**Hempseed protein hydrolysates-antioxidative and anticancer effects**

Višnja Gaurina Srček\*, Kristina Radošević, Marijan Logarušić, Igor Slivac, Ivana Radojčić Redovniković

*University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory for Cell Technology and Biotransformation, Pierottijeva 6, 10000 Zagreb, Croatia*

*\*Corresponding author: vgaurinasrcek@pbf.hr*

**Highlights**

* Hempseed protein hydrolysates possess antioxidative activity.
* Hempseed protein hydrolysates showed antiproliferative effects in HeLa cells.
* Protective effects of hydrolysates were observed during H2O2 induced oxidative stress.
* Enzyme hydrolysis of hempseed proteins resulted in high value-added products.

**1. Introduction**

In recent years, many research groups had focused on the production of peptides/hydrolysates with potential application in food and nutraceutical industry. Protein hydrolysates or bioactive peptides derived from food proteins have been reported to exhibit a wide range of bioactivity including immunomodulatory, anticancer, antihypertensive, antioxidant, osteoprotective and antimicrobial effects [1]. Industrial hemp (*Cannabis sativa* L) seed is by-product obtained after utilization of plant fibers and is used as oil and protein source. It contains 20-25% of proteins and its amino acid profile showed similar or even higher level of essential amino acids (except for lysine) in comparison to soy proteins [2]. Recent studies have reported antioxidative properties of hempseed protein hydrolysates obtained by enzyme *Alcalase® 2.4L* on induced oxidative stress in PC12 cells [3]. However, more studies are needed to investigate effects on hempseed protein hydrolysates obtained by other enzymes on different cells. The aim of this study was to prepare hempseed protein hydrolysate (HPH) from hempseed protein isolate (HPI) by three commercial enzymes: *Alcalase® 2.4L, Neutrase and Protamex*. In addition, biological potential of prepared HPHs was measured by ORAC assay as well as their effects on viability and cellular changes of normal and tumor cells.

**2. Methods**

HPHs were prepared using the commercial enzymes *Alcalase® 2.4L, Neutrase and Protamex* as described by [3]. Antioxidant activity of obtained HPHs was assayed by ORAC method. Effects of HPHs on proliferation of normal (HaCaT) and tumor (HeLa) cells were determined by colorimetric MTS assay while cellular changes were assayed by spectrofluorimeter and MUSE cell analyzer.

**3. Results and discussion**

Prepared HPHs showed strong antioxidant activity (ORAC values in range 576.52±36.5 - 695.2±6.75 μM TE/g protein) in comparison to 32.6±2.35 μM for HPI, indicating releasing of antioxidant peptides during hydrolysis process. Tested hydrolysates showed cytotoxic activities toward tumor HeLa cells with no effects on normal HaCaT cells (Figures 1-2). Similar results of concentration-dependent antiproliferative effects of germinated soybean protein hydrolysates on breast and cervical cancer cell lines with minimal effects on normal cells was reported by [4]. Observed antiproliferative effects of HPHs in tumor cells suggested relationship between cytotoxicity and antioxidant activity also indicating their potential as functional food ingredient in anticancer therapy.

**Figure 1.** Effects of HPHs on HaCaT cells by MTS assay **Figure 2.** Effects of HPHs on HeLa cells by MTS assay

The ability of HPH-N to protect HaCaT and HeLa cells against oxidative stress induced by H2O2 is shown in Figure 3. Pretreatment of cells with HPH-N significantly reduced ROS (+) cells on concentration-dependent manner (29.13-40.5% for HPH-N *vs.* 44.2% for H2O2 in HaCaT cells and 27.03 -32.87% for HPH-N *vs.* 54.8% for H2O2 in HeLa cells) indicating its protective role against ROS formation.

**Figure 3.** Protective effects of HPH-N on induced oxidative stress in HaCaT (left) and HeLa (right) cells.

**4. Conclusions**

The results indicate that hempseed protein hydrolisys results in releasing peptides with antioxidative and anticancer activities and might be considered as functional food ingredient.

**References**

1. M. Chalamaiah, W. Yu, J. Wu, Food Chem. 245 (2018) 205-222.
2. J.C. Callaway, Euphytica 140 (2004) 65-72.
3. C.H. Tang, X.S. Wang, X.Q. Yang, Food Chem. 114 (2009) 1484-1490.
4. M. González Montoya, E. Ramón-Gallegos, M.C. Robles-Ramírez, R. Mora-Escobedo, Plant Foods Hum. Nutr. 71 (2016) 368-374.