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Research Article

Effects of Microwave Irradiation Pre-Treatment of Egg White Proteins on Ant Oxidative Activity of Their Hydrolysates Prepared with Pepsin

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Abstract: The effects of Conventional Heating (CH) and Microwave Irradiation (MWI) pre-treatment of Egg White Proteins (EWPs) on antioxidative activity of their hydrolysates prepared with pepsin were investigated. MWI pre-treatment of EWPs resulted in a higher degree of hydrolysis (DH) and the highest hydroxyl radical scavenging activity (40.67±1.30%), superoxide anion scavenging activity (75.53±2.79%), inhibitory activity of tea oil autoxidation (67.68±2.50%) and reducing power (0.34±0.01) of Egg White Proteins Hydrolysates (EWPHs) in comparison with CH pre-treatment, although there was no significant difference in DPPH radical scavenging activity between the EWPHs originated from the proteins pre-treated by CH and MWI ($p>0.05$). According to the experimental results, MWI pre-treatment could improve the antioxidant activities of EWPHs compared with CH treatment.

Keywords: Antioxidative activity, egg white, egg white protein hydrolysates, microwave irradiation

INTRODUCTION

Egg white is an important source of bioactive proteins, such as ovalbumin, lysozyme, ovoinhibitor etc. Ovalbumin can restrict lipid oxidation and lysozyme is able to suppress reactive-oxygen species and oxidative stress genes; ovoinhibitor is also capable of inhibiting formation of active-oxygen species. This indicates that the proteins have strong antioxidative activity. The antioxidative activity of EWPs are mainly related to the type and number of peptides or amino acid residues on the surface of a protein molecular structure. It is well known that functional properties of protein can be improved by enzymatic hydrolysis under suitable conditions; the DH is an important parameter for determination of functional properties of protein hydrolysates. You *et al*. (2009) found that as DH increased from 18 to 33%, the hydroxyl radical scavenging activity of loach protein hydrolysates increased first and then decreased. Protein hydrolysates from egg white and egg yolk have been found to possess antioxidant activity (Sakanaka *et al*., 2004; Lin *et al*., 2012). The molecular weight and amino acid sequence of peptides were reported to determine the antioxidant activities of protein hydrolysates (You *et al*., 2009). Sun *et al*. revealed that the ant oxidative activity of protein hydrolysates from egg white was determined by the DH (Sun *et al*., 2013).

Enzymatic hydrolysis of proteins after MWI pre-treatment is a new method for accelerating the hydrolysis of proteins. Microwaves are electromagnetic waves and the heating of proteins by microwave energy is fast and uniform throughout the material, which facilitates more enzyme-cutting sites exposure to protease. Numerous studies have dealt with the application of microwave as a preference method to accelerate protein hydrolysis for preparing samples for amino acids analysis (Chen *et al*., 1987; Chiou and Wang, 1989; Marconi *et al*., 1995). Lin *et al*. (2012) also reported the efficacy of this technique for preparing ant oxidative peptides derived from enzyme hydrolysis of bone collagen after microwave assisted acid pre-treatment (Lin *et al*., 2010). According to these results, the denaturation of protein occurs in minutes using this technique, in contrast to the hours required by conventional heat treatments. However, the authors of the present study did not know whether MWI pre-

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treatment could improve the functional properties of protein hydrolysates.

In the present study, we have developed the method of preparation of protein hydrolysates by enzyme hydrolysis of EWPs after irradiation pre-treatment, in the aim to accelerate the enzymatic reactions and also to find whether MWI pre-treatment can improve ant oxidative activity of protein hydrolysates.

MATERIALS AND METHODS

Materials and chemicals. Fresh eggs were obtained from the farmer's market of Center South University of Forestry and Technology (Changsha, Hunan, China). The egg white was freeze-dried after the separation of egg white and egg yolk. Pepsin was purchased from Novozyme Co. (Bagsvaerd, Denmark). All radical testing chemicals including 1.10-Phenanthroline, pyrogallic acid and 1.1-dipheny l-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (Sigma Chemical Co., St. Louis, MO, USA). Other chemicals used were of analytical grade.

Methods: Preparation of EWPs hydrolysates. After the sample (hen's egg white) was diluted with distilled water to the final concentration of 3% (w/v) and heated at 90^{o}C in a water bath for 15 min to denature the egg white protein before the mixed solution was cooled down to room temperature. Then, the pH of the mixed solution was adjusted to 2.0 with 1 M HCl. The mixed solution was hydrolyzed with pepsin at 37^{o}C for 1, 3, 5 and 7 h, respectively, which achieved various DHs. The enzyme to substrate ratio (E/S) was 9000:1 (U/g). The pH of the mixed solution was maintained constant at 2.0 by continuous addition of 1 M HCl or 1 M NaOH. In order to reach complete enzyme inactivation, the samples were treated at 85^{o}C for 30 min. The hydrolysates were centrifuged in a GL-21M refrigerated centrifuge (Xiangyi Instrument Co. Ltd., Changsha, China) at 4000 g for 20 min and the supernatants were freeze-dried with a vacuum freeze dryer (Christ Alpha 1-2 LD, Bioblock Scientific, France) and stored at -18^{o}C for further use.

Determination of the degree of hydrolysis, DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide anion scavenging activity, reducing power and inhibition of linoleic acid autoxidation. The Degree of Hydrolysis (DH), DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide anion scavenging activity, reducing power and inhibition of linoleum acid autoxidation, were determined according to the method of Sun *et al*. (2013).

Statistical analysis. All the tests were conducted in triplicate. The results were expressed as a mean±standard deviation (S.D.) and were subjected to one-way Analysis of Variance (ANOVA). The values $p<0.05$ were regarded as significant. All statistical analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Preparation of EWPHs using pepsin. It has been demonstrated that MWI could be used to enhance the enzymatic proteolysis. Besides, the rate and degree of hydrolysis depend also on the protein substrate used, proteolysis conditions, the specificity of the enzyme used for the proteolysis and the DH. After the EWPs were pre-treated by CH and MWI, respectively, protein hydrolysates with different DH were obtained using pepsin. The hydrolysis curves of EWPs after 1 to 7 h of incubation were shown in Fig. 1, the results showed that the DH of the egg white proteins increased rapidly within the first 2 h, the higher levels of proteolysis were obtained in the MWI digestions after 7 min of digestion in comparison with CH digestion. After 1, 3, 5 and 7 h of hydrolysis, the DHs of EWPs denatured by CH were 6.47%, 13.37%, 16.93% and 18.22%, respectively. However, after 1, 2, 4 and 7 h of hydrolysis, the DHs of EWPs pre-treated by MWI could reach 7.25%, 11.79%, 16.98% and 19.81%, respectively. Increase of enzymatic hydrolysis by MWI has also been reported on the case of preparing samples for amino acids analysis (Chiou and Wang, 1989; Kanatt *et al*., 2007; Lin *et al*., 2012).

DPPH radical-scavenging activity: The DPPH• radical has a single electron and shows maximum absorbance at 517 nm, which decreases significantly on exposure to a proton-donating substrate such as an antioxidant (Tung *et al*., 2007; Singh *et al*., 2009; Shimada *et al*., 1992). The DRSA of the EWPHs were shown in Fig. 2, the results clearly indicated that the EWPHs with different DH exhibited different scavenging activities against DPPH radicals. After the EWPs were treated by CH, the EWPHs with DH 16.93% showed the highest DRSA (96.07±3.84%) among the four protein hydrolysates. However, there was no significant difference in DRSA between the EWPHs originated from the proteins treated by CH and MWI ($p>0.05$), the DRSA of the EWPHs with DH 16.98% was 97.37±1.47%. The results indicated that MWI pre-treatment could not increase the DRSA of EWPHs in comparison with CH pre-treatment. Lin *et al*. (2010) has also reported that microwave assisted acid pre-treatment could not improve the DPPH radical-scavenging activity of three bone collagen hydrolysates.

Hydroxyl radical scavenging activity: The chemical activity of hydroxyl radical is the strongest among reactive oxygen species, it can easily react with biomolecules such as amino acids, proteins and DNA. Therefore, the removal of hydroxyl radical can be beneficial to human health (Cacciuttoloa *et al*., 1993; Qian *et al*., 2008). The hydroxyl radical scavenging activities of the EWPHs with different DH were shown in Fig. 2, the EWPHs with different DH showed

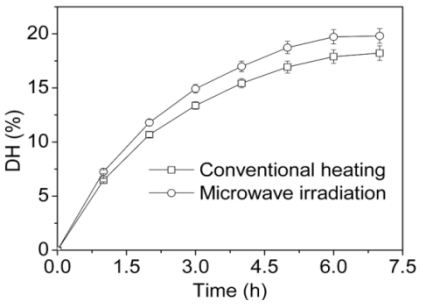

Fig. 1: The hydrolysis curve of EWPs hydrolyzed by using pepsin

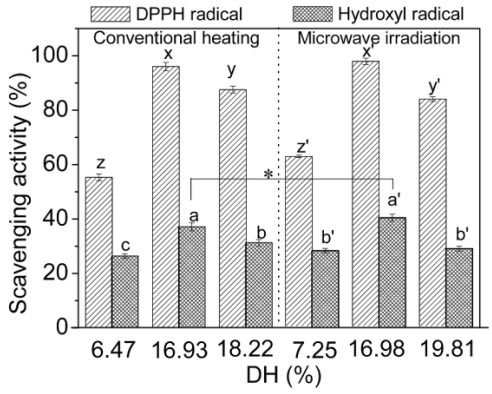

Fig. 2: DPPH and hydroxyl radical-scavenging activity of the EWPHs at different DH. Each result is the average calculated in 95% condense interval of five parallel studies

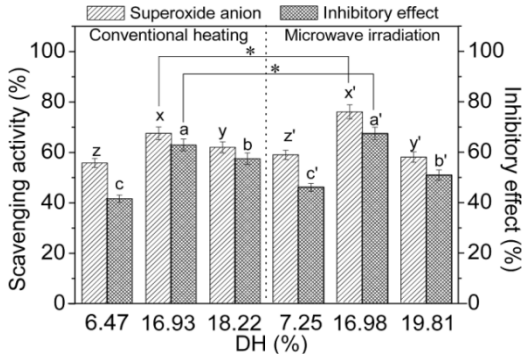

Fig. 3: Reducing power activity and lipid per oxidation inhibition activity of the EWPHs with different DH. Each result is the average calculated in 95% condense interval of five parallel studies

different scavenging activities against hydroxyl radical. After the EWPs were treated by CH, the EWPHs with DH 16.93% exhibited the strongest scavenging activity against hydroxyl radical, reaching 36.82±1.47%. However, the EWPHs (DH 16.98%) originated from the proteins pre-treated by MWI exhibited the higher HRSA than the EWPHs (DH 16.93%) originated from the proteins treated by CH, which could reach 40.67±1.30%. The differences may be attributed to differences in type of amino acids and peptide fragments produced by pepsin hydrolysis. The results also indicated that the EWPHs were an effective electron donor for the reduction of hydroxyl radical.

Superoxide anion scavenging activity: Superoxide anion radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species, which can cause disease and damage to tissues and thus investigation of the free radical scavenging activity is of great importance (Kanatt *et al*., 2007). SASA of the EWPHs with different DH was presented in Fig. 3, all the EWPHs showed considerable scavenging abilities over superoxide anion. The EWPHs with DH 16.93% that originated from the EWPs treated by CH exhibited significant the stronger SASA (67.72±2.51%) than the EWPHs with DH 6.47% and 18.22% ($p<0.05$). However, the EWPHs with DH 16.98% presented the higher scavenging abilities over superoxide anion (75.53±2.79%) than the EWPHs with DH 16.93% and the former was derived from the EWPs pre-treated by MWI. This indicated that MWI treatment could not only promote the proteolysis of the EWPs by pepsin, but also improved SASA of the EWPHs compared with CH treatment.

Inhibition of tea oil autoxidation: Free radical-induced lipid peroxidation is a complex process that involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids (Bougatef *et al*., 2010). The inhibitory effects of the EWPHs as an antioxidant in prevention of tea oil autoxidation were investigated. As shown in Fig. 3, all the tested samples showed strong inhibitory effects. After the EWPs were treated by CH, as the DH increased from 6.47 to 18.22%, the antioxidative activity of the EWPHs first increased and then decreased. The same phenomenon could be observed in the proteolysis of the EWPs by pepsin after the proteins were treated by MWI. The result also showed that the inhibitory activity of the EWPHs with DH 16.98% (67.68±2.50%) was significantly higher than that of the EWPHs with DH 16.93% (62.68±2.32%) ($p<0.05$). This indicated that limited hydrolysis of the EWPs could lead to better antioxidant ability than extensive hydrolysis and MWI pre-treatment could improve inhibitory effects of lipid peroxidation of EWPHs compared with CH treatment.

Reducing power assay is often to evaluate the ability of an antioxidant to donate an electron (Yildirim *et al*., 2000). Many reports have revealed that there is a direct correlation between the ant oxidative activities and the reducing power of bioactive compounds. For the reducing power assay, the presence of antioxidants

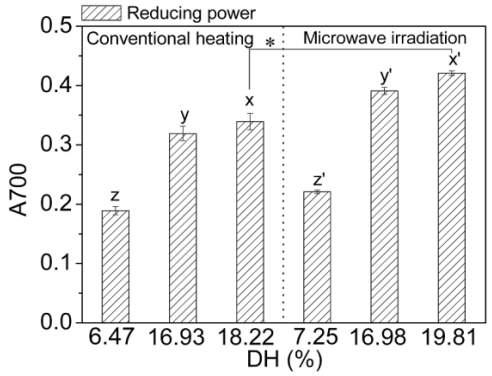

Fig. 4: Reducing power activity of the EWPHs with different DH. Each result is the average calculated in 95% condense interval of five parallel studies

in the tested samples causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. The reducing power of the EWPHs with different DH was showed in Fig. 4. After the EWPs were treated by CH, as the DH increased from 6.47 to 18.22%, the reducing power of the EWPHs increased from 0.19 to 0.34±0.01. However, the EWPHs with DH 19.81% showed higher reducing power than the EWPHs with DH 18.22% and the former was originated from the proteins pre-treated by MWI. The difference might be attributed to the presence of more specific peptides or active amino acids in the EWPHs with high DH, which could react with free radicals to form more stable products.

CONCLUSION

The EWPs were pre-treated by CH and MWI in our study, respectively. As the DH increased from 6.47% to 19.81%, the antioxidant activities of EWPHs first increased and then decreased. When the EWPHs exhibited the strongest DRSA, HRSA, SASA and the inhibitory activity of tea oil autoxidation, the DHs of the EWPs treated by CH and MWI were 16.93% and 16.98%, respectively. MWI pre-treatment could improve the antioxidant activities of EWPHs compared with CH treatment. Therefore, MWI pre-treatment is a useful method for improving the antioxidant activities of proteins.

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REFERENCES

Bougatef, A., N. Nedjar-Arroume, L. Manni, R. Ravallec, A. Barkia, D. Guillochon and M. Nasri, 2010. Purification from enzymatic hydrolysates of sardinelle (*Sardinellaaurita*) by-products proteins. Food Chem., 118(3): 559-565.

Cacciuttoloa, M.A., L. Trinha, J.A. Lumpkina and G. Rao, 1993. Hyperoxiainduces DNA damage in mamm alian cells. Free Radic. Bio. Med., 14(3): 267-276.

Chen, S.T., S.H. Chiou, Y.H. Chu and K.T. Wang, 1987. Rapid hydrolysis of proteins and peptides by means of microwave technology and its application to amino acid analysis. Int. J. Pept. Protein Res., 30(4): 572-576.

Chiou, S.H. and K.T. Wang, 1989. Peptide and protein hydrolysis by microwave irradiation. J. Chromatogram., 49: 424-431.

Kanatt, S.R., R. Chander and A. Sharma, 2007. Antioxidant potential of mint (*Mentha spicata* L.) in radiation-processed lamb meat. Food Chem., 100(2): 451-458.

Lin, Y.J., G.W. Le, J.Y. Wang, Y.X. Li, Y.H. Shi and J. Sun, 2010. Ant oxidative peptides derived from enzyme hydrolysis of bone collagen after microwave assisted acid pre-treatment and nitrogen protection. Int. J. Mol. Sci., 11: 4297-4308.

Lin, S.Y., Y. Guo, Q. You, Y.G. Yin and J.B. Liu, 2012. Preparation of antioxidant peptide from egg white protein and improvement of its activities assisted by high-intensity pulsed electric field. J. Sci. Food Agri., 92: 1554-1561.

Marconi, E., G. Panfili, L. Bruschi, V. Vivanti and L. Pizzoferrato, 1995. Comparative study on microwave and conventional methods for protein hydrolysis in food. Amino Acids, 8(2): 201-208.

Qian, Z.J., W.K. Jung, H.G. Byun and S.K. Kim, 2008. Protective effect of an ant oxidative peptide purified from gastrointestinal digests of oyster, Crassostrea gigas against free radical induced DNA damage. Bioresource Technol., 99: 3365-3371.

Sakanaka, S., Y. Tachibana, N. Ishihara and L.R. Juneja, 2004. Antioxidant activity of egg-yolk protein hydrolysates in linoleum acid oxidation system. Food Chem., 86: 99-103.

Shimada, K., K. Fujikawa, K. Yahara and T. Nakamura, 1992. Ant oxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agri. Food Chem., 40: 945-948.

Singh, U.A.K., S. Singh and M. Rai, 2009. Total phenolics content and free radical scavenging activity of brasslike vegetables. J. Food Sci. Tech. Mys., 46: 595-597.

Sun, S.G., M.H. Ma, Q.L. Lin, T. Yang and H. Niu, 2013. Systematic investigation of antioxidant activity of egg white protein hydrolysates obtained by pepsin. Adv. J. Food Sci. Technol., 5: 57-62.

Tung, Y.T., J.H. Wu, Y.H. Kuo and S.T. Chang, 2007. Antioxidant activities of natural phenol compounds from Acacia confusa bark. Bioresour Technol., 98: 1120-1123.

Yildirim, A., A. Mavi, M. Oktay, A.A. Kara, O.F. Algur and V. Bilaloglu, 2000. Comparison of antioxidant and antimicrobial activities of tilia (Tilia argentea Desf ex DC), sage (Salvia triloba l.) and black tea (Camellia sinensis) extracts. J. Agri. Food Chem., 48(10): 5030-5034.

You, L.J., M.M. Zhao, C. Cui, H.F. Zhao and B Yang, 2009. Effect of degree of hydrolysis on the antioxidant activity of loach (Misgurnus anguillicaudatus) protein hydrolysates. Innov. Food Sci. Emerge., 10: 235-240.