ANTI-OXIDATION AND PROCOLLAGEN ENHANCING EFFECT OF LEG BONE HYDROLYSATES FROM JEJU CORSS BRED HORSES

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Abstract – This study was conducted to evaluate anti-oxidative effects and procollagen enhancing activity of hydrolysates from Jeju cross bred horse. Horse leg bone was obtained from National Institute of Animal Science, RDA in Korea and extracted in three times with hot water (total 24 hours). The extracts were lyophilized and hydrolyzed with Pancripsin for 4, 8, and 12 hrs (HM4, HM8, HM12). Hydrolysates less than 3 kDa (HL) and above 3 kDa (HO) were also separated using centrifugal membrane filtration. Also, anti-oxidative activity of HL increased in a dose dependently and the activity of HL was significantly higher than HM and HO in all hydrolysis time (p<0.05). DPPH scavenging activity of HL4, HL8, and HL12 at 10 mg/mL was 99.57, 95.70 and 96.96 uM Trolox Equivalent (TE) and significantly higher than HM, HO in all hydrolysis time (p<0.05). ABTS radical scavenging activity and FRAP activity of HL4 (10 mg/mL) was 228.63 μM TE, respectively. The oxygen radical absorbance capacity of HL4 at 1 mg/ml was 350.5μM TE. Procollagen type I C-peptide was destroyed and reduced after UV radiation. However, the procollagen type I C-peptide in photodamaged cell was increase significantly with 250 μg/ml of HL4. These results indicated that the small molecular weight hydrolysates of HL, especially after 4 hrs of hydrolysis, has potential to be used as anti-oxidants and skin protect compounds from photodamaging.

Key words: Procollagen type I C-peptide, Horse Leg Bone, Hydrolysates, Antioxidant effect, Anti-ageing

I. INTRODUCTION

The skin of human is constantly being exposed to environmental irritants. These irritants cause free radicals and reactive oxygen species which leave serious damages on the cells of skin. Recently, in the cosmetic and functional food industry, many researchers have paid considerable attention to delay or improve the symptoms of skin aging [3]. The use of certain natural products for protecting against skin aging has recently been proposed these include ginseng, aloe vera, berries, and porcine placenta [5]. Bone broth has been consumed for ages as an important source of nutrients. Many Asian people enjoy consuming bone broth, especially, Korans, who like to have Hanwoo bone broth in winter for nourishment. Equine bone and bone extracts from Jeju island in Korea are being extensively distributed owing to their bioactive function such as bone health and bone density improvement. However, there is still a lack of scientific information about the anti-oxidant and procollagen enhancing activity of hydrolysates from Jeju horse leg bone extracts. Accordingly, this study was performed to experiment tried to find out the anti-oxidation and procollagen enhancing effects of hydrolysates from horse leg bone extracts.

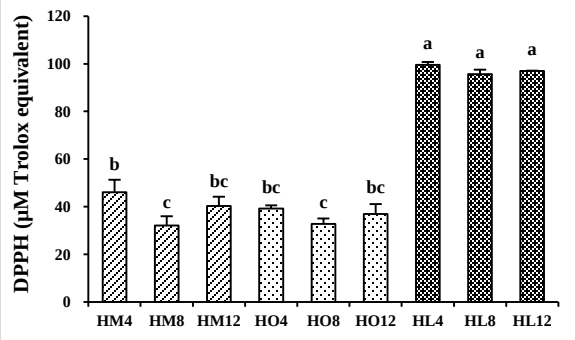
II. MATERIALS AND METHODS

Horse leg bone was extracted with hot water for 24 hrs and all debris was discarded and the solution was lyophilized. Hydrolysis was performed with 0.2% Pancripsin for 4, 8, and 12hrs. The hydrolysates were grouped likely hydrolysates materials (HM), hydrolysates less than 3 kDa (HL) and hydrolysates over 3 kDa (HO). The HL was separated using centrifugal membrane filtration. 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. The normal human newborn foreskin fibroblast cell line, HS68 (ATCC CRL 1635), was purchased from the American Type Culture Collection (ATCC, USA). HS 68 cells were seeded on 48-well tissue culture plates at a density of 10^5 cells/mL in DMEM containing 10% FBS with 1% penicillin/streptomycin and incubated for 24 h. Thereafter, the DMEM was discarded and the cell wells washed with phosphate-buffered saline (PBS). Next, 500 μL of PBS and various concentrations of hydrolysate samples were added to the cell wells and the HS 68 cells were exposed to UVB radiation at dose of 100 mJ/cm^2. After irradiation, cells were treated with hydrolysate samples and incubated for another 24 h and procollagen type I was determined using the Procollagen Type I C-Peptide EIA Kit (Takara, Japan). All of treatments were analyzed by General Linear Model (GLM) procedure of SAS software ($p<0.05$).

III. RESULTS AND DISCUSSION

Figure 1. DPPH radical scavenging activities of horse leg bone hydrolysates

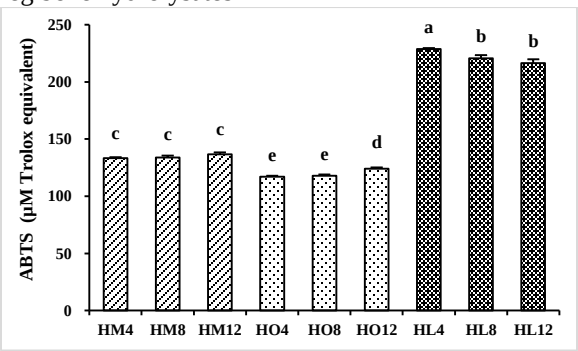


[a-c] Values of bar with different letters among treatments differ significantly at $p< 0.05$

1) HM4, hydrolysates mixture after 4h hydrolysis; HM8, hydrolysates mixture after 8h hydrolysis; HM12, hydrolysates mixture after 12h hydrolysis; HO4, hydrolysates over 3 kDa after 4h hydrolysis; HO8, hydrolysates over 3 kDa after 8h hydrolysis; HO12, hydrolysates over 3 kDa after 12h hydrolysis; HL4, hydrolysates less than 3 kDa after 4h hydrolysis; HL8, hydrolysates less than 3 kDa after 8h hydrolysis; HL12, hydrolysates less than 3 kDa after 12h hydrolysis

Lots of methods have been used to evaluate the antioxidant activity or capacity of food compounds. These method have been classified according to the mechanism of radical deactivation involved and the physiological relevance of the free radical, or according to the competitive or direct approach at the antioxidant reaction. DPPH radical scavenging activity of horse leg bone hydrolysates with different hydrolysis time and molecular weight size was shown in Fig. 1. DPPH scavenging activity of HL4, HL8, and HL12 at 10 mg/mL was 99.57, 95.70 and 96.96 uM Trolox Equivalent (TE), respectively, and it was significantly higher than HM, HO with all hydrolysis time ($p<0.05$).

Figure 2. ABTS radical scavenging activities of horse leg bone hydrolysates

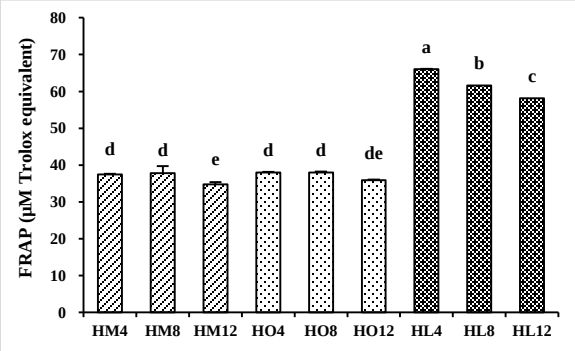


[a-e]Values of bar with different letters among treatments differ significantly at $p< 0.05$.

[1)] Refer to Fig. 1

ABTS radical scavenging activity of horse leg bone hydrolysates with different hydrolysis time is shown in Fig. 2. HL4 showed the highest ABTS scavenging activities compare to HL8 and HL12. HM and HO showed significantly low ABTS radical scavenging activity. Kim et al (2013) reported that hydrolysates lower than 3 kDa (hydrolization by pepsin and pancreatin) of Jeju horse leg bone obtained from local market showed 0.48 mM TE of ABTS radical scavenging activity.

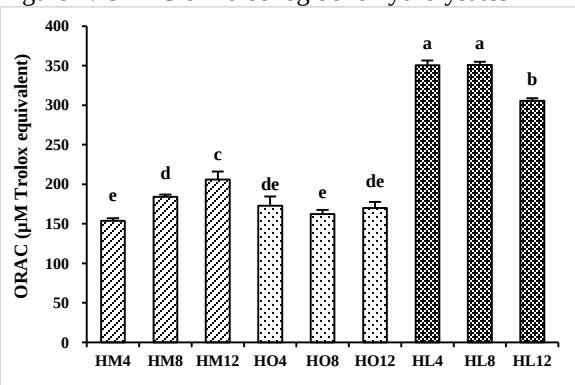
Figure 3. FRAP of horse leg bone hydrolysates



[a-e]Values of bar with different letters among treatments differ significantly at $p< 0.05$
[1)] Refer to Fig. 1

The FRAP value of low molecular weight HL4, HL8, and HL12 was shown as 66.05, 61.66 and 58.13 uM TE, respectively (Fig. 3). Especially, HL4 showed the highest FRAP value. The FRAP analysis presents a similar trends as was observed for the DPPH and ABTS radical scavenging activity.

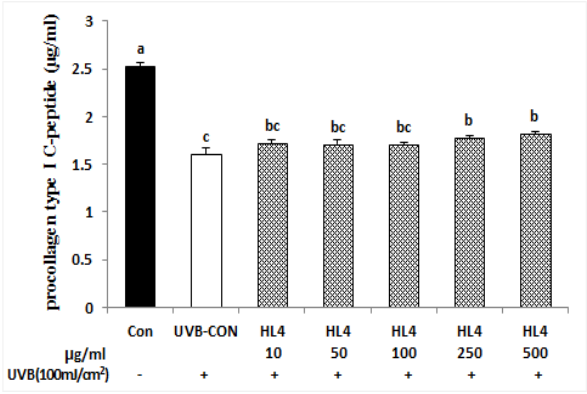
Figure 4. ORAC of horse leg bone hydrolysates



[a-e]Values of bar with different letters among treatments differ significantly at $p< 0.05$
[1)] Refer to Fig. 1

The ORAC assay determines the ability of an antioxidant to quench free radicals by hydrogen donation, and is thus a measure of both general and specific antioxidant actions by using a fluorescent probe and monitoring the change in its fluorescence intensity. The HL4 and HL8 showed the highest ORAC value compare to the hydrolysate HM and HO regardless hydrolysis time (Fig. 4).

Figure 5. Procollagen type I C-peptide of horse leg bone hydrolysates



[a-c]Values of bar with different letters among treatments differ significantly at $p< 0.05$
[1)] Refer to Fig. 1

Type I procollagen is reduced in photodamaged human skin and this reaction can be resulted from increased degradation by metalloproteinase and/or from reduced procollagen synthesis. Procollagen type I C-peptide of UV damaged newborn foreskin fibroblast cell line was increased with dose of 250 and 500 ug/ml compare to UV damaged cell (Fig. 5).

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 collagen enhancing compounds in food industry. Even though, further animal test should be evaluated in the future.

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