Biofumigation: from the “classical” approach to the use of biorefined glucosinolates as natural plant protection agents

N. Bellostas, J. C. Sørensen, H. Sørensen

Department of Natural Sciences, Biochemistry and Natural Product Chemistry, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Abstract

The rising concern about the impact of traditional agriculture on the environment and on human health has boosted the search for more environmental friendly pesticides and herbicides. In this context, Cruciferous crops have been shown to be a viable alternative due to the biological effects displayed by the hydrolysis products of special allelochemicals (glucosinolates) present in tissues of these plants. Since it was first coined, the termed biofumigation relied on the fact that the effect of methyl isothiocyanate (a commercial fumigant) could be mimicked by isothiocyanates derived from glucosinolates in Crucifers. Research on biofumigation has delivered inconsistent results during the past 15 years although recent efforts in overcoming empiricism have shown important progress in enhancing the release of isothiocyanates from plant tissues. Yet, the amount of isothiocyanate released remains in most cases below 50% of the potential releasable amount based on glucosinolate concentration, which limits the applicability of biofumigation. Biorefineries solely based upon aqueous and mechanical processes have now been developed, which allow for the extraction and separation of all biochemical constituents of biological materials, such as seeds of agricultural crops. Rapeseed (*Brassica napus* L.) is a valuable crop from which high quality oils, proteins, fibres and pure glucosinolates have been extracted by biorefining. This opens up the possibility for the targeted use of preparations of biorefined glucosinolates and myrosinases for the control of plant pathogens and weeds without the limitations shown by fresh tissues.

Keywords: Biofumigation, glucosinolates, isothiocyanates, biorefinery, rapeseed.

Introduction

In the search for environmental friendly methods of pest control, members of the Brassicaceae family have attracted special attention. Cruciferous plants contain glucosinolates, which upon hydrolysis by endogenous enzymes (myrosinases) release a series of biologically active products with fungicidal, insecticidal, herbicidal and nematocidal properties (Bjergegaard et al., 1994; Brown and Morra, 1997; Sørensen, 1990; Sørensen et al., 2001). Among the various types of glucosinolate derived products isothiocyanates (ITCs) are almost always claimed to be the most biologically active (Brown and Morra, 1997, Rosa et al., 1997) mainly due to their high reactivity toward electrophilic groups in biomolecules (Bjergegaard et al., 1999; Björkmann, 1973).

The term “biofumigation” was coined in the early 1990s to describe the suppression of soil borne pests by volatile ITCs present in some Brassica crops. However, in the past years the use of Cruciferous tissues to control plant pathogens and weeds has shown inconsistent results (Matthiessen and Kirkegaard, 2006; Morra, 2004).

In the past 20 years the use of enzymes for the extraction of biomolecules from plants using aqueous media (biorefinery) has been developed (Jensen et al., 1990; Bagger et al., 1998, Sørensen, 2001). In this period of time the use of aqueous enzymatic processes for the extraction of oil, protein, fibre and glucosinolates from seeds of rapeseed has been developed in a number of EU-funded project (“The whole crop biorefinery” (AGRE-0061), BOP (FAIR-CT95-0260), ENHANCE (QLRT 1999-01442)) and it has proven successful (Bagger et al., 2003; Bagger et al., 2007; Palmieri et al., 1994). A new scenario has thus opened up in which the direct use of pure glucosinolates and pure myrosinases as isolated from Cruciferous plants is possible.

Glucosinolate hydrolysis products and their biological activity

Glucosinolates are a group of allelochemicals ubiquitous in plants of the order *Capparales* (Kjær, 1960; Sørensen, 1990). Glucosinolate containing plants have long been known for their particular flavour and smell, which are related to the co-occurrence of these compounds with a specific enzyme known as myrosinase (Bellostas, 2007). Myrosinases (EC 3.2.1.147) are β- thioglucoside glucohydrolases that catalyze the hydrolysis of the β-D-thioglucopyranoside bond in glucosinolates forming a variety of products with different structures and properties. ITCs are the most frequent products formed at neutral pH upon the myrosinase catalysed hydrolysis of aliphatic glucosinolates, whereas at acidic conditions nitriles are formed (Bellostas, 2007; Sørensen, 1990). Other compounds with different toxicity and physico-chemical properties are formed from indol-3-ylmethylglucosinolates (Bjergegaard et al., 1994; Buskov et al., 2000).

The biological activity of glucosinolate hydrolysis products towards fungi, nematodes, insects and weeds has been confirmed in a number of experiments *in vitro*, in pot studies in greenhouses and in field trials (reviewed by Brown and Morra, 1997 and Rosa et al., 1997). It is clear that ITCs are or form components toxic against a variety of pathogens and plant species *in vitro*. However, and in the light of the lack of consistency between results under field conditions, the involvement of other compounds has also been suggested.

The “classical” biofumigation approach: use of plant tissues or seed meal amendments

The term “biofumigation” was coined in an attempt to refer to the notion of emulating the well-known fumigant effect of a synthetic ITC (methyl ITC) through biological sources of ITCs (Mathiessen and Kirkegaard, 2006). The need for new soil fumigants due to the phase out of methyl bromide (Anonymous, 1992) boosted research on biofumigation worldwide and in the past 15 years the use of brassicaceous fresh tissues for soil-borne pathogen control has been an object of

increasing attention for many research groups (Brown and Morra, 1997; Mathiessen and Kirkegaard, 2006).

Most research in biofumigation has focused on allyl, but-2-enyl and phenethyl ITCs due to the fact that they are the main glucosinolate hydrolysis product at neutral pH formed from glucosinolates in some Brassica species and that they give basis for components with high biological activity (see above). In the last few years and in the frame of a more systematic approach to research in biofumigation (Mathiessen and Kirkegaard, 2006) efforts have been directed towards the maximization of ITC release from the cruciferous tissue. However, the maximum release remains at levels between 25-50% of the potential release based on glucosinolate concentration, with large differences having been documented between Brassica species (Gimsing and Kirkegaard, 2006, Morra and Kirkegaard, 2002). The suboptimal level of ITC liberation limits the applicability of the "classical" biofumigation approach and it has been suggested that it could be used in combination with other techniques for pathogen control (Mathiessen and Kirkegaard, 2006).

The "biorefinery" concept – use of glucosinolates as natural plant protection agents

The use of aqueous solutions to extract biomolecules from agricultural crops (biorefining) has proven successful since it was first tested 20 years ago (Hillemann et al., 1988; Jensen et al., 1990). *B. napus* L. (oilseed rape) is one of the most documented examples and high quality oils, proteins, fibres and glucosinolates have been extracted on successive generations of the bioprocessing technique (Jensen et al., 1990; Bagger et al., 1998; Bagger et al., 2003; Bagger et al., 2007, Sorensen, 2001; Figure 1). Other cruciferous crops have been tested with equal success and further progress has been found to be possible by combination with firmly controlled cold-pressing and supercritical fluid techniques for oil extraction.

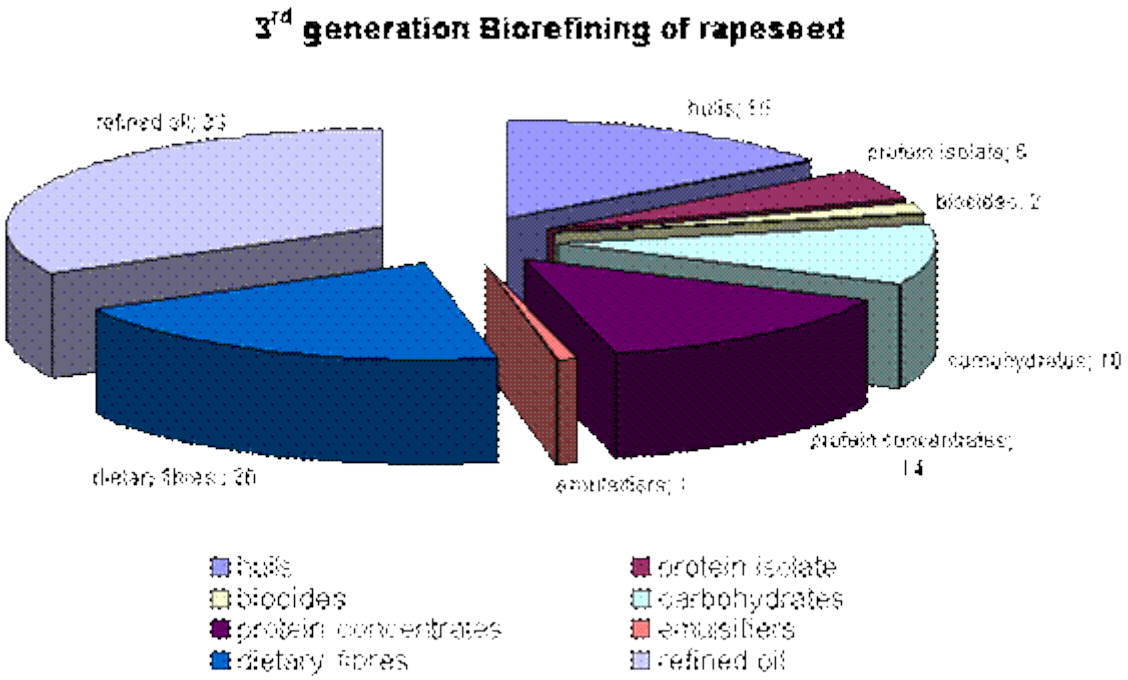

Figure 1. Fractionation achieved by last (third) generation of the aqueous-based bioprocessing technology.

Biorefining is thus based upon environmental friendly and mild processes that avoid the use of organic solvents. Among other things this prevents quality problems normally observed in oil and pressed cake upon rapeseed crushing in traditional solvent extraction oil-mills, which have been related to appreciable glucosinolate degradation (Bellostas et al., 2007). Furthermore, biorefining allows for the full fractionation of the pressed cake after oil extraction, yielding high quality products readily usable in the food, non-food and feed industry, which gives an increased overall value to the cruciferous crop. In this context, glucosinolates are no longer regarded as a waste product, but instead, as a product with a high value potential. A new scenario has thus opened up in which the use of pure glucosinolates and pure myrosinases isolated directly from cruciferous crops is possible with the use of biorefining/bioprocessing technologies (Bagger et al., 2007). A more targeted use of glucosinolates could thus be made by choosing the most efficient glucosinolate against the particular pathogen as found by *in vitro* studies. Similarly, the use of cofactors that have proven successful in directing the hydrolysis of glucosinolates towards the desired compound *in vitro* (Bellostas, 2007), allows the hydrolysis reaction to be targeted towards the production of the most active compounds derived from glucosinolate hydrolysis.

Conclusions

The glucosinolate-myrosinase system has proven to be an efficient means of plant pathogen and weed control. However, the “classical” biofumigation approach seems to be limited in the control achieved. Using biorefined glucosinolates and myrosinases in a formulation could help to overcome the limitations of

biofumigation by developing a more targeted application of the specific glucosinolate/s as well as optimizing the hydrolysis to direct it to the most active compounds. In any case, despite the biodegradability of glucosinolate hydrolysis products has been demonstrated by their short residence times in soil, only those compounds that show to be active at concentrations that are not harmful to the environment and to humans and animals should be used in this approach (Sørensen et al. 2001).

References

Anonymous, 1992. United Nations Environment Programme 1992. Montreal Protocol Assessment. Methyl bromide: its atmospheric science, technology and economics. Synthesis Report of the Methyl Bromide Interim Scientific Assessment and Methyl Bromide Interim Technology and Economic Assessment, pp. 41.

Bagger, C.L., Sørensen, H. and Sørensen, J.C. 1998. High-quality oils, proteins, and bioactive products for food and non-food purposes based on biorefining of cruciferous oilseed crops. Plant Proteins from European Crops. J. Gueguen and Y. Popineau (Eds.) Springer Verlag, 272-278.

Bagger, C.L., Sørensen, H., Sørensen, J.C. and Sørensen, S. Biorefining – The soft Processing Alternative. In: Proceedings of 11th International Rapeseed Congress, 6-10 July 2003 Copenhagen, Denmark.

Bagger, C., Bellostas, N., Jensen, S.K., Sørensen, H. Sørensen, J. C., Sørensen, S. Processing -bioprocessing of oilseed rape in bioenergy production and value added utilization of remaining seed components. In: Proceedings of the 12th International Rapeseed Congress, 26-29 March 2007, Wuhan, China.

Bellostas, N. Glucosinolates and myrosinases in Cruciferous species – mechanisms of formation of glucosinolate transformation products and perspectives for their utilization as natural plant protection agents. PhD Thesis, Department of Natural Sciences, Faculty of Life Sciences, University of Copenhagen,Denmark.

Bellostas, N., Bjergegaard, C., Jensen, S.K., Sørensen, H. Sørensen, J.C., Sørensen, S. 2007. Nutritional value of cruciferous oilseed crops in relation to profile of accumulated biomolecules with especial regard to glucosinolates transformation products. In: Proceedings of the 12th International Rapeseed Congress, 26-29 March 2006, Wuhan, China.

Bjergegaard, C., Li, P.W., Michaelsen, S., Møller, P., Otte, J. and Sørensen, H. Glucosinolates and their transformation products - compounds with a broad biological activity. Bioactive Substances in Food of Plant Origin (1994) 1, 1-15.

Bjergegaard C, Møller P, Sørensen H., Sørensen J. C., Sørensen S. 1999. Micellar electrokinetic capillary chromatography of thiocarbamoyl derivatives produced in reactions between isothiocyanates and amino acids. J. Chrom. A. 836, 115-127.

Björkman, R. 1973. Interaction between proteins and glucosinolate isothiocyanates and oxazolidinethiones from Brassica napus seed. Phytochem. 12, pp. 1585-1590.

Brown, P. D., Morra, M. J. 1997. Control of soil-borne plant pests using glucosinolate containing plants. Advances in Agronomy, 61, 167-231.

Buskov, S., Olsen, C.E., Sørensen, H. and Sørensen, S. Supercritical fluid chromatography as basis for identification and quantitative determination of indol-3-ylmethyloligomers and ascorbigens. J. Biochem Biophys Methods (2000) 43, 307-327.
Gimsing, A. L.; Kirkegaard, J. A. Glucosinolate and isothiocyanate concentration in soil following incorporation of Brassica biofumigants. Soil Biology and Biochemistry 2006, 38 (8), 2255-2264.
Hillemann, G., Jensen, S.K. og Sørensen, H.: "Novo-rapsmel" som proteinkilde til minkfoder. Dansk Pelsdyravlerforening. Faglig Årsberetning (1988) 156-162.
Jensen, S.K., Olsen, H.S. and Sørensen, H. 1990. Aqueous Enzymatic Processing of Rapeseed for Production of High Quality Products. In: Rapeseed/Canola: Production, Chemistry, Nutrition and Processing Technology (Ed. F. Shahidi), Van Nostrand Reinhold Publisher, pp. 331-343.
Kjær, A. 1960. Naturally derived isothiocyanates (mustards oils) and their parent glucosinolates. In: Hetz W, Grisenbach H, and Kirby GW (Eds.), Progress in the chemistry of organic natural products. Springer-Verlag, Berlin, New York, pp. 122-176.
Matthiessen, J. N.; Kirkegaard, J. A. Biofumigation and Enhanced Biodegradation: Opportunity and Challenge in Soilborne Pest and Disease Management. Critical Reviews in Plant Sciences, 2006, 25 (3), 235-265.
Morra, M. 2004.Controlling soil-borne plant pests using glucosinolate-containing tissues. Agroindustria. 3 (3), 251-255.
Morra, M. J.; Kirkegaard, J. A. Isothiocyanate release from soil-incorporated Brassica tissues. Soil Biology and Biochemistry 2002, 34 (11), 1683-1690.
Palmieri S, Rollin P, Sørensen H and Sørensen S. Myrosinase technology for a potential glucosinolate utilization in Agro- Industry. Agro Food Ind High Tech 9:24–27 (1998).
Rosa, E. A. S., Heaney, R. K., Fenwick, R. G., and Portas, C. A. M. 1997. Glucosinolates in crop plants. In: Janick, J. (Eds.), Horticultural Reviews, Volume 19. John Wiley & Sons, Inc., United States of America, pp. 99-215.
Sørensen, H. Glucosinolates: Structure-Properties-Function. In: Rapeseed/Canola: Production, Chemistry, Nutrition and Processing Technology (Ed. F. Shahidi) Van Nostrand Reinhold publisher (1990), Chapter 9, 149-172.
Sørensen J. C. 2001. Biorefined oilseed products – high quality products with added value. PhD Thesis. Chemistry Department, KVL, Denmark.
Sørensen, H., Sørensen, J.C. and Sørensen, S. Phytochemicals in food: The plant as chemical factories. In: Biologically-active Phytochemicals in Food. Analysis, Metabolism, Bioavailability and Function. The Royal Society of Chemistry, UK.