**This project will provide new insights into the mechanisms and impact of high pressure processing technology on vegetable and plant matrices that are necessary for the development of viable future industrial applications.**

The objectives (Figure 1) of the present project were 1. To establish the HP processing parameters to control the activity of glucosinolate degrading enzyme (myrosinase) determined from glucosinolate degradation and/or the formation of glucosinolates derived compounds (isothiocyanates). 2. To reveal the mechanistic effect on and control of myrosinase activity during HP and 3. To establish the mechanistic description of HP processing on bioactive compounds and enzymes in plant cells using the cruciferous system of myrosinase (enzyme) – glucosinolates (bioactive compounds) as model system.



Figure 1.

Standardized and optimal conditions for growing sprouts of Brassica oleracea var. gemmifera in the laboratory were investigated. Seedlings were analysed with respect to glucosinolate profile and content as well as to myrosinase activity content. Optimal methods for packaging of the sprouts for HP processing were developed. Afterwards, the methodologies to determine myrosinase activity, and bioactive compounds, in particular, glucosinolate profile, but also other antioxidant bioactive compounds (eg. polyphenols, carotenoids, and chlorophylls) were optimized. The effect of different HP processing conditions (pressure level, temperature, and time) on the myrosinase activity, glucosinolate profiles and content, and antioxidants were investigated.

11 glucosinolates were identified and distributed in three groups 1) aliphatic, 2) aromatic and 3) indol-3-ylmethylglucosinolates (IMGs). The profile and concentration of glucosinolate from seeds and seedlings were found to change upon growing times, and the total glucosinolate concentration was highest for the Day 7 seedlings and, thus, chosen for future investigations. Packaging the seedlings in plastic bag at 40 % vacuum was the best solution for preserving intact seedlings. HP treatment had a major effect on the glucosinolate concentrations resulting in a significant decrease corresponding to a degradation of 70%. The myrosinase activity decreased for both myrosinase systems upon increasing pressure to 800 MPa. Applying first-order kinetic to determine activation volumes ((∆V#) revealed a linear relationship from 400-600 (∆V#= −19.04 mL/mole) and 450-600 MPa (∆V#= −37.79 mL/mole) 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 counteracted by myrosinase inactivation. A response surface method study revealed that the highest glucosinolate concentration and lowest myrosinase activity were obtained at 600 MPa, 60 °C, and 10 min. This study indicates that the combination of pressure, time and temperature has an important role in glucosinolate-myrosinase system. This may be a result of two opposing mechanisms i.e. pressure induced disruption of membrane leading to increased hydrolysis of glucosinolate and pressure induced inactive of myrosinase leading to decreased hydrolysis of glucosinolate.

So far, the study with parallel determination of residual enzyme activity as well as degradation of the natural enzyme substrates present in the matrix shows that the evaluation of the effect of HP processing of plant matrices should not solely be based on the residual enzyme activity. The combined effects on enzyme activity and residual level of substrate/bioactive compounds are important as considerable substrate conversion may occur during the HP processing, thus affecting the applicability of HP treatment for preservation of bioactive compounds.

HP processing and subsequent refrigerated storage had a significant effect on free amino acids, although the behavior differed according to the individual compound, pressure and storage time. The individual content of certain amino acids such as alanine, glycine, leucine, serine, proline, threonine, valine and phenyl alanine were significantly decreased at 600 MPa. In contrast to untreated samples, a significant increase in umami specific amino acid such as aspartic and glutamic acids was found in HP-treated seedlings, while asparagine and glutamine were reduced. Total free amino acids content was increased in untreated and HP-treated samples during storage, although the extent of increase was higher when HP was applied. These results suggest that HP treatment affects proteolysis and/or certain amino acids metabolism pathways in Brussels sprouts seedlings after HP treatment and during subsequent storage.

It can be also highlighted that HP has the potential to control the extent of radical formation in seedlings of Brussels sprouts most probably due to the improved extractability of antioxidant bioactive compounds (chloropylls, polyphenols and carotenoids) which can scavenge the radicals or by non-thermal inactivation of enzymes, thus avoiding the subsequent production of reactive species.

The results obtained in the present project helped to give one step in the knowledge of HP processing technique. Although the potential of this HP technology to preserve food is clearly demonstrated by the wide range of products commercialized, its potential in food processing is still in the beginning. This project allowed us to reach the overall aim – preserving bioactive compounds in cruciferous vegetables by creating new processing solutions through controlled enzyme inactivation. Therefore, HP can be considered not only as a tool for food preservation but also for obtaining a new generation of convenience foods based on plant matrices, with their subsequent socio-economic impact in the development of new food industries based on this technology.

The address of the project public website should also be indicated, if applicable.

The internet address should be active.

http://food.ku.dk/english/projects/fc/65.hpbioactive/