High Pressure Processing Assisted Enzymatic Hydrolysis of 1 Corbicula fluminea and Evaluation of Its Anti-cancer Activity 2 3 陳佳琳 (5107) 4 2022/04/27 Outline 5 6 1. Introduction 7 2. The optimum product conditions for high pressure processing assisted enzymatic 8 hydrolysis of *Corbicula fluminea* hydrolysate (pCFH) 9 3. Effects of pCFH on cell survival and chemosensitivity of human breast cancer 10 MDA-MB-231 cells Effects of pCFH on cell apoptosis and migration of human breast cancer MDA-MB-11 4. 12 231 cells 13 5. Summary Abstract 14 15 Corbicula fluminea hydrolysate shows physiology functions such as 16 hepatoprotection, antioxidant, and anticancer. High pressure processing (HPP) can 17 improve the degree of hydrolysis and increase the biological activity of peptides. This 18 study aims to use HPP-assisted enzymatic hydrolysis of C. fluminea (pCFH), and to 19 evaluate pCFH effects on anticancer ability in human triple negative breast cancer 20 (TNBC) MDA-MB-231 cells. Response surface methodology found that the optimum condition for pCFH preparation was 40 grams of C. fluminea powder hydrolyzed with 21 22 2% of Umamizyme G for 3 hours, 1% of pepsin for 1 hour, and extracted under 450 MPa 23 for 15 minutes. The contents of soluble protein and peptide were 1.98 mg/mL and 450.98 24 mg/mL, respectively; and the IC<sub>50</sub> of pCFH on MDA-MB-231 cells was 0.89 mg/mL. 25 Subsequently, treatment with doxorubicin (IC<sub>50</sub> 215 nM) and supplied with pCFH (0.45, 26 0.90, and 1.80 mg/mL) inhibited the cell viability to 86.2%, 73.1%, and 62.8% of control. 27 Treatment with paclitaxel (IC<sub>50</sub> 45.6 nM) and supplied with pCFH (0.45, 0.90, and 1.80 28 mg/mL) also decreased the cell viability to 85.8%, 70.8%, and 58.3% of control. Annexin 29 V and PI double staining demonstrated that treatment with pCFH (0.45, 0.90, and 1.80 30 mg/mL) for 36 hours increased early apoptotic cells to 188.4%, 224.0%, and 259.8% of 31 control. Furthermore, treatment with pCFH (0.45, 0.90, and 1.80 mg/mL) reduced wound 32 healing (90.2%, 55.3%, and 49.3% of control), and cell migration (67.8%, 60.4%, and 33 40.4% of control). In summary, pCFH increased chemosensitivity, and inhibited cell 34 migration, as well as induced cell apoptosis of TNBC cells. Hence, pCFH is a promising 35 dietary agent for attenuation of TNBC.

1	References
2	Chen, T. Y., Lin, B. C., Shiao, M. S., & Pan, B. S. (2008). Lipid-lowering and LDL-
3	oxidation inhibitory effects of aqueous extract of freshwater clam (Corbicula
4	fluminea)-using tilapia as an animal model. Journal of Food Science, 73(7), H148-
5	54.
6	Liao, N., Chen, S., Ye, X., Zhong, J., Wu, N., Dong, S., Yang, B., & Liu, D. (2013).
7	Antioxidant and anti-tumor activity of a polysaccharide from freshwater clam,
8	Corbicula fluminea. Food & Function, 4, 539-548.
9	Franck, M., Perreault, V., Suwal, S., Marciniak, A., Bazinet, L., & Doyen, A. (2019).
10	High hydrostatic pressure-assisted enzymatic hydrolysis improved protein digestion
11	of flaxseed protein isolate and generation of peptides with antioxidant activity. Food
12	Research International, 115, 467-473.