Dry-curing ham derived peptide (Asp-Leu-Glu-Glu) regulated the antioxidant enzymes activities in Caco-2 cells

Lujuan Xing, Wangang Zhang

College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China,

Introduction: Dry-cured hams are well-known and highly appreciated products in Mediterranean area and China. The long term fermentation endows the unique flavor and quality of dry-cured hams. In our previous study on Chinese Xuanwei hams, antioxidant peptide Asp-Leu-Glu-Glu (DLEE) was identified with significant activity on scavenging free radicals (Xing, Hu, Hu, Ge, Zhou & Zhang, 2016). In current study, the Caco-2 cells were cultured *in vitro* and treated with different dose of DLEE and the cellular ROS level and antioxidant enzymes activities were determined to investigate the intracellular protection effect of DLEE.

Materials and methods:

1. The cellular antioxidant activity

   The cell-based cellular antioxidant activity (CAA) of hydrolysates was determined using the method described by Torres-Fuentes, Maria, Recio, Alaiz & Vioque (2015). The CAA value was calculated based on the following equation:

   CAA unit= 100 - (∫ SA/ ∫ CA) *100

   Where ∫ SA is the integrated area of the sample curve and ∫ CA is the integrated area of the control curve.
2. Reactive oxygen species (ROS)

   The amount of ROS in Caco-2 cell was investigated following with the DCFH-DA method. The detection of fluorescence was measured with 485 nm excitation wavelength and 535 nm emission wavelength.
3. The antioxidant enzyme activity

   The activities of catalase (CAT), glutathione S transferase (GST) and glutathione peroxidase (GPx) were measured using the assay Kit (Nanjing Jiancheng Bioengineering Institute) according to the protocol.

Results:

1. The cellular ROS detection in Caco-2 cell.

   The fluorescent images of Caco-2 cells were presented with treatment of 1.0 mg/mL of DLEE and GSH. Compared with control group, the fluorescence spots of H_2O_2 stimulated group were improved. In the treatment group of DLEE, the spots were reduced significantly indicating that the ROS generation could be inhibited by DLEE incubation.
2. The cellular antioxidant activity of DLEE in Caco-2 cell model.

   With the increase of concentration, the cellular radical scavenging activities of DLEE and GSH were all shown to be enhanced slightly. Under the same concentration of 0.5 mg/mL, DLEE was measured to have the CAA unit of 32.4, which was nearly same with that of protein hydrolysates from eggshell membrane of 32.2 (Shi, Kovacs-Nolan, Jiang, Tsao & Mine, 2014). The CAA unit indicates that DLEE peptide would also exhibit free radical scavenging activity in intracellular condition.
3. The activities of antioxidant enzymes

   Stimulated by H_2O_2, the activity of GPX, GST and CAT all decreased compared with control group. After incubated by DLEE, the enzyme activities were posed to be alleviated in dose manner. In special of CAT, the activity increased from 32.5 U/mg to 38.7 U/mg under the concentrations of 0.5 and1.5 mg/mL DLEE incubation. Overall, the pretreatment of DLEE showed the favorable effect on the cytoprotection on Caco-2 cells under the oxidative stress condition.

Conclusions: The cellular ROS level was shown to be reduced whereas the antioxidant enzymes activities were improved in DLEE treatment. These results suggest that DLEE could have remarkable antioxidant capacity in Caco-2 cells.

Acknowledgements and Financial support statement: This work was financially supported by the National Natural Science Foundation of China (32001720).

References:

Shi, Y., Kovacs-Nolan, J., Jiang, B., Tsao, R., & Mine, Y. (2014). Antioxidant activity of enzymatic hydrolysates from eggshell membrane proteins and its protective capacity in human intestinal epithelial Caco-2 cells. Journal of Functional Foods, 10, 35-45.

Torres-Fuentes, C., Contreras, M. D. M., Recio, I., Alaiz, M., & Vioque, J. (2015). Identification and characterization of antioxidant peptides from chickpea protein hydrolysates. Food Chemistry, 180, 194-202.

Xing, L. J., Hu, Y. Y., Hu, H. Y., Ge, Q. F., Zhou, G. H., & Zhang, W. G. (2016). Purification and identification of antioxidative peptides from dry-cured Xuanwei ham. Food Chemistry, 194, 951-958.