World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:7, No:4, 2013

Production of WGHs and AFPHs using Protease Combinations at High and Ambient Pressure

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***Abstract*—**Wheat gluten hydrolyzates (WGHs) and anchovy fine powder hydrolyzates (AFPHs) were produced at 300 MPa using combinations of Flavourzyme 500MG (F), Alcalase 2.4L (A), Marugoto E (M) and Protamex (P), and then were compared to those produced at ambient pressure concerning the contents of soluble solid (SS), soluble nitrogen and electrophoretic profiles. The contents of SS in the WGHs and AFPHs increased up to 87.2% according to the increase in enzyme number both at high and ambient pressure. Based on SS content, the optimum enzyme combinations for one-, two-, three- and four-enzyme hydrolysis were determined as F, FA, FAM and FAMP, respectively. Similar trends were found for the contents of total soluble nitrogen (TSN) and TCA-soluble nitrogen (TCASN). The contents of SS, TSN and TCASN in the hydrolyzates together with electrophoretic mobility maps indicates that the high-pressure treatment of this study accelerated protein hydrolysis compared to ambient-pressure treatment.

***Keywords*—**Production, Wheat gluten hydrolyzates, Anchovy fine powder hydrolyzates, Protease combinations.

I. Introduction

ONE important new enzyme technology that gains recent attention is enzyme reaction at high pressure. Stability and activity of several enzymes are increased at specific conditions of high pressure due to modification of catalytic behavior, modulation of enzyme selectivity or increase in Vmax, which results in the increases in product yield and reaction rate [1-3]. In light of these reports, application of high pressure to enzymes such as proteases is expected to provide considerable advantages in some food processes such as production of protein hydrolyzates with improved sensory property and functionality, and synthesis of functional peptides. Meanwhile, high-pressure treatment can influence on hydrogen bonds and alter three-dimensional configuration of enzyme molecules.

Therefore, the effect of high-pressure treatment on enzyme inactivation has to be studied in details and appropriate selection for high-pressure tolerant enzymes seems to be required to prevent possible enzyme inactivation caused by high pressure [4-6]. Medium high pressure that is normally referred to the pressure range of 100-400 MPa is important for high-pressure enzyme processes because enzyme activity may be maintained or even enhanced without significant inactivation caused by change in covalent bonding [7, 8]. Therefore, it is quite recommendable that purposed high-pressure treatment on food enzymes be done at the above pressure range.

Proteases belong to the most important class of food enzymes that have a share about 60% in the world industrial enzyme market [9, 10]. They are, in most cases, produced from natural resources and are used in a number of biotechnological processes, including baking, fermentation, cheese preparation and ripening, meat tenderization and production of protein hydrolyzates [10-13]. As a previous work to apply high-pressure technology to protease-related food processes, we tested pressure susceptibility of various reagent-grade and industrial proteases at the hydrostatic high pressure of 100 and 300 MPa, and found that some proteases such as trypsin and Alcalase 2.4L held good pressure tolerance [6].

In this study, we prepared hydrolyzates from wheat gluten (WG) and anchovy fine powder (AFP) by the high-pressure treatment at 300 MPa, exploiting combinations of Flavourzyme 500MG (F), Alcalase 2.4L (A), Marugoto E (M) and Protamex (P). The reaction variables of the hydrolyzates produced at 300MPa, such as soluble solid (SS) and soluble nitrogen, and electrophoretic profiles were compared to those of the hydrolyzates produced at ambient pressure.

II. Materials and Methods

A. Reagents and Materials

Flavourzyme 500MG (aminopeptidase, from Aspergillus oryzae), Alcalase 2.4L (subtilisin, from Bacillus licheniformis), Marugoto E and Protamex (from B. licheniformis and B. amyloliquefaciens) were used to produce hydrolyzates from WG and AFP. From these enzymes, Flavourzyme 500MG, Alcalase 2.4L and Protamex were products of Novozymes A/S (Bagsvaerd, Denmark) and Marugoto E was a product of Supercritical Technology Research Corporation (Hiroshima, Japan). WG was obtained from Sigma (St. Louis, MO, USA) and AFP was purchased from a local market. All other chemicals used were guaranteed reagents from various suppliers and double distilled water was used. Vinyl pouch was used for the production of protein hydrolyzates at hydrostatic high pressure of 300MPa.

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B. Operating Procedure of High-Pressure Equipment

A vinyl pouch filled with a protein suspension and the enzyme(s) was treated using single-vessel (150 mL capacity) high-pressure equipment (DP-SHPL-015L-400, Dima Puretech, Incheon, Korea) [6]. The pouch was submerged in the pressure vessel of the equipment, equilibrated with distilled water that was used as the pressure-transmitting fluid at 50°C and then the vessel was closed with a vessel cover. After a water-feeding valve was opened, pressure was built up with a hand-pressurizing pump up to 10 MPa within 10 s. Then, an automatically pressurizing pump that was controlled with a controller of temperature and pressure was used to build up pressure slowly at a constant rate of 100MPa/min to minimize adiabatic heating until the preset pressure of 300MPa was reached. To conduct protein hydrolysis, the vessel was maintained at this pressure for a pre-determined time and then was decompressed within a few seconds by opening an air vent after closing the water-feeding valve. The pouch in the vessel was taken and was treated as described in C.

C. Production of Protein Hydrolyzates

Wheat gluten hydrolyzates (WGHs) and anchovy fine powder hydrolyzates (AFPHs) were produced using combinations of F, A, M and P as follows. A 12% (w/v) substrate suspension was made in 0.1 M phosphate buffer (pH 7.0). After adjusting temperature of the suspension to 50°C, single or combined enzymes was individually added to the suspension. After hydrolyzing it for 1 h at 300 MPa or ambient pressure, the reaction mixture was heat-treated to remove the remaining enzyme activity and was centrifuged immediately at 10,000 ×g at 10°C for 30 min. The supernatant was used as the corresponding protein hydrolyzate.

D. Analysis of Protein Hydrolyzates

SS, total soluble nitrogen (TSN) and TCA-soluble nitrogen (TCASN) were determined as indices of enzyme hydrolysis. Also, 12% SDS-PAGE was conducted to trace progression of enzyme hydrolysis.

III. Results and Discussion

When electrophoretic analysis using 12% SDS-polyacrylamide gel was conducted for the protein hydrolyzates prepared from WG and AFP, exploiting combinations of F, A, M and P, fast-migrating major peptide bands around several thousand daltons were found for all hydrolyzates produced. For examples, Fig. 1 shows electropherograms and the corresponding electrophoretic mobility maps of the WGHs and AFPHs prepared by one- and three-enzyme hydrolysis.

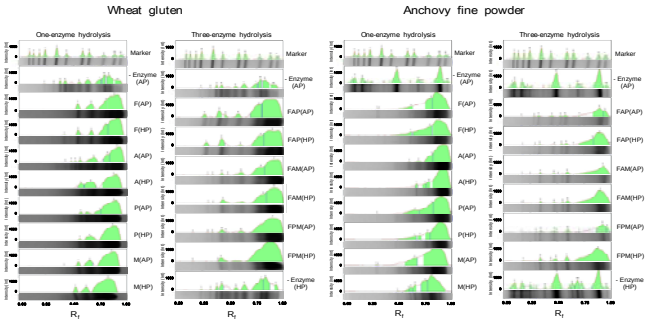

Fig. 1 Electropherograms and the corresponding electrophoretic mobility maps of the WGHs and AFPHs prepared by one- and three-enzyme hydrolysis at ambient pressure (AP) and 300 MPa (HP) at 50°C for 1 h. The molecular masses, in kDa, of peak 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 (from left to right) in the electrophoretic mobility map of Marker lane were 250, 150, 100, 75, 50, 37, 25, 20, 15, 10, 5 and 2, respectively.

As evident from Fig. 1, electrophoretic mobility of the hydrolyzates greatly increased after the enzyme treatments at high and ambient pressure. As a result, the protein bands with good mobility up to the Rf of 1 became predominant for both hydrolyzates from WG and AFP.

The triplicate SS of the WGHs and AFPHs increased up to 87.2% according to the increase in enzyme number used for hydrolysis both at high and ambient pressure. Particularly, it was found that high-pressure enzyme hydrolysis increased SS content compared to ambient-pressure hydrolysis. This fact clearly indicates that proteolytic cleavage was favored at high pressure. Based on SS content, the optimum enzyme combinations for one-, two-, three- and four-enzyme hydrolysis were determined as F, FA, FAM and FAMP, respectively.

For the enzyme combinations of F, FA, FAM and FAMP for one-, two-, three- and four-enzyme hydrolysis, the contents of TSN and TCASN were measured. The same trend as SS content was found. That is, the contents of TSN and TCASN obtained by the high-pressure treatment at 300 MPa were conspicuously higher than those obtained by ambient-pressure treatment. Also, the increase in enzyme number used for hydrolysis increased the contents of TSN and TCASN both at high and ambient pressure. Degree of hydrolysis nitrogen (DHN) that was expressed as the percentage ratio of TCASN against total nitrogen also increased from 3.0 to 72.0% and 1.8 to 64.0% at the high-pressure treatment at 300 MPa and ambient-pressure treatment, respectively.

IV. Conclusion

Protein hydrolyzates were produced from WG and AFP at the high pressure of 300 MPa and ambient pressure, exploiting combinations of F, A, M and P. Comparison of SS, TSN and TCASN, together with electrophoretic analysis, indicates that the high-pressure process of this study accelerated protein hydrolysis conspicuously compared to the ambient-pressure counterpart. Therefore, the process might be applied efficiently to other biotechnological processes, such as biotransformation, to improve conversion efficiency by increasing reaction rate at

high-pressure condition.

ACKNOWLEDGMENT

This study was carried out as one part of the research project of Development of High Pressure Process for Foods, Korea Food Research Institute, Republic of Korea..

REFERENCES

[1] Balny, C. (2006). What lies in the future of high-pressure bioscience? Biochim. Biophys. Acta 1764:632-639.
[2] Heremans, K. and Smeller, L. (1998). Protein structure and dynamics at high pressure. Biochim. Biophys. Acta 1386:353-370.
[3] Vila Real, H.J., Alfaia, A.J., Calado, A.R.T., and Ribeiro, M.H.L. (2007). High pressure temperature effects on enzymatic activity: Naringin bioconversion. Food Chem. 102:565-570.
[4] Katsaros, G.I., Katapodis, P., and Taoukis, P.S. (2009). High hydrostatic pressure inactivation kinetics of the plant proteases ficin and papain. J. Food Eng. 91:42-48.
[5] Borda, D., Indrawati, Smout, C., Van Loey, A., and Hendrickx, M. (2004). High pressure thermal inactivation of a plasmin system. J. Dairy Sci. 87:2351-2358.
[6] Kim, N., Maeng, J.-S., and Kim, C.-T. (2013). Effects of medium high pressure treatments on protease activity. Food Sci. Biotechnol. 22:289-294.
[7] Curl, L. and Jansen, E.F. (1950). The effect of high pressure on pepsin and chymotrypsinogen. J. Biol. Chem. 185:716-723.
[8] Yaldagard, M., Mortazavi, S.A., and Tabatabaie, F. (2008). The principles of ultra high pressure technology and its application in food processing/preservation: A review of microbiological and quality aspects. Afr. J. Biotechnol. 7:2739-2767.
[9] El Enshasy, H., Abuoul-Enein, A., Helmy, S., and El Azaly, Y. (2008). Optimization of the industrial production of alkaline protease by Bacillus licheniformis in different production scales. Aust. J. Basic Appl. Sci. 2:583-589.
[10] Kumar, C.G. and Takagi, H. (1999). Microbial alkaline proteases: from a bioindustrial viewpoint. Biotechnol. Adv. 17:561-594.
[11] Dayanandan, A., Kanagaraj, J., Sounderraj, L., Govindaraju, R., and Rajkumar, G.S. (2003). Application of an alkaline protease in leather processing: an ecofriendly approach. J. Clean. Prod.11:533-536
[12] Gusek, T.W. and Kinsella, J.E. (1987). Purification and characterization of the heat-stable serine proteinase from Thermomonospora fusca YX. Biochem. J. 246:511-517.
[13] Sugiura, M., Suzuki, M., Ishikawa, M., and Sasaki, M. (1976). Pharmaceutical studies on aminopeptidase from Aspergillus japonica. I. Chem. Pharm. Bull. 24:2286-2293.