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Potential Antioxidant Peptides in Rice Wine

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Introduction

Food protein, especially the hydrolysate type, has been considered to be a health-benefiting functional food. In particular, the peptides with N-terminal leucine/valine of beta conglycinin found in soybean, histidine in the second residue of 3 types of peptide in white egg albumin and hydrolysate of bovine skin have all been shown to have antioxidant properties. In food, the safety problem of synthetic antioxidants like BHA and BHT needs to be resolved. Therefore, the application of natural antioxidants such as peptides in food and other health products are important, and currently research is being carried out by scientists to elucidate these antioxidant peptides. In vivo studies have shown that peptides from soybean increased the immune response of rats and a peptide extracted from Japanese rice wine, Sake, had an antihypertensive effect on spontaneous hypertensive rat (SHR). For instance, a Japanese group has identified the structure of angiotensin converting enzyme (ACE) inhibition peptide in Sake. In Europe, the consumption of red wine is favorably regarded, as it has been claimed to be health promoting due to high polyphenol content. Likewise, in many Asian countries, consumption of rice wine is common. In Korea, a variety of rice wine is found and Chongju for example is regularly taken with meals and has been traditionally alleged to maintain health, but scientific evidence is scarce. The fine form of Chongju (Samhaeju) is made by fermentation at a low temperature and excels in protein content compared to other alcoholic beverages such as wine and liquors. In order to understand the rationale of Samhaeju in the aspect of their health benefits and long shelf-life, we have assessed the antioxidant property of the peptides produced during fermentation of traditionally made Samhaeju and identified the amino acid sequences for the responsible antioxidant peptide.

Materials and Methods

Preparation of rice wine sample The alcohol portion of the rice wine was removed by adding 50 ml of rice wine to 50 ml of distilled water and concentrated to the original volume through vacuum rotary evaporator at 40°C.

Free radical scavenging activity The overall antioxidant activity of the prepared sample was assessed by DPPH method. As positive control, a-tocopherol (500 μ M) was used. The percentage of inhibition, which

represents the scavenging ability of the sample on DPPH radical, was calculated as follows;

Antioxidant Activity (AOA) = 100-[(absorbance increase of sample/absorbance increase of control) x 100]

Lipid peroxidation The antioxidant activity against oxidation of lipid by the samples was analyzed by beta-carotene bleaching test (BCBT) and oxidation of erythrocytes. The degree of lipid peroxidation was determined by measuring thiobarbituric acid reactive (TBARS). The antioxidant activity of the sample against lipid oxidation is expressed antioxidant Activity Coefficient (AAC) in which,

$AAC = [(AA_{105} - AB_{105}/AB_0 - AB_{105})] \times 1000$

AA_{105} and AB_{105} are the absorbencies of the test and blank sample at t=105 min, and AB_0 is the absorbance of the blank sample at t=0 min.

Sample purification The sample separation was carried out prep liquid chromatography equipped with a RP C_{18} column (Zorbax RX-C_{18}, Hewlett Packard, USA). The flow rate was adjusted to 15 ml/min. The eluent A (0.1% TFA in Distilled water) and B (0.086 TFA in 80% ACN) were also filtered before use. The fractionated samples from prep-HPLC showing high antioxidant activity was dissolved in distilled water and loaded on C_{18} column (Nova-Pak C_{18}, Waters, USA).The column was then eluted with a gradient of 5% of 0.086% TFA in 80% ACN. The fractions showing antioxidant activity were pooled and lyophilized. These fractions were eluted and further purified by consecutive chromatographic methods using different gradient conditions.

Identifications of antioxidant peptides Edman degradation with an automated protein sequencer equipped with on-line HPLC (Hewlett Packard G1005A protein sequencing system) was used for analysis of the amino acid sequence of antioxidant peptides.

Results and Discussion

Rice wine concentrate was separated by prep liquid chromatography (Table 1) and 55 fractions were evaluated for antioxidant activity. Of the fractions, 6 (F4, F5, F10, F11, F31, F33) had AOA value over 30,

Table 1. Antioxidant Activity of rice wine fractionated by prep HPLC

Fraction	F4	F5	F10	F11	F16	F19	F31
*AOA	31.5±4.8	43.6±2.5	46.8±4.2	32.3±2.4	15.8±0.7	15.2±1.0	32.93±2.6
**AAC	472.7±5.5	405.9±46.7	317.9±11.0	312.6±10.5	328.4±36.2	468.1±57.6	72.7±0.1

Original rice wine ; 56.5±4.7(AOA), 808±9.9(AAC)
Vit E (500 μ M) ; 77.1±2.2(AOA), 572±2.7(AAC)
*AOA: Antioxidant activity as determined by DPPH method
**AAC: Antioxidant activity coefficient as determined by BCBT method

and 7 fractions (F3, F4, F5, F10, F11, F16, F19) had AAC value of over 300 demonstrating high antioxidant activity of the fractions.

Of the samples, 4 fractions, which had leading antioxidant activity of each test, were analyzed again in order to reconfirm their antioxidant activity. The protective effect of the selected fractions against t-butyl hydroperoxide (t-BHP) induced oxidation was investigated in normal human erythrocytes and determined by malondialdehyde (MDA) formation. The amount of MDA formed from t-BHP induced erythrocytes was considerable but was inhibited by rice wine extract. The inhibition rate of the fractionated samples ranged from 15.6 to 35.7%. Fraction number 5 (F5) showed the greatest inhibition rate (35.7%) and was comparable to the positive control, 75mmol ascorbic acid (25.8%) (Figure 1).

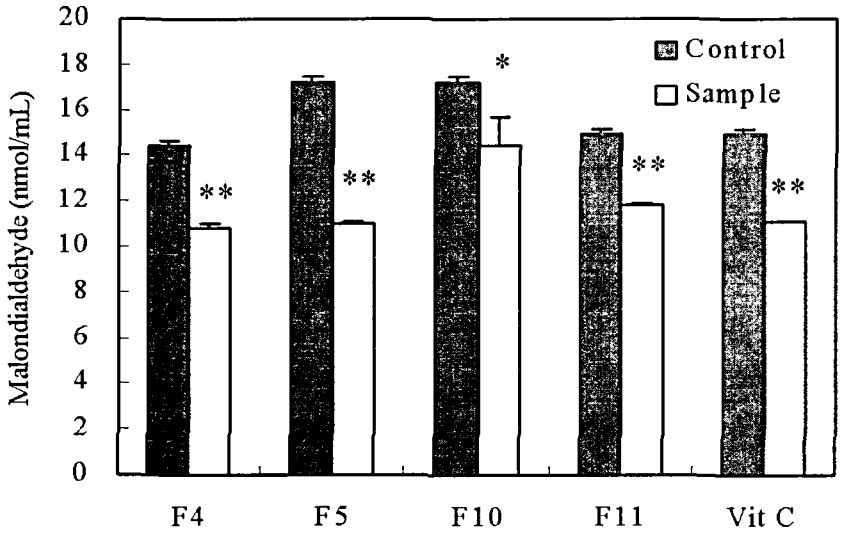

Fig. 1 Antioxidant effect of fractionated samples against erythrocyte oxidation.
* P<0.05 control vs sample, ** P<0.01 control vs sample

As F5 showed high antioxidant activity it was further separated by reverse phase liquid chromatography. AAC was widely observed for F5-3 to F5-12 fractions, in particular, F5-7 and F5-11 were 435 and 423, respectively which surpassed other fractions. In order to elucidate the peptide responsible for the antioxidant activity, further purification was made by HPLC for the sample fraction with the highest antioxidant activity (F5-11) (Figure 2). Two of the fractions, F5-11-10 and F-5-11-12 showed high AAC, in which the value (153) was the same. As the 2 fractions (F5-11-10 and F-5-11-12) of the highest AAC value was not pure enough for the elucidation of the peptide, it was further separated by HPLC at different condition.

Four peptides(P1, P2, P3, P4) were obtained from F5-11-10 and 2 peptides (P5, P6) from F5-11-12 (Figure 2). The amino acid composition(sequences) of P4 and P3 were identified as Ile-His-His and Val-Val-His(Asn) respectively. The sequences for P1 and P2 were not identified. The sequence of peaks P5 and P6 were found to be Leu(Val)-Lys-Arg-Pro and Leu-Val-Pro (Table 2). The peptides with identified sequence were synthesized and tested for antioxidant activity.

The AAC activity of the synthetic peptide is shown in Figure 3. Peptides with histidine in the sequence

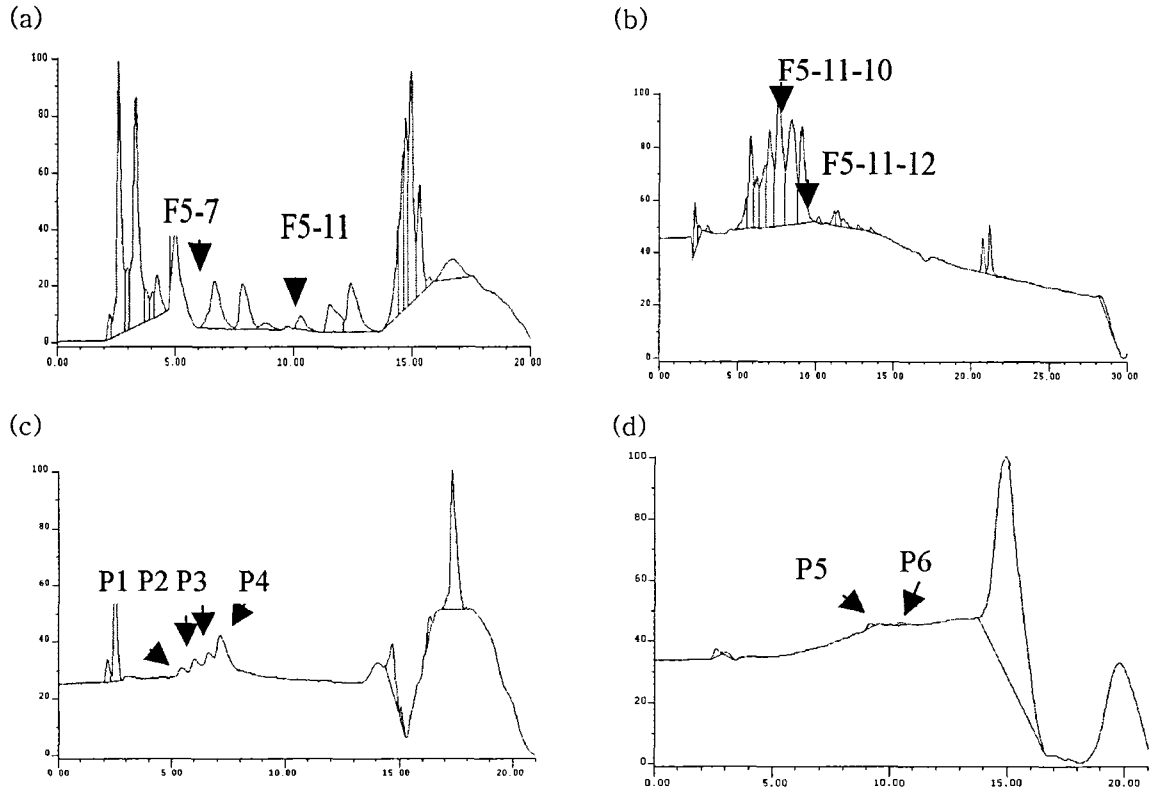

Fig. 2 Purification of antioxidant peptides from rice wine. The sample fractions were collected at the retention time presented in the chromatogram. Fractions showing antioxidant activity were indicated by arrow. Figures represent (a) the separated fraction of sample F5, (b) the separated fraction of sample F5-11, (c) the separated fraction of sample F5-11-10 and (d) the separated fraction of sample F5-11-12.

Table 2. Amino acid sequences of isolated antioxidant peptides from rice wine.

peak	Sequence
P1	Not identified
P2	Not identified
P3	Val-Val-His(Asn)
P4	Ile-His-His
P5	Leu(Val)-Lys-Arg-Pro
P6	Leu-Val-Pro

(VVH and IHH) showed high AAC activity. The AAC activity of peptide sequence with Leu and Val at the N-terminal (LKRP and VKRP) augmented considerably when the concentration increased from 1mM to 4mM, while the LVP sequence appear to be unaffected by the dose of the peptide.

The ability of the synthetic peptides to scavenge radicals formed from t-BHP-induced erythrocytes was weak and the most effective peptide was VKLP.

In general the findings from this study indicate that like antioxidant rich red wine, which is health-promoting when consumed in moderate amount, rice wine has the potential to have similar effect.

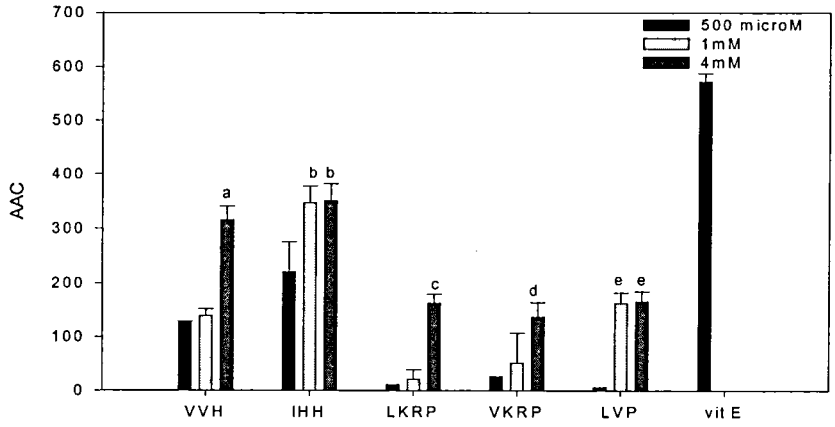

Fig. 3 Antioxidant activity of synthetic peptides.
a ; p<0.01 4mM vs 500 μ M, 1mM
b ; p<0.01 500 μ M vs 1mM, 4mM
c ; p<0.01 4mM vs 500 μ M, 1mM
d ; p<0.01 4mM vs 500 μ M, 1mM
e ; p<0.01 500 μ M vs 1mM, 4mM

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