CELLULAR ANTIOXIDANT PROPERTIES OF *Brassica napus* PROTEIN HYDROLYSATES

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Introduction. Bioactive peptides as products of the hydrolysis of diverse food proteins by microbial fermentation, enzyme digestion or proteolysis *in vitro* by different enzymes have unique intrinsic properties that make them attractive therapeutic agents (1). Such functionalities may include antioxidative (2). Sequences of potential bioactive peptides encrypt within a food protein can be predicted by an *in silico* analysis using different databases to achieve a desirable activity. The processing of Canola (*Brassica napus*) for oil production generates an industrial by-product which can be used as an important protein source (˜34%) for bioactive peptides production.
Thus the aim of this work was to evaluate cellular and *in vitro* antioxidant properties of eighteen protein hydrolysates obtain from the hydrolysis of *Brassica napus* protein isolate with two different enzyme combinations, and correlated with their amino acid composition, peptidic profile, MW distribution and the *in silico* bioactivity prediction.

Methods. Bioinformatic analysis was carried using the Biopep database of bioactive peptides, and protein sequences from Protein Data Bank database. Then different hydrolysates were prepared by the treatment of canola isolate with Alcalase and Flavourzyme or Pepsin and Pancreatin. Antioxidant properties were evaluated in cell line Caco-2 by the DCF assay (3) and correlated with the activity previously observed by *in vitro* assays (TEAC by ABTS radical decolorization assay, DPPH radical scavenging, reducing power and β-carotene bleaching). Peptidic and aminoacid profile was obtained by HPLC (4) and MW distribution by FPLC (5).

Results. Analysis using a bioactive peptides database showed 10 different potential biological activities in *Brassica napus* proteins (Fig. 1).

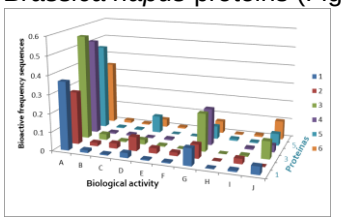

FIg. 1 Predicted profiles of bioactive peptides in main Brassica napus storage proteins (1-4), and reference proteins (5,6). A = ACE inhibitor; B = activating ubiquitin-mediated proteolysis; C = antiamnestic;D = antioxidative; E = antithrombotic; F = hypotensive; G = immunomodulating; H = inhibitor (dipeptidyl-aminopeptidase IV, CaMPDE and neuropeptide inhibitor are included); I = regulating; J = opioid; K = bacterial permease ligand; L = immunostimulating.

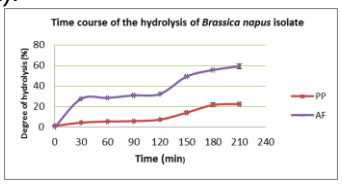

FIg. 2 Time course of the hydrolysis of: PP) *Brassica napus* isolate by Pepsin (added at time 0) and Pancreatin (added 120 min later), AF) *Brassica napus* isolate by Alcalasa (added at time 0) and Flavourzyme (added 120 min later) Data correspond to the average ±SD of three determinations.

Experimental and *in silico* data also indicates that both enzymatic systems were successful in release peptides (Fig 2) capable of preventing oxidative stress in the cells at a concentration- dependent way, the best respond were found with the hydrolysate obtain with Alcalasa (60 min) with a 50% of inhibition achieved with 4.5 ug/ul, this hydrolysate also showed the highest activity in the β-carotene bleaching inhibition assay (76.59%), and antiradical activity against DPPH (36.20% ARA) in comparison to the other hydrolysates, when assayed at a concentration of 5ug/ul.

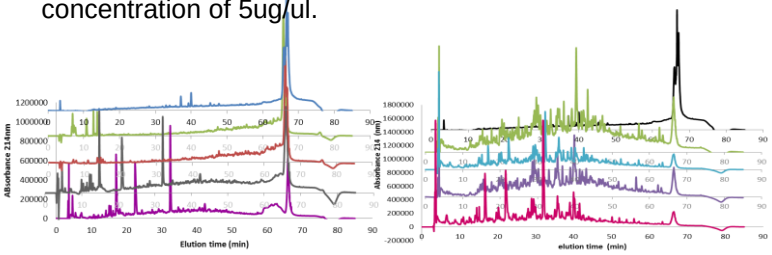

Fig 3 Analytical C18 reverse-phase HPLC time course of the peptidic profile of *Brassica napus* hydrolysis with a)pepsin-pancreatin enzymes and b)alcalase-flavourzyme.

Some others hydrolysates were found with a significant *in vitro* antioxidant activity but less effective in decreased the reactive oxygen species (ROS) in the cell line, these can be explain by the differences found in their peptidic profile and MW distirbution Fig. 3. So these results generated useful information for production of bioactive ingredients and their further application in health-promoting foods.

Conclusions. Antioxidant peptides are abundant in B*rassica napus* enzyme produced hydrolysates therefore value-adding to canola meal is possible via generation of bioactive peptides for eventual use as dietary supplements.
Bioinformatic analysis was a useful tool for explaining how released peptides may be contributing to antioxidant properties of protein hydrolysates.
Further studies are needed to analyze interactions between peptide, structure-activity relationships and *in vivo* futures of peptides.

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