Assessing the impact of high pressure processing on selected physical and biochemical attributes of white cabbage (*Brassica oleracea* L. var. *capitata alba*)

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Abstract

The aim of this study was to investigate the effect of combined pressure/temperature treatments (200, 400 and 600 MPa, at 20 and 40 °C) on key physical and chemical characteristics of white cabbage (*Brassica oleracea* L. var. *capitata alba*). Thermal treatment (blanching) was also investigated and compared with high pressure processing (HPP). HPP at 400 MPa and 20-40 °C caused significantly larger colour changes compared to any other pressure or thermal treatment. All pressure treatments induced a softening effect whereas blanching did not significantly alter texture. Both blanching and pressure treatments resulted in a reduction in the levels of ascorbic acid, effect that was less pronounced for blanching and HPP at 600 MPa and 20-40 °C. HPP at 600 MPa resulted in significantly higher total phenol content, total antioxidant capacity and total isothiocyanate content compared to blanching. In summary, the colour and texture of white cabbage were better preserved by blanching. However, HPP at 600 MPa resulted in significantly higher levels of phytochemical compounds. The results of this study suggest that HPP may represent an attractive technology to process vegetable-based food products that better maintains important aspects related with the content of health-promoting compounds. This may be of particular relevance to the food industry sector involved in the development of convenient novel food products with excellent functional properties.

Keywords

White cabbage, high pressure processing, blanching, texture, antioxidants, isothiocyanates

1. **Introduction**

In recent years there has been an increasing demand by consumers for food products of high nutritional quality, with desirable organoleptic qualities and appropriate shelf-life. Typically, a thermal treatment such as water blanching is employed to extend the shelf-life of certain vegetable-based foods such as ready meals and frozen vegetables, since blanching inactivates enzymes responsible for food deterioration and it also reduces microbial counts (Bahceci et al. 2005; Olivera et al. 2008; Volden et al. 2009). However, thermal processing such as blanching can induce losses of important compounds due to thermal degradation and leaching into cooking water (Rungapamestry et al. 2007; Olivera et al. 2008; Volden et al. 2008, 2009). In this respect, a number of novel food processing technologies have emerged, such as high pressure processing (HPP), which represent potential alternatives to traditional thermal technologies. Several authors have studied the effects of HPP on microbial and vegetable enzyme inactivation and have suggested that it can be used as a non-thermal blanching method for vegetable processing (Eshtiaghi and Knorr 1993; Rastogi et al. 2007; Hsu et al. 2008). In addition to the possibility of low temperature treatment, some advantages of the use of HPP for food applications include the independence of size and geometry of the sample during processing and the fact that is represents a waste-free environmentally friendly technology (Norton and Sun 2008).

HPP at moderate temperature does not affect covalent bonds, therefore important quality attributes such as colour, flavour and nutritional quality may be maintained after HP treatment (Oey et al. 2008b). However, significant differences can be observed in the effect of HPP on the physical properties and chemical composition of vegetables with the observed effect depending on the type of product and the temperature/pressure applied. In the review by Oey et al. (2008b),

HPP was observed to only affect the ascorbic acid content of fruit and vegetable products at extreme pressure and temperature combinations. Several authors have also shown that the effect of high pressure processing on the vitamin and phytochemical content varies depending on fruit or vegetable type (McInerney et al. 2007; Wolbang et al. 2008; Patras et al. 2009; Keenan et al. 2010). Thus besides pressurization level and temperature applied, factors such as the structure and composition of the food matrix may be of great significance when assessing the impact of HPP on the nutritional quality of vegetables (Fuchigami et al. 1998). Similarly, the effect of HPP on important quality properties such as colour and texture of fruit and vegetable cannot be generalized as it ultimately depends on the temperature/pressure applied and on the intrinsic properties of the product under study (Oey et al. 2008a).

White cabbage (*Brassica oleracea* L. var. *capitata alba*) is a vegetable belonging to the Brassicaceae family. This family includes vegetables such as broccoli, cauliflower, brussels sprouts and kale which are characterised by the presence of high levels of phytochemicals such as carotenoids, phenolics and glucosinolates (Fahey et al. 2001; Lee et al. 2011). In addition, several epidemiological studies have linked consumption of cruciferous vegetables with reduced risk of cancer, a topic which has been reviewed extensively in the literature (Zhang and Tang 2007; Jeffery and Keck 2008; Jeffery and Araya 2009). The beneficial effects associated with cruciferous consumption have been attributed to a particular type of compounds named isothiocyanates which are formed in cruciferous vegetables upon hydrolysis of precursor compounds glucosinolates by the endogenous enzyme myrosinase (Bones and Rossiter 2006). The effect of HPP on the myrosinase-glucosinolate system in broccoli has been previously investigated by other authors (Van Eylen et al. 2007, 2008a, 2009). The enzyme myrosinase was

found to be resistant to HP treatment. Moreover, myrosinase activity was found to be higher following HPP at 200-400 MPa and 50-60 °C compared to treatment at the same temperature but atmospheric pressure (Van Eylen et al. 2008). Also, HP treatment of broccoli heads (200-500 MPa at 40 °C for 15 min) was reported to favour the formation of bioactive isothiocyanate compounds by the enzyme myrosinase compared to processing at atmospheric pressure (Van Eylen et al. 2009).

No studies have been published to date on the impact of high pressure processing on the quality of white cabbage. The objective of this study was to assess the impact of combined pressure/temperature treatments as an alternative to thermal blanching on key physical and chemical characteristics of white cabbage.

2. Materials and methods

2.1 Chemicals

Benzene-1,2-dithiol (BDT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2,5,7,8-tetramethylchromane-2 carboxylic acid (Trolox), Folin Ciocalteu Reagent (FCR), L–ascorbic acid, silica gel (Grade 9385, pore size 60 Å, 230-400 mesh), sodium tetraborate and DL-sulforaphane were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). Diatomaceous earth was purchased from Dionex (Idstein Germany). HPLC grade water and methanol were purchased from BDH Chemicals Ltd (Poole, England).

2.2 White cabbage samples

Ten kilograms of white cabbage (*Brassica oleracea* L. Var. *capitata alba* cultivar *Sicilian*) were purchased from a local supplier and stored at 4 ˚C for 24 hours. External cabbage leaves and stalk were removed and discarded using a sharp stainless steel knife. Two outer cabbage leaves per head of white cabbage were separated for colour analysis. Cabbage heads were subsequently cut into four sections and each of the sections was manually shredded (2-3 mm width) using a stainless steel knife. The resultant shredded cabbage was thoroughly mixed and subsequently vacuum packed, together with cabbage leave sections for colour measurements, into high oxygen barrier pouches (oxygen transmission rate 8 cc/m^2/24 h at 23 °C, 1 bar and 75 % RH; Versatile Packaging Ltd, Monaghan, Ireland). A total of twenty-four packages were produced, three packages per treatment, each package typically containing approximately 220 g of white cabbage. Packages destined to high pressure processing were double vacuum packed using two pouches per package to prevent breakage during pressurization.

2.3 High-pressure and thermal processing treatments

Samples for high-pressure treatments were subjected to 200, 400 or 600 MPa for 5 min at two temperatures, 20 °C or 40 °C, in a Stansted Fluid Power Iso-Lab 900 Power High Pressure Food Processor (Stansted Fluid Power Ltd., Stansted, UK). The pressure-transmitting medium used was a mixture of ethanol–castor oil (90:10). The pressure vessel had a 2 L capacity and an internal diameter of 10 cm. The compression and decompression rates were both 300 MPa/ min. The temperature of the chamber was thermostatically controlled at 20 or 40 °C during the treatment. Due to compressive heating, an increase in the temperature of the pressurising fluid, by up to a maximum of 12 °C at 600 MPa, was observed.

Samples destined for blanching treatment were placed in pierced pouches and submerged in a water bath set at 90-95 ˚C for 3 min. Pouches were subsequently immersed in a mixture of water/ice to promote rapid cooling.

Non-treated (NT) samples were kept as controls. Each treatment was carried out in triplicate resulting in a total of three samples per treatment. Texture and colour analysis were performed one day after treatment. Samples for chemical analysis were frozen at -20 °C for 24 hr and subsequently freeze dried at -50 °C and 0.03 mbar (Frozen in Time Ltd., York, UK). Lyophilised samples were vacuum-packed and stored at -80 °C until analysis.

2.4 Texture analysis

The texture of white cabbage shreds was measured with the Kramer Shear Force test using an Instron texture analyser (Instron 4464 Universal Testing Machine, Canton MA, USA) equipped with a 2 KN load cell and a 10 blade Kramer shear cell. Shreds of white cabbage (10 g) were placed uniformly across the bottom of the slotted box attachment. The test speed was set to 200 mm/min. Compressive load (N) at maximum load was recorded and Kramer-shear values were reported as Newton per gramme. Five measurements were taken per sample and the results were averaged.

2.5 Colour measurements

White cabbage colour was measured using a Hunterlab spectrophotometer (Ultrascan XE, Hunter Associates Laboratory, Inc., Reston, VA, US), with a D65 illuminant and 10° standard observer angle. Colour coordinates were determined using the 1976 CIELAB system and the results were

160 expressed as L* (lightness), a* (redness) and b* (yellowness). The instrument was calibrated
161 before each series of measurements using white (L* = 100) and black (L* = 0) standard tiles. A
162 numerical total colour difference was also calculated as suggested by Jung et al. (2003) using the
163 formula:
164 $\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2}$ where L_0^*, a_0^* and b_0^* (the colour values of non-
165 treated samples) were used as reference values. In addition, the Hue angle (hue°) was used as a
166 parameter to describe colour and was calculated using the formula:
167 $hue^\circ = \tan^{-1}(b^*/a^*)$. Hue angle is defined as a colour wheel, with red-purple at an angle of 0º,
168 yellow at 90º, bluish-green at 180°, and blue at 270º (McGuire 1992) and is commonly employed
169 to describe the tone or tint of the sample under study.
170 Three pieces of cabbage leaves per sample were employed for colour measurements and the
171 results were averaged.
172
173 2.6 Ascorbic acid analysis
174 Ascorbic acid determination was carried out by reverse phase high performance liquid
175 chromatography (RP-HPLC) according to the method previously described by Tiwari et al.
176 (2008). Briefly, 0.2 g of freeze-dried powder were dissolved in 10 ml of 6 % metaphosphoric
177 acid, vortexed with a V400 Multituve Vortexer (Alpha laboratories, North York, Canada) for 1
178 min and subsequently centrifuged for 10 min at 2000 g and 4 ˚C (MSE Mistral 3000i, Sanyo
179 Gallenkamp, Leicestershire, UK). The final extracts were obtained by filtering 5 ml of the
180 supernatant through 0.45 μm PTFE syringe filters (Sigma Aldrich, St Louis, USA). Sample
181 extracts were stored at -80 ˚C until analysis. Three extracts were prepared per sample. The
182 chromatographic system consisted of a Waters 600s controller, a Waters 717 plus autosampler

and a Waters 616 pump. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA, USA) at 245 nm. Separations were conducted on a Supelco hypersil ODS column (Supelco, USA) with dimensions 150 x 4.6 mm and 5 μm particle size, fitted with hypersil ODS guard column (Gemini C18 [4.0 mm × 3.0 mm], Phenomenex, UK). The mobile phase consisted of 25 mM monobasic potassium phosphate adjusted to pH 3 at a flow rate of 1 $mLmin^{-1}$. Column temperature was set at 40 °C, injection volume was 20 μL and total run time was 5 min. For identification purposes, the retention times of ascorbic acid under the chromatographic conditions specified above was determined. Also, a standard calibration curve for ascorbic acid in 6 % metaphosphoric acid at concentrations ranging from 25 μg/L to 700 μg/L was prepared and used for quantitative analyses.

2.7 Sample extraction for total phenols and antioxidant capacity assays

Sample extracts for the measurement of total phenol content and antioxidant capacity were prepared by pressurized liquid extraction (PLE) using an Accelerated Solvent Extractor (ASE 200, Dionex, Idstein, Germany) equipped with a solvent controller. One gram of freeze-dried white cabbage powder was mixed with 4 g of silica as dispersant (Merck grade, 60 Å, Sigma Aldrich, St Louis, USA) and packed into 22 ml extraction cells in the following order: (i) filter paper (Dionex, Idstein, Germany), (ii) 0.4 g diatomaceous earth (iii), 1 g freeze dried sample mixed with 4 g silica, and (iv) filter paper. The PLE method used to prepare the samples extracts was based on the method by Mohn et al. (2007). In particular, the extraction solvent consisted of 70 % methanol in water, and pressure and temperature were set at 120 bars and 50 ˚C respectively. Extraction time consisted of 3 cycles of 5 min each and nitrogen purge time was 80 seconds. The obtained extracts were subsequently filtered through 0.45 μm PTFE syringe filters

206 (Sigma Aldrich, St. Louis, USA) and stored at -80 ˚C until analysis. Three extracts were
207 prepared per sample.
208
209 2.8 Total phenols assay by Folin-Ciocalteau Reagent
210 Total phenolic content of methanolic white cabbage extracts was evaluated using a modified
211 version of the Folin-Ciocalteu assay as described by Singleton et al. (1999). Briefly, 100 µL of
212 methanolic white cabbage extract or standard, 100 µL of MeOH and 100 µL of Fiolin-Ciocalteu
213 reagent and 700 µL of Na_2CO_3 were added into a 1.5 mL micro-centrifuge tube. The samples
214 were vortexed immediately and the tubes were incubated in the dark for 20 min at room
215 temperature. After incubation all samples were centrifuged at 16250 g for 3 min. The absorbance
216 of the supernatant was then measured at 735 nm in 1 mL plastic cuvettes using a
217 spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Japan). Gallic acid was used as a
218 standard and a calibration curve was built with concentrations between 10 and 200 mgL^{-1}. The
219 results are expressed in mg gallic acid equivalent per 100 g dry-weight basis (mg GAE/100g
220 DW).
221
222 2.9 Antioxidant capacity by the radical DPPH scavenging capacity assay
223 The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to measure the free radical
224 scavenging capacity of the sample extracts as described by Wijngaard et al. (2009). The reaction
225 mixture consisted of 500 µL of diluted sample (serial dilutions of the white cabbage extracts
226 were prepared prior analysis) and 500 µL of a freshly made DPPH methanolic solution (0.05
227 mg/mL) and was prepared in 1.5 mL microcentrifuge tubes. The absorbance of the freshly
228 prepared DPPH solution was measured prior to analysis and absorbance values were in the range
229 1.2-1.3. After vortexing, the tubes were left in the dark for 30 min at room temperature. The

absorbance was then measured against methanol at 515 nm in 1 mL cuvettes using a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Milton Keynes). As the DPPH is reduced by the antioxidants present in the sample, the solution colour fades in a way that is proportional to the antioxidant concentration. The sample concentration that causes a decrease in the initial DPPH concentration by 50% is defined as the IC_{50} and is used to calculate the antioxidant capacity. The IC_{50} of Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxilic acid), a synthetic hydrophilic vitamin E analogue, is also calculated and the antioxidant capacity of the sample is then expressed as Trolox equivalent antioxidant capacity values (TEAC) using the formula $TEAC = (IC50_{Trolox} / IC50_{Sample}) \times 10^5$ as previously outlined by Hagen et al. (2007).

2.10 Total isothiocyanate content

The total amount of isothiocyanates was determined by UV-Vis spectrophotometry according with the standard assay system developed by Zhang et al. (1992). Briefly, 900µL of 100mM K_2PO_4 (pH 8.5), 100µL of sample or ITC standard (sulforaphane, Sigma Aldrich Chemical Co., St Luis, USA) and 1mL of MeOH containing 80 mM benzene-1,2-dithiol (BDT, Sigma Aldrich Chemical Co., St Luis, USA) were added to a 2.0 mL centrifuge tube. The mixture was then incubated for 90 min at 60° C. Samples were then cooled at room temperature and their absorbance was measured spectrophotometrically at 365 nm. Quantification was achieved by means of a calibration curve built using different concentrations of sulforaphane standard (2.8-280 µM). Total isothiocyanate content (TICN) was expressed as µmol sulforaphane equivalents per gram of dry weight sample.

2.11 Statistical analysis

253 Data was analysed using the general linear model (GLM) procedure from the SAS statistical
254 package (SAS 9.1 version, SAS Institute Inc., Cary, NC, USA). Two different models were
255 applied. The first model included treatment (non-treated, blanching, 200 MPa at 20 ºC, 200 MPa
256 at 40 ºC, 400 MPa at 20 ºC, 400 MPa at 40 ºC, 600 MPa at 20 ºC, 600 MPa at 40 ºC) as a fix
257 effect. The second model only considered pressurised samples, and included temperature,
258 pressure, and temperature × pressure interaction as fixed effects. No significant interactions
259 between temperature and pressure were excluded from the model. Differences were assessed
260 using the Tukey test. The level of significance was set at $p<0.05$. Pearson correlation analysis
261 was used to examine the relationships amongst the different parameters under study.
262
263 **3. Results and discussion**
264 3.1 Colour parameters
265 The instrumental colour parameters showing significant differences among non-treated, blanched
266 and pressurised white cabbage samples are presented in Table 1. No colour changes were
267 observed between control (non-treated) and blanched samples ($p>0.05$). All pressure treatments
268 resulted in a decrease in L* values in comparison with the non-treated samples. Also, HPP at 400
269 MPa (20 ºC and 40 ºC) and 600 MPa (20 ºC) resulted in lower L* values compared to those of
270 blanched white cabbage, whereas the rest of the pressure treatments were not significantly
271 different. In the case of a* and b* coordinates, both blanched and pressurised samples showed no
272 differences in comparison with non-treated samples (data not shown).
273
274 Total colour difference (ΔE) was used to express the degree of colour change induced by
275 processing (blanching and HPP) in white cabbage. Pressurised samples showed larger colour

changes (ΔE) compared with blanched samples, however only in the case of 400 MPa at 20 ºC and 40 ºC these differences were significant ($p<0.05$) (Table 1).

The decrease in L* values obtained in the present study following HPP is in agreement with previously published studies of products such as pressurised swede, green beans and melon (Krebbers et al. 2002; Wolbang et al. 2008; Clariana et al. 2011). Colour changes in HP treated fruits and vegetables can be related to changes in structural properties (Oey et al. 2008a). Texture modification may result in changes in the nature and extent of internally scattered light and the distribution of surface reflectance, which in turn may produce changes in lightness (MacDougall 2002). In the present study, L* values were inversely correlated ($p<0.001$, $r = -0.775$) with the Kramer Shear values obtained in the texture measurements. This result would support the idea that textural changes caused by HPP may somewhat have influenced changes of colour appearance, and may help to explain the higher degree of colour change (ΔE) observed in pressurised samples compared with blanched samples.

Among pressurised samples, no interaction between the pressure level and the pressurisation temperature was observed for any of the studied colour parameters, therefore these interactions were dropped from the models. Colour parameters showing significant differences among the pressure levels applied are shown in Table 2. HPP at 200 and 600 MPa showed a greener appearance (lower a* and higher hue° values) in comparison with processing at 400 MPa. These colour differences were nonetheless of a small magnitude and may not result in an apparent change in the tone of the white cabbage.

299 *3.2 Texture*

300 Kramer shear test involves a complex form of mechanical loading, including shear, compression
301 and extrusion. Shearing results in breaking of the cell walls during the test, and shear force is
302 used as an index of product firmness (Bourne 2002). The Kramer shear values of non-treated,
303 blanched and pressurised white cabbage samples are shown in Figure 1. Blanching was the only
304 treatment that induced no significant changes in cabbage texture ($p>0.05$). The lack of texture
305 degradation following blanching of white cabbage does not agree with the softening effect
306 observed in swede following blanching at the same conditions (Clariana et al. 2011). These
307 results show the effect of the thermal treatment applied varies depending on the vegetable under
308 study.

309

310 On the other hand, HPP induced an increase in the Kramer Shear force, indicating increased
311 shear strength in pressurised samples ($p<0.001$). An increase in the Kramer shear force has also
312 been related to an increase in flexibility or a decrease in crispiness of the vegetable under study
313 (Martin-Diana et al. 2006).

314

315 Through cell disruption, HP processing facilitates the occurrence of enzymatic and non-
316 enzymatic reactions. Substrates, ions and enzymes which are located in different compartments
317 in the cells may become liberated during HP treatment and may thus interact with each other. At
318 the same time, pressure can enhance the action of pectinmethylesterase (PME), lower the
319 polygalacturonase (PG) activity, and retard β-elimination (Oey et al. 2008a). The degree of cell
320 disruption is not only dependent on the applied pressure but also on the type of plant cell. Similar
321 to our results, Préstamo and Arroyo (1998) reported a firming effect of HPP on spinach leafs due

to the collapse of spinach cells resulting in a more rigid structure. On the contrary, other authors have reported firmness loss after HPP of vegetables such as swede (400 MPa and 20-40 ºC for 5 min) (Clariana et al. 2011) and cherry tomatoes (200-400 MPa and 20 ºC for 20 min) (Tangwongchai et al. 2000). On the other hand, upon HP treatment PME may become liberated and may come into contact with its substrate pectin resulting in the demethylation of the latter. The de-esterified pectin is in turn capable of forming a gel network that may contribute to the observed increased firmness.

Among HPP treatments, no interaction ($P>0.05$) between pressure and temperature was observed for Kramer shear values, indicating, that both parameters had an independent effect on this variable. No significant effect of the pressure level applied was observed for Kramer shear values (data not shown). Other authors have reported that pressurisation at 600 MPa resulted in greater preservation of texture compared with HPP at lower pressures in different vegetables such as swede and cherry tomatoes (Tangwongchai et al. 2000; Clariana et al. 2011). On the other hand, the effect of the pressurisation temperature on KS values was significant ($p<0.001$). Pressurisation at 20 ºC resulted in higher KS values in comparison with HPP at 40 ºC (148.19 ± 1.22 and 139.32 ± 1.22 respectively). Similar results were found for swede samples (Clariana et al. 2011). This effect may be attributed to the inactivation of the pectin degrading enzyme polygalacturonase (PG) by combinations of moderate pressures and temperatures as previously suggested by Crelier et al. (2001) in the case of tomato PG.

3.4 Ascorbic acid

The ascorbic acid content of the non-treated, blanched and pressurised white cabbage samples is presented in Figure 2. Ascorbic acid content of white cabbage was significantly reduced after

346 blanching and all high pressure processing treatments. Ascorbic acid is a heat-sensitive
347 compound and thus losses in blanched samples may be attributed to thermal degradation. In
348 addition, blanching is also known to cause losses in ascorbic acid due to leaching into the
349 cooking water as previously reported for other *Brassica* vegetables such as Brussels sprouts
350 (Olivera et al. 2008) and swede (Clariana et al. 2011).
351
352 Blanching and HPP at 600 MPa (20 ºC and 40 ºC) presented similar levels of ascorbic acid,
353 while HPP at lower pressures levels induced stronger losses (Figure 2). These results are in
354 agreement with previous published results showing that ascorbic acid losses in swede samples
355 were significantly lower at 600 MPa compared with 400 MPa (5 min, 20 and 40 °C) (Clariana et
356 al., 2011). Also, according to Patras et al. (2009), HPP of strawberry purees at 600 MPa (15 min,
357 20 °C) allowed better ascorbic acid retention in comparison to treatment at 400 MPa. Similarly,
358 Quaglia et al. (1996) observed that increasing the pressure level applied to green peas up to 900
359 MPa (5 min, 33.5-42.5 °C) resulted in significantly higher ascorbic acid content in the processed
360 samples compared to lower levels of pressure. The better preservation of ascorbic acid at higher
361 pressures has been previously associated with texture measurements (Krebbers et al. 2002;
362 Clariana et al. 2011). In particular, Clariana et al. (2011) observed a strong correlation between
363 the loss of texture in swede samples following high-pressure treatment and losses of ascorbic
364 acid. It has been hypothesized that cell damage associated with loss of texture causes increased
365 release of oxidative species thus accelerating ascorbic acid destruction (Krebbers et al. 2002). In
366 the present study only a mild negative correlation ($r = -0.56$, $p<0.005$) was found between
367 Kramer shear values and ascorbic acid content. Another factor that affects the stability of
368 ascorbic acid is oxidation by enzymes such as peroxidase (POD) (Davey et al. 2000). POD has

been shown to become inactivated following blanching (Castro et al. 2011). The pressure stability of POD however depends on the type of vegetable or fruit under study, and also on the pressure treatment applied. A number of studies have shown that the activity of POD may be significantly decreased following high pressure treatment (Cano et al. 1997; Krebbers et al. 2002) thus resulting in a subsequent higher retention of ascorbic acid (Quaglia et al. 1996).

The higher levels of ascorbic acid measured at 600 MPa compared to lower pressures in the present study may thus be related to a higher pressure-induced inactivation of POD compared to 400 and 200 MPa. Interestingly, samples pressurised at 200 MPa and 400 MPa, showed significantly higher ascorbic acid levels when HPP was performed at 40 ºC compared with 20 ºC. This result is somewhat unexpected due to the characteristic low thermal stability of ascorbic acid. This effect may however be attributed to an enhanced effect on the oxidative enzyme peroxidase when the pressure treatment is combined with thermal treatment, thus resulting in a higher retention of ascorbic acid, as previously reported for green peas by Quaglia et al. (1996).

3.5 Total phenol content of white cabbage

Polyphenols are secondary plant metabolites that take part in the protection of plants against ultraviolet radiation, pathogens and herbivores (Harborne and Williams 2000). During the last decade, a large amount of research has been conducted on the polyphenol composition of foods. The interest in polyphenols in the past decade has been stimulated by epidemiological studies linking diets rich in plant foods with reduced risk of diseases associated with oxidative stress, such as cancer and cardiovascular disease (Scalbert et al. 2005).

392 *Brassicas* including white cabbage are known to contain high levels of phenolic compounds (Lee
393 et al. 2011). In the case of white cabbage, the main phenolic acids present include sinapic,
394 caffeic, ferulic and p-coumaric acid (Lee et al. 2011). Processing can affect the polyphenol
395 content of foods in several ways depending on factors such as the type of substrate and the
396 processing conditions (Sensoy et al. 2006). In the present study, samples processed at 200 and
397 400 MPa showed total phenol contents similar to non-treated samples, while blanched samples
398 showed reduced levels and samples pressurised at 600 MPa increased levels of total phenols.
399 However, none of these differences were significant (Table 3). Some differences were however
400 observed among treatments. HPP at 600 MPa at 20 and at 40 ºC resulted in samples with higher
401 ($p<0.001$) total phenol content compared to blanching.
402
403 With regards to blanching, total phenol levels in blanched samples were lower compared with
404 non-treated samples, however the losses were not significant. Previously published studies on the
405 effect of blanching on the total phenol content of *brassica* vegetables reported significant losses
406 in total phenol content following blanching of cauliflower (Volden et al. 2009), red cabbage
407 (Volden et al. 2008) and swede (Clariana et al. 2011). Total phenol loss following blanching can
408 be attributed to thermal degradation and leaching into the cooking water (Rungapamestry et al.
409 2007) as endogenous oxidative enzymes are typically inactivated at these conditions.
410
411 Among pressurised samples, no interaction between the pressure level and the pressurisation
412 temperature was observed for total phenol content; therefore these interactions were dropped
413 from the models. Pressurisation of white cabbage at 600 MPa resulted in higher levels of total
414 phenols ($p<0.01$) than HPP at 200 and 400 MPa (Table 2). The results obtained in the present

study are in agreement with previous studies which also found an increase of total phenol values in swede roots treated at 600 MPa (Clariana et al. 2011) and strawberry purees (Patras et al. 2009) in comparison with lower pressure treatments. The observed effect on total phenol values after processing at 600 MPa has been attributed to a possible increased extractability of polyphenol compounds at this pressure level (Patras et al. 2009; Clariana et al. 2011).

3.6 Total antioxidant capacity

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical was used to measure the antioxidant capacity of the sample extracts and the results were expressed as Trolox equivalent antioxidant capacity values (TEAC). Significant reductions in total antioxidant capacity of the white cabbage extracts were detected after all studied treatments, except for HPP at 600 MPa (20 ºC and 40 ºC) (Table 3). HPP at 600 MPa and 20 ºC resulted in samples with higher antioxidant capacity ($p<0.001$) than blanched samples. HPP at 200 and 400 MPa induced the strongest reduction of total antioxidant capacity.

When considering only high pressure processed samples, no significant interaction between pressure and temperature was observed for total antioxidant capacity, as measured by the DPPH method, therefore interaction was dropped from the model. No significant effect of the pressurisation temperature was detected on total antioxidant capacity. However, a pressure effect was detected and pressurising at 600 MPa resulted in samples with higher ($p<0.001$) total antioxidant capacity in comparison with pressurising at 200 or 400 MPa (Table 2).

Similar results have been previously reported for swede roots (Clariana et al. 2011), green beans and carrots (McInerney et al. 2007), and blackberry purees (Patras et al. 2009). No correlation

438 was obtained in the present study between antioxidant capacity and total phenol content.
439 However, a strong correlation ($p<0.001$) was observed between ascorbic acid and antioxidant
440 capacity ($r = 0.982$), thus indicating that the observed changes in TEAC values might be mainly
441 attributed to changes in ascorbic acid levels.
442
443 *3.7 Total isothiocyanate content of white cabbage*
444 Isothiocyanates are not present as such in *brassica* vegetables. The precursors of isothiocyanates
445 are a type of secondary metabolites named glucosinolate compounds (β-D-thioglucose linked to
446 a sulfonated oxime and a variable side chain). The formation of isothiocyantes from
447 glucosinolate precursors is mediated by an endogenous thioglucosidase enzyme named
448 myrosinase (EC 3.2.3.1)**.** Although in intact plant tissues glucosinolates are physically separated
449 from myrosinases, upon tissue disruption, such as cutting, or mastication, glucosinolate
450 compounds become in contact with the endogenous enzyme myrosinase (EC 3.2.3.1), which in
451 turn hydrolyses glucosinolates to form isothiocyanates (Bones and Rossiter 2006). Processing
452 may affect the levels of total isothiocyanates in a number of ways such as by inducing changes in
453 glucosinolate levels and/or myrosinase activity.
454
455 In blanched white cabbage samples, the total isothiocyanate content measured was very low
456 (0.41 ± 0.04 μmol sulforaphane equivalent/g dry weight sample) and was not significantly
457 different from that of untreated white cabbage (Table 3). This was to be expected since
458 blanching may result in thermal inactivation of the enzyme myrosinase, thus resulting in limited
459 or no conversion of glucosinolates into health beneficial isothiocyanates (Matusheski et al. 2004;
460 Matusheski et al. 2006). On the other hand, pressurisation induced an increase in total

461 isothiocyanate content, although the increases were only significant for HPP at 400 and 600 MPa
462 (Table 3). Cell permeability has been observed to occur during high pressure processing and
463 permeabilisation rates have been found to increase with increasing pressure (Van Eylen et al.
464 2009). Pressure induced changes in cell permeability may in turn increase isothiocyanate levels
465 by facilitating contact between enzyme and substrate. In the present study, total isothiocyanate
466 content showed a positive correlation with Kramer shear values ($r = 0.69$, $p<0.0001$), thus
467 suggesting that the effect of HPP on levels of total isothiocyanate might be partly explained by
468 HPP induced changes in texture. Previous studies by other authors also attributed the beneficial
469 effect on isothiocyanate levels to increased cell permeabilisation following HPP (Van Eylen et
470 al. 2008b, 2009).
471
472 Among pressurised samples, no interaction between the pressure level and the pressurisation
473 temperature was observed. HPP at 400 and 600 MPa resulted in significantly higher levels of
474 total isothiocyanates in white cabbage compared to 200 MPa independently on the pressurisation
475 temperature (Table 2). These results suggest that the enzyme myrosinase remained active during
476 the combinations of pressure/temperature under study. In this sense, myrosinase has been
477 reported to be pressure resistant in buffer systems, broccoli juices and broccoli heads, under
478 several combinations of pressure and temperature treatments (Van Eylen et al. 2007, 2008a,
479 2009). It thus appears that pressure-induced cell damage rather than myrosinase activity may
480 represent a key factor in the conversion of glucosinolates into isothiocyanates by myrosinase
481 during HPP.
482

These results indicate that this processing technique can be employed as a food processing and/or preservation technique to increase the levels of some of its key phytochemicals such as isothiocyanates.

4. Conclusions

From the experimental results obtained in the present study it can be concluded that the use of high pressure processing may induce significant changes in the quality of white cabbage. However, while colour and texture were better preserved by blanching, HPP treatments such as 600 MPa resulted in significantly higher levels of antioxidant activity and important phytochemicals such as total phenols and total isothiocyanates, while obtaining the same levels of ascorbic acid than blanching. In summary, HPP may represent an attractive technology to process vegetable-based food products that better maintains important aspects related to the content in health-promoting compounds. This may be of particular relevance to the food industry sector involved in the development of convenient novel food products with excellent functional properties.

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648

649

Table 1

Instrumental colour analysis of non-treated, blanched and high pressure processed white cabbage

	Non-treated	Blanching	200 MPa		400 MPa		600 MPa		P [1)]
			20 ºC	40 ºC	20 ºC	40 ºC	20 ºC	40 ºC	
L* [2)]	73.99 ± 1.00[a]	69.02 ± 1.05[ab]	63.64 ± 2.62[bc]	64.81 ±2.03[bc]	60.56 ± 4.41[c]	60.78 ± 1.63[c]	62.26 ± 1.48[c]	63.15 ± 2.00[bc]	<0.001
ΔE [3)]	-	5.14 ± 0.95[b]	10.40 ± 2.57[ab]	9.22 ± 2.06[ab]	13.5 ± 4.38[a]	13.35 ± 1.82[a]	11.75 ± 1.47[ab]	10.86 ± 2.00[ab]	<0.05
hue° [4)]	101.15 ± 0.68[abc]	102.03 ± 0.23[abc]	102.91 ± 0.50[ab]	103.55 ± 2.47[a]	99.42 ± 0.39[c]	100.24 ± 0.64[bc]	101.86 ± 0.75[abc]	103.55 ± 0.55[a]	<0.0001

Only colour parameters showing significant differences among treatments are shown. Results represent least square means of three replicates ± standard deviation. Different letters within a row are used to indicate significant differences among treatments.

[1)]Significance

[2)]Lightness

[3)]Total colour difference

[4)]Hue angle

Table 2

Quality parameters of high pressure processed white cabbage

	200 MPa	400 MPa	600 MPa	P [1)]
a* [2)]	-3.49 ± 0.56[b]	-2.65 ± 0.38[a]	-3.40 ± 0.25[b]	<0.01
hue[o] [3)]	103.23 ± 1.63[a]	99.83 ± 0.65[b]	102.7 ± 1.1[a]	<0.001
Total phenols [4)]	325.06 ± 33.19[b]	330.15 ± 26.22[b]	393.01 ± 18.41[a]	<0.01
TEAC [5)]	91.5 ± 17.04[b]	80.36 ± 4.98[b]	313.09 ± 26.0[a]	<0.0001
TICN [6)]	1.86 ± 0.58[b]	5.14 ± 0.26[a]	5.10 ± 0.39[a]	<0.0001

Only parameters with non-significant interaction between the pressure level and the pressurisation temperature are shown. Results represent least square means of six replicates ± standard deviation. Different letters within a row are used to indicate significant differences among treatments.

[1)]Significance

[2)]Redness

[3)]Hue angle

[4)]Total phenol content expressed as mg Gallic acid equivalent/100 g dry weight sample

[5)]Total antioxidant capacity expressed as Trolox equivalent antioxidant capacity and calculated as TEAC = (IC_{50}Trolox/ IC_{50}Sample) × 10^5

[6)]Total isothiocyanate content expressed as µmol Sulforaphane equivalent/g dry weight sample

Table 3

Total phenol content, antioxidant capacity and total isothiocyanate content of non-treated, blanched and high pressure processed white cabbage

	Non-treated	Blanching	200 MPa		400 MPa		600 MPa		P[1)]
			20 ºC	40 ºC	20 ºC	40 ºC	20 ºC	40 ºC	
Total phenols[2)]	338.27 ± 17.93^{abc}	282.47 ± 7.14^{c}	310.04 ± 43.26^{c}	340.09 ± 14.31^{abc}	319.32 ± 34.84^{bc}	340.97 ± 12.40^{abc}	384.55 ± 18.57^{ab}	401.47 ± 16.97^{a}	<0.001
TEAC[3)]	354.16 ± 38.06^{a}	258.39 ± 17.1^{b}	82.63 ± 10.37^{c}	100.37 ± 19.56^{c}	80.95 ± 6.63^{c}	79.77 ± 4.11^{c}	322.42 ± 13.80^{a}	303.76 ± 35.18^{ab}	<0.001
TICN[4)]	0.76 ± 0.03^{bc}	0.41 ± 0.04^{c}	2.34 ± 0.31^{b}	1.38 ± 0.23^{bc}	5.18 ± 0.08^{a}	5.11 ± 0.4^{a}	5.07 ± 0.49^{a}	5.12 ± 0.38^{a}	<0.0001

Results represent least square means of three replicates ± standard deviation. Different letters within a row are used to indicate significant differences among treatments.

[1)]Significance

[2)]Total phenol content expressed as mg Gallic acid equivalent/100 g dry weight sample

[3)]Total antioxidant capacity expressed as Trolox equivalent antioxidant capacity and calculated as TEAC = (IC_{50}Trolox/ IC_{50}Sample) × 10^5.

[4)]Total isothiocyanate content expressed as µmol Sulforaphane equivalent/g dry weight sample

Fig 1. Kramer shear values (N/g) of non-treated, blanched and high-pressure-processed white cabbage. Results represent least square means of three replicates. Error bars represent standard deviations. Different letters indicate significant differences among treatments ($p<0.001$).

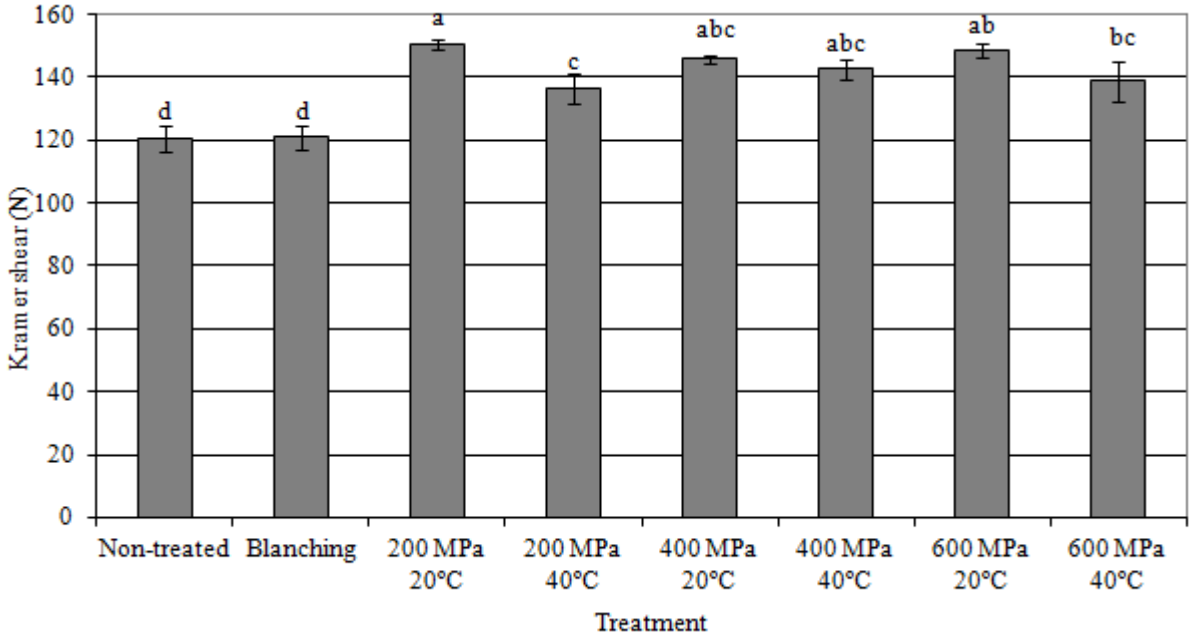

Fig 2. Ascorbic acid content of non-treated, blanched and high-pressure-processed white cabbage. Results represent least square means of three replicates. Error bars represent standard deviations. Different letters indicate significant differences among treatments ($p<0.001$).