Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 109 (2008) 595–605
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Effect of thermal treatment on glucosinolates and antioxidant-related parameters in red cabbage (*Brassica oleracea* L. ssp. *capitata* f. *rubra*)

Jon Volden [a,b,*], Grethe Iren A. Borge [b], Gunnar B. Bengtsson [b], Magnor Hansen [c], Ingrid E. Thygesen [a], Trude Wicklund [a]

[a] *Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Aas, Norway*
[b] *Matforsk AS – Norwegian Food Research Institute, Osloveien 1, N-1430 Aas, Norway*
[c] *Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Aas, Norway*

Received 7 September 2007; received in revised form 19 November 2007; accepted 14 January 2008

Abstract

The effects of various thermal processing treatments (blanching, boiling and steaming) of red cabbage, *Brassica oleracea* L. ssp. *capitata* f. *rubra* cv. 'Autoro', were assessed for the levels of glucosinolates (GLS), total phenols (TP), total monomeric anthocyanins (TMA), L-ascorbic acid (L-AA) and soluble sugars, as well as for the antioxidant potential by the ferric reducing ability power (FRAP) and oxygen radical absorbance capacity (ORAC) assays. Individual native GLS were determined by ion-pair HPLC-MS/DAD. There were significant ($p < 0.05$) losses in blanched red cabbage: TP, 43%, TMA 59%, FRAP 42%, ORAC 51%, L-AA 48% and soluble sugars 45%. Boiling gave less extensive reductions: TP 16%, TMA 41%, FRAP 17%, ORAC 19%, L-AA 24% and soluble sugars 19%. Steaming caused no losses for TP, ORAC, FRAP or soluble sugars. However, significant reductions were found for TMA and L-AA, with 29% and 11%, respectively. In general losses were accounted for in the processing waters; however, TMA was not fully recovered, indicating degradation. Total GLS were severely affected by processing, with reductions of 64%, 38% and 19% in blanched, boiled and steamed red cabbage, respectively. Total aliphatic and indole GLS were similarly affected. Lost GLS were partially recovered in the processing water.

Keywords: Red cabbage; Blanching; Steaming; Boiling; Glucosinolates; Flavonoids; Anthocyanins; Total phenols; Antioxidant power; FRAP; ORAC; Sucrose, glucose, fructose; L-Ascorbic acid

1. Introduction

There is solid evidence that a diet rich in vegetables and fruits is beneficial to the well-being and health of humans. However, it is not well understood how particular constituents are responsible for these effects or, alternatively, whether these effects are a consequence of synergistic action of several constituents. Vitamins, flavonoids and glucosinolates (GLS) have been subjects of particular interest in this respect. Flavonoids can act *in vitro* as scavengers of active oxygen species and electrophiles, and as chelators of metal ions, and they may, therefore, be beneficial *in vivo* to reduce the risk of cardiovascular diseases (Hollman, 2001; Pietta, 2000; Rice-Evans, Miller, & Paganga, 1996). Isothiocyanates, which are degradation products from GLS, have been linked to a reduced risk of cancer in lungs and the alimentary system, by inhibiting phase I and inducing phase II enzymes in the metabolism of xenobiotics (van Poppel, Verhoeven, Verhagen, & Goldbohm, 1999).

Vegetables are frequently subjected to various forms of processing to make them more suitable for consumption, as well as more resilient to long term storage. Wet thermal

* Corresponding author. Address: Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-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.2008.01.010

processing can affect the phytochemicals by thermal breakdown and, as the integrity of the cell structure is lost, by migration of components, leading to losses by leakage or breakdown by enzymatic action, and by non-enzymatic factors such as light and oxygen (Davey et al., 2000). However, when optimised, thermal treatment will rapidly inactivate enzymes, prevent oxygen access and minimise leakage, thus increasing the retention of (labile) constituents. Zhang and Hamauzu (2004) boiled broccoli florets for 5 min with a vegetable–water ratio of 1:20, finding significant reductions of 72%, 66% and 65% for total phenols, L-ascorbic acid and antioxidant activity, respectively. Halvorsen et al. (2006) reported large increases in antioxidant activity for several vegetables after microwaving, steaming and boiling. Wennberg, Ekvall, Olsson, and Nyman (2006) investigated the levels of carbohydrates and GLS in two cultivars of blanched white cabbage, finding substantial reductions compared to fresh. Carbohydrates were reduced by 45% and 47% and the total GLS content by 50% and 74%. Obvious differences in contents of phytochemicals due to different cultivars and growing conditions, as well as due to differences in execution and descriptions of the processes, make interpretations and comparisons between studies difficult. In addition, the sample preparation step is of pivotal importance when examining labile constituents and constituents related to enzymes. Lacks of standardized analytical methods also contribute to these difficulties.

As processing of vegetables is likely to affect the phytochemicals present, knowledge regarding the fate and final concentrations of important constituents is important for complementing epidemiological tools in relating the levels of GLS and flavonoids ingested to the incidence of various detrimental diseases. Processed vegetables constitute a substantial dietary proportion and knowledge of the effects of wet thermal treatments is important. Various assays for measuring "antioxidant power", based on hydrogen atom transfer or electron transfer reactions, can be utilized (Huang, Ou, & Prior, 2005), such as the ferric reducing ability power (FRAP) assay (Benzie & Strain, 1996) and the oxygen radical absorbance capacity (ORAC) assay (Ou, Hampsch-Woodill, & Prior, 2001). The content of total phenols (TP) by the Folin-Ciocalteu reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999) and total monomeric anthocyanins (TMA) by the pH-differential method (Giusti & Wrolstad, 2001) may be implemented as indicators of the integrated potential of specific compounds.

In most studies on thermal processing effects in brassicas, only one type of phytochemicals has been investigated and, therefore, the behaviour of and relations between the various constituents under identical experimental conditions are not known. The objective of the present study was to determine the effects of commonly used thermal processing methods on a range of important and potentially health-promoting constituents of red cabbage, that is glucosinolates (GLS), flavonoids with related parameters (TMA and TP), L-ascorbic acid (L-AA), soluble sugars, as well as antioxidant power (FRAP and ORAC values).

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, K_2HPO_4, $NaH_2PO_4 \cdot H_2O$ and L(+)-ascorbic acid were obtained from Merck KGaA (Darmstadt, Germany). Benzylglucosinolate, prop-2-enylglucosinolate, 4-methylsulfinylbutylglucosinolate and 3-methylsulfinylpropylglucosinolate were purchased from C_2 Bioengineering (Karlslunde, Denmark). Sucrose, D-glucose and D-fructose were supplied by ChemService (West Chester, PA, USA). 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 Hydro Gas and Chemicals AS (Oslo, Norway). All chemicals and gases were of analytical grade.

2.2. Plant material

Red cabbages (*Brassica oleracea* L. ssp. *capitata* f. *rubra* cv. 'Autoro') were sown in 96 well plug trays with peat-based soil, germinated in greenhouse (20 °C day, 18 °C night, 16 h light) and transplanted after 28 d into a 100 m^2 open field with 60 cm row distance and 40 cm plant spacing at the Norwegian University of Life Sciences in Aas, Norway (59.66°N, 10.78°E, altitude 88 m). After planting, the area was fertilised and treated with Gusathion® (Bayer, Leverkusen, Germany) against cabbage flies, and throughout the growing season with Fastac® (BASF AG, Ludwigshafen, Germany) against *Lepidoptera* pests. Mechanical weeding was done. The cabbages were harvested 129 d after transplanting and placed in a storage chamber at 0–1 °C at 98% relative air humidity.

2.3. Processing

Five cabbage heads were randomly selected and trimmed of their outer leaves and the stem. The heads were then divided into four segments and the central core removed. The segments were chopped into 1 × 1 cm pieces, using a Braun CombiMax K 700 food processor (Braun

GmbH, Kronberg, Germany). A pooled batch of 5000 g was made up of 1000 g from each of the cabbage heads and stored in a dark refrigerated enclosure as raw material for all treatments. Samples (300 g) were taken from the pooled batch at regular intervals (three times) throughout the experiment as reference for fresh, unprocessed cabbage.

Blanching was carried out by immersing 300 g of cut cabbage in 3000 g of boiling water for 3 min. The recorded temperature range in the process water was 94–96 °C. The blanched material was drained, cooled in ice water (1000 g ice, 2000 g water) for 1 min and then allowed to drain for 1 min. Boiling was carried out by immersing 300 g of cut cabbage in 300 g of boiling water, which was immediately brought to the boil again. The cabbage was boiled for 10 min, drained and left to cool in a plastic box placed in ice water. Steaming was carried out using a steam insert with 300 g of cabbage suspended above 300 g of boiling water for 10 min under a lid, and then left to cool in the same manner as for the boiled material. Each process was performed three times in a randomised way for all treatments during one session. All processed material was submerged in liquid nitrogen and ground to a coarse powder in a porcelain mortar and stored in plastic containers at –80 °C until further treatment. Process water samples were dripped into liquid nitrogen and stored in the same manner as for the solid material.

2.4. Sample treatment for chemical analyses

Further grinding to a finer particle size was carried out on the frozen material using a pre-cooled Braun Combi-Max K 700 food processor at speed 10 for 1 min. Great care was taken to prevent thawing of the cabbage material and all equipment in contact with the cabbage material was held at subzero temperatures. The resulting powder was stored in 100 g plastic boxes with tight fitting lids at –80 °C until extraction.

2.5. Soluble sugars

Glucose, sucrose and fructose were determined according to a slightly modified method described by Sharma, D'Souza, McConnell, and Mazza (1988). Briefly, 3.0 g frozen, ground red cabbage was weighed in a tube and kept on ice for 30 min; then 14 ml of 85 °C distilled water were added prior to incubation for 15 min at 85 °C and cooling on ice for 30 min. The samples were homogenized for 60 s at 20,000 rpm, using a Polytron PT 3000, and the homogenate filtered and centrifuged (Heraeus Biofuge Fresco, Osterode, Germany) at 12,800*g* for 7 min. Separation and detection were performed using a Agilent 1100 Series LC (Agilent Technologies, Waldbronn, Germany) HPLC with main column Hyper REZ XP Carbohydrate Pb (300 × 7.8 mm i.d.) with a guard column: 50 × 30 mm i.d. (Thermo Electron corp., Cheshire, UK). The mobile phase was water and the separation operated at 80 °C at a flow of 0.4 mL/min. A Gilson 132 RI detector (Gilson Medical Electronics Inc., Middleton, WI, USA) were used for detection. Quantification was based on external standards and quoted as g sugars per 100 g FW.

2.6. L-ascorbic acid

Sampling for the L-AA determination was performed as described by Wold et al. (2004). Briefly; 25 g of frozen red cabbage material were added to 50 ml 1.0% (w/v) oxalic acid and homogenized for 1 min using a Braun MR 400 hand processor, then filtered through a Whatman 113 V folded filter paper (Whatman International Ltd., Brentford, UK) then applied onto an activated (5 ml methanol + 5 ml water) Sep-Pak C18 from Waters Corp. (Milford, MA, USA). The first three ml were discarded and the eluent to be analyzed by HPLC was filtered through a 0.45 μm Millex-HA filter from Millipore Corp (Bedford, MA, USA) prior to injection. All samples was analyzed in duplicate and injected in triplicate. Isocratic HPLC separation and detection were performed according to Williams, Baker, and Schmit (1973), using an Agilent 1100 Series LC system (Agilent Technologies) equipped with a quaternary pump, an inline degasser, an autosampler, a column oven and a UV detector. The separation was conducted with a Zorbax SB-C18 (250 × 4.6 mm, 5 μm) column with a complementary Zorbax XDB C18 (4 × 4 mm, 5 μm) guard column (Agilent Technologies). Injection volume was 5 μl, the flow was 1 ml min^{-1} of 0.05 M KH_2PO_4 at 25 °C and detection was performed at 254 nm. L-AA was quantified by external calibration and results are reported as mg L-AA acid per 100 g FW.

2.7. Sample preparation for spectroscopic analyses

Five grammes of frozen ground cabbage material were weighed into pre-cooled 50 ml centrifuge tubes prior to addition of 15 ml of cold acidified (10 mM HCl) methanol and homogenization 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 J2-21M/E centrifuge (GMI Inc., Ramsey, MIN, USA). The supernatant was decanted and the pellet resuspended with 10 ml of acidified methanol and centrifuged. The supernatants were combined, mixed and distributed fully filled and tightly capped in 5 ml plastic tubes especially designed low temperature use and stored at −80 °C prior to analysis of antioxidant power, total phenols and total monomeric anthocyanins. Storage under these conditions renders the samples stable over time. All samples for spectroscopic analysis were analyzed within three months of sample preparation. All samples were extracted in duplicate and analyzed in triplicate.

2.8. Total phenols

TP was determined using a Konelab 30i (Thermo Electron Corp., Vantaa, Finland) clinical chemical analyser.

The procedure was based on using the Folin-Ciocalteu reagent (FCR), as described by Singleton et al. (1999). In brief, 20 μl of sample were added to 100 μl 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 samples were again mixed and incubated at 37 °C for 15 min prior to absorbance reading at 765 nm. TP were assessed against a calibration curve of gallic acid, and the results presented as mg gallic acid equivalents (GAE) per 100 g fresh weight (FW).

2.9. Total monomeric anthocyanins

The TMA concentration was assayed using the pH-differential method using a Konelab 30i instrument. The method implemented is a modification, based on the method of Giusti and Wrolstad (2001). 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.

2.10. Ferric reducing ability power (FRAP)

The FRAP assay, described by Benzie and Strain (1996), was carried out, modified, using a Konelab 30i. 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 (595 nm). Trolox (Vit. E analogue) was used as control. Results are expressed as mmol Fe^{2+} per 100 g FW.

2.11. Oxygen radical absorbance capacity (ORAC)

The ORAC assay, developed and validated by Ou et al. (2001), was performed, as described by Davalos, Gomez-Cordoves, and Bartolome (2004), with minor adjustments. Measurements were carried out on a FLUOStar Optima instrument, 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). 20 μl of diluted samples and 120 μl of 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. A calibration solution, consisting of Trolox, was used in each assay. Calibrations were performed in triplicate and samples in duplicate. Readings were carried out from the bottom of the wells at intervals of 3 min over a period of 92 min until the fluorescence was less than 5% of the initial reading. The areas under the fluorescence decay curve (AUC), based on fluorescence values relative to the initial reading, were calculated for standards and samples. The AUC of blank was subtracted and the results were expressed as mmol Trolox equivalents (TE) per 100 g FW.

2.12. Glucosinolates

The analysis of intact GLS was carried out using ion-pair high-performance liquid chromatography for improving the separation of the charged GLS molecules, negating the need for desulfation. Confirmation of identity was accomplished using both negative-ion electrospray mass spectrometry and ultraviolet spectroscopy. Samples were freeze-dried, milled and stored at −40 °C prior to extraction. Extraction was performed as follows: 4.5 ml of 70% (v/v) methanol, which was heated to the boiling point (73 °C) in a water bath, was added to 200 mg of sample and kept at the same temperature for 3 min in a plastic tube with a cap; 200 μl of 2 mM benzylglucosinolate were added as control standard. Homogenization was performed using an Ultra-Turrax T25 Basic from IKA-Werke GmbH & CO (Staufen, Germany) at 21,500 rpm using a S25N-8G dispersion tool for 1 min, followed by centrifugation at 4300*g* for 15 min. The supernatant was decanted and the pellet re-suspended with 3 ml of 70% methanol and centrifuged before the two supernatants were combined. Aliquots of 1.5 ml were evaporated to dryness at 45 °C using a Savant SPD131DDA Speed Vac Concentrator (Thermo Electron Corp., Vantaa, Finland). The sample was re-dissolved with an appropriate amount of water and filtered using 0.45 μm PVDF Millex-HV (Millipore) before LC–MS analysis. The separation and detection were performed on an Agilent 1100 Series LC/MSD Trap XCT system with a photodiode array detector, using a Betasil C18 (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). Injection volume was 5 μl and column flow was 0.2 ml min^{-1}. Mobile phases used were A, 0.1% TFA in ultra pure water and B, 0.1% TFA in methanol, with a gradient programme of 0–5 min, 100% A; 5–30 min, 0–80% B; 30–35 min, 80–100% B; 35–40 min, 100% B and 40–42 min, 100–0% B and re-equilibrating of the column at 100% A for 3 min prior to the next run. 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). The mass spectrometer was used in a negative mode of polarity with a capillary voltage of 3500 V, drying temperature of 350 °C and a nebulizer pressure of 40 psi. The nitrogen drying gas flow was set at 10 l min^{-1}. Ion trap software version used was LC/MSD Trap Software Version 5.2 SR1. The results are reported as μmol GLS per 100 g FW.

2.13. Statistical analysis

In all experiments, the general linear model procedure was used to conduct a one-way analysis of variance (ANOVA, $p < 0.05$) combined with Tukey's test for individual comparisons to evaluate the effect of the different thermal treatments, blanching, boiling and steaming, on the resultant mean recoveries of the analytes and assays. ANOVA was performed using MINITAB® Release 14.20 Statistical Software (Minitab Inc. State College, PA, USA).

3. Results

3.1. Soluble sugars and dry matter

Contents of soluble sugars and dry matter in fresh and the relative recoveries in processed red cabbage are shown in Table 1. Sucrose, glucose and fructose were significantly ($p < 0.05$) reduced, by 42%, 46% and 47%, in blanched cabbage, respectively. Recoveries (plant matrix + process water) of 92–94% in blanching were not significantly different from untreated samples. Sugars present in the ice water were not analyzed. Boiling significantly reduced glucose and fructose (by 20%) and sucrose (by 15%). Lost carbohydrates were recovered in the boiling water. Steaming did not affect the sugar contents. The dry matter content (Table 1) declined significantly in blanched and boiled material with losses of 31% and 11%, respectively, but was not affected by steaming (Table 1). All losses of dry matter were recovered in the processing water.

3.2. Antioxidant-related parameters

The levels in untreated red cabbage and the effects of blanching, boiling and steaming of red cabbage on the antioxidant-related parameters are shown in Table 2. Blanching and boiling gave significant losses of 59–42% and 41–16%, respectively, for all parameters. The effects of steaming were less marked, with no statistically significant reductions observed for TP, ORAC or FRAP. TMA was not fully recovered in the processing waters and all cumulative values were significantly lower than those found in raw cabbage. Total values for TP in boiled and steamed samples were found to be statistically different from untreated samples but >100% was recovered and this was also the case for FRAP in blanched and boiled as well for ORAC in boiled.

3.3. Glucosinolates

The ion-pair separated aliphatic, aromatic and indole GLS, as detected by UV and MS, and their corresponding levels, are shown in Table 3. Neo-glucobrassicin was also detected but co-eluted with anthocyanins and omitted from further study. Aliphatic GLS were the dominant GLS group with 78% of the total molar concentration, and glucoraphanin as the major constituent at 41% (32% of the total). Indoles accounted for 19% of the total GLS with 4-methoxyglucobrassicin as the most abundant at 55% (10% of the total). The distribution of total aliphatic and indole did not change in the processed solid material (Table 3). All GLS were significantly reduced, except for gluconasturtiin in steamed cabbage. Total GLS were significantly ($p < 0.05$) reduced: blanched > boiled > steamed at 64%, 38% and 19%, respectively. Blanching reduced the total aliphatics, aromatics (gluconasturtiin) and indoles by 66%, 61% and 67%, respectively, and boiling by 40%, 28% and 42%. The reductions in steamed samples were 22% and 25% in total aliphatics and indoles, respectively. The losses of progoitrin, glucoerucin and 4-methoxyglucobrassicin were notably higher in all processes compared to the other GLS. The recoveries of total GLS after blanching (excluding ice water) and steaming were 82% and 96%, respectively. Boiling water was not analyzed, due to limited samples. The higher losses of progoitrin, glucoerucin and 4-methoxyglucobrassicin were matched by higher recoveries in the processing water and the total values were similar for all types of GLS. The recoveries of aliphatics, aromatics and indoles in the blanching experiment were 78%, 88% and 78%, respectively and 93%, 101% and 86% in the steaming experiment.

4. Discussion

4.1. General

The present study investigated the effects of blanching, boiling and steaming on the levels of several phytochemicals and health-related parameters in red cabbage. We also examined the recoveries in the processing waters, in order try to determine the types of loss during processing.

4.2. Soluble sugars and dry matter

The levels of soluble sugars found in untreated red cabbage, as shown in Table 1, correspond well with those reported by the United States Department of Agriculture (US Department of Agriculture, 2006): 0.60, 1.74 and 1.48 g/100 g FW, for sucrose, glucose and fructose, respectively. Wennberg et al. (2006) reported reductions of 34% and 30% in dry matter in blanched cabbage, similar to ours, attributing the 82–90% of the losses to the soluble sugar content and the rest to dietary fibre. In our study, about half of the dry matter loss to the blanching water could be accounted for as soluble sugars. The leakage of dry matter is likely to be a function of the osmotic gradient of soluble constituents as the blanching experiment gave the highest loss. Time seems to be of less importance because, even if the boiling experiment was much longer (10 min), the loss of DM was less than that of the blanching experiment (3 min). The treatment times were not long enough to reach diffusion equilibrium: at equilibrium 9.1% and 52.5% of a soluble component would have been recovered in the solid matter after blanching and boiling,

respectively, if losses in the ice water after blanching are ignored. Martin-Villa, Vidal-Valverde, and Rojas-Hidalgo (1982) investigated the effect of cooking on sugars in red cabbage and found reductions of 50%, 57% and 17% for glucose, fructose and sucrose, respectively. In our study, the reductions found are not that dramatic for glucose and fructose, with 20% losses (Table 1). We determined the reduction in sucrose to be 13% which is more in line with Martin-Villa et al. (1982). The cumulative values of recovered soluble sugars in processed vegetable material and water were not significantly low compared to untreated samples, indicating that all was recovered. The total recoveries for fructose and total sugars in boiled were significantly higher than 100%. The reason for this is not clear.

4.3. L-ascorbic acid

The level of vitamin C in red cabbage has been reported by others to vary considerably. Proteggente et al. (2002) found 37 mg/100 g FW and Podsedek, Sosnowska, Redzynia, and Anders (2006) 62 and 73 mg/100 g FW. Wold, Rosenfeld, Lea, and Baugerød (2006) investigated the L-ascorbic acid content over two seasons, finding an average value of 36.7 mg/100 g FW for the same cv., 'Autoro', as used by us. Our values (Table 2) are similar to that found in the literature and it is likely that the determined contents differ due to effects of environmental factors, cultivars and analytical approaches. It must also be mentioned that our results comprise L-AA only and not dehydroascorbic acid (DHAA), which normally contributes <10% of the total vitamin C content in many horticultural crops (Wills, Wimalasiri, & Greenfield, 1984). Tentative investigations into the contents of DHAA of untreated red cabbage revealed that DHAA constituted 11% of the total (result not shown). Processing methods utilizing heat can irreversibly inactivate ascorbate oxidase, thus counteracting enzymatic breakdown; however, reduction of L-AA may also occur as a function of thermal breakdown and leaching into the cooking medium (Davey et al., 2000). L-AA was significantly reduced ($p < 0.05$), by 48%, 24% and 11%, in

Table 1
Effect of heat treatment on glucose, fructose, sucrose, total sugars and dry matter in red cabbage. Values for untreated red cabbage are given in actual values, g per 100 g FW, and for the processed samples (blanched, boiled, steamed) as percentage recovery[a]

Fresh/100 g		Blanched, recovery (%)[b]				Boiled, recovery (%)			Steamed, recovery (%)		
		Solid	Water	Ice water	Σ[c]	Solid	Water	Σ	Solid	Water	Σ
0.76 ± 0.07	Sucrose	58.6 ± 12.0*	33.1 ± 1.0	na[d]	91.6 ± 13.0	86.5 ± 6.8	29.7 ± 0.6	116 ± 7	96.5 ± 2.6	7.4 ± 1.7	104 ± 2
1.58 ± 0.04	Glucose	53.8 ± 5.2*	39.2 ± 1.6	na	93.0 ± 3.8	79.7 ± 2.9*	29.6 ± 1.8	109 ± 5	97.2 ± 0.6	7.5 ± 1.0	105 ± 2
1.32 ± 0.02	Fructose	53.2 ± 5.2*	40.6 ± 1.8	na	93.9 ± 3.1	79.7 ± 2.2*	28.1 ± 1.5	108 ± 4*	99.7 ± 0.5	7.3 ± 0.7	107 ± 1
3.65 ± 0.02	Total sugars	54.6 ± 6.3*	38.4 ± 1.2	na	93.0 ± 5.4	81.1 ± 1.0*	29.1 ± 1.4	110 ± 2*	97.9 ± 0.3	7.4 ± 1.0	105 ± 1
9.44 ± 0.11	Dry matter	68.6 ± 2.3*	30.7 ± 3.3	3.0 ± 0.3	102 ± 3	89.5 ± 2.3*	10.8 ± 1.9	100 ± 1	98.0 ± 3.7	3.7 ± 1.5	102 ± 2

[a] Data presented as arithmetic means ± SD ($n = 3$).
[b] Recovery relative to untreated.
[c] Sum of the recoveries in both solid and water.
[d] Not analyzed.
* Significantly different from untreated at the $p = 0.05$ probability level.

Table 2
Effect of heat treatment on L-ascorbic acid (L-AA), total phenols (TP), total monomeric anthocyanins (TMA), FRAP and ORAC in red cabbage. Values for untreated red cabbage are given per 100 g FW and for the processed samples (blanched, boiled, steamed) as percentage recovery[a]

Fresh/100 g		Blanched, recovery (%)[b]				Boiled, recovery (%)			Steamed, recovery (%)		
		Solid	Water	Ice water	Σ[c]	Solid	Water	Σ	Solid	Water	Σ
54.4 ± 2.1[d]	L-AA	52.3 ± 2.4*	na[i]	na	–	75.8 ± 2.6*	na	–	88.7 ± 1.4*	na	–
210 ± 9[e]	TP	57.0 ± 1.2*	40.9 ± 2.2	nd[j]	97.9 ± 2.0	84.1 ± 3.3*	40.7 ± 2.0	125 ± 4*	102 ± 4.2	8.9 ± 0.3	111 ± 4*
114 ± 5[f]	TMA	41.1 ± 0.6*	38.2 ± 1.5	2.6 ± 0.8	81.9 ± 1.3*	59.4 ± 2.5*	29.2 ± 1.5	88.5 ± 2.7*	71.4 ± 3.0*	6.3 ± 0.3	77.7 ± 3.2*
2.94 ± 0.11[g]	FRAP	58.3 ± 1.5*	57.0 ± 2.6	4.4 ± 0.6	120 ± 3*	82.9 ± 3.9*	45.2 ± 4.4	128 ± 2.0*	98.1 ± 3.4	9.5 ± 0.4	108 ± 4
3.10 ± 0.09[h]	ORAC	49.0 ± 1.0*	46.4 ± 2.1	nd	95.4 ± 3.0	80.9 ± 4.7*	50.7 ± 3.4	132 ± 7.2*	87.5 ± 5.2	24.4 ± 1.4	120 ± 7

[a] Data presented as arithmetic means ± SD ($n = 3$).
[b] Recovery relative to untreated.
[c] Sum of the recoveries in both solid and water.
[d] mg.
[e] mg GAE.
[f] mg cy-3-gluE.
[g] mmolFe^{2+}.
[h] mmolTE.
[i] Not analyzed.
[j] Not detected.
* Significantly different from untreated at the $p = 0.05$ probability level.

blanched, boiled and steamed red cabbage. The water contents were not analyzed due to shortage of sample; therefore, an unequivocal conclusion regarding degree of thermal breakdown is not possible. Puupponen-Pimiä et al. (2003) found that vitamin C in blanched white cabbage (8 mm slices, 96 °C, 3 min, <5 min cooling) was reduced by 30%. The losses in our study indicate a 47.7% loss. It is not clear whether the vegetable–water ratio used by the authors is comparable to ours. Differences in exposed surface area, i.e. their use of 8 mm slices vs our 1 × 1 cm pieces, might also contribute to the differences observed. Zhang and Hamauzu (2004) found reductions in L-AA of 66% in 5 min boiled broccoli florets. Our losses (24.2%) were much lower than those reported by Zhang and Hamauzu (2004) and even though the boiling time and degree of exposed leaching were larger. They did, however, process the broccoli using a vegetable to water ratio of 1:20, contrary to our 1:1, which may indicate that the degree of leaching is a function of the amount of water used and perhaps to a lesser extent the cooking time.

4.4. Total phenols

The phenolic compounds are the major antioxidant constituents present in red cabbage (Podsedek et al., 2006). Levels reported in the literature vary. Proteggente et al. (2002) found 158 mg GAE/100 g FW, Podsedek et al. (2006) 135 and 171 mg GAE/100 g FW, and Wu et al. (2004) 254 mg GAE/100 g FW. These values correspond well to ours (Table 2). Blanching and boiling significantly ($p < 0.05$) reduced the contents in our experiment by 43% and 16%, respectively. Zhang and Hamauzu (2004) found a 72% reduction in TP in broccoli florets boiled for 5 min using a vegetable–water ratio of 1:20. This is much larger than the losses found in our study for both blanched (3 min, vegetable–water ratio 1:10) and boiled (10 min, vegetable–water ratio 1:1) samples. The deviations might partly be explained by the differing vegetable–water ratios used. Wu et al. (2004) reported the opposite effect in boiled (3–4 min) red cabbage, with an increase of 26% calculated on "as is weight basis", thus with no normalisations made for differing moisture content. The physical attributes of the processed cabbage were not described. Puupponen-Pimiä et al. (2003) reported a similar increase of 21% in TP in blanched sliced white cabbage. The cumulative levels of total phenols in both water and solids in our study were also assessed and no overall degradations seemed to have occurred; actually the cumulative values for boiling and steaming (both 10 min) were significantly higher than those of fresh samples, indicating an overall increase in the content of phenols. In some respects this is in conjunction with the increases reported by others in solid material (Puupponen-Pimiä et al., 2003; Wu et al., 2004). Puupponen-Pimiä et al. (2003) speculated that the heat treatment facilitated release from the cellular matrix, resulting in a higher extraction yield. The highest recovery was found for boiled samples where the exposure to water was higher than for steamed, although the exposure time remained the same. It is assumed that the heat transfer was faster and more homogeneous during boiling than steaming.

4.5. Total monomeric anthocyanins

The monomeric anthocyanins in red cabbage were measured by means of their inherent pH-dependent colour characteristics (Giusti & Wrolstad, 2001) and the results shown in Table 2. The anthocyanin literature levels for red cabbage vary. Podsedek et al. (2006) found 41 and 76 mg cy-3-gluE/100 g FW, Piccaglia, Marotti, and Baldoni (2002) 125 mg cy-3-gluE/100 g FW and Wu et al. (2006) 322 mg cyanidin glucosides/100 g FW. Our results are intermittent to these. Flavonoids other than anthocyanins are more or less absent in red cabbage (US Department of Agriculture, 2007). Indeed, Wu et al. (2006) identified cyanidin as the dominant anthocyanin aglycone, of which 85% was acylated. All manners of processing affected anthocyanins significantly ($p < 0.05$) with reductions of 59%, 41% and 29% for blanching, boiling and steaming, respectively, as shown in Table 2. The reductions observed in the processed cabbage were not fully recovered in the processing waters, fitting the notion that anthocyanins are degraded by heat (Markakis, 1982). However, the actual extent of heat degradation might depend on various factors related to anthocyanin structure and the pH value. In acidic to neutral media, anthocyanins exist in four equilibrium configurations (Markakis, 1982; Mazza & Miniati, 1993). The more stable red-coloured structure exists at pH < 3 and, as the pH is raised to 4–6, the colourless structures predominate. The equilibrium reactions towards the colourless forms are endothermic, i.e. favoured by heating. The reverse reaction is slow and if insufficient time is allowed, underestimation of anthocyanins present might occur (Markakis, 1982). No attempts were made to stabilize the anthocyanins by adjusting the pH. Kirca, Ozkan, and Cemeroglu (2007) investigated the effect of pH on black carrot anthocyanins and found that the stability of the anthocyanins decreased significantly at pH > 5. This is approximately the same pH area as in our samples. The reason for the overall apparent loss of TMA might thus be related to the thermal aspects which might have led to an underestimation, not necessarily only due to thermal degradation but to the pH level and limitations in sample assay.

4.6. FRAP and ORAC

FRAP and ORAC values found in this study (Table 2) corresponded well with those determined by others but were somewhat higher. For FRAP, Proteggente et al. (2002) found 1.87 mmol Fe^{2+}/100 g FW and Wold et al. (2006) found (in the identical cultivar averaged over two consecutive years) 2.09 mmol Fe^{2+}/100 g FW. For ORAC, Proteggente et al. (2002) found 2.12 mmol TE/100 g FW and Wu et al. (2004) 2.23 mmol TE/100 g FW. Deviations

Table 3
Effect of heat treatment on GLS (μmol/100 g FW) in red cabbage. Values for untreated red cabbage are given in μmol per 100 g FW and for the processed samples (blanched, boiled, steamed) as percentage recovery[a]

Fresh			Blanched, recovery (%)[b]				Boiled, recovery (%)			Steamed, recovery (%)		
μmol/100gFW	%-of total[c]		Solid	Water[d]	Ice water[e]	Σ[f]	Solid	Water	Σ	Solid	Water[d]	Σ
462 ± 6	100	GLS (total)	35.8 ± 2.0*	47.2 ± 0.5	–	82.0 ± 0.8*	62.4 ± 2.4*	–	–	81.2 ± 2.2*	16.5 ± 1.5	96.4 ± 1.1*
361 ± 5	78	Alip. (total)	34.5 ± 1.9*	44.0 ± 0.5	–	77.5 ± 0.9*	60.1 ± 2.3*	–	–	78.1 ± 1.4*	15.4 ± 1.5	92.7 ± 1.6*
37.0 ± 0.5	8	IB[i]	39.4 ± 3.0*	38.8 ± 0.8	nd[g]	76.7 ± 1.3*	69.2 ± 2.5*	na[h]	–	89.6 ± 0.5*	5.9 ± 0.1	95.1 ± 0.1
91.7 ± 4.0	20	PROG[j]	21.6 ± 0.1*	52.0 ± 0.3	nd	73.6 ± 0.3*	34.8 ± 2.3*	na	–	45.5 ± 3.6*	38.6 ± 5.6	82.2 ± 3.7*
40.4 ± 2.5	9	SIN[k]	38.7 ± 2.0*	32.8 ± 1.3	nd	71.1 ± 1.3*	68.4 ± 3.7*	na	–	88.3 ± 0.3*	6.3 ± 0.1	94.4 ± 0.2
148 ± 1.9	32	RAPH[l]	39.3 ± 3.6*	45.3 ± 0.3	nd	82.6 ± 1.4*	69.6 ± 1.9*	na	–	90.8 ± 1.4*	7.7 ± 0.1	97.8 ± 1.3
35.3 ± 1.5	8	NAP[m]	39.2 ± 1.4*	36.1 ± 1.4	nd	75.7 ± 0.2*	70.6 ± 4.6*	na	–	90.2 ± 0.6*	5.9 ± 0.1	96.2 ± 0.7
8.6 ± 0.3	2	ERU[n]	29.1 ± 1.2*	43.0 ± 2.3	nd	71.5 ± 3.1*	44.2 ± 2.0*	na	–	60.2 ± 3.5*	24.5 ± 2.4	83.2 ± 0.1*
13.1 ± 0.8	2.8	Aro./NASTU[o]	39.1 ± 1.6*	49.1 ± 0.0	nd	87.5 ± 1.6	71.6 ± 3.7*	na	–	91.3 ± 2.8	11.2 ± 0.8	101.2 ± 1.9
88.0 ± 1.5	19	Indo. (total)	33.5 ± 1.7*	45.4 ± 0.6	–	78.0 ± 0.6*	57.7 ± 2.2*	–	–	75.1 ± 4.1*	12.6 ± 0.5	85.7 ± 2.3*
8.6 ± 0.2	2	4-OHGB[p]	9.7 ± 0.7*	69.3 ± 0.5	nd	78.6 ± 0.5*	22.8 ± 1.3*	na	–	26.0 ± 2.1*	66.3 ± 6.0	91.2 ± 5.2*
31.3 ± 0.7	7	GB[q]	37.4 ± 1.4*	49.6 ± 0.2	nd	86.3 ± 0.7*	66.8 ± 2.8*	na	–	87.3 ± 4.2*	7.6 ± 0.1	92.7 ± 1.9
48.1 ± 1.0	10	4-metGB[r]	35.2 ± 2.1*	38.4 ± 0.9	nd	72.6 ± 0.7*	58.0 ± 2.0*	na	–	75.9 ± 4.3*	6.3 ± 0.3	80.1 ± 3.8*

[a] Data presented as arithmetic means ± SD ($n = 3$).
[b] Recovery in relation to fresh matter content.
[c] Relative amounts to total GLS content.
[d] $n = 2$.
[e] Ice water.
[f] Sum of the recoveries in both solid and water.
[g] Not determined.
[h] Not analyzed due to shortage of sample.
[i] Glucoiberin.
[j] Progoitrin.
[k] Sinigrin.
[l] Glucoraphanin.
[m] Gluconapin.
[n] Glucoerucin.
[o] Gluconasturtiin.
[p] 4-Hydroxyglucobrassicin.
[q] Glucobrassicin.
[r] 4-Methoxyglucobrassicin.
* Significantly different from untreated at the $p = 0.05$ probability level.

in reported values are likely to be caused by differences in cultivars, growing conditions and analytical aspects. Processing resulted in significant losses in both FRAP and ORAC values for red cabbage undergoing blanching and boiling (Table 2). It seems that the amount of water used has a profound effect on the degree of leaching as the reductions in blanched samples were more than twice those in boiled. Steamed red cabbage was unaffected for both assays. The effect of processing reported in the literature varies greatly and is to some extent confusing. Wu et al. (2004) found a 41% increased ORAC value in cooked (3–4 min) red cabbage and Halvorsen et al. (2006) an increase of 270% in FRAP. Both of these studies were reported on an "as eaten" basis with no corrections for deviations in moisture content, and in addition the water–vegetable ratio used was not stated. Puupponen-Pimiä et al. (2003) found that the antioxidant activity measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method in cauliflower decreased by 23%, but increased by 9% in cabbage during blanching. Zhang and Hamauzu (2004) found that, after both microwave and conventional boiling for 5 min (vegetable–water ratio of 1:20), the florets and stems of broccoli had lost 65% of the antioxidant power as measured by DPPH. Wu et al. (2004) also investigated cooked broccoli and found a decrease of 16% for hydrophilic ORAC value. It might seem that the results of processing are not only dependent on the manner in which it is performed but also the type of vegetable used. Significantly higher recoveries (plant matrix + water) than for untreated material were found in the boiling experiment, with 128% and 132% for FRAP and ORAC, respectively. The recovery in blanching was 120% ($p < 0.05$) for FRAP whereas no change could be found in ORAC. The 120% recovery for ORAC in steamed was not deemed to be statistically significant. The reasons for these deviations are not known. Both steaming and boiling were performed for 10 min, and thus more than three times longer heat exposure compared to blanching, with no water cooling afterwards. The same pattern was seen for TP, as well, with the overall highest recovery when boiled. These variable results might be due to differences in sample preparation and treatment parameters, for instance piece size of the cut vegetable.

4.7. Glucosinolates

Ten GLS (six aliphatic, one aromatic and three indoles) were determined in fresh and processed red cabbage (Table 3) and results correspond with those found by others (Ciska, Martyniak-Przybyszewska, & Kozlowska, 2000; Oerlemans, Barrett, Suades, Verkerk, & Dekker, 2006; Verkerk, Dekker, & Jongen, 2001). It is speculated that higher levels found in fresh red cabbage might be attributed to the limited sample preparation when analyzing intact GLS, yielding higher values for contents of GLS. In addition, differences in cultivars and growing conditions could also explain the relatively large differences in levels found (Ciska et al., 2000).

Cooking of brassica affects glucosinolates, depending on the manner of processing, time used, type of vegetable and the degree of cellular damage. Factors within the plant matrix, such as ascorbic acid, epithiospecifier protein and ferrous iron, may affect the extent of hydrolysis of GLS by myrosinase (Bones & Rossiter, 1996). External factors, such as temperature and pH, can also affect the hydrolysis (Ludikhuyze, Ooms, Weemaes, & Hendrickx, 1999). Yen and Wei (1993) investigated myrosinase from red and white cabbage, finding increase in activity up to 60 °C, followed by a dramatic decrease with rising temperature. Rungapamestry, Duncan, Fuller, and Ratcliffe (2006) investigated the effects of steaming on myrosinase activity and showed a loss of 90% after 7 min. Hence, it is likely that complete thermal inactivation had occurred in boiled and steamed red cabbage after 10 min in the present study. It is also assumed that the enzymatic activity in blanched red cabbage was terminated, even though the time was only 3 min, due to the large excess of already boiling water (1:10) as well as the physical size of the cabbage pieces (small). Naturally, the actual effects of this are dependent on the efficacy of heat transfer and physical properties of material in question. As myrosinase is partially or totally inactivated during heat treatment, it is prudent to assume that potential loss of GLS is a function of leaching into the processing waters as well as thermal and/or chemical degradation (Dekker, Verkerk, & Jongen, 2000; Rosa & Heaney, 1993).

The most dramatic decrease in solid cabbage was noted in blanching, followed by boiling and steaming, with 64%, 38% and 19%, respectively, ($p < 0.05$), as shown in Table 3. Wennberg et al. (2006) blanched shredded cabbage at a vegetable–water ratio of 1:1 for 5 min with consecutive 15 min air cooling, finding losses of 50% and 74% in total GLS for the two cv. investigated. This is in some respects similar to our findings. Sones, Heaney, and Fenwick (1984) found that total GLS in cooked whole leaves (10 min, 0.05% salt solution, unknown vegetable–water ratio) of red cabbage was reduced by 18% compared to untreated. This is lower than the loss found in our experiment and may partly be explained by the different physical areas exposed, i.e. intact leaves as against small pieces. Rosa and Heaney (1993) boiled shredded cabbage for 10 min (vegetable–water ratio 1:5), finding reductions in excess of 50% which is similar to the findings in our study. Boiling, in our experiment, was also conducted for 10 min but with a vegetable–water ratio of 1:1 and blanching was conducted for 3 min with a ratio of 1:10, indicating that the amount of water is likely to play an important role in the reductions. Ciska and Kozlowska (2001) found a reduction of 49% after 10 min of boiling sliced cabbage (vegetable–water ratio 1:3), corresponding well to our findings of 38% loss, taking into account differences in water used. Ciska and Kozlowska (2001) also found that the largest decrease occurred during the first 5 min and this observation was related to the results obtained for blanching for 3 min which, with a much higher water quantity, might explain the higher losses.

The losses of the individual GLS varied (Table 3) and this result complies with other findings (Rosa & Heaney, 1993; Sones et al., 1984; Wennberg et al., 2006). Variations in individual diffusion and hydrophilic properties of individual GLS are likely to be of importance. The major individual GLS distributions of glucoraphanin, progoitrin and 4-methoxyglucobrassicin in our study (Table 3) did not change as a result of the investigated cooking methods and remained between 59% and 62% of the total. Reductions in progoitrin, glucoerucin and 4-hydroxyglucobrassicin were more striking for all processes compared to the other GLS. This is somewhat contradictory to Rosa and Heaney (1993) who found that the highest individual reductions were for glucoiberin, sinigrin and glucobrassicin.

The cumulative recoveries, i.e. plant matrix + water, for the GLS most dramatically reduced (progoitrin, glucoerucin, 4-OH-glucobrassicin) were significantly lower than those determined in untreated cabbage for both blanching and steaming. All total recoveries for all individual GLS for blanching were significantly lower than the original contents in raw material. Steaming gave total recoveries statistically lower for total GLS, total aliphatic and total indole with 96%, 93% and 86%, respectively. In addition the total recoveries, in steaming, for 4-methoxyglucobrassicin were lower than that in fresh material. The level of aromatic gluconasturtiin was not different from the level found in fresh material. The total recoveries of compounds with the largest reductions, i.e. progoitrin, glucoerucin and 4-hydroxyglucobrassicin, in our study were 82%, 83% and 91%, respectively, for steaming. The overall total recovery of GLS was 96% for steamed red cabbage, related to total aliphatic, total aromatic (gluconasturtiin) and total indole at 93%, 101% and 86%, indicating some unaccounted losses. Rosa and Heaney (1993) also investigated the GLS in the processing water, finding high degrees of recoveries of 90%, 96% and 91% for total aliphatic, total indole and total GLS and consequently recovered a total of 90%, 89% and 97% for glucoiberin, sinigrin and glucobrassicin, respectively, those which showed the most dramatic decrease in the solid material. This is somewhat in conjunction with Ciska and Kozlowska (2001) who found that indole GLS were more efficiently lost compared to aliphatic as a result of greater diffusion properties. This is contradictory to Rungapamestry et al. (2006) who did not find that indole GLS were any more thermolabile or more prone to loss as a result of diffusivity than others. Oerlemans et al. (2006) investigated the effect of thermal degradation in GLS in myrosinase-deactivated red cabbage, finding that GLS are relatively heat-stable at temperatures <100 °C; however, they indicated that indoles were more thermolabile than were aliphatic. GLS levels in blanching water indicate that most of the GLS have leached into the cooking medium; however, it is difficult to assess the degree of thermal degradation, if any, due to our limited data from process waters. Notably, in the steaming experiment, it seems that 7% of total aliphatic and 14% of total indole GLS are unaccounted for.

The findings in this study indicate that the method of processing significantly influences the concentrations of phytochemicals and antioxidant parameters in red cabbage. The impact of processing was the least for steaming. Losses of health-related photochemicals are thus likely to be a function of type and physical form, and cooking parameters, such as time, temperature, amount of water and degree of wounding. The major factor, in this study, determining the extent of losses of phytochemicals seems to be the degree of water exposure. If an increase in the intake of GLS and antioxidants can contribute to an overall positive effect on the general health in the population, a better knowledge of how processing affects the compounds of interest is of pivotal importance.

Acknowledgements

The Research Council of Norway are acknowledged for their funding of the project entitled "Bioactive phytochemicals (flavonoids) in fruit and vegetables: storage, processing and rapid sensor-based analytical methods" (NFR146579/140, 2002–2006). Grete Skrede, Mona Ringstad and Berit Karoline Martinsen at Matforsk AS – Norwegian Food Research Institute and Karin Haffner(†), Anne-Berit Wold, Kari Grønnerød, Signe Hansen and Liv Berge at the Norwegian University of Life Sciences are acknowledged for their valuable assistance.

References

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.

Bones, A. M., & Rossiter, J. T. (1996). The myrosinase–glucosinolate system, its organisation and biochemistry. *Physiologia Plantarum, 97*(1), 194–208.

Ciska, E., & Kozlowska, H. (2001). The effect of cooking on the glucosinolates content in white cabbage. *European Food Research and Technology, 212*(5), 582–587.

Ciska, E., Martyniak-Przybyszewska, B., & Kozlowska, H. (2000). Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. *Journal of Agricultural and Food Chemistry, 48*(7), 2862–2867.

Davalos, A., Gomez-Cordoves, C., & Bartolome, 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., Inze, 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.

Dekker, M., Verkerk, R., & Jongen, W. M. F. (2000). Predictive modelling of health aspects in the food production chain: A case study on

glucosinolates in cabbage. *Trends in Food Science and Technology, 11*(4–5), 174–181.

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 & Sons, Inc.

Halvorsen, B. L., Carlsen, M. H., Phillips, K. M., Bohn, S. K., Holte, K., Jacobs, D. R., et al. (2006). Content of redox-active compounds (i.e., antioxidants) in foods consumed in the United States. *American Journal of Clinical Nutrition, 84*(1), 95–135.

Hollman, P. C. H. (2001). Evidence for health benefits of plant phenols: Local or systemic effects? *Journal of the Science of Food and Agriculture, 81*(9), 842–852.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry between antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841–1856.

Kirca, A., Ozkan, M., & Cemeroglu, B. (2007). Effects of temperature, solid content and pH on the stability of black carrot anthocyanins. *Food Chemistry, 101*(1), 212–218.

Ludikhuyze, L., Ooms, V., Weemaes, C., & Hendrickx, M. (1999). Kinetic study of the irreversible thermal and pressure inactivation of myrosinase from broccoli (*Brassica oleracea* L-Cv. *Italica*). *Journal of Agricultural and Food Chemistry, 47*(5), 1794–1800.

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

Martin-Villa, C., Vidal-Valverde, C., & Rojas-Hidalgo, E. (1982). High-performance liquid-chromatographic determination of carbohydrates in raw and cooked vegetables. *Journal of Food Science, 47*(6), 2086–2088.

Mazza, G., & Miniati, E. (1993). *Anthocyanins in fruits, vegetables and grains*. Boca Raton, FL, USA: CRC Press.

Oerlemans, K., Barrett, D. M., Suades, C. B., Verkerk, R., & Dekker, M. (2006). Thermal degradation of glucosinolates in red cabbage. *Food Chemistry, 95*(1), 19–29.

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.

Piccaglia, R., Marotti, M., & Baldoni, G. (2002). Factors influencing anthocyanin content in red cabbage (*Brassica oleracea* var. *capitata* L f *rubra* (L) Thell). *Journal of the Science of Food and Agriculture, 82*(13), 1504–1509.

Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of Natural Products, 63*(7), 1035–1042.

Podsedek, A., Sosnowska, D., Redzynia, M., & Anders, B. (2006). Antioxidant capacity and content of *Brassica oleracea* dietary antioxidants. *International Journal of Food Science and Technology, 41*(Suppl. 1), 49–58.

Proteggente, A. R., Pannala, A. S., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., et al. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research, 36*(2), 217–233.

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.

Rosa, E. A. S., & Heaney, R. K. (1993). The effect of cooking and processing on the glucosinolate content – studies on 4 varieties of Portuguese cabbage and hybrid white cabbage. *Journal of the Science of Food and Agriculture, 62*(3), 259–265.

Rungapamestry, V., Duncan, A. J., Fuller, Z., & Ratcliffe, B. (2006). Changes in glucosinolate concentrations, myrosinase activity, and production of metabolites of glucosinolates in cabbage (*Brassica oleracea* var. *capitata*) cooked for different durations. *Journal of Agricultural and Food Chemistry, 54*(20), 7628–7634.

Sharma, D. D., D'Souza, V. F., McConnell, M. B., & Mazza, G. (1988). Identification and quantification of sugars in Winter-Hardy apples by high-performance liquid-chromatography. *Canadian Institute of Food Science and Technology Journal – Journal de l Institut Canadien de Science et Technologie Alimentaires, 21*(4), 435–437.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In Oxidants and antioxidants, Pt. A. *Methods in Enzymology* (pp. 152–178). San Diego, CA, USA: Academic Press.

Sones, K., Heaney, R. K., & Fenwick, G. R. (1984). An estimate of the mean daily intake of glucosinolates from cruciferous vegetables in the UK. *Journal of the Science of Food and Agriculture, 35*(6), 712–720.

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.

US Department of Agriculture, Agricultural Research Service (2006). USDA Nutrient Database for Standard Reference, Release 19. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl.

US Department of Agriculture, Agricultural Research Service (2007). USDA Database for the Flavonoid Content of Selected Foods, Release 2.1. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/nutrientdata.

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.

Verkerk, R., Dekker, M., & Jongen, W. M. F. (2001). Post-harvest increase of indolyl glucosinolates in response to chopping and storage of Brassica vegetables. *Journal of the Science of Food and Agriculture, 81*(9), 953–958.

Wennberg, M., Ekvall, J., Olsson, K., & Nyman, M. (2006). Changes in carbohydrate and glucosinolate composition in white cabbage (*Brassica oleracea* var. *capitata*) during blanching and treatment with acetic acid. *Food Chemistry, 95*(2), 226–236.

Williams, R. C., Baker, D. R., & Schmit, J. A. (1973). Analysis of water-soluble vitamins by high-speed ion-exchange chromatography. *Journal of Chromatographic Science, 11*(12), 618–624.

Wills, R. B. H., Wimalasiri, P., & Greenfield, H. (1984). Dehydroascorbic acid levels in fresh fruit and vegetables in relation to total vitamin-C activity. *Journal of Agricultural and Food Chemistry, 32*(4), 836–838.

Wold, A. B., Rosenfeld, H. J., Holte, K., Baugerød, H., Blomhoff, R., & Haffner, K. (2004). Colour of post-harvest ripened and vine ripened tomatoes (*Lycopersicon esculentum* Mill.) as related to total antioxidant capacity and chemical composition. *International Journal of Food Science and Technology, 39*(3), 295–302.

Wold, A. B., Rosenfeld, H., Lea, P., & Baugerød, H. (2006). The effect of CA and conventional storage on antioxidant activity and chemical composition in red and white cabbage (*Brassica oleracea* var. *capitata* L.) and savoy (*Brassica oleracea* var. *sabauda* L.). *European Journal of Horticultural Science, 71*(5), 212–216.

Wu, X. L., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry, 52*(12), 4026–4037.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2006). Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *Journal of Agricultural and Food Chemistry, 54*(11), 4069–4075.

Yen, G. C., & Wei, Q. K. (1993). Myrosinase activity and total glucosinolate content of cruciferous vegetables, and some properties of cabbage myrosinase in Taiwan. *Journal of the Science of Food and Agriculture, 61*(4), 471–475.

Zhang, D. L., & Hamauzu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chemistry, 88*(4), 503–509.