä et al. (2003) reported that the effect of long-term freezer storage on the TP content of different vegetables was minimal in comparison to the blanching step.

3.4. Antioxidant capacities

The measured FRAP values, for all cultivars, were more or less similar but notably higher (×2) for the purple variety (Table 3), most probably due to the presence of polyphenolic pigments. The ORAC values were more variable but, also here, 'Grafitti' had the highest value. The white cultivars had the lowest antioxidant capacity values. The levels correspond well with values found by Ou, Huang, Hampsch-Woodill, Flanagan, and Deemer (2002) for ORAC, where the antioxidant levels in 57 cultivars of cauliflower were determined. However, the FRAP values found by us were 2–3 times higher than those found by Ou et al. (2002). The reason for this could be related to the different incubation intervals; Ou et al. (2002) used 4 min as opposed to our 10 min. Our results show that almost all antioxidant capacities were significantly reduced by blanching: FRAP and ORAC by 10–21% and 25–31%, respectively (Table 3). These losses correspond fairly well to the 23% losses determined by Puupponen-Pimiä et al. (2003). Using the DPPH· method, Gębczyński and Kmiecik (2007) found reductions in antioxidant capacity of 6% and 9% for white and green cauliflower, respectively, but these cultivars were different from those in our study. Only the ORAC value in 'Aviso' was unaffected by blanching.

Freezer storage did not affect the FRAP levels to a large extent. However, significant declines of 15–16% were found for the white cultivars ('Aviso', 'Flamenco') at 12 months compared to blanched. Larger losses of 28–36% ($p < 0.05$) were found for ORAC values in the green ('Celio', 'Emeraude') and purple cultivars ('Grafitti') after 12 months when compared to blanched samples. Also, a significant reduction (28%) in 'Aviso' was found in the 12 compared to the 3 month sample. Puupponen-Pimiä et al. (2003) stored blanched frozen cauliflower for 6 and 12 months, finding that the antioxidant capacity, measured by the DPPH· method, remained stable. Our results showed that losses in antioxidant capacities occurred mostly towards the end of the one year period, corresponding to similar reductions in L-AA and TP.

3.5. Total monomeric anthocyanins

The levels of TMA in raw, blanched and freezer-stored purple cauliflower (cv. 'Grafitti') are depicted in Fig. 2. No anthocyanins were detected in the other cultivars. Untreated purple cauliflower had 73.9 ± 2.8 cy-3-gluE/100 g FW, which is intermediate to what has been determined by others (Chiu, Prior, & Wu, 2005; Lo Scalzo, Genna, Branca, Chedin, & Chassaigne, 2008). The anthocyanin content was significantly reduced by 38% when blanched (Fig. 2). Lo Scalzo et al. (2008) blanched purple cauliflower (2 min, ratio cauliflower-water 1:10), finding a mean anthocyanin loss of 77%. This dramatic loss might, however, be related to the use of 10 min cooling in ice-water after blanching, allowing additional leaching. The loss inflicted by blanching was higher than that observed for L-AA, TP and the antioxidant capacities and is likely to be linked to the localisation of the pigments in the axis cells of the curd tissue (Chiu et al., 2005), allowing efficient leaching. L-AA, on the other hand, was lost at a lower rate as it is more evenly distributed throughout the plant. Anthocyanins are known to be degraded by heat (Markakis, 1982). However, the short time interval used when blanching is unlikely to have a substantial effect in this respect. Others, e.g. Puupponen-Pimiä et al. (2003), have investigated the

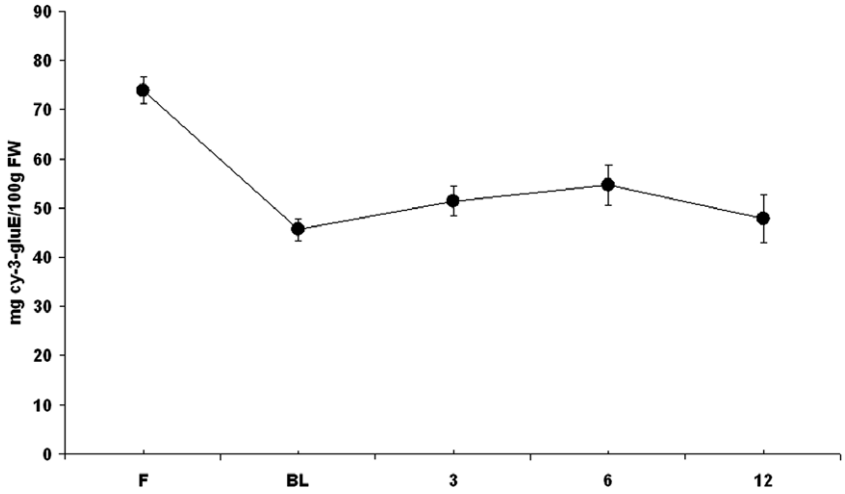

Fig. 2. Effects of blanching and long-term freezer storage on TMAs in purple cauliflower, Cv. 'Grafitti'. F = fresh, BL = blanched cauliflower. 3, 6 and 12 denote the number of months of frozen storage. Vertical bars indicate standard deviation, $n = 3$.

loss of TP in blanched cauliflower, finding losses closer to what has been determined in this study. Freezer storage did not have any effect on the content of TMA in purple cauliflower.

3.6. Colour

L^*, C^* and h_{ab} values in untreated, blanched and long-term frozen cauliflower are presented in Fig. 3. The colour coordinates were in the yellow part of b^* (positive) and green part of a^* (negative) for all cultivars, except for the purple cultivar 'Grafitti'. Lightness was markedly higher in the white cultivars 'Aviso' and 'Flamenco' than in the coloured. The largest values for chromaticity were found in the green cultivars, i.e., in 'Celio' and 'Emeraude'. Purple cauliflower had a hue value of 340° and the other cultivars were in the range 93–104°. The results determined accord well with those of Schonhof et al. (2004) who investigated the colour coordinates

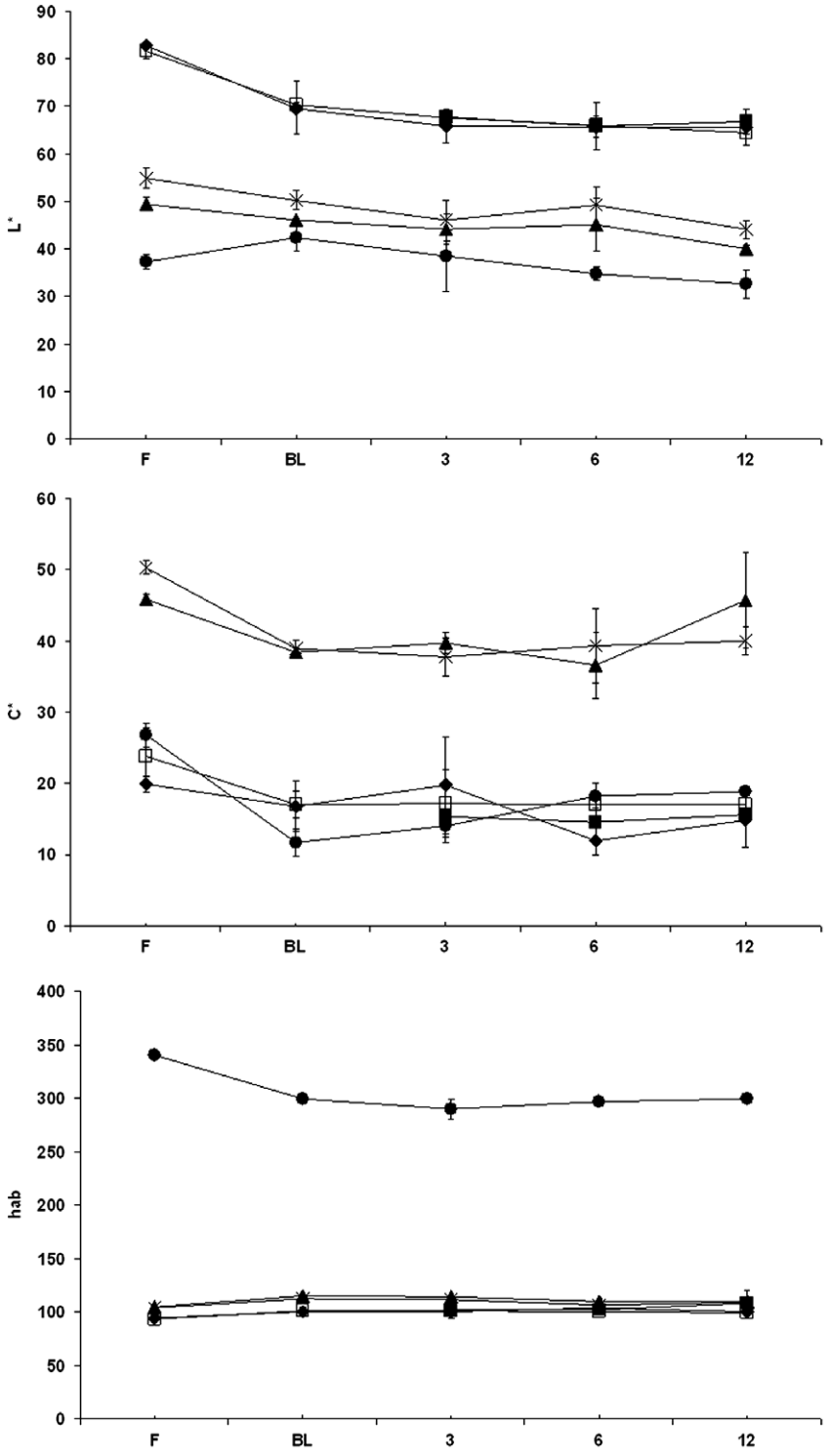

Fig. 3. Effects of blanching and long-term freezer storage on lightness (L^*), chromaticity (C^*) and hue angle (h_{ab}) in cauliflower. F = fresh, BL = blanched cauliflower. 3, 6 and 12 denote the number of months of frozen storage. Cultivars: ◆ = 'Aviso', ■ = industrial 'Aviso', □ = 'Flamenco', ▲ = 'Celio', x = 'Emeraude' and ● = 'Grafitti'. Vertical bars indicate standard deviation, $n = 3$.

of white, green, purple and green pyramidal cauliflower over three consecutive years. Blanching significantly ($p < 0.05$) decreased the lightness in the white cultivars ('Aviso'; −16%, 'Flamenco'; −14%) and in romanesco ('Celio'; −7%). The degree of colour saturation, C^*, was significantly reduced by blanching in the coloured cultivars ('Celio'; −16%, 'Emeraude'; −23%) with the most prominent effect in purple cauliflower ('Grafitti'; −56%). No significant change in C^* was found in the white cultivars. Blanching significantly increased the tonality (h_{ab}) in all cultivars on average by 9%, except for purple cauliflower where a decrease of 12% was found. The decrease in hue in purple cauliflower indicates a shift in the colour from red to blue. The effect of blanching on the hue in purple cauliflower is likely to be caused by leaching. Also, for the remaining anthocyanins within the plant cells, alterations in the pH value caused by blanching could have affected the hue caused by anthocyanins (Giusti & Wrolstad, 2001). The reductions in hue and chroma correspond well with the leaching losses of anthocyanins (Fig. 2).

Overall, freezer storage did not affect the colour coordinates in a major way (Fig. 3); L^* remained relatively stable throughout the storage period, except for the 'Flamencó samples, where significant decreases were detected in the 6 and 12 month samples. Only chromaticity in 'Grafitti' was significantly affected by freezer-storage with increased values at months 6 and 12, reflecting a detected significant decrease in b^*, i.e. more blue (results not shown). In general, storage did not affect the hue angles except for significant reductions at 6 and 12 months for 'Celio' and month 6 in 'Emeraude'. Mondragón-Portocarrero, Pena-Martínez, Fernández-Fernández, Romero-Rodríguez, and Vázquez-Odériz (2006) investigated the effects of blanching and freezing of *Brassica rapa* leaves and found that the colour parameters were relatively stable in storage, which is in accordance with our findings. The colour coordinates were unaffected by long-term freezer storage for cv. 'Aviso' and the industrial supplied sample ('Aviso'IND).

It is concluded that blanching caused significant reductions in the levels of most phytochemicals and antioxidant-related parameters and led to changes in colour. In general, freezer storage gave diminishing levels of antioxidant-related compounds over time. Colour and GLS were not severely affected by freezer-storage. The findings support the notion that frozen cauliflower can serve as an excellent supply of important health-related compounds, even after storage for one year.

Acknowledgement

The presented study was undertaken with financial support from the Research Council of Norway, Project No. NFR146579/140, "Bioactive phytochemicals (flavonoids) in fruit and vegetables: storage, processing and rapid sensor-based analytical methods". Magnor Hansen, Kari Grønnerød, Signe Hansen, Karin Haffner(†), Liv Berge, Karin Svinnset and May Helene Aalberg at the Norwegian University of Life Sciences and Grethe Iren A. Borge, Grete Skrede, Mona Ringstad and Berit Karoline Martinsen at Matforsk AS are acknowledged for their help. Eva Ackermann is also accredited for her valuable assistance.

References

Andersen, Ø. M., & Jordheim, M. (2006). The anthocyanins. In Ø. M. Andersen & K. R. Markham (Eds.), *Flavonoids: chemistry, biochemistry, and applications* (pp. 471–551). Boca Raton, FL: CRC Press.

Barbosa-Cánovas, G., Altunakar, B., & Mejía-Lorío, D. J. (2005). *Freezing of fruits and vegetables. An agribusiness alternative for rural and semi-rural areas. FAO Agricultural Services Bulletin 158*. Rome, Italy: FAO – Food and Agriculture Organization of the United Nations.

Bennett, R. N., Mellon, F. A., & Kroon, P. A. (2004). Screening crucifer seeds as sources of specific intact glucosinolates using ion-pair high-performance liquid chromatography negative ion electrospray mass spectrometry. *Journal of Agricultural and Food Chemistry, 52*(3), 428–438.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76.

Cano, M. P. (1996). Vegetables. In L. E. Jeremiah (Ed.), *Freezing effects on food quality* (pp. 247–298). New York: Marcel Dekker Inc.

Chiu, L. W, Prior, R. L., & Wu, X. L. L. (2005). *Toward identification of the candidate gene controlling anthocyanin accumulation in purple cauliflower* (*Brassica Oleracea* L. var. *botrytis*). American Society of Plant Biologist Annual Meeting, July 16–20, 2005, Seattle, USA, pp. 628.

CIE, Commision Internationale de l'Eclairage, Colorimetry (3rd ed.), Pub. 015:2004.

Cieślik, E., Leszczyńska, T., Filipiak-Florkiewicz, A., Sikora, E., & Pisulewski, P. M. (2007). Effects of some technological processes on glucosinolate contents in cruciferous vegetables. *Food Chemistry, 105*(3), 976–981.

Dávalos, A., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay. *Journal of Agricultural and Food Chemistry, 52*(1), 48–54.

Davey, M. W., Van Montagu, M., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., et al. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture, 80*(7), 825–860.

Gębczyński, P., & Kmiecik, W. (2007). Effects of traditional and modified technology, in the production of frozen cauliflower, on the contents of selected antioxidative compounds. *Food Chemistry, 101*(1), 229–235.

Giusti, M. M., & Wrolstad, R. E. (2001). Unit F1.2: Anthocyanins. Characterization and measurement of anthocyanins by UV–visible spectroscopy. In R. E. Wrolstad (Ed.), *Current protocols in food analytical chemistry* (pp. 1–13). New York: John Wiley and Sons.

Goodrich, R. M., Anderson, J. L., & Stoewsand, G. S. (1989). Glucosinolate changes in blanched broccoli and Brussels-sprouts. *Journal of Food Processing and Preservation, 13*(4), 275–280.

Hertog, M. G. L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., et al. (1995). Flavonoid intake and long-term risk of coronary-heart-disease and cancer in the 7 countries study. *Archives of Internal Medicine, 155*(4), 381–386.

Knekt, P., Jarvinen, R., Reunanen, A., & Maatela, J. (1996). Flavonoid intake and coronary mortality in Finland: a cohort study. *BMJ (British Medical Journal), 312*(7029), 478–481.

Lisiewska, Z., & Kmiecik, W. (1996). Effects of level of nitrogen fertilizer, processing conditions and period of storage of frozen broccoli and cauliflower on vitamin C retention. *Food Chemistry, 57*(2), 267–270.

Lo Scalzo, R., Bianchi, G., Genna, A., & Summa, C. (2007). Antioxidant properties and lipidic profile as quality indexes of cauliflower (*Brassica oleracea* L. var. *botrytis*) in relation to harvest time. *Food Chemistry, 100*(3), 1019–1025.

Lo Scalzo, R., Genna, A., Branca, F., Chedin, M., & Chassaigne, C. H. (2008). Anthocyanin composition of cauliflower (*Brassica oleracea* L. var. *botrytis*) and cabbage (*Brassica oleracea* L. var. *capitata*) and its stability in relation to thermal treatments. *Food Chemistry, 107*, 136–144.

Markakis, P. (1982). Stability of anthocyanins in foods. In P. Markakis (Ed.), *Anthocyanins as food colors* (pp. 163–180). New York: Academic Press.

Mondragón-Portocarrero, A. D. L., Pena-Martínez, B., Fernández-Fernández, E., Romero-Rodríguez, A., & Vázquez-Odériz, L. (2006). Effects of different pre-freezing blanching procedures on the physicochemical properties of *Brassica rapa* leaves (Turnip Greens, *Grelos*). *International Journal of Food Science and Technology, 41*(9), 1067–1072.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*(10), 4619–4626.

Ou, B. X., Huang, D. J., Hampsch-Woodill, M., Flanagan, J. A., & Deemer, E. K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *Journal of Agricultural and Food Chemistry, 50*(11), 3122–3128.

Puupponen-Pimiä, R., Häkkinen, S. T., Aarni, M., Suortti, T., Lampi, A. M., Eurola, M., et al. (2003). Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. *Journal of the Science of Food and Agriculture, 83*(14), 1389–1402.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*(7), 933–956.

Rodrigues, A. S., & Rosa, E. A. S. (1999). Effect of post-harvest treatments on the level of glucosinolates in broccoli. *Journal of the Science of Food and Agriculture, 79*(7), 1028–1032.

Schonhof, I., Krumbein, A., & Brückner, B. (2004). Genotypic effects on glucosinolates and sensory properties of broccoli and cauliflower. *Nahrung-Food, 48*(1), 25–33.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Tian, Q. G., Rosselot, R. A., & Schwartz, S. J. (2005). Quantitative determination of intact glucosinolates in broccoli, broccoli sprouts, Brussels sprouts, and cauliflower by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. *Analytical Biochemistry, 343*(1), 93–99.

van Poppel, G., Verhoeven, D. T. H., Verhagen, H., & Goldbohm, R. A. (1999). Brassica vegetables and cancer prevention – Epidemiology and mechanisms. *Advances in Nutrition and Cancer, 2*(472), 159–168.

Verhoeven, D. T. H., Verhagen, H., Goldbohm, R. A., van den Brandt, P. A., & van Poppel, G. (1997). A review of mechanisms underlying anticarcinogenicity by brassica vegetables. *Chemico–Biological Interactions, 103*(2), 79–129.

Volden, J., Borge, G. I. A., Bengtsson, G. B., Hansen, M., Thygesen, I. E., & Wicklund, T. (2008). Effect of thermal treatment on glucosinolates and antioxidant related parameters in red cabbage (*Brassica oleracea* L ssp. *capitata* f. *rubra*). *Food Chemistry, 109*, 595–605.

World Cancer Research Fund/American Institute for Cancer Research (2007). *Food, nutrition, physical activity and the prevention of cancer: a global perspective*. Washington, DC: AICR.

World Health Organization (2003). *Diet, nutrition and the prevention of chronic diseases*. Geneva, Switzerland: WHO Technical Report Series 916.