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Enzymatic hydrolysis of protein-rich biomass waste for the production of biostimulants

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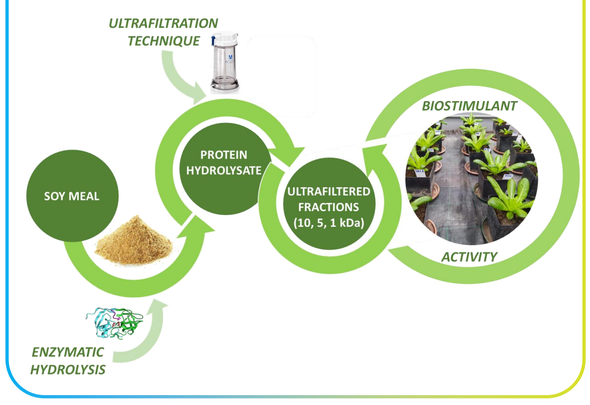
SPOKE, WP E TASK DI APPARTENENZA

Spoke: 8
WP: 8.1 «Producing new products to upgrade waste value»
Task: 8.1.1

ABSTRACT

After decades of agricultural innovation and practices primarily focused on increasing crop yields, current efforts in food systems are directed toward enhancing product quality and promoting the sustainability of food production. In this context, the identification of new inputs to address modern agricultural challenges has become a critical issue. Among technological innovations proposed by scientific community to improve the quality of agricultural products and make agriculture more sustainable, biostimulants represent a promising innovation [1]. This research project aims at developing straightforward, greener and cost-effective route for biostimulant preparation based on enzymatic hydrolysis of protein-rich biomass waste. Protein Hydrolysates (PHs) are mixtures of peptides and amino acids with a wide range of applications in many industrial sectors [2]. In particular, they can be used as biostimulants in horticulture due to their capacity to enhance crop quality parameters, nutrient efficiency and abiotic stress tolerance [1]. In order to develop an efficient protocol for PHs production starting from soymeal (SM), different commercial enzymatic mixtures (carbohydrases and proteases) and experimental conditions (T, pH, incubation time) were used to set-up SM hydrolysis and the process was scaled-up, using a 10L reactor. Soymeal hydrolysates (SMHs) obtained after centrifugation, filtration and concentration steps were characterized (%N and %C recovered, pH, salinity, degree of hydrolysis (DH), free and total amino acids). Moreover, their auxino-like activity on mung bean and biostimulant properties on lettuce were evaluated.

OVERVIEW OF THE PROJECT



MATERIALI/METODI/WORKFLOW

1. Enzymatic Hydrolysis. A screening of different carbohydrases formulations (Viscozyme® L, Ceremix® Plus MG, Celluclast® 1.5L and Ultraflo®) was preliminary performed on soymeal (SM) [3]. As second step, SM was suspended in distilled H_2O in a 10L reactor, and the resulting mixture was treated with a pool of carbohydrase and protease formulations, under mechanical stirring. Reaction conditions were set according to optimal temperature and pH of enzyme, while regarding enzymes combination, an average of temperature and pH optimal values for single enzymes were used [3,4]. At the end of the hydrolysis, each reaction mixture was centrifuged to separate the supernatant, *i.e.* the Soymeal Hydrolysate (SMH), from the solid residue, *i.e.* materials not hydrolysed.

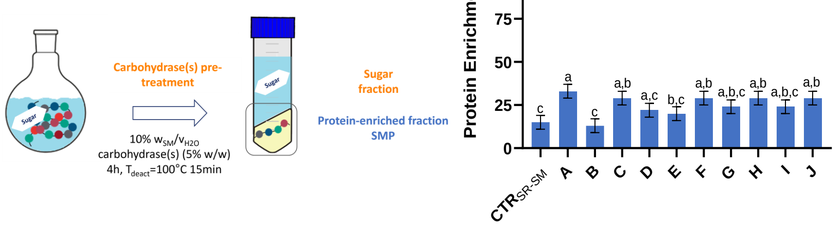
2. Characterization of SMHs obtained. SMHs obtained using different experimental conditions, *i.e.* P2303 and P2304, were characterized. Yield (Y, w/w % dry matter basis) was calculated with respect to the mass of SM introduced at the beginning of the hydrolysis; % N and % C recovered in the final SMHs were calculated with respect to N and C of the starting SM, by elemental analysis. Degree of hydrolysis (DH) was determined using the OPA-NAC method [5], with some modifications. The free and total amino acid content was determined by HPLC.

3. Auxino-like activity and biostimulant properties of SMHs obtained. Auxino-like activity was evaluated on Mung Bean, according to [6]. For biostimulants assay, lettuce plants were grown in an experimental greenhouse under controlled conditions (10 plants per m^2). Nutrients were directly added to the inhert substrate by providing different amount of slow release granular fertilizer (composition 14:7:17 N:P:K); in the case of nutritional stress conditions (CTR 70), the amount of fertilizer was reduced to 70%. SMHs A (lab-scale) and B (industrial-scale) were sprayed on each plant by foliar application (dose 2 kg/ha, number of applications 2). Post harvest, yield was calculated considering a plant density of 10 plants per square meter.

RISULTATI

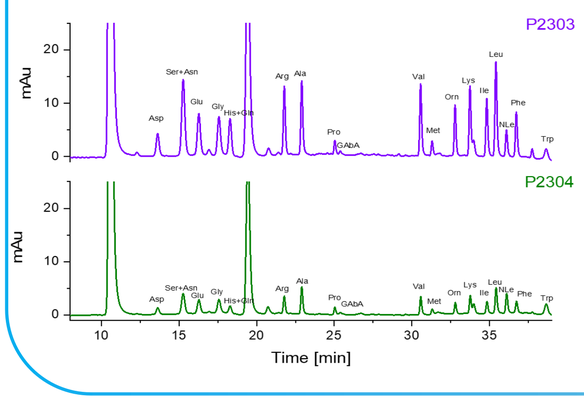
1. Enzymatic Hydrolysis: carbohydrases pre-treatment

Enzymatic hydrolysis	Enzyme trade name	pH	Temperature (°C)
A	Viscozyme® L	5.0	50
B	Ultraflo® L	4.5	60
C	Celluclast® 1.5L	4.5	50
D	Ceremix® Plus MG §	6.0	50
E	Viscozyme® L + Ultraflo® L	4.7	55
F	Viscozyme® L + Celluclast® 1.5L	5.0	50
G	Viscozyme® L + Ceremix® Plus MG §	5.5	50
H	Ultraflo® L + Celluclast® 1.5L	4.5	55
I	Ultraflo® L + Ceremix® Plus MG §	5.3	55
J	Celluclast® L + Ceremix® Plus MG §	5.3	50



The Protein Enrichment after carbohydrases(s) pre-treatment was evaluated based on the formula reported above. A statistical factor of 6.25 was used to convert the results from elemental analysis into the percentage of protein (%P). A control (CTR_{SR-SM}) was also prepared (T=50°C, pH=5), without the addition of any enzyme. Differences between enzymes employed for SM hydrolysis resulted statistically irrelevant, as all carbohydrases were able to partially hydrolyze carbohydrates contained in SM.

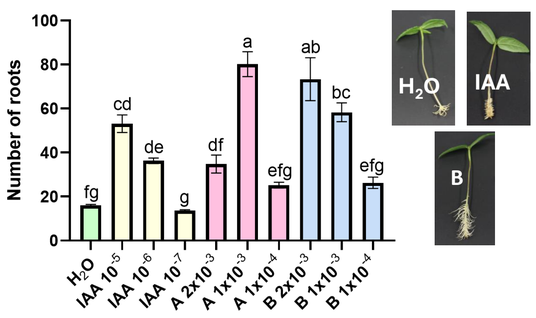
2. Characterization of SMHs obtained



Reaction Code	Y (%)	% N	% C
P2303	42	56	41
P2304	37	57	40

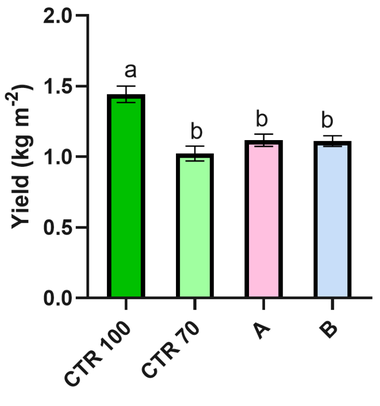
Reaction Code	pH	Salinity (dS/m)	DH (%)	Free AA % (w/w)	Total AA % (w/w)
P2303	6.0	2.9	59	22	31
P2304	6.4	2.6	40	8	33

3. Auxino-like activity



As reported in graph, both **A (lab-scale SMH)** at concentration $1x10^{-3}$ v/v and **B (industrial-scale SMH)** at concentration $2x10^{-3}$ v/v and $1x10^{-3}$ v/v present a higher mean number of adventitious roots than control (H_2O) and hormone Indole-3-Acetic-Acid (IAA). SMHs tested at specific concentration have an auxin-like effect on mung bean.

3. Biostimulant properties – nutritional stress condition



As reported in graph, there are significative differences between control and nutritional stress in terms of yield. Nevertheless, the application of both **A** and **B** does not improve significantly the productivity.
Moreover, other parameters such as carotenoids, chlorophyll *a+b* and total and reducing sugars in lettuce leaves do not show any significant differences in response to the treatment.

REFERENZE

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