High pressure processing of swede (*Brassica napus*): Impact on quality properties

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ABSTRACT

The effects of combined pressure/ temperature treatments (200, 400 and 600 MPa, at 20 and 40 ºC) on the physical and nutritional properties of swede roots (*Brassica napus* var. *napobrassica*) were assessed. Changes induced by high pressure processing (HPP) on the original properties of swede samples were compared with those produced by thermal treatment (blanching). All studied treatments altered the physical properties of swede, resulting in a loss of hardness and water binding capacity. The strongest alteration of texture was observed after HPP at 400 MPa, while 600MPa was the treatment that better preserved the texture properties of swede. Blanching caused less total colour changes (ΔE) than HPP. Antioxidant properties of swede were measured as total antioxidant capacity, ascorbic acid and total phenol content. All treatments caused a loss of antioxidant capacity, which was less pronounced after HPP at 600 MPa and 20 ºC and blanching. Four glucosinolates were detected in swede roots, glucoraphanin, progoitrin, glucobrassicanapin and glucobrassicin. Glucobrassicanapin and glucobrassicin contents were reduced with all studied treatments. Progoitrin content was not affected by blanching and HPP at 200 MPa. HPP at higher pressure levels (400 and 600 MPa), though, induced an increase of progoitrin levels. The results indicated that blanching and HPP at 600 MPa and 20ºC were the treatments that better preserved the original quality properties of swede.

Keywords: High pressure processing; Swede; Texture; Antioxidants; Glucosinolates.

1. Introduction

Over the last years there has been an increase in consumer demand for minimally processed foods, more similar to fresh products, without the presence of additives. At the same time and due to new consumption habits, there is an increase in consumption of ready to eat products. One of the main problems in manufacturing healthy ready to eat products, such as fresh-cut vegetables is their short shelf life. Therefore, extension of food shelf life using mild processing technologies that minimally affect the sensory and texture of the products is a challenge for the food industry.

Traditionally, foods of vegetable origin are submitted to a thermal treatment (water blanching) to reduce the microbial load and inactivate deleterious enzymes responsible of quality deterioration during storage (Cano, Hernandez, & Ancos, 1997; Lee & Coates, 1999). However, heat treatment has some detrimental effects on the texture, sensory and nutritional value of vegetables (Podsedek, 2007; Roy, Takenaka, Isobe, & Tsushida, 2007). In the last years, the application of high pressure processing (HPP) on products of vegetable origin as an alternative to blanching processing to inactivate microorganisms and enzymes responsible of food deterioration with minimal alteration of quality has been extensible studied (Castro et al., 2008; Eshtiaghi & Knorr, 1993; Hendrickx, Ludikhuyze, Van den Broeck, & Weemaes, 1998). HPP mainly affects non-covalent bonds, allowing a better preservation of micronutrients such as hydrophilic vitamins, pigments and flavour (Oey, Van der Plancken, Van Loey, & Hendrickx, 2008). The effects of HPP on the colour and texture is dependent on the type of vegetable (Oey, Lille, Loey, & Hendrickx, 2008).

Swede (*Brassica napus* var. *napobrassica*) commonly known as rutabaga in America, and turnip in Ireland, is a vegetable from the *Brassicaceae* family (Grubben & Denton, 2004). Early, small swedes can be shredded and served raw. Larger specimens can be cut into pieces and consumed in soups or as a side dish in the roast. Swede is known to have a high content of beneficial health compounds including phenolic compounds, glucosinolates and vitamin C (Paul & Southgate, 1978; Podsedek, 2007). Some of these compounds can be lost during the thermal treatment, depending on the processing conditions (Podsedek, 2007; Roy et al., 2007). HPP has been assessed as a mild technology to process other vegetables from the *Brassica* family such as broccoli and cauliflower (Prestamo & Arroyo, 1998; Van Loey et al., 1998). HPP have shown

minimal effects on the pigments and antioxidant capacity (Oey, Van der Plancken, et al., 2008; Oey, Lille, et al., 2008). Moreover, HPP proved to provide beneficial health benefits by inducing hydrolysation of glucosinolates (Van Eylen et al., 2009). However, HPP can also have a detrimental impact on the quality of vegetables. Therefore, it is essential for consumer acceptance to assess the effects of HPP on the quality. The objective of the present study was to investigate the effect of combined pressure/temperature treatments on the texture, colour, antioxidant properties and glucosinolate profile taking as a reference a non-treated swede and swede processed with a traditional thermal treatment (blanching).

2. Material and methods

2.1 Chemicals

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2, 5, 7, 8- tetramethylchromane-2-carboxylic acid (Trolox), pyrogallol, Folin-Ciocalteu reagent (2 N), sodium carbonate, gallic acid, tetradecylammonium bromide (TDAB), potassium phosphate monobasic (KH_2PO_4), phosphoric acid (H_3PO_4), L-ascorbic acid and silica gel were obtained from Sigma Aldrich (St. Louis, USA). Diatomaceous earth was obtained from Dionex (Idstein, Germany). Metaphosporic acid and solvents HPLC grade: methanol, acetonitrile and water were purchased from BDH Ltd. (Poole, UK).

2.2 Sample preparation

Swede roots (*B. napus* var. *napobrassica*) were purchased from a local Irish distributor. Inedible parts were removed with a sharp knife, obtaining a central cube that was further cut into two slices of approximately 15 mm. Swede cylinders were obtained by punching each slice with a cork borer.

2.3. High pressure processing (HPP) and thermal treatment

Swede cylinders were double vacuum packed in 250g portions into high oxygen barrier pouches (Versatile Packaging Ltd, Monaghan, Ireland). Packed samples were placed in a 1 litre high pressure unit with an internal size of 100 mm diameter× 254 mm height (Pressure Engineered System, Temse, Belgium). The pressurisation fluid was a mixture of water and rust inhibitor (Dowcal N, 60% v/v). The samples were subjected to

pressures of 200, 400 and 600 MPa for 5 min at two temperature levels 20 °C and 40 °C.

Swede samples for the thermal treatment (blanching) were placed in drilled bags to allow contact with hot water, and immersed in a water bath at 90 °C for 3 min. After blanching, samples were immersed in cold water to promote rapid cooling. Each treatment was repeated three times. Non-treated (NT) samples were kept as control.

Physical measurements were performed on the same day of treatment. Samples for chemical analysis were frozen at -20°C 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. Three samples of each treatment were used for all analysis.

2.4. Texture analysis

Texture measurements were performed with a TAXT2i texture analyzer equipped with a 250 N cell (Stable Micro Systems, Surrey, England). Parameters for texture analysis were set according to Trejo-Araya et al. (2009) with some modifications.

A compression test was performed on swede cylinders of 14×15 mm (diameter × length) placed in vertical position using a 20 mm perspex cylindrical probe. Hardness was measured as the peak force (N) delivering 30% strain at a compression rate of 1 mm/s. A cutting test was performed on 14 mm diameter swede cylinders placed in horizontal position using a stainless steel blade. The test was performed at a penetration rate of 3 mm/s and 75% strain. Results for cutting test were expressed as peak force (N) and distance of displacement produced at maximum cutting force (mm). Ten cylinders per sample were analysed for each test.

2.5. Expressible moisture

Expressible moisture (EM) of swede roots cylinders was determined according with Trejo-Araya et al. (2009). Swede cylinders of 11×11 mm (diameter × length) were used to measure EM. The surface of each sample was dried using tissue paper and weighed before analysis. Filter papers (Whatman No. 1, 55 mm diameter) employed to absorb the released water were also weighed. EM was measured with a TA-XT2i texture analyser by compressing a cylinder placed in vertical position between two filter papers. The test was performed at a compression rate of 1 mm/s and 70% strain. After compression, filter papers were weighed immediately. EM was measured as the weight

difference of the filter papers before and after compression divided by the original sample weight. Five cylinders per sample were analysed.

2.6. Colour measurements

Swede colour was measured with a HunterLab spectrophotometer (Ultrascan XE, Hunter Associates Laboratory, Inc., Reston, VA) with a D65 illuminant and 10° standard observer angle. Colour coordinates were determined using the 1976 CIELAB system and the results were expressed as L* (lightness), a* (redness) and b* (yellowness). The instrument was calibrated before each series of measurements using white (L* = 100) and black (L* = 0) standard tiles. A numerical total colour difference (ΔE) was calculated as suggested by Jung Ghoul & de Lamballerie-Anton (2003):

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

The colour values of non-treated samples (L_0^*, a_0^*, and b_0^*) were used as reference values for ΔE calculation. Three colour measurements per sample were taken.

2.7. Sample extraction

Lyophilised samples were milled to a fine powder using a blender (BL440001, Kenwood limited, Hampshire, U.K.) and extracted by pressurised liquid extraction (PLE) using an Accelerated Solvent Extractor (ASE 200, Dionex, Idsteinn, Germany) equipped with a solvent controller. Cellulose filters (Dionex, Idsteinn, Germany) were inserted at top and bottom of 22 ml extraction cells. One gramme of sample powder mixed with 4 g of silica (Merck grade, 60Å, sigma Aldrich, St. Louis, USA) was packed on top of 0.4 g of diatomaceous earth into each cell. PLE variables were set according to Mohn, Cutting, Ernst & Hamburger (2007); preheat time: 1 min; static extraction per cycle: 5 min; flush: 100 % of cell volume; purge: 80 s with nitrogen; pressure: 120 bar , temperature: 50 °C, extraction time: 3 × 5 min cycles; 70% methanol in water was used as extraction solvent. After collection in 60 ml vials the extracts were filtered through PTFE syringe filters (pore size 0.45 µm, Sigma Aldrich, St. Louis, USA) and stored at -80 °C until analysis. Three extracts of each sample were obtained for further analysis.

2.8. Ascorbic acid analysis

Ascorbic acid content in root swedes was determined as was described by Tiwari, O'Donnell, Patras & Cullen (2008). Extracts were obtained by dissolving 0.15 g of freeze dried powder in 10 ml of 6% metaphosphoric acid. After vortexing

mixture was centrifuged at 2,000g for 10 min at 4 °C (Sanyo MSE Mistral 3000ii, Sanyo). Five millilitres of the supernatant was filtered through PTFE syringe filters (pore size 0.45 µm, Sigma Aldrich, St. Louis, USA) and placed in an autosampler vial. The chromatographic system was composed by a Waters (Milford, MA, USA) 600s controller, a Waters 717plus autosampler, a Waters 616 pump equipped with a hypersil ODS guard column (Gemini C18, Phenomenex., UK), and an hypersil ODS column (15 cm× 4.6 cm, 5 µm, Supelco, US). Elution took place at 40 °C with 25 mM KH_2PO_4 adjusted to pH 3 with H_3PO_4, at a flow rate of 1 ml/min. The eluate was monitored with a Waters 486 turnable absorbance detector (Milford, MA, USA) set at 245 nm. Millennium 32 software from Waters (Milford, MA, USA) was used for peak integration. A calibration curve of ascorbic acid (25-500 µg/ml) in metaphosphoric acid (6%) was used for quantification. Each extract was analysed in triplicate and average was employed for calculation.

2.9. Total phenol analysis

Total phenolic content of swede root was assessed using a modified version of the Folin–Ciocalteu assay (Singleton, Orthofer, & Lamuela-Raventos, 1999). 100 µl of PLE extract or gallic acid standard, 100 µl of methanol, 100 µl of Folin-Ciocalteu reagent and 700 µl of Na_2CO_3 were mixed in 1.5 ml centrifuge tubes. Vortexed samples were left in the dark for 20 min at room temperature. After centrifugation at 13,000 rpm for 3 min the absorbance of supernatant was measured at 735 nm using a spectrophotometer (UV- 1700 Pharma Spec, Shimadzu, Japan). Total phenol concentration was calculated using a standard calibration curve with gallic acid (10-400 mg/l), and expressed as mg gallic acid equivalent/100g dry weight (mg GAE/100g DW). Each extract was analysed in triplicate and average was employed for calculation.

2.10. Total antioxidant capacity

Total antioxidant capacity was measured using the DPPH assay described by Wijngaard, Rossle & Brunton (2009). At first serial dilutions of swede PLE extracts with methanol were prepared. Analysis was realised by adding 500 µl of diluted extracts to 500 µl of DPPH working solution (0.048 mg/ml), and after vortexing, the samples were left in the dark for 30 min at room temperature. Absorbance was measured against methanol at 515 nm using a spectrophotometer (UV- 1700 Pharma Spec, Shimadzu,

Milton Keynes). Antioxidant capacity was referred to a synthetic antioxidant, Trolox, and expressed as Trolox equivalent antioxidant capacity value (TEAC), using the formula TEAC = ($IC50_{Trolox}/IC50_{Sample}$) × 10^5 where IC_{50} is the concentration of sample extract needed to obtain a depletion of 50% in the original absorbance of DPPH. Each extract was analysed in triplicate and average was employed for calculation.

2.11. Glucosinolate analysis

Two millilitres of PLE extract was evaporated to dryness under nitrogen and redissolved in 0.5 ml of 70% methanol in water. Concentrated extract was filtered through hydrophilic polyethersulfone membrane (Millex MP, pore size 0.22 µm, Millipore, Massachusetts, US), and analysed according to Prestera et al. (1996). Glucosinolates were analysed with paired-ion chromatography (Agilent series 1100 HPLC) using a µ-Bondapak C18 reverse phase column (3:9 x 300 mm) connected to a µ-Bondapak C18 Guard-pak (Waters, Melford, MA, USA) set at 3 ml/min and monitoring at 235 and 245 nm. The mobile phase employed was 0.005 M TDAB dissolved in acetonitrile/water (1:1). Sinigrin was employed as external standard, a calibration curve of sinigrin was made (0.016 - 1 mg/ml). The quantification of each glucosinolate was estimated as suggested by Fahey, Zhang & Talalay (1997) and Shapiro et al. (2001). Each extract was analysed in triplicate and average was employed for calculation.

2.12. Data analysis

All statistical analyses were performed using the SAS Enterprise Guide version 4 (Statistical Analytical Systems Institute, Cary, NC, USA). Two different models were applied. The first model included treatment (NT, blanching, 200 MPa at 20ºC, 200MPa at 40ºC, 400 MPa at 20ºC, 400MPa at 40ºC, 600 MPa at 20ºC, and 600MPa at 40ºC) as a fix effect. The second model only considered pressurised samples, and included temperature, pressure and temperature × pressure interaction as fixed effects. No significant interactions ($p > 0.05$) were dropped from the model. Differences were assessed by the Tukey test ($p < 0.05$). Pearson correlation analysis was used to investigate the relationship among the studied parameters.

3. Results and discussion

3.1. Texture

Food processing is known to critically affect the texture of vegetables. Processing of vegetables brings on mechanical damage that leads to turgor loss and induces alteration of pectin structure and function caused by both enzymatic and chemical processes, such as ß-elimination (Buggenhout, Sila, Duvetter, Loey, & Hendrickx, 2009; Greve, McArdle, Gohlke, & Labavitch, 1994). The impact of processing on swede texture was assessed using a compression and a cutting test. The compression test gives a measure of hardness related to the force needed to compress a tissue. After processing, the hardness of swede decreased ($p<0.001$) with all treatments assayed (Fig. 1). Pressurisation at 200 MPa and 40ºC allowed better hardness retention than all other treatments but 600 MPa and 40 ºC. Blanching and HPP at 200 and 600 MPa and 20 ºC showed similar hardness values ($p>0.05$). Finally, the softest texture was achieved at 400 MPa. Softening of vegetables induced by heat and pressure treatments seems to follow different patterns. Texture changes in blanched vegetables are not associated with enzyme activity, as most vegetable enzymes are inactivated at the conditions used for blanching. The initial loss of hardness induced by thermal processes such as blanching is related with loss of turgor due to membrane disruption (Greve et al., 1994). However, the main contributing factor to tissue softening during thermal processing (Sila, Smout, Elliot, Loey, & Hendrickx, 2006; Vu, Smout, Sila, Loey, & Hendrickx, 2006) is the reduction of adhesion between cells due to the solubilisation of pectin by ß-elimination reaction. Similar texture losses than those obtained in the present study were reported by Moreira, Oliveira, Oliveira & Singh (1994) after thermal treatment of turnip (*Brassica rapa*).

Differently, high pressure processing results in minimal pectin solubilisation (Oey, Lille, et al., 2008). Textural changes in vegetables induced by HPP are mainly caused by mechanical damage and changes on enzyme activity. The results of the compression test suggest that the degree of cell disruption was dependent on the applied level of pressure. These results are in agreement with previous observations reporting strong firmness loss after HPP in a range of 100-400 MPa of different vegetables (Basak & Ramaswamy, 1998). Trejo-Araya et al. (2007) reported no further reduction of hardness in carrots above 300 MPa. The authors suggested that above a certain pressure threshold (which will be product structure dependent) tissue might not further compress or be

disrupted. The reported results, showing a decrease of hardness with pressures up to 400 MPa (Fig. 1) suggest that the compression threshold for swede would be higher than in the case of carrots. After processing at 600 MPa, higher values of hardness were observed compared to 400 MPa. Similarly, Tangwongchai, Ledward, and Ames (2000) also reported less apparent damage in tomato at pressure levels above 400 MPa. The authors reported inactivation of polygalacturonase (PG) at 500 MPa and above. Inactivation of enzymes related to softening of vegetables such as PG would explain the apparent texture recovery at 600 MPa.

Changes on texture were also measured with the cutting test. The cutting test can give information on other parameters such as the resistance of a tissue to fracture, determined by the maximum force during the cutting test, and tissue elasticity, measured by the increase of both cutting force and displacement (Trejo-Araya et al., 2007). The results of the cutting test are shown in Fig. 2. HPP at 600 MPa presented similar ($p>0.05$) values of cutting force and displacement than non-treated (NT) samples (Fig. 2), suggesting that this pressure level would not alter the cutting properties of swede. HPP at 400 MPa induced the highest increase in cutting force and displacement values. On the contrary, blanching and HPP at 200 MPa induced a reduction of cutting force compared to NT samples. Higher values of cutting force have been related with more deformable materials, with less cell integrity and hence more rubbery-like texture, as part of the cutting force is used to deform the material (Dowgiallo, 2005; Trejo-Araya et al., 2007).

From the reported results it can be extracted that HPP at 400 MPa would have induced the greater textural changes in swede, resulting in a softer and more rubbery-like texture. On the contrary, HPP at 600 MPa proved to be the treatment that better preserved the texture of swede. In agreement to our results, other authors have reported less alteration of texture after processing carrots at 600 MPa compared to a mild heat treatment (Trejo-Araya et al., 2009).

On the other hand, a significant effect of the pressurisation temperature on swede texture was observed at 200 and 400 MPa (Fig. 1). Swede samples treated at 40ºC were harder than those treated at 20ºC. These results would suggest greater enzyme inactivation at higher pressurisation temperature. In agreement with this, Fachin, Smout, Verlent, Ly-Nguyen, Van Loey, and Hendrickx (2004) reported higher inactivation of PG by increasing the pressurisation temperature in pressurised purified tomato PG

treated at fixed pressure. On the contrary, no differences were observed between both pressurisation temperatures at 600 MPa. This observation would be consistent with higher enzyme inactivation at higher pressure levels, independently on the pressurisation temperature. This result would be consistent with the observed inactivation of PG reported previously in diced tomato with HPP at 600 MPa/25 ºC during 3 min (Shook, Shellhammer, & Schwartz, 2001).

3.2. Expressible moisture

Expressible moisture (EM) of swede was measured as the water released from swede upon compression at 70% strain. EM would be a measure of the water binding capacity (WBC) of swede; a higher EM would indicate a lower WBC of the product. The EM of vegetables is related to the cellular structure, turgidity, integrity and cell wall strength (Trejo-Araya et al., 2009). Fig. 3 shows an increase of EM of swede ($p<0.001$) after all studied treatments, indicating a loss of WBC as a consequence of processing. Pressurisation at 600 MPa and 20 ºC was the treatment that altered the EM of swede to a lesser extent. Blanching exhibited higher EM than pressurised samples, with the exception of HPP at 400 MPa and 20 ºC. Other authors have reported increases in EM in blanched and pressurised vegetables (Trejo-Araya et al., 2009). A soaked appearance has been observed in pressurised vegetables (Prestamo & Arroyo, 1998; Tangwongchai et al., 2000). This phenomenon has been related to the disturbance of cell permeability, permitting transport of water from inside to outside of the cell (Prestamo & Arroyo, 1998). Results of EM of samples HPP at 20 ºC are in agreement with those of Prestamo and Arroyo (1998) and Tangwongchai et al. (2000), who reported higher water losses in a pressure range of 200-400 MPa than at 500-600 MPa.

Among pressurised samples, the lower pressures used (200-400 MPa) showed lower EM after processing at 40 ºC than at 20ºC. The better retention of water binding properties observed when processing at 40ºC compared to 20ºC at 200 and 400 MPa, suggest that HPP at higher temperature would induce less structural changes in swede. These results agree with those reported for hardness, where swede treated at 200 and 400 MPa showed less alteration of texture at 40ºC compared to 20ºC. Moreover, a significant negative correlation observed between EM values and the compression force ($p<0.0001$; $r= -0.86690$) would further confirm this relationship.

315 *3.3. Colour parameters*

316 Processing of swede (HPP and blanching) induced a significant reduction of most
317 colour coordinates (Table 1). The changes in L* (lightness) values were the most
318 pronounced. Table 1 shows lower total colour changes (ΔE) in blanched swede
319 compared to HPP. Among pressurised samples, no significant interaction between the
320 pressure level and the pressurisation temperature was observed (data not shown).
321 Therefore, interaction between pressure and temperature was dropped from the model
322 for colour values. Statistical analysis showed a significant effect of the pressure level
323 applied on colour coordinates (Table 2). HPP at 200 MPa showed the highest a*
324 (redness) and ΔE values and the lowest L* values among pressure treatments. On
325 contrary, no differences among pressure treatments ($p>0.05$) were observed for the b*
326 (yellowness) coordinate (data not shown).

327 The influence of HPP on the L* coordinate of vegetables can be partly related to
328 structural changes, since texture alterations may affect the extent of internally scattered
329 light and the distribution of surface reflectance (Oey, Lille, et al., 2008). Results for
330 compression force were positively correlated with L* ($p<0.001$; $r = 0.839$), while values
331 of displacement at maximum cutting force were negatively correlated with L* values
332 ($p<0.01$; $r = –0.608$). Confirming that pressure induced changes on the texture of swede
333 could have influenced colour changes to some extent. Other possible causes of colour
334 changes after processing of vegetables may be due to changes in the activity of enzymes
335 such as peroxidases (Burnette, 1977; Ueno, Hayashi, Shigematsu, & Fujii, 2009). In this
336 sense, swede roots are rich in peroxidases (Baardseth & Slinde, 1980). Eisenmenger and
337 Reyes-De-Corcuera (2009) reported activation of peroxidases in carrots HPP between
338 300 and 500 MPa. According to this, stronger alteration of swede colour (a* coordinate)
339 at 400 and 600 MPa could be related to activation of enzymes such as peroxidases. In
340 contrast, swede peroxidases have been reported to be inactivated by blanching
341 (Baardseth & Slinde, 1980). This fact would explain the better colour retention
342 observed in blanched swede compared to HPP. Ueno et al. (2009) reported the
343 formation of green-blue compounds in pressurised turnip (*B. rapa)* after one week of
344 storage. The authors related its formation either to the partial destruction of cellular
345 membrane structures by pressure or to the activity of enzymes such as peroxidases.

346

3.4. Ascorbic acid

Among root vegetables, swedes are known to be a valuable source of ascorbic acid (Paul & Southgate, 1978). However, ascorbic acid is a labile compound greatly affected during vegetable processing. All studied treatments induced a significant decrease in ascorbic acid (AA) content of swede (Table 3). Maximum retention of AA was observed after blanching and HPP at 600 MPa (ca. 81-67% retention). HPP at 200 MPa showed lower values, although not significant, of AA than pressurisation at 600 MPa. Swede samples pressurised at 400 MPa resulted in the lowest values in AA content (Table 3). No significant effect of the pressurisation temperature on the AA content of swede was observed ($p>0.05$).

AA is an antioxidant compound very soluble in water and with low thermal stability (Podsedek, 2007). Losses of vitamin C occur primarily by chemical degradation, which is speed at higher temperatures (Dewanto, Wu, Adom, & Liu, 2002). Therefore, losses of AA due to thermal degradation and leaching were expected in blanched swede. Similarly, previous studies have reported heat induced losses of AA in other species of *Brassica* (Olivera et al., 2008). The effect of HPP in AA content has also been reported previously, although sensitivity of AA towards pressure and temperature has proved to be dependent on the environment (Oey, Van der Plancken, et al., 2008). Our results are in agreement with other authors that observed more pronounced AA losses at 400 MPa than at 600 MPa in strawberry purées (15 min at 20 ºC) and in green peas (5 min at 33.5 - 42.5 ºC) (Patras, Brunton, Da Pieve, & Butler, 2009; Quaglia, Gravina, Paperi, & Paoletti, 1996).

Krebbers et al (2002) suggested that the breakdown of AA after HPP was mainly the result of chemical breakdown. Disruption of cell walls by HPP would release oxidative species and subsequently would increase diffusion and reaction rate of substrates. Strong correlations ($p<0.001$) between texture measurements and AA content were observe in both compression force ($r = 0.72673$) and displacement at maximum cutting force ($r = –0.810$), confirming the relationship between cell damage and AA content.

3.5. Total Phenol

Vegetables from the *Brassica* family are known to present strong antioxidant properties (Podsedek, 2007). Table 3 shows the total phenol (TP) content present in swede samples. Pressurisation at 600 MPa and 20 °C had no significant effect on TP values

compared to NT samples. All other treatments induced a significant reduction of TP content of swede. HPP at 200 MPa and 40 ºC, and 400 MPa at both temperatures resulted in the lowest values of TP.

The decrease of the TP content of vegetables due to thermal treatment is a well known phenomenon (Roy et al., 2007). It could be due either to leaching losses or to chemical degradation of phenols, being their losses dependent on both the vegetable studied and the intensity of the treatment (Roy et al., 2007) The effect of HPP on TP content have also shown very different responses depending on the type of vegetable and the pressure treatment applied (Oey, Van der Plancken, et al., 2008). Decrease in TP content has been reported in strawberries treated at 300-600 MPa and 20-60 ºC for 2-10 min (Terefe, Matthies, Simons, & Versteeg, 2009). Other studies have shown no TP differences in pressurised onions (400 MPa/ 5-50 ºC /30 min) (Roldan-Marin, Sanchez-Moreno, Lloria, de Ancos, & Cano, 2009), while HPP of strawberry purée (600 MPa/ 15 min/ 22 ºC) showed increased TP values (Patras et al., 2009). The decrease of phenolic compounds observed with pressure may be related to the enhancement of the chemical oxidation of polyphenols, since swede does not contain the polyphenol oxidase system (Boswell, 1950). As previously stated, cell disruption entailed by HPP would release substrates and promote changes in TP content, as shown by significant correlations ($p<0.001$) with both compression force ($r = 0.650$) and displacement at maximum cutting force ($r = –0.649$). Another contributory factor to changes on TP content may be related to changes on AA, since this molecule can react with the reactive Folin-Ciocalteu reagent (Prior, Wu, & Schaich, 2005). TP content showed a strong correlation ($p<0.001$) with AA content ($r = 0.752$). Therefore, the reported reduction of AA could have influenced the reduction of TP content to some extent. On the contrary, increase of TP content at 600 MPa has been related with increased extractability at higher pressures (Patras et al., 2009). According to this, the little effect on the TP content observed after processing at 600 MPa and 20 ºC may be due to increased extractability at this pressure level, which could have counterbalanced the losses of phenols caused by pressure induced oxidation.

3.6. Total antioxidant capacity

Table 3 shows results for the total antioxidant capacity of swede, measured as DPPH scavenging capacity and referred as Trolox equivalent antioxidant capacity value (TEAC). All treatments produced a significant decrease of antioxidant capacity of

swede, except HPP at 600 MPa and 20 ºC. This treatment together with blanching showed higher antioxidant capacity ($p<0.001$) than other pressure treatments. The loss in antioxidant capacity due to thermal treatment it is well known (Roy et al., 2007). This decrease, as previously stated, can be due to leaching losses or to the degradation of antioxidant compounds accelerated by high temperatures (Roy et al., 2007).

Among pressurised samples, no significant interaction between the pressure level and the pressurisation temperature was observed (data not shown). Therefore, interaction between pressure and temperature was dropped from the model for total antioxidant analysis. Statistical analysis revealed that the pressure level applied had a significant effect ($p<0.001$) on TEAC values of swede. HPP at 600 MPa showed the highest total antioxidant capacity among pressurised swede (Table 2). Pressurisation at 400 MPa proved to be most severe pressure treatment with regard to TEAC values. No studies about the effect of HPP on the antioxidant capacity of swede roots have been found. In agreement with our findings, McInerney, Seccafien, Stewart, and Bird (2007) and Patras et al. (2009) observed a decrease in antioxidant activity of carrots and strawberry purée, respectively, treated at 400 MPa, while no variations were reported at 600 MPa. However, HPP at 600 MPa of other vegetables and fruits (green beans and blackberry purée) induced an increase in antioxidant capacity (McInerney et al., 2007; Patras et al., 2009). According to that, the effect of high pressure on antioxidant capacity would depend not only on the pressurisation conditions but also on the type of vegetable studied.

Total antioxidant capacity showed a positive correlation ($p<0.001$) with both AA content ($r= 0.880$) and total phenol content ($r = 0.650$). This result suggests that changes in total antioxidant capacity during processing of swede were related to changes in both compounds with antioxidant properties and textural changes.

3.7. Glucosinolate content

Glucosinolates (GLS) are a group of secondary plant metabolites found in high concentrations in *Brassica* vegetables (Oerlemans, Barrett, Suades, Verkerk, & Dekker, 2006). Glucosinolates are of particular interest in food research because of their health benefits. The products derived from the hydrolysis of GLS have a potential anticarcinogenic effect and beneficial effects for the health (Fahey, Zalcmann, & Talalay, 2001). However, high concentrations of some GLS in vegetables, such as

444 progoitrin, can have toxic effects. Therefore, monitoring the behaviour of glucosinolates
445 after vegetable processing becomes essential (Van Eylen et al., 2009).

446 Four glucosinolates were found in sufficient amount for quantification in non-treated
447 swede (Table 3): glucoraphanin, progoitrin and glucobrassicanapin, that are aliphatic
448 GLS, and glucobrassicin, an indol GLS. Processing of swede caused no significant
449 effect in glucoraphanin content (Table 3). Likewise, progoitrin content was not affected
450 ($p>0.05$) by blanching and HPP at 200 MPa. HPP at higher pressure levels (400 and 600
451 MPa), though, induced an increase of progoitrin levels ($p<0.001$). It should be noted
452 that despite the increased presence of progoitrin, all samples analysed showed
453 progoitrin levels below the threshold that could involve a health risk to consumers
454 (Fahey et al., 2001; Oerlemans et al., 2006). Glucobrassicanapin content was reduced
455 ($p<0.001$) with all studied treatments. Similarly, processing of swede at all conditions
456 studied induced a significant reduction of glucobrassicin content. However, blanching
457 was the treatment that better preserved the content of this indol GLS (Table 3). Similar
458 to our results, Van Eylen et al. (2009) reported losses of glucobrassicanapin and
459 glucobrassicin glucosinolates after conventional treatments of broccoli heads (100 ºC).
460 The authors related the losses to thermal degradation of GLS or to leaching losses. Our
461 results confirm the different thermolability among the studied GLS. As previously
462 observed, progoitrin and glucoraphanin proved to be less thermolabile than
463 glucobrassicin and glucobrassicanapin (Oerlemans et al., 2006). These results suggest
464 that the observed decrease of GLS in blanched swede would be more likely due to
465 thermal degradation.

466 On the contrary, the decrease of GLS content with high pressure processing has been
467 attributed to their hydrolysis. A higher cell disruption favoured by HPP would allow
468 contact of enzyme myrosinase with GLS, resulting in their hydrolysis into health
469 beneficial compounds (Van Eylen et al., 2009). Therefore, the effect of HPP on GLS is
470 dependent on the changes induced on cell structure and on myrosinase activity (Van
471 Eylen et al., 2009). From the above mentioned, it derives that the reported decrease of
472 glucobrassicanapin and glucobrassicin in pressurised samples would be related to
473 enhanced hydrolysis of these glucosinolates by high pressure. Glucobrassicanapin and
474 glucobrassicin contents showed a positive correlation ($p<0.001$) with swede hardness
475 ($r= 0.766$ and $r = 0.744$, respectively), suggesting the relationship between higher cell
476 disruption and higher GLS hydrolysis.

The reported results would suggest the potential health benefit of HPP of swede by increasing hydrolysis of GLS. Further study on the hydrolysis compounds derived of HPP of swede would help to shed light on the exact mechanisms promoting pressure induced hydrolysis of GLS.

4. Conclusions

From the reported results, it can be concluded that both processing technologies (blanching and HPP) altered the physical and nutritional properties of swede. High pressure processing at 400 MPa induced the strongest alteration on swede quality. The results suggest that the strong structural modifications induced by HPP at 400 MPa would have played a role in the alteration of antioxidant properties of swede. In general, blanching (thermal treatment) and HPP at 600 and 20ºC were the treatments that better preserved swede quality traits. Therefore, this pressure treatment could be considered as a possible alternative to blanching for swede processing. Moreover, the possible hydrolysis of glucosinolates by high pressure, could promote beneficial health effects on pressurised swede. Further study on this direction would be necessary to confirm this hypothesis.

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Figure 1. Compression force of non-treated and processed (blanching and high pressure processing) swede roots. Different letters indicate significant differences among treatments ($p<0.001$).

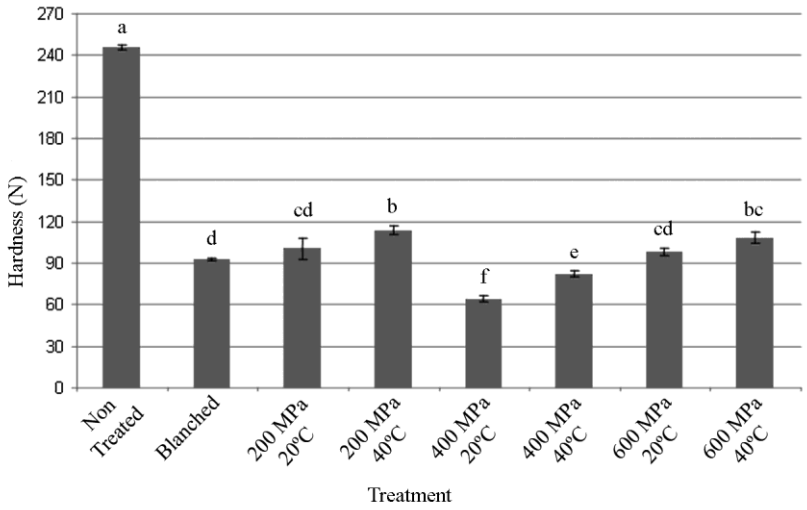

Figure 2. Cutting force and displacement at maximum cutting force of non-treated and processed (blanching and high pressure processing) swede roots. Bars with different letters indicate significant differences on cutting force among treatments ($p<0.001$). Upper points with different letters indicate significant differences on displacement values among treatments ($p<0.01$).

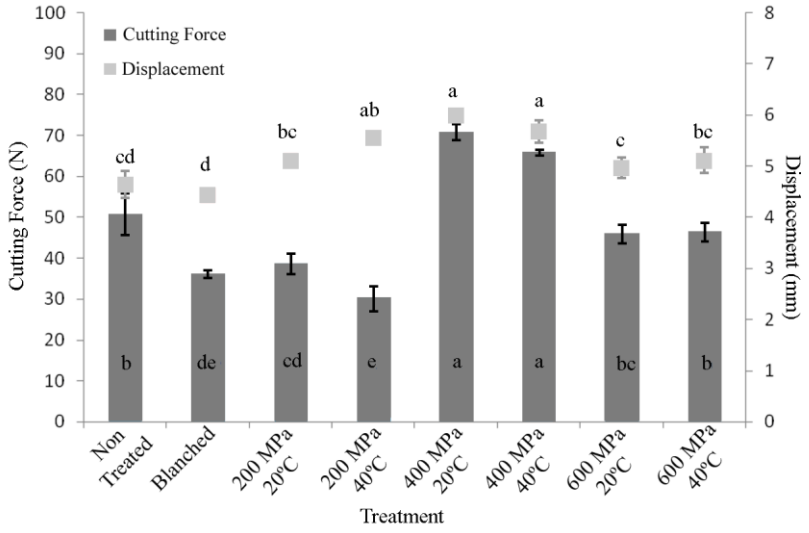

Figure 3: Expressible moisture of non-treated and processed (blanching and high pressure processing) swede roots. Bars with different letters are significantly different ($p<0.001$).

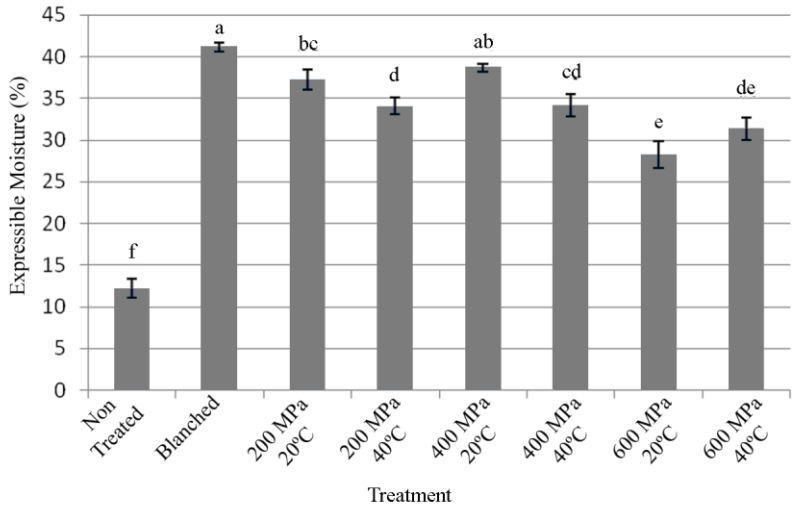

Table 1.
Instrumental colour parameters of non-treated and processed (blanching and high pressure processing) swede roots.

	Non-treated	Blanching	200 MPa		400 MPa		600MPa		SE [1]	p [2]
			20 °C	40 °C	20 °C	40 °C	20 °C	40 °C		
L*	66.59^{a}	44.96^{b}	26.32^{de}	25.86^{e}	28.77^{cd}	27.72^{cde}	29.51^{c}	26.50^{de}	0.52	<0.001
a*	3.86^{a}	2.74^{ab}	3.40^{ab}	2.73^{ab}	1.88^{b}	2.04^{b}	2.18^{ab}	2.34^{ab}	0.31	<0.01
b*	16.81^{a}	16.75^{a}	10.71^{b}	9.66^{b}	11.32^{b}	11.32^{b}	12.02^{b}	10.68^{c}	0.64	<0.001
ΔE	-	21.72^{d}	40.74^{ab}	41.37^{a}	38.29^{bc}	39.30^{abc}	37.44^{c}	40.59^{ab}	0.53	<0.001

Values are Least-square means (LS Means) of three replicates. Different letters within a row indicate significant differences among values.
[1] Standard error.
[2] Significance.

Table 2.
Effect of the pressure level on quality parameters of pressurised swede roots

	200 MPa	400 MPa	600 MPa	SE [1)]	p [2)]
L*	26.09[b]	28.25[a]	28.00[a]	0.34	<0.01
a*	3.07[a]	1.96[b]	2.26[b]	0.20	<0.01
Δ*E*	41.06[a]	38.79[b]	39.01[b]	0.38	<0.01
Total phenols [3)]	349,4[b]	353,6[b]	399,3[a]	11,2	<0.05
TEAC [4)]	78.8[b]	55.1[c]	143.6[a]	5.4	<0.001

Results are Least-square means (LS Means) of six replicates. Different letters within a row indicate significant differences among values.
[1)] Standard error.
[2)] Significance.
[3)] Expressed as mg gallic acid equivalent/100 g dry weight sample.
[4)] Expressed as trolox equivalent antioxidant capacity and measured as ($IC50_{Trolox}/IC50_s$) x 10^5.

Table 3.
Antioxidant indices and glucosinolate content of non-treated and processed (blanching and high pressure processing) swede roots.

	Non-treated	Blanching	200 MPa		400 MPa		600MPa		SE [1)]	p [2)]
			20 °C	40 °C	20 °C	40 °C	20 °C	40 °C		
Ascorbic acid [3)]	846.72^{a}	685.29^{b}	526.68^{c}	529.62^{c}	257.49^{d}	314.93^{d}	664.60^{bc}	568.15^{bc}	31.63	<0.001
Total Phenols [4)]	436.25^{a}	367.68^{cd}	384.89^{bcd}	325.99^{e}	319.02^{e}	347.63^{de}	419.96^{ab}	387.82^{bc}	7.69	<0.001
TEAC [5)]	215.18^{a}	157.03^{b}	76.30^{cd}	81.20^{cd}	51.76^{d}	58.45^{d}	182.91^{ab}	104.27^{c}	7.37	<0.001
Glucoraphanin [3)]	0.46^{ab}	0.43^{b}	0.50^{ab}	0.43^{b}	0.64^{a}	0.54^{ab}	0.58^{ab}	0.54^{ab}	0.04	<0.05
Progoitrin [3)]	0.33^{d}	0.37^{cd}	0.31^{d}	0.36^{d}	0.63^{a}	0.48^{bc}	0.53^{ab}	0.52^{b}	0.02	<0.001
Glucobrassicanapin [3)]	1.04^{a}	0.72^{b}	0.52^{bc}	0.56^{bc}	0.62^{bc}	0.42^{c}	0.53^{bc}	0.51^{bc}	0.05	<0.001
Glucobrassicin [3)]	0.68^{a}	0.55^{b}	0.41^{c}	0.36^{cd}	0.28^{d}	0.38^{cd}	0.39^{c}	0.36^{cd}	0.02	<0.001

Values are Least-square means (LS Means) of three replicates. Different letters within a row indicate significant differences among values.
[1)] Standard error.
[2)] Significance.
[3)] Expressed as mg /g dry weight sample.
[4)] Expressed as mg gallic acid equivalent/100 g dry weight sample.
[5)] Expressed as trolox equivalent antioxidant capacity and measured as ($IC50_{Trolox}/IC50_{s}$) x 10^{5}.