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Influence of pressure/temperature treatments on glucosinolate conversion in broccoli (*Brassica oleraceae* L. *cv Italica*) heads

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ABSTRACT

Glucosinolates are a group of secondary plant metabolites that are found in the Brassicaceae family. Upon hydrolysis by the endogenous enzyme myrosinase, a large number of compounds can be formed of which some are potentially anticarcinogenic, while others are largely inactive. Furthermore, some bioactive compounds are unstable. Therefore, it is not only important to determine the type and amount of glucosinolates present or hydrolysed in a given plant, but it is also relevant to investigate the type of hydrolysis products. In this research, the effect of combined pressure–temperature treatments (100–500 MPa, 20–40 °C) on the glucosinolate conversion and the kind of hydrolysis products was studied in broccoli, both during treatment and after autolysis. The results, showed that high pressure can induce glucosinolate hydrolysis during treatment, promote the formation of isothiocyanates after treatment and that relatively more indole oligomers are formed during treatment than during autolysis. These results indicate that pressure treatment limits the loss of glucosinolates and its health beneficial products.

1. Introduction

Glucosinolates are a group of secondary plant metabolites that are found in relatively high concentrations in the Brassicaceae family (Fahey, Zalcmann, & Talalay, 2001). Over 120 different glucosinolates are known, which all have a common basic structure comprising a β-D-thioglucose group, a sulphonated oxime moiety and a variable side chain derived from amino acids. According to this side chain, glucosinolates can be divided into an aliphatic, e.g. glucoraphanin; indole, e.g. glucobrassicin; and aromatic, e.g. glucotropaeolin, groups.

In all glucosinolate containing plants, myrosinase (EC 3.2.1.147) is also found. Upon tissue disruption, this enzyme hydrolyses glucosinolates releasing glucose and an unstable aglycon, that spontaneously rearranges to form different products depending on the reaction conditions, such as pH, presence of Fe^{2+} and ascorbic acid (Bones & Rossiter, 2006). In recent years, glucosinolates and especially their hydrolysis products received a lot of attention because of their potential anticarcinogenic effect (Conaway, Yang, & Chung, 2002; Das, Tyagi, & Kaur, 2000; Holst & Williamson, 2004). Some of these products – e.g. sulforaphane, an isothiocyanate that can be formed after hydrolysis of glucoraphanin, the major glucosinolate in broccoli – are considered to be very potent inducers of phase II detoxifying enzymes such as glutathione S-transferase, while phase I enzymes are reduced, such as cytochrome P450 enzymes (Zhang, Cho, Posner, & Talalay, 1992). In this way, the initial stage of the carcinogenic sequence associated with DNA damage is blocked and hence these substances are good blocking agents.

Apart from the health beneficial effect, isothiocyanates also have a bacteriocidal effect against food borne pathogens (Brandi, Amagliani, Schiavano, De Santi, & Sisti, 2006). However, not only sulforaphane, but also sulforaphane nitrile, a much weaker inducer of phase II enzymes (Matusheski & Jeffery, 2001), can be formed in broccoli upon glucoraphanin hydrolysis (Matusheski, Juvik, & Jeffery, 2004; Matusheski et al., 2006). An important factor in determining whether the nitrile or the isothiocyanate is formed, e.g. in broccoli (*Brassica oleracea* L. *cv Italica*), is the epithiospecifier protein which has been shown to be more thermolabile than myrosinase (Matusheski et al., 2004, 2006).

Besides formation, it is also important that the health related compounds are retained in the food until consumption. Isothiocyanates, are volatile and temperature labile (Jin, Wang, Rosen, & Ho, 1999; Van Eylen, Oey, Hendrickx, & Van Loey, 2007a) and can also react with amino acids (Bjergegaard, Moller, Sorensen, Sorensen, & Sorensen, 1999). Consequently, it is not only important to study the type of glucosinolates present and whether or not they are hydrolysed but it is also relevant to investigate the type of products present after glucosinolate hydrolysis.

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Besides aliphatic glucosinolates, broccoli also contains indole glucosinolates (Hansen, Moller, Sorensen, & Detrejo, 1995; Kushad et al., 1999; Vang et al., 2001). Although the formation of some indol-3-ylmethylglucosinolate hydrolysis products has been determined in model systems (Buskov, Olsen, Sorensen, & Sorensen, 2000), information about what happens in food products during or after processing is still limited. This is important since e.g. 3,3'-diindolylmethane may reduce the risk of cancer (Chen & Andreasson, 2001; Chen, McDougal, Wang, & Safe, 1998; Nachshon-Kedmi, Fares, & Yannai, 2004).

In order for myrosinase to hydrolyse the glucosinolates and form the health beneficial compounds, tissue disruption has to occur, for example by cutting or mastication. However, before consumption, vegetables are often heat treated which can result in myrosinase inactivation (Van Eylen, Indrawati, Hendrickx, & Van Loey, 2006; Van Eylen et al., 2007a), so that the health beneficial compounds are no longer formed. A heat treatment often has a negative impact on colour, texture and vitamin content. Therefore, alternatives such as a high pressure/temperature treatment, are developed that have less impact on quality attributes, but can still provide a microbial safe food product and permitting the inactivation of enzymes that cause product deterioration during storage (Butz & Tauscher, 2002; San Martin, Barbosa-Canovas, & Swanson, 2002).

In this research, high pressure/temperature processing conditions were chosen for which it has previously been shown that it should be possible to bring an active form of myrosinase into contact with the glucosinolates (Van Eylen, Oey, Hendrickx, & Van Loey, 2007b) and the impact of such processing on the content of broccoli glucosinolates, isothiocyanates and indole degradation products was investigated. Since myrosinase can remain active after treatment, also the autolysates (incubated homogenates) of the treated samples were investigated.

2. Materials and methods

2.1. Preparation and treatment of broccoli heads

One batch of broccoli (*B. oleracea* L. *var Italica* subvar. Monaco, Greenpartners, Belgium) was used for the treatments. For each treatment condition, 50 g of randomly mixed small broccoli head pieces (≈2 cm from top) were vacuum packed in polyethylene bags and heat sealed. Thermal treatments (30–100 °C) were performed in a water bath, with samples starting at room temperature.

Treatments at elevated pressure (100–500 MPa) were performed in a single vessel (590 ml) high pressure apparatus (EPSI, Temse, Belgium) with temperature control in the vessel mantle and automatic pressure build-up (16.7 MPa/s). The pressure transmitting fluid was ethylene glycol (60%, Dowcal, The Dow Chemical Co., Horgen, Switzerland). Before pressurisation, the vessel was first equilibrated at the desired temperature (20 or 40 °C). The adiabatic change in temperature during compression and decompression is about 3 °C/100 MPa. After compression, the temperature quickly decreases to achieve near isothermal–isobaric conditions after about 5 min (Weemaes et al., 1997). The adiabatic heating was partly compensated by loading the samples at an initial temperature of 4 °C. The treatment time was 15 or 35 min. Decompression was done instantaneously.

After treatments, samples were immediately frozen in liquid nitrogen and stored at −40 °C until freeze-dried (24 h). The freeze drying resulted in 89% loss of mass. The freeze-dried broccoli was ground to a fine powder and used for analysis.

2.2. Identification and quantification of the glucosinolates

Freeze-dried broccoli powder, (200 mg) was extracted (3 × 2 min) in a boiling 70% methanol solution (5 ml) with addition of two internal standards (glucotropaeolin and sinalbin; 1.4 μmol; from the laboratory collection (Denmark)). The sample was centrifuged for 3 min between each extraction. The collected supernatant was dried under a constant N_2 flow and re-dissolved in deionised water (5 ml).

An aliquot (1 ml) of this crude extract was applied to a DEAE Sephadex A-25 column (0.5 ml) and the unbound material was removed by washing with deionised water (2 × 1 ml) and sodium acetate buffer (2 × 0.5 ml; 20 mM, pH 5.0).

After washing, purified sulfatase (75 μl, Type H-1 from Helix pomatia, Sigma, MO, USA) was added and the columns were incubated overnight at room temperature. The sulfatase was purified by dissolving the sulfatase powder (70 mg) in deionised water (3 ml) and adding ethanol (3 ml). This solution was centrifuged (10 min, room temperature (RT)) and to the supernatant ethanol (9 ml) was added after which the solution was centrifuged (10 min, RT) again. The pellet was dissolved in deionised water (2 ml) and this sulfatase solution was subsequently passed through a 0.5 ml DEAE Sephadex A-25 and a 0.5 ml SP Sephadex C-25 column.

After overnight incubation, the desulphoglucosinolates (dGLS) were eluted from the columns with deionised water (3 × 1 ml). The collected eluent was dried under constant N_2 ow and redissolved in deionised water (200 μl) and centrifuged prior to analysis. Analyses were performed using a Hewlett-Packard HP3 D CE capillary electrophoresis system (Agilent, Waldbronn, Germany) equipped with diode array detector.

All separations were performed on a fused silica capillary (Agilent, Stevens Creek, CA; 75 μm ID, 64.5 cm total length, 56 cm effective length). Samples were injected from the anodic end of the capillary (vacuum injection, 40 mbar, 1 s). The separation buffer consisted of sodium cholate (250 mM) and boric acid (200 mM) at pH 8.5. The separation was carried out at 11 kV and 60 °C. The capillary was used between each run sequentially with 1.0 M NaOH (5 min), water (2 min) and separation buffer (5 min). Detection was performed oncolumn at 230 and 280 nm. Data processing was carried out by use of a HP Vectra 5/100 mHz Pentium with HP Chemstation V 6.01. The quantity of the dGLS was estimated as the average between the quantities calculated from the two internal standards, taking into account the relative response factors.

2.3. GC/MS determination of isothiocyanates (ITC) and sulforaphanenitrile

The concentration of isothiocyanates (ITC) and nitrile metabolites from the glucosinolates was determined by gas chromatography with mass spectrometer (GC/MS) (PolarisGCQ, ThermoFinnigan, Austin) based on the method described by Chiang, Pusateri, and Leitz (1998). Isothiocyanates were extracted with ethylacetate from 0.3 g of the powder of three untreated samples and of all the treated broccoli powder samples, as well as from the powder autolysates (3 ml of water added to 0.3 g powder and incubated for 1 h at RT).

For the broccoli powder samples, 3 extractions (5 ml ethylacetate) were carried out, while for the autolysates 5 extractions (9 ml ethylacetate) were performed by mixing the sample with the ethylacetate, centrifuging and collecting the ethylacetate phase. After extraction, the ethylacetate volume was reduced to 0.5 ml in a rotary evaporator.

An external standard curve was prepared from myrosinase converted glucoraphanin for quantification of sulforaphane. The GC column was a 30 m × 0.25 mm ID, 0.25 μm film thickness

Rtx-5MS (Restek, Bellefonte, PA, USA) with He carrier gas at constant flow of 1.0 ml min^{-1}. The temperature program was 40 °C (2 min), 10 °C min^{-1} to 270 °C (1.67 min), transfer line 275 °C, MS ion source 200 °C and scan range 50–200 m/z. Injector temperature was 200 °C in splitless mode, and the injection volume was 1 μl sample and 1 μl air with hot needle. The autosampler was programmed to do three solvent pre-washes of the syringe, three sample washes, three sample pull-ups and three solvent washes after injection. The limit of detection was approx. 40 μmol l^{-1} for the GC/MS analysis. 4-methylsulphinylbutyl ITC (sulforaphane) was quantified by selective ion monitoring (SIM) of the 160 m/z fragment and the external standard curve. For 3-methylsulphinylpropyl ITC (116 m/z), 4-methylsulphinylbutane nitrile (128 m/z), 3-butenyl ITC (72 m/z) and 2-propenyl-ITC (72 m/z) determined by GC/MS relative ratios were estimated in comparison to the sample treated at 30 °C after autolysis (1 h) of the lyophilised broccoli powders (=100%).

2.4. Identification of the degradation products of the indole glucosinolates

The type and amount of products formed upon degradation of indol-3-ylmethyl-glucosinolates were determined by the method described by Buskov et al. (2000). Broccoli powder (0.5 g) from three untreated samples, all treated samples and the autolysates (broccoli powder (0.5 g) incubated for 1 h with 5 ml water) were extracted with ethylacetate (3 × 5 ml). The sample was centrifuged and the ethylacetate phase collected and evaporated under constant air flow. The residue was then dissolved in acetone (300 μl).

The compounds were separated by supercritical fluid chromatography (SFC) on a 200 × 4.6 mm (ID) Hypersil column (5 μm particles, Hewlett–Packard, Waldbronn, Germany). The flow rate was set at 2 ml min^{-1}, column temperature at 50 °C and the column back pressure was 20 MPa. The initial mobile phase was carbon dioxide with 7 vol.% (7.4 mol%) methanol as modifier, after a 13 min run at isocratic conditions, the modifier concentration was increased linearly to 15 vol.% (15.8 mol%) in 7 min, followed by a 6 min isocratic run at this condition. Afterward, the modifier was increased to 25 vol.% (26.2 mol%) in 3 min and returned to initial conditions after a 3 min hold at 25%. Detection was performed at 217 and 280 nm. Identification was done by comparison of the peak retention time with previously injected standards.

3. Results and discussion

3.1. Effect of processing on the glucosinolate content of broccoli heads

An electropherogram of the desulphoglucosinolates, with the names of the identified compounds, from one of the untreated samples is shown in Fig. 1. The total amount of the glucosinolates found in the untreated samples, was 22.4 ± 3.3 μmol/g broccoli powder, with 13.8 ± 3.5 μmol aliphatic (50–70%) and 8.6 ± 1.0 μmol indole (30–50%) glucosinolates. The aliphatic glucosinolate fraction consisted of glucoiberin (12–19%) and glucoraphanin (81–88%), while the indole glucosinolate fraction consisted of glucobrassicin (68–76%), 4-methoxyglucobrassicin (11–18%) and neo-glucobrassicin (12–19%). These glucosinolates are found in most broccoli cultivars (Hansen et al., 1995; Kushad et al., 1999; Vang et al., 2001). The amounts found in our study are similar to those reported by Kushad et al. (1999), but are lower than the amounts reported by Hansen et al. (1995) and Vang et al. (2001). These latter authors found however, a similar glucosinolate ratio to the one reported here.

During treatment, glucosinolate degradation occurred, as shown by the relative amount of glucosinolates, i.e. the amount in sample divided by the amount of the corresponding blank, after treatment (Fig. 2). At atmospheric pressure, the highest glucosinolate degradation is observed at 100 °C. Myrosinase, is denatured at this temperature, thus, the decrease is probably due to leaching and/or thermal degradation. Thermal degradation normally results in a higher decay of indole glucosinolates than aliphatic glucosinolates (Oerlemans, Barrett, Suades, Verkerk, & Dekker, 2006). This is however not observed in the results (not shown) so the decline in glucosinolate content is likely due to leaching. At 20 °C and elevated pressure (100–500 MPa), there seems to be no degradation after 15 min, whereas after a 35 min treatment at elevated pressure (200 or 300 MPa), about 20% less glucosinolates are present. At 40 °C and elevated pressure (100–500 MPa), there is already a clear effect on glucosinolate degradation after 15 min. After 35 min this effect is even more pronounced, with a maximum at 300 MPa where 63% of the glucosinolates are degraded.

Myrosinase and glucosinolates are separated in intact tissue, thus for myrosinase-catalysed hydrolysis of glucosinolates to occur, an active form of the enzyme and the substrate have to be brought together. Cell permeabilisation has been shown to occur during a pressure treatment (Van Eylen et al., 2007b), with the

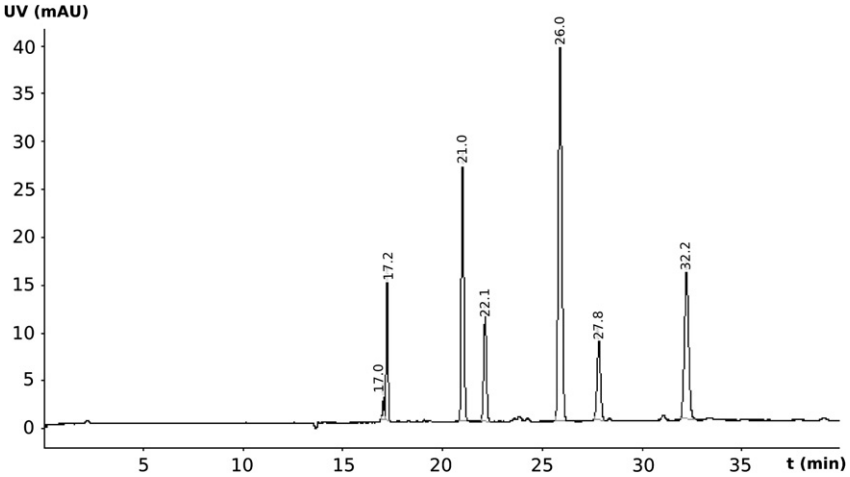

Fig. 1. Electropherogram of desulphoglucosinolates isolated from lyophilised broccoli powder. The peaks identified as glucosinolate were glucoiberin (MT 17.0), glucoraphanin (MT 17.2), glucosinalbin (IS, MT 21.0), glucotropaeolin (IS, MT 22.1), glucobrassicin (MT 26.0), 4-methoxyglucobrassicin (MT 27.8) and neoglucobrassicin (MT 32.2 min). MT: migration time and IS: internal standard.

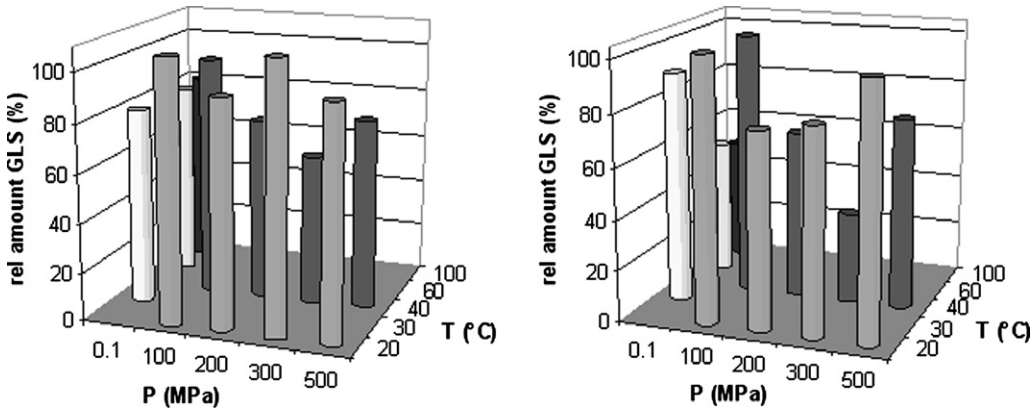

Fig. 2. Relative amount of total glucosinolates after 15 min (left) and 35 min (right) treatments at different HHP/T conditions.

permeabilisation rate increasing with pressure. This is probably the reason why a larger proportion of glucosinolates are hydrolysed at higher pressures. At 40 °C, myrosinase inactivated slowly at 300 MPa, while at 500 MPa the inactivationn is much faster (Van Eylen et al., 2007a) and myrosinase has only limited activity. This may explain why at 500 MPa a lower amount of glucosinolates is hydrolysed than at 300 MPa. At 100 MPa, the limiting factor is probably cell disintegration, which is necessary for the contact between myrosinase and glucosinolates.

These results show that glucosinolate hydrolysis can be induced during processing. This is important since not the intact glucosinolates, but only some of the hydrolysis products, such as isothiocyanates, have a health beneficial effect (Conaway et al., 2002; Das et al., 2000; Holst & Williamson, 2004). However, some hydrolysis products can have a negative effect on taste and aroma if present in high quantities (Drewnowski & Gomez-Carneros, 2000). The present results show that by varying the process parameters, e.g. time, pressure, temperature, the extent to which glucosinolates are hydrolysed can be altered, thus leading to different amounts of health beneficial and/or taste/aroma affecting products.

3.2. Effect of processing on isothiocyanate formation

It is generally acknowledged that isothiocyanates (ITC) and particularly sulforaphane can induce a number of anticarcinogenic effects, such as apoptosis and induction of phase 2 xenobiotic metabolising enzymes (Holst & Williamson, 2004). Therefore, it was to be verified whether these isothiocyanates were formed during or after treatment by GC/MS. Sulforaphane (4-(methylsulphinyl)butyl ITC) was obtained upon myrosinase-catalysed hydrolysis of pure glucoraphanin and the mass spectrum was compared with literature (Al-Gendy & Lockwood, 2003; Chiang et al., 1998; Matusheski & Jeffery, 2001). The spectrum obtained was similar, but in our study the 160 m/z fragment peak (100%) was higher than the 72 m/z fragment peak (45%), while the opposite is found in the literature. The isothiocyanates derived from glucoiberin (iberin or 3-(methylsulphinyl)propyl ITC), sinigrin (2-propenyl ITC) and gluconapin (3-butenyl ITC) were also identified, based upon comparison of the mass spectra and their retention times with the ones described by Al-Gendy and Lockwood (2003). Besides the isothiocyanates, also sulforaphane nitrile, the nitrile formed from glucoraphanin, could be identified.

Neither the isothiocyanates nor the nitrile were present in the freeze dried broccoli powder samples, probably due to volatilisation during the freeze drying and grinding. In all of the autolysed samples, except for the cooked (100 °C) sample, sulforaphane was identified and quantified (Fig. 3). After the treatments at atmospheric pressure a higher amount of sulforaphane was formed in broccoli heads treated at 60 °C than broccoli heads kept at 30 °C or untreated broccoli heads as could be expected from literature (Matusheski et al., 2004). In the autolysates of the cooked samples, no sulforaphane or other isothiocyanates were detected, which indicates that myrosinase is inactivated. For the treatments at elevated pressure, no trend can be deduced from the absolute concentrations (Fig. 3), due to the differences in glucosinolate content before the autolysis induced by the treatments (Fig. 2) and the variability in the glucosinolate amount in the starting material. Fig. 4 shows the relative amount

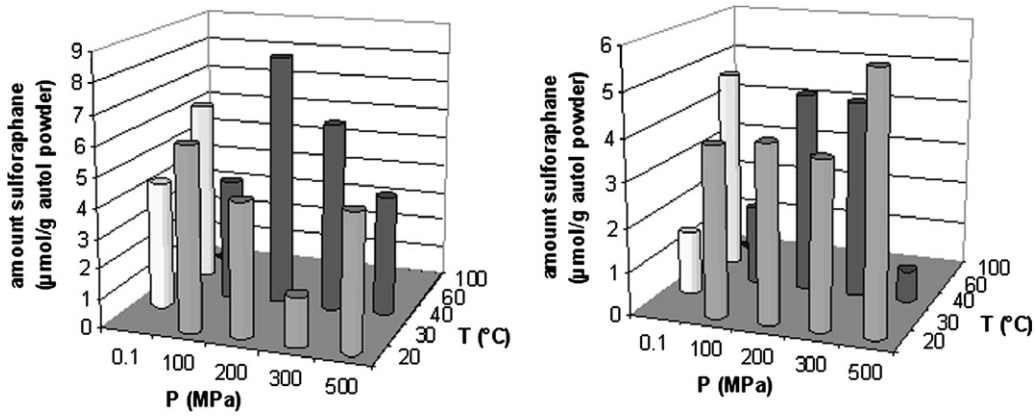

Fig. 3. Amount of sulforaphane present after autolysis (1 h) of the lyophilised broccoli powders obtained from the 15 min (left) and 35 min (right) treated samples at different HHP/T conditions.

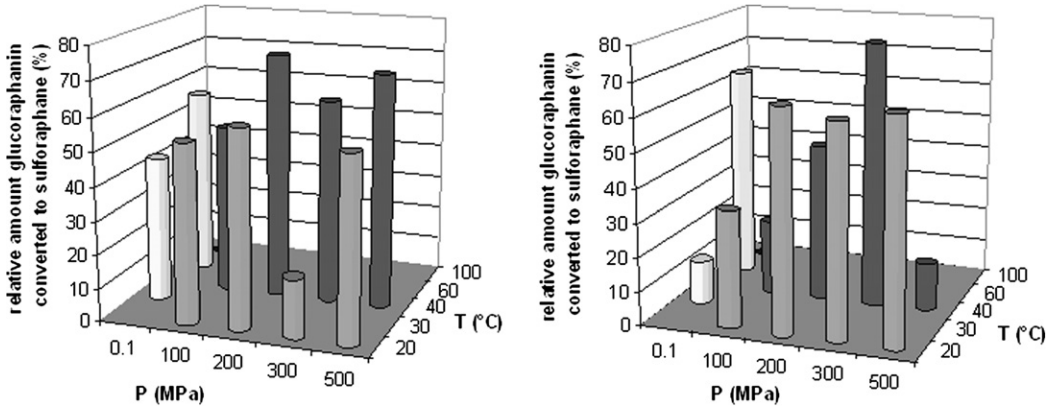

Fig. 4. Relative amount of glucoraphanin present as sulforaphane after autolysis (1 h) of the lyophilised broccoli powders obtained from the 15 min (left) and 35 min (right) treated samples at different HHP/T conditions.

of glucoraphanin present as sulforaphane after autolysis of the lyophilised broccoli powders. At 20 °C, pressure treatments from 200 MPa upto 500 MPa seem to have a similar effect as a temperature treatment at 60 °C as the treatments result in a higher percentage of the glucoraphanin that is converted to sulforaphane. After the pressure treatments (200–500 MPa) at 40 °C for 15 min, the amount of glucoraphanin converted to sulforaphane seems even higher than after the 60 °C treatment. After a 35 min treatment, the highest amount of glucoraphanin converted to sulforaphane is observed after a treatment at 300 MPa, the conditions at which the highest glucosinolate conversion took place during treatment. After the 500 MPa/40 °C/35 min treatment, only little sulforaphane is formed, probably due to myrosinase inactivation (Van Eylen et al., 2007a). The GC/MS results obtained for the sample treated at 300 MPa/20 °C/15 min are probably outliers since all values obtained for this sample seem to be too low compared to the observed trend.

For iberin, a relative comparison was conducted, where the SIM peak area of the treated samples is compared with that of the sample treated at 30 °C, taking the amount of glucoiberin present before autolysis into account. For iberin, as for sulforaphane, an effect of processing on the amount of glucoiberin converted to iberin can be observed (Fig. 5). After a 15 min treatment there is no clear trend, however, after a 35 min treatment, it can be derived that increasing pressure up to 300 MPa cause a higher degree of iberin formation. After autolysis of the 500 MPa treated samples, no iberin could be detected. This could be due to a diminished activity of the myrosinase or instability of the iberin. After the 500 MPa/20 °C treatment this is unexpected, since a high amount of sulforaphane is present after autolysis. For 2-propenyl ITC and 3-butenyl ITC it was clear that a higher amount was present in the 60 °C treated sample as compared to the other samples, but no clear effect of the pressure treatments can be observed. This could be due to their low amount, since the glucosinolate from which these isothiocyanates are formed are only present in very low amounts (below the limit of quantification in the analysis of the glucosinolates).

The results obtained from the isothiocyanates are confirmed by investigating the sulforaphane nitrile in the same way as the iberin (Fig. 6). It can be seen that when processing conditions become more intensive and where more sulforaphane is formed, less glucoraphanin is converted to sulforaphane nitrile. After the 35 min treatment there is a clear trend. These results indicate that in broccoli the epithiospecifier protein is not only less thermostable (Matusheski et al., 2004), but also less resistant to pressure treatments than myrosinase.

The results obtained indicate that a pressure treatment is a valuable alternative for a temperature treatment, e.g. to blanch broccoli before freezing, to increase the formation of health beneficial compounds. It is documented in literature that thermal blanching results in substantial loss of sulforaphane forming capability (Galgano, Favati, Caruso, Pietrafesa, & Natella, 2007). From the results presented here, it can be concluded that HHP/T conditions that are adequate for blanching, e.g. 500 MPa/20 °C, even result in an increased formation of sulforaphane. This is beneficial because sulforaphane is a much more potent inducer

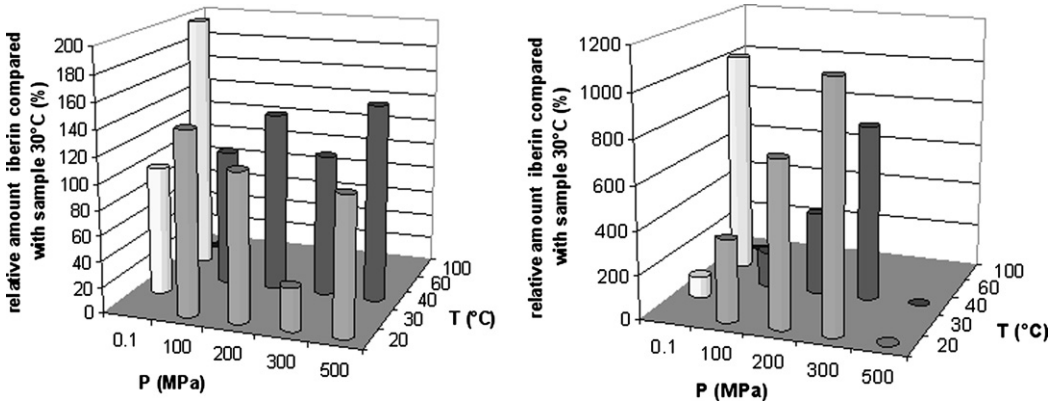

Fig. 5. Relative amount of iberin present compared with the sample treated at 30 °C after autolysis (1 h) of the lyophilised broccoli powders obtained from the 15 min (left) and 35 min (right) treated samples at different HHP/T conditions and taking the amount of glucoiberin before autolysis into account.

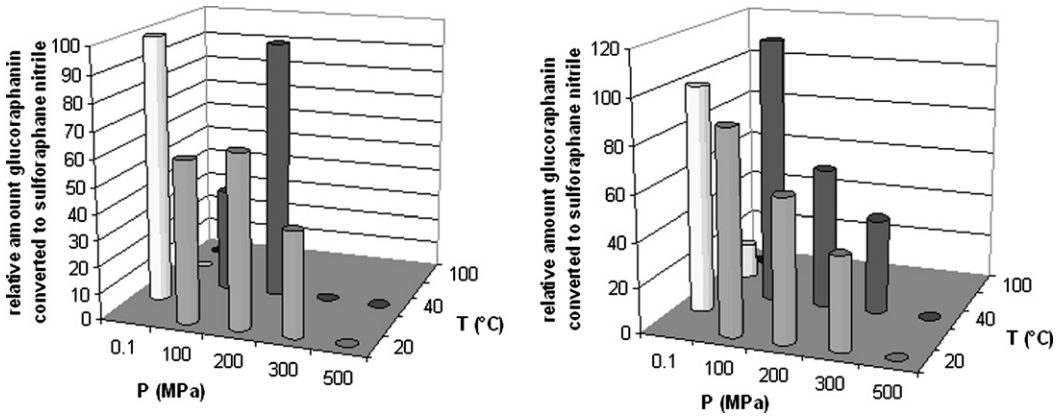

Fig. 6. Relative amount of sulforaphane nitrile present compared with the sample treated at 30 °C after autolysis (1 h) of the lyophilised broccoli powders obtained from the 15 min (left) and 35 min (right) treated samples at different HHP/T conditions and taking the amount of glucoraphanin before autolysis into account.

of xenobiotic metabolising enzymes than the corresponding nitrile (Matusheski & Jeffery, 2001). The products from the aliphatic glucosinolates were no longer present after freeze drying and grinding of the samples because of their volatile nature. This indicates that also products that are formed, e.g. during blanching, are probably lost before consumption thus limiting their potential positive effect on health. However, a beneficial effect may still be obtained due to their bactericidal properties against food borne pathogens, which can result in an extended shelf-life of isothiocyanate containing vegetables (Brandi et al., 2006). Moreover, disappearance of isothiocyanates in intact vegetables is likely to occur slower as observed in this study, where the samples are freeze dried and ground to a powder, so a fraction of isothiocyanates can still be present at the time of consumption. For some glucosinolate hydrolysis products, e.g. goïtrin, it is known that they can have a toxic effect. However, the aliphatic compounds studied in this paper are considered anticarcinogenic at the concentrations in which they are present in broccoli (Vang et al., 2001).

3.3. Effect of processing on formation of indole products

In recent years, products of aliphatic glucosinolates have received more attention than the indol-3-ylmethylglucosinolate products; nevertheless, these indole products are also present in significant amounts in broccoli. In this study 30–50% of the total glucosinolates present were indol-3-ylmethylglucosinolates. Indol-3-ylmethylglucosinolate degradation products were identified in the freeze dried broccoli powders (Fig. 7) and autolysates of the broccoli powder (Fig. 8). The main indol-3-ylmethylglucosinolate products identified are ascorbigens 2-C-(indol-3-ylmethyl)-β -L-xylo-3-hexulofuranosonic acid γ-lactone derivatives and the dimeric and trimeric products. The pH of broccoli head tissue is 6.5 and at this pH it is expected that the products formed after hydrolysis of the indol-3-ylmethylglucosinolates (glucobrassicin, neoglucobrassicin, and 4-methoxyglucobrassicin) react with ascorbic acid to form the corresponding ascorbigens (Buskov et al., 2000).

In the untreated samples, some ascorbigen and di-(indol-3-yl)methane (DIM) were present although in very low amounts.

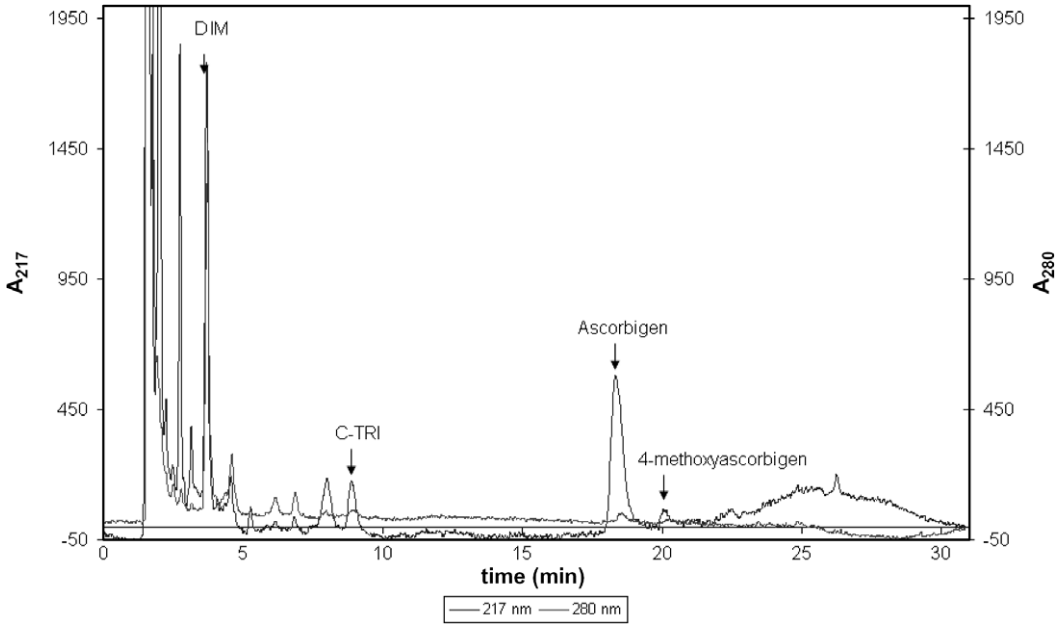

Fig. 7. Chromatogram acquired after SFC of a broccoli powder extract obtained after treatment at 40 °C and 300 MPa for 35 min (λ = 217 & 280 nm). DIM: di-(indol-3-yl)methane, C-TRI: (5,6,11,12,17,18-hexahydroclona[1,2-b4,5-b′7,8-b″]tri-indole).

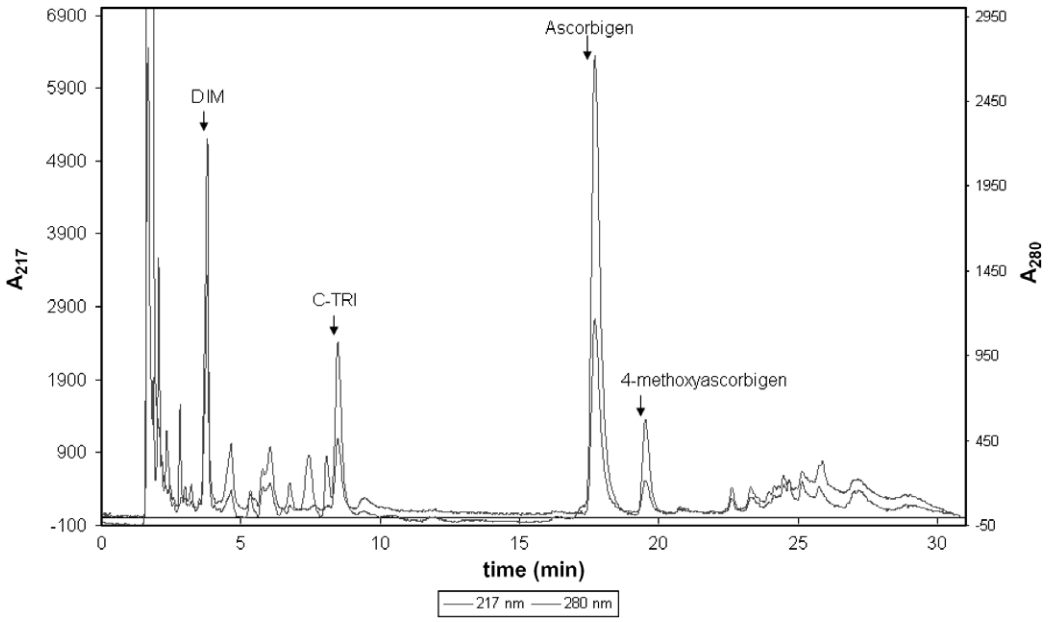

Fig. 8. Chromatogram acquired after SFC of an autolysed broccoli powder sample (1 h) after treatment at 20 °C and 500 MPa for 15 min (λ = 217 & 280 nm). DIM: di-(indol-3-yl)methane, C-TRI: (5,6,11,12,17,18-hexahydroclona[1,2-b4,5-b′7,8-b″]tri-indole).

This could be due to the handling of the samples before processing, e.g. cutting. The presence of ascorbigen and DIM in cauliflower samples without incubation time for myrosinase activity was previously observed (Buskov et al., 2000). After treatments at 100 °C, the non-autolysed extracts contained some more ascorbigen and DIM than the untreated samples, although there was no difference between 15 and 35 min treatment. This is probably due to some myrosinase activity occurring during heating followed by inactivation of the enzyme so upon autolysis, indol-3-ylmethylglucosinolate degradation products were no longer formed. The large glucosinolate decrease that was observed in the cooked samples is thus probably mostly due to leaching into the cooking water. At a 60 °C treatment, ascorbigen, DIM and (5,6,11,12,17,18)-hexahydroclona[1,2-b4,5-b′7,8-b″]tri-indole (C-TRI) are formed in a larger amount than at 30 °C, where no C-TRI is formed, in agreement with the glucosinolate analysis. During elevated pressure treatments at 20 °C (100–500 MPa), only small increases in ascorbigen and DIM are detected, and at 300 MPa also of some C-TRI. From the glucosinolate analysis it was already clear that there was little glucosinolate conversion at these temperature–pressure conditions. In contrast to 20 °C, at 40 °C and elevated pressures above 100 MPa, there is a clear increase in C-TRI in the treated samples with a decline in glucosinolates.

Upon autolysis, a large increase in indol-3-ylmethylglucosinolate products, especially the ascorbigens, occured. The amounts of ascorbigen formed upon autolysis are similar for all samples, except for the 100 °C treated sample as indicated before, and the 500 MPa/35 min treated samples, where less indole products are present. This is probably due to myrosinase being already largely inactivated (Van Eylen et al., 2007a), as also seen in the sulforaphane formation (Fig. 4). From these results, it can be concluded that myrosinase was still active after treatment and able to convert the glucosinolates, except for the aforementioned samples.

Ascorbigen and DIM are formed in similar amounts during treatment and in samples with higher glucosinolate conversion, C-TRI is also formed, although in lower amounts. During autolysis, the major product is ascorbigen, with only limited amounts of DIM or C-TRI. Formation of ascorbigen, DIM and C-TRI is a chemical process and it is probable that in the autolysed samples, which are homogenised, ascorbic acid is more readily available than during treatment of intact tissue leading to a higher formation of ascorbigen. The type of products formed during treatment were similar to the ones formed during autolysis, only in different relative quantities. The results obtained in the present study for broccoli are in agreement with a previous study, where it was shown that in other *Brassica* autolysates ascorbigens are the main indole glucosinolate derived products (Buskov et al., 2000).

Determining the type of products formed upon hydrolysis of the indol-3-ylmethylglucosinolates is of great importance since e.g. indol-3-ylmethanol and DIM have been shown to have an anticarcinogenic effect, both in vitro and in vivo in rats (Chen & Andreasson, 2001; Chen et al., 1998; Nachshon-Kedmi et al., 2004) while for ascorbigen, there is no evidence of a health related effect. Therefore, it might be advantageous to limit the formation of ascorbigen, which will also result in the preservation of ascorbic acid, a well known anti-oxidant. Thus, converting the indol-3-ylmethylglucosinolates during processing, which results in formation of DIM and C-TRI, might be a good way to limit the formation of ascorbigens. Although for the indolic compounds indol-3-ylmethanol and DIM, an anticarcinogenic and therapeutic effect has been shown, it is likely that indol-3-ylmethanol both inhibits and, given post initiation, potentially promotes carcinogenesis (Stoner et al., 2002). However, it is shown here that in *Brassica* vegetables the extent of indol-3-ylmethanol formation is low, because of the formation of dimers and ascorbigen, and it is likely that the bioavailability of indol-3-ylmethanol is also low (Holst & Williamson, 2004) causing the dietary dose to correspond to the physiological, protective dose.

4. Conclusion

In this work, the effect of temperature and high pressure in combination with mild temperature on glucosinolate conversion and on the type of hydrolysis products formed was investigated. During mild pressure treatments glucosinolate hydrolysis takes place. The hydrolysis products of the aliphatic glucosinolates seem to disappear from the broccoli after treatment, while the hydrolysis products of the indol-3-ylmethylglucosinolates formed during treatment are contained in the broccoli. During the treatment, ascorbigen and indol-3-ylmethylglucosinolate oligomers appeared to be formed in similar amounts. During autolysis of the treated

samples, glucosinolates were hydrolysed, which indicates that there was still enough active myrosinase. This was not the case for the cooked samples. A high pressure treatment has a positive effect on the health potential of broccoli since more of the glucosinolates are turned over into the beneficial isothiocyanates afterwards. During autolysis, the main indol-3-ylmethylglucosinolate hydrolysis product formed is ascorbigen. Processing can have an effect on the type of products formed, which is of great relevance due to the fact that different glucosinolate hydrolysis products have different anticarcinogenic properties and it is shown here that the process intensity influences the type of products formed. Depending on which products are more beneficial, it may be possible to choose the appropriate process conditions. This study indicates that processing conditions can have an effect on the kind of products that are formed from the glucosinolates. This is an important issue to evaluate the health effect of glucosinolate hydrolysis products that are consumed in the human diet.

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