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# FUNGAL PROTEASE INDUCED HYDROLYSIS OF PUMPKIN SEED AND RAPESEED CAKE EFFECTS THEIR ANTIOXIDATIVE ACTIVITY AND DIGESTIBILITY

Sandra Balbino, Marija Barišić, Marko Obranović, Klara Kraljić, Dubravka Škevin, Duška Ćurić
*Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia*

## INTRODUCTION

Nowadays, with growing demand for animal protein alternatives, new potential sources are being evaluated. Plant proteins are however usually deficient substitutes mainly because of their limited low solubility and related digestibility. Oil seed cakes are one of the materials with great potential as sources of plant protein due to the high content and also low market value that enables very high added-value of possible products. Pumpkin seed and rapeseed cake remain as by-products in the process of oil production and are usually used for feed. However, due to their high protein levels as well as the content of antioxidants and other important bioactive compounds they can potentially be used in the production of dietary supplements and functional food. The aim of this study was to perform enzymatic hydrolysis of pumpkin seed and rapeseed cakes and to determine its effect on the degree of hydrolysis, antioxidant activity and *in vitro* digestibility.

## RESULTS

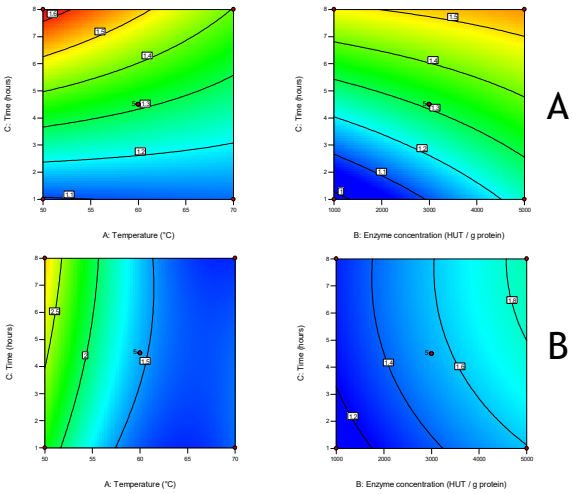



Figure 1. Contour plots for the effect of enzyme concentration, time of treatment and incubation temperatures on degree of hydrolysis of fungal protease treated pumpkin seed (A) and rapeseed (B) cake samples

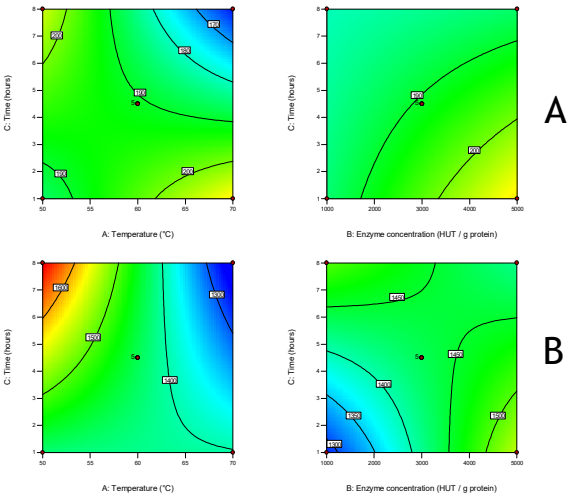
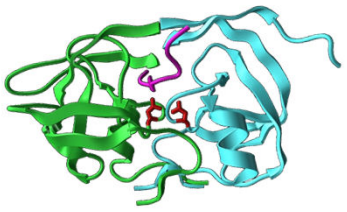



Figure 3. Contour plots for the effect of enzyme concentration, time of treatment and incubation temperatures on antioxidant activity measured by FRAP (mmol Trolox / g) of fungal protease treated pumpkin seed (A) and rapeseed (B) cake samples



## MATERIALS AND METHODS

Pumpkin seed and rapeseed cake samples were grinded in "CryoMill" (Retsch, Germany) for 12 minutes and defatted for 5 hours by petroleum ether in Soxhlet apparatus. The fungal protease enzymatic hydrolysis was carried out with varying enzyme concentration (1000, 3000 and 5000 HUT/g protein), time of treatment (1, 4.5, 8 hours) and at different incubation temperatures (50, 60 and 70 °C). A total of 17 experiments according to the Box-Behnken experimental design was performed in two batches. First batch of hydrolyzed samples was centrifuged and supernatants were used for the determination of degree of hydrolysis and antioxidant activity. Degree of hydrolysis was determined using formol titration method (AOAC, 1995) while the antioxidant activity was measured by DPPH and FRAP methods (Čukelj et al., 2015) Second batch of samples was lyophillized and used for the determination of *in vitro* digestibility performed by Megazyme kit (K-PDCAAS 06/18).

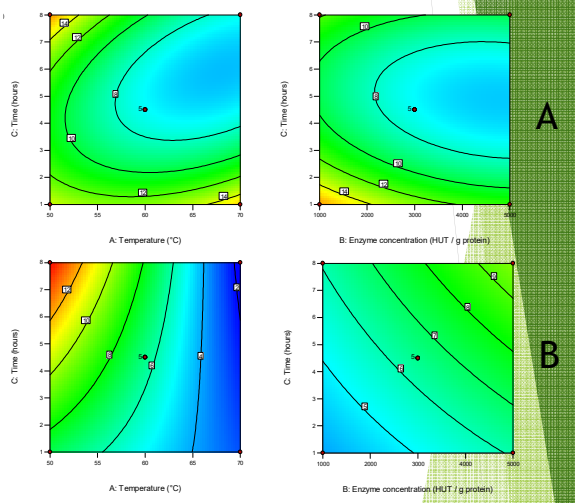



Figure 4. Contour plots for the effect of enzyme concentration, time of treatment and incubation temperatures on protein digestability measured as amine concentration (mM/g) of fungal protease treated pumpkin seed (A) and rapeseed (B) cake samples

## CONCLUSIONS

1. Degree of hydrolysis showed that enzyme treatments were efficient in all samples. Hydrolysis was however higher in rapeseed cake in comparison to pumpkin seed cake. Degree of hydrolysis also increased with longer treatment duration and higher enzyme concentration and decreased with higher incubation temperatures.
2. Rapeseed cake also had better antioxidative activity measured by both DPPH (dana not shown) and FRAP method. Highest antioxidative activity measured by DPPH method was determined in rapeseed cake sample treated at 60 °C with 1000 HUT/g protein for 1 hour, while FRAP method showed highest result for rapeseed cake incubated for 4.5 hours at 50 °C and 5000 HUT/g protein. Higher enzyme concentrations significantly increased antioxidative activity of hydrolysates.
3. Digestibility was higher in all hydrolysed samples compared to the control. It was however decreased with higher enzyme concentrations and temperatures.
4. Overall, these results show that fungal protease enzymatic treatment can efficiently increase nutritional properties of pumpkin seed and rapeseed oilcakes.