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Isolation and Properties of *B. carinata* Myrosinase

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To improve biofumigation effectiveness and its reproducibility outcome, the availability of plant material appears to be essential; this should show a stable composition of the starting bioactive molecules year after year. The possibility to control the kinetics of the glucosinolate-myrosinase system contained in Brassicaceae defatted seed meals, when this material is used as a biofumigant, is thus of fundamental importance, given its role in the production of the final active compounds.

Aim

The aim of this work was the study both of the physico-chemical properties of *B. carinata* (Bc) myrosinase (MYR), and its activity throughout the various phases of preparation and application of amendments for optimizing the biofumigant properties of the final product.

EXPERIMENTAL

To achieve the fixed aim we have:

- Purified the Bc MYR
- Determined the main properties of soluble and insoluble Bc MYR
- Defined a practical method to evaluate the enzyme activity in the biofumigant material and the release kinetics of the active compounds
- Used the results and information to optimize the amendment formulations based on Bc defatted meals

Soluble Bc MYR was extracted homogenizing the seeds in water, keeping the slurry in an ice-bath and then separating the solid phase by centrifugation. This procedure was repeated until no MYR activity was found in the surnatant (spectrophotometric method).

The pooled liquid extract was used as starting material to purify the soluble MYR whereas the solid residue, that still contains MYR activity, was used, without further manipulations, to characterize the insoluble enzyme (pH-Stat method).

The soluble MYR fraction appears to be about 40% of the total MYR activity contained in the seed and it was purified using the usual procedure.

Brassica carinata Myrosinase Purification

Step of purification	Activity (U mL^{-1})	Proteins (mg mL^{-1})	Specific Activity (U mg^{-1})	Purification fold
Crud extract	0.20	2.8	0.07	1
dialysis	0.13	1.00	0.13	2
Con-A-Sepharose	1.50	0.45	3.40	48
SP Sepharose	3.00	0.17	17.70	253
Superdex 200	37.1	1.85	20.10	287

The Mw of the soluble Bc MYR is similar to the well known Mw of *Sinapis alba* MYR, ranging around 140 kD, determined by gel filtration chromatography, with 2 subunits of ca. 70 kD (Fig. 1).

The activity of Bc MYR in its soluble and insoluble form has a similar trend as regards changing pH and temperature (Fig. 2 and 3).

In addition, no differences were found in enzyme activity on different substrates (sinigrin, nasturtin and sinalbin) for either of the enzyme forms.

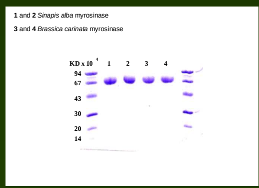

Figure 1. SDS-PAGE Electrophoresis pH 12 of MYRs

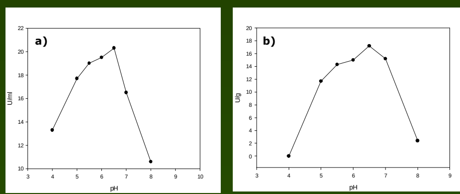

Figure 2. pH activity profiles of soluble (a) and insoluble (b) Bc MYR

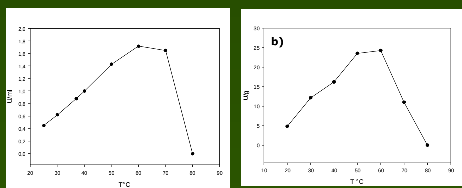

Figure 3. Temperature activity profiles of soluble (a) and insoluble (b) Bc MYR

These findings were essential in choosing the best parameter to control the glucosinolate hydrolysis and to define the best biofumigant formulation

In addition, using purified MYR and sinigrin, we defined an analytical procedure based on the headspace analysis of the allylisothiocyanate released from a well defined amount of meal to which water was added. This method is useful to explore the influence of different parameters on MYR activity and, in particular, the kinetics of allyl-isothiocyanate release (Figure 4).

Figure 4. Flask with defatted meal and water used to evaluate the allyl-isothiocyanate release over time by the headspace analysis

Using this procedure it was possible to verify the rate of allyl-isothiocyanate release from the defatted meals when pure Bc MYR (Fig. 5) or lab defatted meals containing MYR (Fig. 6) were added to the mixture. The aim of this experiment was to obtain the maximum isothiocyanate yield in a defined time, as well as the behaviour of the amendment when some parameters, such as the temperature, were changed (Fig 7).

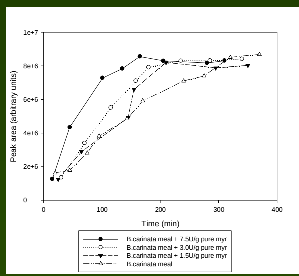

Figure 5. AITC released from industrial Bc meal with and without addition of pure Bc MYR in different amounts

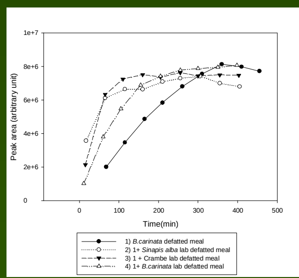

Figure 6. AITC from industrial Bc meal with and without addition of lab-defatted meal containing MYR from different Brassica seeds

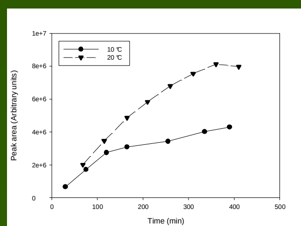

Figure 7. AITC released from industrial Bc meal at different temperatures

CONCLUSIONS AND PERSPECTIVES

Although the molecular properties of soluble Bc MYR appear similar to those of mustard MYR, the former seems to be more resistant to temperature. Comparable characteristics were also observed for the insoluble form. These findings are certainly an important item that should be considered in the amendment formulation groundwork process. To understand the behaviour of Bc MYR in defatted meals, especially when it is working in its natural conditions, a number of experiments are still in progress to optimize the amendment composition also in relationship with the target pathogen.

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