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The impact of high pressure on glucosinolate profile and myrosinase activity in seedlings from Brussels sprouts

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ABSTRACT

The potential of high pressure (HP) to control bioactive components using seedlings of Brussels sprouts as a simple non-chopped vegetable system was examined. Enzyme activity *in situ* compared to purified enzyme and residual enzyme substrate *in situ* are used as three complementary measures for the HP effect. Purified myrosinase and seedlings of Brussels sprouts were submitted to HP 200–800 MPa at 5 °C for 3 min. The myrosinase activity decreased for both myrosinase systems upon increasing pressure to 800 MPa. Applying first-order kinetic to determine activation volumes revealed a linear relationship from 400 to 600 ($\Delta V^{\#} = -19.04$ mL/mol) and 450–600 MPa ($\Delta V^{\#} = -37.79$ mL/mol) for seedlings and purified myrosinase, respectively, indicating a protective effect of the plant matrix against enzyme inactivation. Purified myrosinase was activated at 200 MPa but at 800 MPa the glucosinolate degradation due to pressure induced disruption of the plant matrix seems to be partly counter-acted by myrosinase inactivation.

Industrial relevance: High Pressure (HP) processing is an effective non-thermal preservation treatment for liquid and solid food. Moreover, over the last years, the potential of this technology to improve health and safety attributes of foods has been demonstrated. In particular, the ability of HP to preserve bioactive compounds has been established. There are only few studies evaluating the impact of HP on the complex bioactive glucosinolates-myrosinase. Therefore, this study opens the doors through the application of HP to preserve the bioactive glucosinolates in cruciferous vegetables by creating new processing solutions through controlled enzyme inactivation. Thus, HP could be an effective tool to achieve more effective solutions to obtain the new generation of convenient food and meet the need for new bioactive food products.

1. Introduction

Cruciferous vegetables are distinguished by their content of glucosinolates present in all plants of the order *Capparales* and only in few other plants (Bellostas, Kachlicki, Sørensen, & Sørensen, 2007; Fahey, Zalcmann, & Talalay, 2001). The intact glucosinolates and their transformation products are bioactive compounds responsible for smell and taste with the isothiocyanates contributing to the typical pungency and bitterness of *Brassica* vegetables and condiments. The glucosinolates and their transformation products are also associated with metabolic effects as reviewed by Holst and Williamson (2004) and Jeffery and Araya (2009) including health beneficial effects related to reduced risk of cancer and the prevention of some degenerative diseases (Bhattacharya et al., 2010; Dinkova-Kostova & Kostov, 2012; Egner et al., 2011; Jeffery et al., 2003).

A range of different glucosinolate transformation products are formed when tissue disruption exerted by e.g. cutting, chewing or processing cruciferous vegetables leads to interaction between the endogenous active form of the enzyme myrosinase (EC.3.2.1.147) and the glucosinolates (Bones & Rossiter, 2006; Deng et al., 2015). The glucosinolate degradation products are defined by the reaction conditions and the parent intact glucosinolate structures (Bones & Rossiter, 2006) and intact glucosinolates as well as their degradation products show a highly specific relationship between structure and biological effects (Razis, Iori, & Ioannides, 2011). In addition, it has been shown that the glucosinolate profile and active myrosinase varies between species, cultivars of a single species and between tissues of a single plant (Bellostas et al., 2007; Clossais-Besnard & Larher, 1991; Rosa, Heaney, Portas, & Fenwick, 1996; Sang, Minchinton, Johnstone, & Truscott, 1984). Therefore, it is of a paramount importance

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for exploitation of food sources for glucosinolates as bioactive compounds to know the glucosinolate profile as well as being able to control the myrosinase activity in the plant tissue or food system.

Thermal treatment is a traditional way to control enzyme activity. However, it can result in losses not only of sensorial but also nutritional quality attributes (Barba, Esteve, & Frígola, 2012; Ghawi, Methven, Rastall, & Niranjan, 2012; Ludikhuyze, Ooms, Weemaes, & Hendrickx, 1999). High pressure (HP) processing is a non-thermal technology that is based on applying pressures between 100 and 800 MPa at low or moderate temperatures (<40 °C). Still, HP has been shown to result in microbial death and extended shelf-life of various foods. In addition, HP does not affect covalent bonds, so important quality attributes such as colour, flavour, and nutritional quality can be maintained and therefore high pressure treatment provides a technological alternative to control enzyme activities decisive for food quality.

The effects of the myrosinase-glucosinolate system after applying HP conditions (pressure, time and temperature) have been studied in broccoli juice and heads (Van Eylen, Oey, Hendrickx, & Van Loey, 2007b; Van Eylen et al., 2009). In these studies, HP parameters had an impact on the myrosinase activity and the extent of glucosinolate hydrolysis. The effects from the HP treatment may however be blurred by pre-processing effects on enzymes and bioactive compounds upon disruption of plant cell tissue (Frandsen et al., 2014). This stresses the importance of investigating the myrosinase-glucosinolate interactions in a well-defined and homogenous plant matrix in order to provide basic knowledge of the HP impact on the health beneficial compounds from cruciferous vegetables.

For this purpose, seedlings of *Brassica oleracea* var. *gemmifera* constitute a well-defined and homogenous sample matrix with simple cell structures suitable for mechanistic studies at a molecular level and, importantly, it is possible to HP treat the entire plant material, thereby avoiding autolytic reactions from cutting the plant. This model matrix therefore serves as a base for transferring the obtained knowledge to the next level of studying/using bioactive compounds from complex structured cruciferous vegetables.

The aims of the present work are *i*) to evaluate the optimal growing stage of seedlings from Brussels sprouts based on their glucosinolate composition; *ii*) to examine sprouts as a model system for HP effect on enzyme activity and *iii*) to study the impact of HP processing on myrosinase activity and preservation of intact glucosinolates.

2. Materials and methods

2.1. Materials and reagents

Brussels sprouts (*Brassica oleracea* L.var. *gemmifera* cv Evesham special) seeds were purchased from B&T World seed (Aigues-Vives, France). Germination trays (Bergs Bio Salad) were obtained from plantefrø.dk (Copenhagen, Denmark). Sulfatase from *Helix pomatia*, methyl-α-D-mannopyranoside, Sephadex DEAE A25 and Sephadex C-25 (Na-form) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), Concanavalin-A-Sepharose was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). All other chemicals and reagents were of analytical grade. Glucosinolates were from the laboratory collection of purified reference compounds (Sørensen, Sørensen, Bjergegaard, & Michaelsen, 1999).

2.2. Sprouting of seedlings

Seeds (25 g) were imbibed in water (48 mL) with aeration for 24 h (termed day 1) and subsequently transferred to germination trays. Each tray was added 50 mL water twice a day at room temperature (23 ± 1 °C). After 1, 3, 5, and 7 days seedlings were collected (without cutting) and freeze-dried (Heto FD3, Heto-Holten, Denmark). Fresh seeds and dried seedlings were analysed for glucosinolate concentration and profile to select the optimal harvesting time for HP treatment.

2.3. Packaging and HP treatment of seedlings

Seedlings (3 g) were packaged into plastic bag (PA/PE 20/70, 32 oxygen cm^3/m^2 d bar at 23 °C and 75% RH, SFK, Hvidovre, Denmark) at 40% vacuum and subjected to high pressure (Food Processing Press QFP-6 Avure Technologies AB Vesterås, Sweden) between 200 and 800 MPa for 3 min at an initial temperature of 5 °C. The pressurization liquid was a mixture of water and sodium benzoate (5%). The pressure level, pressurization time, and temperature were controlled automatically. During HP treatment, the temperatures in the pressure chamber increased and the maximum temperatures were up to 40 °C at 800 MPa and lower for the lower pressures, as measured by a built-in thermocouple. The depressurizing instantly resulted in a decrease in temperature. The temperature fluctuation in the seedling samples was anticipated to be quite similar to the temperature increase during pressure build-up and temperature decrease during depressurization. The treatment time (3 min) did not include the pressure build-up (compression rate is ~450 MPa/min) and depressurization <20 s was initiated automatically after the 3 min. Immediately after pressurization, the samples were transferred to an ice/water bath and freeze-dried, then stored at room temperature until further chemical analyses.

2.4. Preparation of desulfoglucosinolates

Flour (100 mg) of whole seeds or freeze-dried seedlings of Brussels sprouts were extracted three times with boiling methanol–water (3 mL, 70%) and centrifuged for 10 min at 1000 *g* as described by Bjerg and Sørensen (1987). The supernatants were pooled, evaporated to dryness and redissolved in 5 mL Milli-Q water (termed crude extract). Glucotropaeolin (100 μL, 7 mM) and sinalbin (100 μL, 7 mM) were added as internal standards. Preparation of desulfoglucosinolates was done according to Bjerg and Sørensen (1987) with minor changes. In brief, column material was prepared according to Sørensen et al., (1999) and crude extract (1 mL) was applied to a DEAE Sephadex A-25 column (0.5 mL column material). The column was washed with Milli-Q water (2 mL) and subsequently buffered with 1 mL 0.02 M acetate buffer, pH 5.5. The column was incubated overnight with sulfatase (75 μL) prepurified from 70 ± 0.1 mg sulfatase subjected to ethanol precipitation followed by anion (DEAE Sephadex A25) and cation (Sephadex C-25) exchange with a final volume of 2 mL (Bjerg & Sørensen, 1987). Desulfoglucosinolates were eluted with 3 × 1 mL Milli-Q water, evaporated and re-dissolved in 100 μL Milli-Q water.

2.5. Quantification of desulfoglucosinolate by micellar electrokinetic capillary chromatography (MECC)

The samples were analysed by capillary electrophoresis (Agilent, 3DCE Waldbronn, Germany) as described by Bjergegaard, Michaelsen, Møller, and Sørensen (1995) using the following conditions; 60 °C, 12 kV, 645 mm × 75 μm I.D. fused silica capillary. Detection was performed on a column at 230 nm with diode array detection at 200–400 nm. The buffer consisted of sodium cholate (250 mM) and borate (200 mM), pH 8.5. Samples were injected from the anionic end of the capillary (vacuum injection 50 mbar, 1 s) and the electrophoresis was performed for 40 min. For the HP-treated samples, additional analyses were made due to co-elution of glucoiberin and glucoraphanin as well as the internal standard glucotropaeolin and glucoerucin in the desulfoanalysis. The crude extract was up-concentrated 5 times, and the samples were analysed as intact glucosinolates in the CTAB (cetyltrimethylammonium-bromide) system according to Michaelsen, Møller, and Sørensen (1992). Normalized areas (NA) and relative normalized areas (RNA) were calculated relative to the reference glucosinolate sinalbin (Michaelsen et al., 1992)