EFFECT OF JEJU HORSE LEG BONE HYDROLYSATES ON ANTI-OXIDATION AND WRINKLE PROTECTION ACTIVITY

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Abstract – This study focused on the anti-oxidative and collagenase- and elastase inhibition effects of low molecular weight peptides (LMP) from commercial Jeju horse leg bone hydrolysates (JHLB) on pancreatin, via enzymatic hydrolysis. Cell viability of dermal fibroblasts exposed to UVB radiation upon treatment with LMP from JHLB was evaluated. Determination of the antioxidant activity of various concentrations of LMP from JHLB were carried out by assessing 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethybenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity, ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC). The DPPH radical scavenging activity of LMP from JHLB (20 mg/mL) was 92.21% and ABTS radical scavenging activity (15 mg/mL) was 99.50%. FRAP activity (30 mg/mL) was 364.72 μM/TE and ORAC activity (1 mg/mL) was 101.85 μM/TE. The anti-wrinkle potential was assessed by evaluating the elastase- and collagenase inhibition potential of these LMP. We found that 200 mg/mL of LMP from JHLB inhibited elastase activity by 41.32%, and 100 mg/mL of LMP from JHLB inhibited collagenase activity by 91.32%. These results indicate that LMP from JHLB has potential utility as an anti-oxidant and anti-wrinkle agent in the food and cosmetic industry. Additional *in vivo* tests should be carried out to further characterize these potential benefits.

Key words: horse leg bone, hydrolysates, collagenase, elastase, antioxidant effect

I. INTRODUCTION

Human skin is constantly being exposed to environmental irritants, and this can lead to the production of free radicals and reactive oxygen species that cause serious damage to the skin cells. Intracellular and extracellular oxidative stress initiated by reactive oxygen species (ROS) accelerates skin aging, which is characterized by wrinkle formation and atypical pigmentation [9]. These changes are induced by alterations in dermal connective tissues such as collagen, elastin fibrillin, and proteoglycans [7]. Considerable research efforts in the cosmetic industry have focused on the mechanisms by which the symptoms of skin aging can be delayed or improved [4]. The use of certain natural products for protecting against skin aging has recently been proposed; these include ginseng, aloe vera, berries, and porcine placenta [6]. .Bone broth has been consumed for ages as an important source of nutrients. It is used as a traditional folk medicine across cultures for the sick and weak, especially for ailments affecting connective tissues such as the gastrointestinal tract, joints, skin, lungs, muscle, and blood [11]. Equine bone and bone extracts from Juju island in Korea are being extensively distributed owing to their bioactive function. One of the health benefits of these equine bone extracts is that they cause an increase in bone density and improve bone health.

In our previous study, a large antioxidant effect was observed in the low molecular weight peptide fraction of less than 3 kDa isolated from leg bone extracts via enzyme hydrolysis [8]. However, there is still a lack of scientific information about the anti-oxidant and anti-wrinkle activities of enzyme hydrolysates from horse leg bone extracts. This study aimed to evaluate the characteristics of horse bone hydrolysates generated by pancreatin treatment on anti-oxidant activity as well as inhibition of collagenase and elastase, which are some of the key causes of skin aging.

II. MATERIALS AND METHODS

Commercial horse leg bones were obtained from an on-line supplier. After removing all visible impurities and debris, leg bones were washed three times with water, which was subsequently discarded. The extraction process was carried out by adding water to the bones at a ratio of 6:1 (v:v) and allowed to proceed for 8 h and repeated twice to produce gelatin. The extracted liquid was freeze-dried and the fat was removed by Folch's method and freeze-dried. Hydrolysis for 4hrs was performed by the addition of 0.2% pancreatin (Bision Co. Korea) after reconstitution of the freeze-dried extracts in distilled water. Hydrolysates were then subjected to centrifugal filtration (Amicon® Ultra-15 centrifugal filter units, Millipore, USA) to separate low molecular weight (<3 kDa) hydrolysate fractions. The filtered hydrolysates (JHLB) were collected and lyophilized for use in subsequent experiments (Fig. 1).

DPPH radical scavenging activity was estimated according to the method of Blois [2] with slight modification. ABTS scavenging activity was determined by method of Re *et al* [7]. FRAP (ferric reducing antioxidant power) was determined by method of Benzie and Strain [1]. ORAC (oxygen radical absorbance capacity) determined by method of Gillespie *et al* [4]. Oxygen radical absorbance capacity calculated as mM trolox equivalent radiation at dose of 100 mJ/cm^2. Elastase inhibitory activity was determined by the method of Cannell *et al.* [3]. Collagenase inhibitory activity was determined by the method of Wűnsch and Heidrich [13] with slight modification. All data collected were subjected to one-way analysis of variance (ANOVA) according to the general linear model procedures for SAS software (ver. 9.SAS Institute Inc., USA). Mean values and standard error of the sample were reported. When analysis of variance indicated a significant treatment effect, Duncan's multiple range test was used to compare the mean values, and a p-value < 0.05 was considered statistically significant.

III. RESULTS AND DISCUSSION

Figure. 1. Preparation procedure of LMP from JHLB extracts hydrolyzed by pancreatin

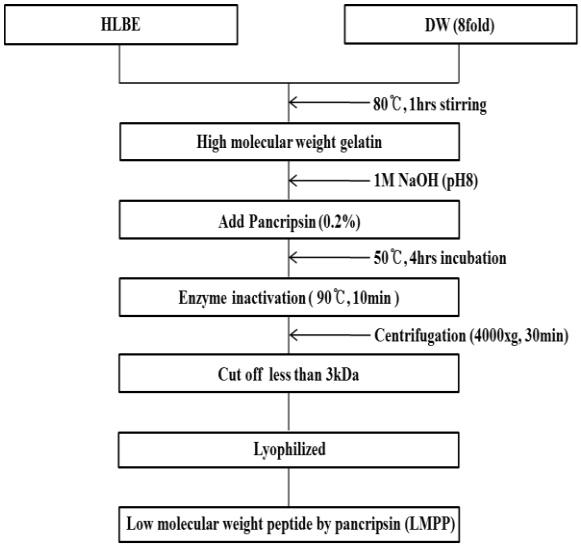
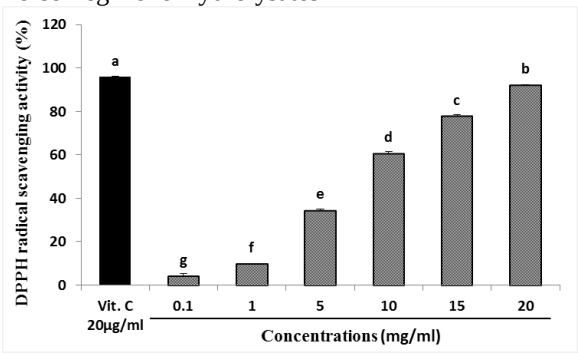


Figure 2. DPPH radical scavenging activities of Horse Leg Bone Hydrolysates



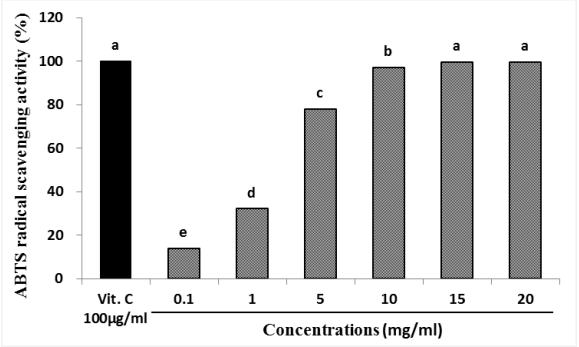
[a-c] Values of bar with different letters among treatments differ significantly at $p< 0.05$

The DPPH radical scavenging activity of LMP-JHLB with various concentrations is shown in Fig. 2. Vitamin C was used as a positive control for comparison of the DPPH radical scavenging activity of LMP from JHLB. As shown, the DPPH radicals were significantly scavenged by LMP from JHLB in a dose dependent fashion ($p < 0.05$). In our previous study, low molecular weight (less than 3 kDa) peptides from Jeju horse showed higher DPPH radical scavenging activity than hydrolysates over 3 kDa [8]. The ABTS radical scavenging activity of LMP from JHLB is shown in Fig. 3. LMP from JHLB significantly reduced ABTS radicals in a dose dependent manner at concentrations ranging from 0.1 to 15 mg/mL. 15 and 20 mg/mL of

LMP from JHLB also had ABTS radical scavenging effects that are equivalent to 100 µg/mL of vitamin C.

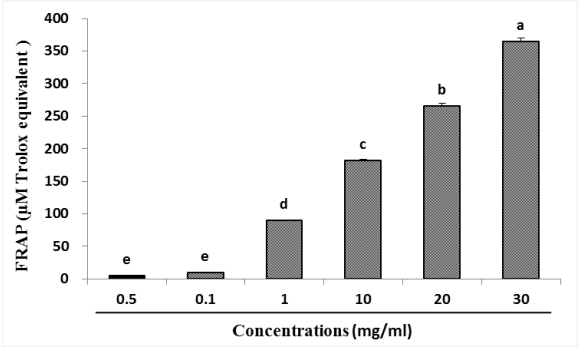
The initial antioxidant value of the low molecular weight peptides (0.1 mg/mL) was 9.46 µM TE whereas the FRAP value of the peptides at 1, 10, 20, and 30 mg/mL was 90.41, 181.59, 266.11, and 364.72 µM TE, demonstrating a significant increase in antioxidant activity that was dose dependent (Fig. 4). The ORAC value of LMP from JHLB significantly increased in a dose-dependent manner (Fig. 5). Similar results were reported by Kim *et al.* [8]. who found that the ORAC activity of pig skin gelatin hydrolysates increased with dose, and 1 mg/mL of pig skin gelatin hydrolysates had 141.39 µM TE/g of ORAC activity. The elastase inhibition activity of the low molecular weight peptides from JHLB is shown in Fig. 6. As a positive control, ursolic acid at 100 µg/mL was used and showed approximately 37% elastase inhibition activity. The low molecular weight peptides from JHLB at 25, 50, 100, and 200 mg/mL had increased elastase inhibition activity in a dose dependent manner ($p < 0.05$). The collagenase inhibition activity of low molecular weight peptides from JBLH is shown in Fig. 7. Collagenase inhibition activity of the hydrolysates at 1, 10, 25, 50, and 100 mg/mL significantly increased in a dose dependent manner.

Figure. 3. ABTS radical scavenging activity (%) of LMP from JHLB extracts hydrolyzed by pancreatin



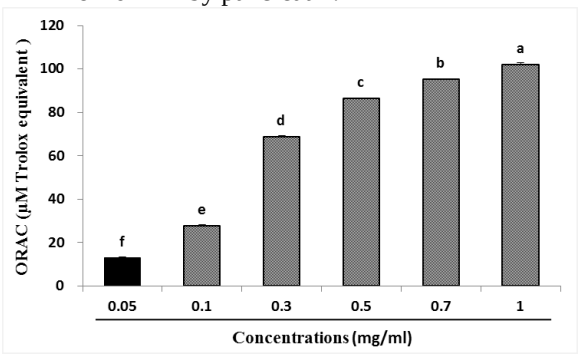
[a-e]Values of bar with different letters among treatments differ significantly at $p < 0.05$.

Figure. 4. Ferric reducing antioxidant power of LMP from Jeju JHLB extracts hydrolyzed by pancreatin.



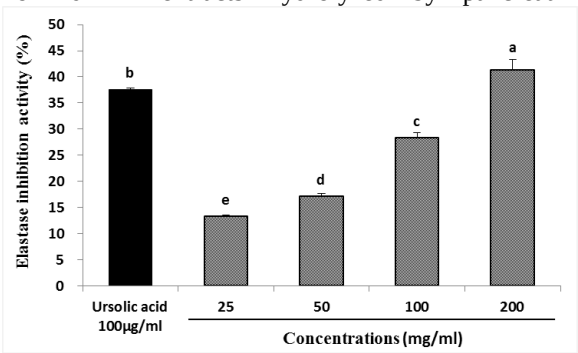
[a-e]Values of bar with different letters among treatments differ significantly at $p < 0.05$

Figure. 5. Oxygen radical absorbance capacity of LMP from JHLB by pancreatin.



[a-e]Values of bar with different letters among treatments differ significantly at $p < 0.05$

Figure. 6. Elastase inhibition activity (%) of LMP from JHLB extracts hydrolyzed by pancreatin



[a-c]Values of bar with different letters among treatments differ significantly at $p < 0.05$

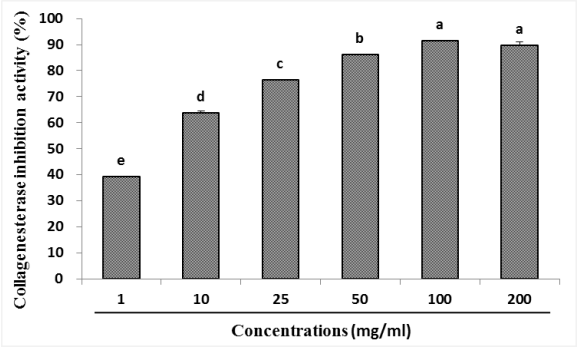


Figure. 7. Collagenase inhibition activity (%) of LMP from JHLB hydrolysates with pancreatin.

IV. CONCLUSION

These results suggest that hydrolysates of horse leg bone extracts smaller than 3 kDa have potential to be used as anti-oxidative and anti-wrinkling compounds in food industry. Even though, further animal test should be evaluated in the future.

ACKNOWLEDGEMENTS

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REFERENCES

1. Benzie, I. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. 239, 70-76.
2. Blois, M. S. (1958). Antioxidant determination by the use of a stable free radical. Nature 181, 1199-1200.
3. Cannell, R.J.P., Kellan, S.J., Owsianks, A.M., Walker, J.M. (1988) Results of a large scale scrren of microalgae for the production of protease inhibitors. *Planta Med*. **54**, 10-14.
4. Cheon, S. J., Jang, M. J., Jang, Y. A., Choi, E. Y., Jun, D. H., Kim, Y. H., Cho, W. A., Jeong, Y. S., Kwon, H, B., Kim, T. H., Choi, K. L., Do, J. R., Lee, C. E. and Lee J. T. (2008) Anti-wrinkle Effect of Cambodian *Phellinus linteus* Extracts. J. of Life Sci. 18(12), 1718-1722.
5. Gillespie, K. M., Chae, J. M. and Ainsworth, E. A. (2007). Rapid measurement of total antioxidant capacity in plants. Nature Protocols 2, 867-870.
6. Han, J.H., Kim, M.R., Park, Y., Hong, Y.H., Suh, H.J. (2013) Skin permeability of porcine placenta extracts and its physiological activities. *Korean J. Food Sci. An*. **33**, 356-362.
7. Jeon, H.S., Seo, J.E., Kim, M.S., Kang, M.H., Oh, D.H., Jeon, S.O., Jeong, S.H., Choi,Y.W., Lee, S. (2013) A retinyl palmitate-loaded solid lipid nanoparticle system: Effect of surface modification with dicetyl phosphate on skin permeation in vitro and anti-wrinkle effect in vivo. *Inter. J. Pharmaceu*. **452**, 311-320.
8. Kim, D. W., Park, K. M, Ha, G. E., Jung, J. R., Chang, O. K., Ham, J. S., Jeong, S. G., Park, B. Y., Song, J., and Jang, A. (2013). Anti-oxidative and neuroprotective activities of pig skin gelatin hydrolysates. Korean J. Food Sci. An. 33, 258-267.
9. Masaki, H. (2010) Role of antioxidants in the skin: anti-aging effects. *J. Dermatol Sci.* **58**, 85-90.
10. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying and improved ABTS radical action decolorization assay. Free. Radical. Biol. Med. 26**,** 1231-123.
11. Siebecker, A. (2005). Traditional bone broth in modern health and disease. Accessed from http://www.townsendletter.com/FebMarch2005/broth0205.htm
12. Varani, J., Spearman, D., Perone, P., Fligiel, S.E.G., Datta, S., Wang, Z.Q., Shao, Y., Kang, S., Fisher, G.J., Voorhees, J. J., (2001). Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro. Am. J. Pathol. 158, 931–942.
13. Wűnsch, E. and Heidrich, H.G. (1963) On the quantitative determination of collagenase. *Hoppe Seylers Z Physiol Chem*. **333**, 149-151.