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Barley Malt Treated with Enzymes Increases Polyphenol Content and Antioxidant Activity

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Abstract - The purpose of this study was to improve the functionality of a healthy drink with examining the possibility of manufacturing different enzymes (alpha-, beta-, glucose-amylase) in barley malts (BM) produced in various malting periods. The study showed that enzyme treatment increased significantly total polyphenol content (TPC), DPPH radical scavenging activity and hydroxly radical scavenging activity in malted liquid samples (MLS) which obtained from various malting periods. The highest of TPC were found in Gluco-24M with 1.981 ㎎TAE/㎖, followed by Beta-24M and Alpha-72M with 1.878 ㎎TAE/㎖ and 1.845 ㎎TAE/㎖, respectively. The DPPH result revealed that percent of inhibition increased by 71-75% compared to the control. No statistical difference was found between MLS obtained by 24 hr of malting (24 M) and 72 hr of malting (72 M) after enzyme treatment. In addition, an increasing of hydroxyl radical was in the same trend to the TPC and DPPH. The hydroxyl radical scavenging activity of enzyme treated samples was 1,5 times higher than the control. These results suggest the possibility of enzyme application to barley malts obtained in various germination periods for improving quality and functionality of barley malts.

Key words - Barley malt, Malting period, Enzymatic treatment, Antioxidant, Total polyphenol

Introduction

Barley (*Hordeum vulgare* L.) is a well known cereal grain, cultivated throughout the world and one of the widely consumed cereal among the most ancient cereal crops. About 80~90% of barley production is used for food and animal feed, but now barley is gaining renewed interest as an ingredient for the production of functional foods due to their absorption of bioactive compounds such as β-glucan, arabinoxylan, and tocopherols (Lee *et al*., 2010).

Recently, food processing technology has played a very important role in the food industry. In among these, instant food manufacturing for long term storage purpose has been also developing. In addition, smoking, excessive drinking, stress like cause atherosclerosis, coronary heart disease and other number of lifestyle-related diseases such as diabetes, so there is an increase in interest in the prevention and treatment of such diseases. Accordingly, research and development on the functional food and natural functional materials for health promotion have been made actively (Seog, 2002). Stout is a beverage obtained from the alcoholic fermentation of roasted and entrusted barley malt to which hops have been added (Lasekan, 1999).

The application of beta-amylase, alpha-amylase and gluco-amylase was reported to be favorable and application of dual enzyme systems for the production of sweeteners had been considered. When two enzymes are simultaneously added to starch solution the conversion was more efficient than successive processes (Bryjak, 2003). The purpose of malting is to create these enzymes, break down the matrix surrounding starch granule, prepare the starches for conversion, and then stop this action until the brewer is ready to utilize the grain. The kiln drying of the new malt denatures (destroys) lots of different enzymes, but several others remains, including the ones necessary for starch conversion. The amount of enzymatic

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starch conversion potential that a malt has been referred to as its “diastatic power” (Lee *et al*., 1999).

The antioxidants present in plants limit the free radical initiation in the body and lower the incidence of various diaseases such as cancers and diabetes. The methanol extracts of rice, wild rice, wheat, oat and barley were found to be effective in DPPH free radical scavenging activity (Ryu *et al*., 2002; Bu *et al*., 2002; Chon and Kim, 2006). Eight Jeju native plants were also reported to show strong DPPH free radical scavenging effect (Jang *et al*., 2015) and the methanol extracts of the eight medicinal palnts were investigated for phytochemicals and antioxidant activities (Chon *et al.,* 2008).

This study was to determine the antioxidant capacity of the barley malts (BM) with different enzyme treatments such as alpha-, beta-, and glucose-amylase by measuring total polyphenol content, DPPH radical scavenging activity, and hydroxyl radical scavenging activity for producing better quality beaverage by using barley malts .

Materials and Methods

Enzymes and reagents

Alpha-amylase (EC 3.2.1.1) from a selected strain of *Bucillus amyloliquefaciens*, beta-amylase 1500 EL from a *Semviscous liquid* and gluco-amylase (EC 3.2.1.3) from a selected strain of *Aspergillus niger*, DPPH_(2,2-diphenyl-1-picril-hydrazyl radical), Tannic acid, Phenol reagent were purchased from the Sigma–Aldrich Chemical Co. All solvents and reagents were of analytical, spectrometric or chromatographic grade.

Preparation of barley malts

Barley seeds were germinated in the cylindrical germination chamber for 24 and 72 hr (Fig. 1). This device is useful for controlling the environment due to maintain uniformity of barley malt. 50 ㎏ of barley seed can be used at one time of germination. The malts were produced under 18 to 20℃, seeds were humidified by water once a day. The cylinder rotation rate was 3 times per minute under ventilated conditions.

Enzyme treatment

24 hr and 72 hr-germinated barley malts (BM) were divided into four groups. 50 g of each BM groups was mixed with 150 ㎖ deionized water, then the mixtures was ground by blender to obtain malted liquid samples (MLS). After the MLS was obtained, alpha-amylase, beta-amylase and gluco-amylase were applied, then incubated at 60-75℃ for 24 hr. For a control, MLS was used without adding enzyme and incubated at room temperature. After 24 hr of incubation, all samples were filtered through adventures 5B filter paper (Tokyo Roshi Kaissha Ltd, Japan), evaporated to adjust the sugar content to 10 brix° by evaporator (EYLA N-1000, Tokoy, Japan) in a 40℃ water bath. Then, the enzyme activity was stopped by boiling at 99℃ for 15 min in a water bath, and rapidly cooled in ice.

Determination of total polyphenol content

The total polyphenol content of the samples was analyzed according to Folin-Ciocaltues method described by Singleton and Rossi (1965). In brief, samples aliquot of 0.2 ㎖ were added to test tubes containing 0.2 ㎖ of phenol reagent (1M). The volume was made up by adding 1.8 ㎖ of distilled water and the solution was vortexed for 3 min for the reaction. Then, 0.4 ㎖ of Na_2CO_3 (10% in water, v/v) was added and the volume was brought to 4 ㎖ by adding 0.6 ㎖ of distilled water. The mixtube was allowed to stand at room temperature for 1h

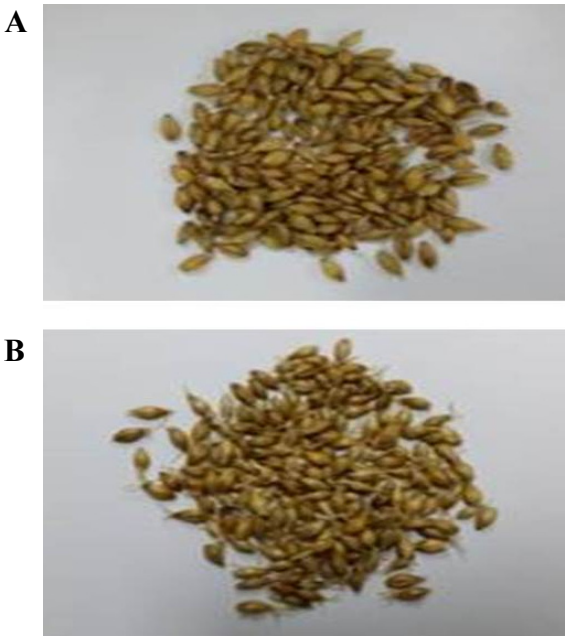

Fig. 1. Barley malts obtained at 24 hr (A) and 72 hr (B) malting.

and absorbance was measured at 725 ㎚ in a spectrophotometer (Model UV-1800. Shimadsu Corporation, Kyoto, Japan). The total phenolic content was calculated from a calibration curve (R^2 = 0.999) using tannic acid equivalent (TAE) per g dry weight (dw).

HPLC quantification of phenolic compounds

To quantify the phenolic compounds in all enzyme treated malt liquid samples (EMLS), a high-performance liquid chromatography (HPLC) system (Agilent 1260 series) with 2 gradient pump system (Agilent Technologies, product No G1311C. made in Germany), an auto sample injector (Agilent Technologies, Product No G1329B. made in Germany), a UV- detector (Agilent Technologies, Product No G4212B. made in Germany.) and a column oven (Agilent Technologies, Product No G1316A. made in Germany) were used. The separation was performed in 4.6×250 ㎜ reversed phase, Eclipse XDB - C18 5 c㎛olumn. HPLC conditions were as follows: Solvent A (water in 0.1% Trifluoroacetic acid) and solvent B (methanol) was prepared. Gradient elution used was 0- 40 min, 20% B; 40-50 min, 40% B; 40-50 min, 40% B; 50-55 min, 40% B; 55-60 min, 20% B; 60 min, 20% B. The flow rate of the mobile phase solution was 1.0 ㎖/min, and detection was carried out in 280, 320 ㎚. Twenty microliter of each sample was injected. 10 standards (*p*-hydroxybenzoic acid, 3-hydroxybenzoic acid, Vanilic acid, *o*-coumaric acid, Syringic acid, Ferulic acid, Caffeic acid, Sinapic acid, *p*-Coumaric acid, Protocatechuic acid) were used as an external standard to monitor phenolic compounds production.

DPPH radical scavenging activity

The antioxidant activity of the treated samples was measured on the basis of the scavenging activity of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the method described by Braca *et al.* (2003) with slight modifications. Samples of aliquot (no diluted) and diluted sample (50% diluted by distilled water) were prepared. One ㎖ of each sample was respectively mixed with 3 ㎖ of DPPH solution (0.15 mM). The mixtures were left to stand at room temperature for 30 min in the dark before the absorbance was measured at 517 ㎚ using a spectrophotometer and against a methanol blank. The scavenging activity was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = (1 - A_{sample} / A_{control}) \times 100$$

Where A_{sample} represents the observance of the experimental sample and $A_{control}$ represent the observance of the control.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the treated samples was measured according to the method of Halliwell *et al.* (1987) with modification. Briefly, 0.1 ㎖ of various concentrations of the samples was added to test tubes consisted of 10 mM $FeCl_3$, 10 mM EDTA, 10 mM H_2O_2, and 10 mM deoxy-ribose in potassium phosphate buffer (pH 7.4) (0.9 ㎖). The reaction mixture was incubated for 1 h at 37℃, and boiling in water bath 95℃ for 15 min after added 0.5 ㎖ of 10% (w/v) trichloroacetic acid and 0.5 ㎖ of 1% (w/w) 2-thiobarbituric acid. The color development was measured of 532 ㎚ against a blank containing phosphate buffer.

Statistical analysis

All experiments were processed in triplication and the average values are reported as mean values with standard deviation. The differences of mean values amongst samples were determined using one-way analysis of variance (ANOVA) at $p \leq 0.05$ significance level. All statistical analysis was performed using GraphPad instant software (version 3.06 for Windows; GraphPad software, San Diego, CA)

Results and Discussion

Sugar content (Brix°) of Enzyme treated malt liquid samples (EMLS) at the different malting periods

The sugar content of EMLS after different malting periods 24 hr (24 M), 48 hr (48 M), 72 hr (72 M) and 96 hr (96 M) was obtained. The result revealed that; gluco-amylase treated MLS obtained from 48 M and 72 M showed higher sugar content with 3.0 and 3.5 Brix°, respectively (Table 1).

The enzymatic hydrolysis of 96 M was showed lower Sugar content with 1-2 brix° compared to other treatments. The decreasing of the sugar content of 96 M might be due to loss of starch was converted to sugar during enzyme hydrolysis.

Table 1. Comparison of brix enzyme treated malt liquid samples (EMLS) at the different malting periods.

Treatment	Malting periods (hr)			
	24	48	72	96
Control	0.8	1.3	1.3	1.0
Alpha-amylase	3.0	3.0	2.5	1.5
Beta-amylase	1.7	2.8	2.7	1.5
Gluco-amylase	3.0	3.0	3.5	2.0

According to previous reports, after 24 hr of germination, all kernels contained areas of starch degradation adjacent to the crushed layer of cells. These degraded areas were concentrated at the crease end of the endosperm-embryo junction, and some of the starch granules have been partially degraded (Evers and McDermot, 1970). Our result showed an increase of sugar content after 24 hr of germination, this might be due to the initial stages of malting for the contained starch to be hydrolyzed and converted to sugar.

Total polyphenol content

The content of total polyphenol in all enzyme treated malt liquid samples (EMLS) showed a higher TPC content after the 24 hr of malting (24 M) in comparison to 72 hr of malting (72 M). The gluco-amylase treated MLS obtained from 24 M (Gluco-24M) showed the highest TPC with 1.981 ㎍/㎖, followed by Beta-amylase treated MLS obtained from 24 M (Beta-24M) with 1.878 ㎍/㎖. However, amongst the EMLS of 72 M, Alpha-amylase treated MLS from 72 M (Alpha-72M) showed significantly higher TPC than that from Beta-72 M and Gluco-72M with 1.8 ㎍/㎖ (Fig. 2). Maillard *et al*.(1996) mentioned that TPC of barley malt was increased at the 24hr of germination, thus, the increase in TPC in our study might be due to the enhanced hydrolytic enzyme activity, thereby leading to the release of bound phenolic compounds and increasing of TPC.

HPLC quantification of phenolic compounds

The phenolic acid content of the control and enzyme treated malt liquid samples (EMLS) at the different malting periods on HPLC is presented in Table 2. The results showed

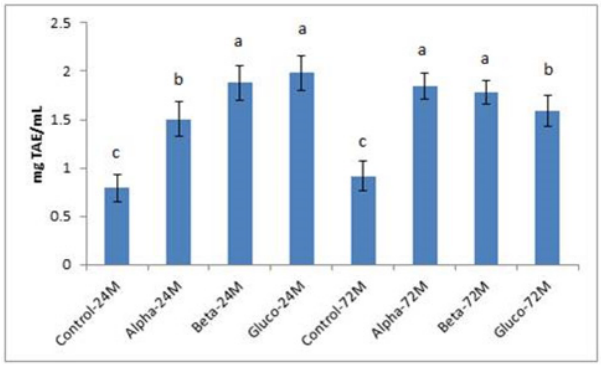

Fig. 2. Total polyphenol content of enzyme treated malted liquid samples (EMLS) at the different malting periods. Means with same letter in column are not significantly different at $p < 0.05$ level by Duncan's multiple range test. The bars represent the standard error.

Table 2. Phenolic acid (㎍/g) identified in all enzyme-treated malt liquid samples (EMLS).

Treatment	24 hr Malting (24M)				72 hr Malting (72M)			
	Control	Alpha amylase	Beta amylase	Gluco amylase	Control	Alpha amylase	Beta amylase	Gluco amylase
p-hydroxybensoic acid	ND	ND	ND	ND	ND	ND	ND	ND
3-hydroxybenzoic acid	0.018	0.035	0.044	0.046	0.021	0.043	0.041	0.037
vanilic acid	0.046	0.058	0.061	0.061	0.028	0.057	0.055	0.049
o-cumaric acid	0.122	0.128	0.059	0.128	0.059	0.119	0.115	0.103
Syringic acid	0.216	0.100	0.201	0.216	0.100	0.201	0.194	0.173
Ferulic acid	9.487	19.067	18.396	20.473	9.487	19.067	18.396	16.391
Caffeic acid	ND	ND	ND	ND	ND	ND	ND	ND
Sinapic acid	0.254	0.226	0.215	0.283	0.131	0.263	0.254	0.226
p-Cumaric acid	0.009	0.215	0.011	0.012	0.005	0.011	0.010	0.009
Protocatechic acid	0.003	0.006	0.007	0.007	0.003	0.007	0.007	0.006

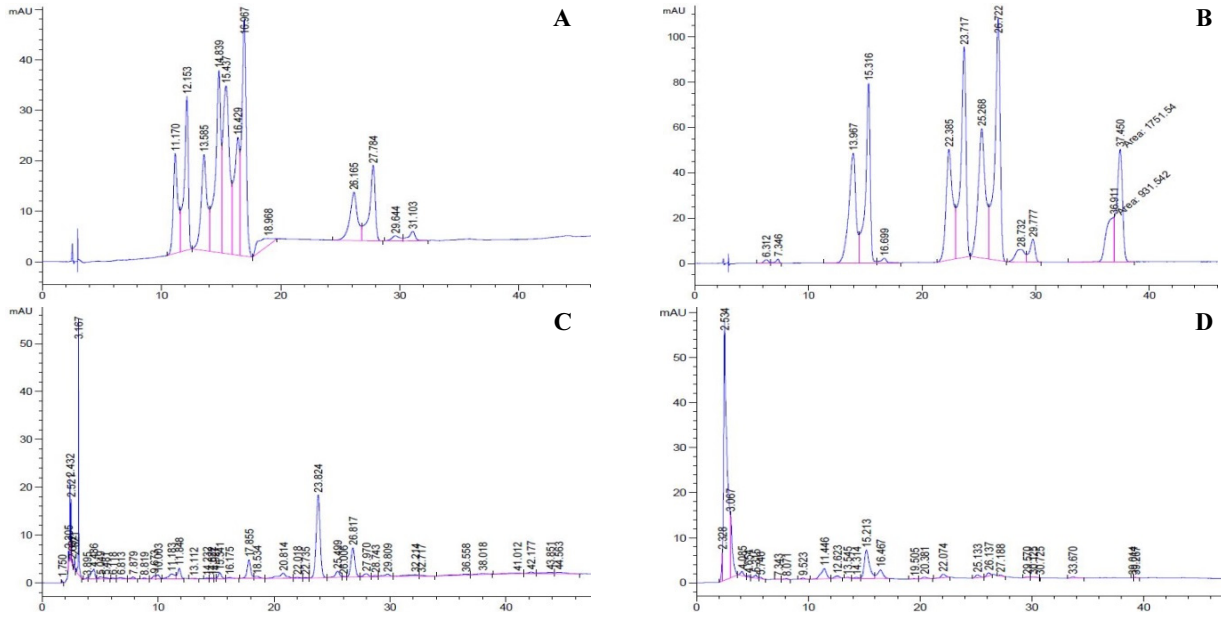

Fig. 3. HPLC-chromatogram of phenolic compounds. A and B: standard, C: control in 24M, and D: control in 72 M.

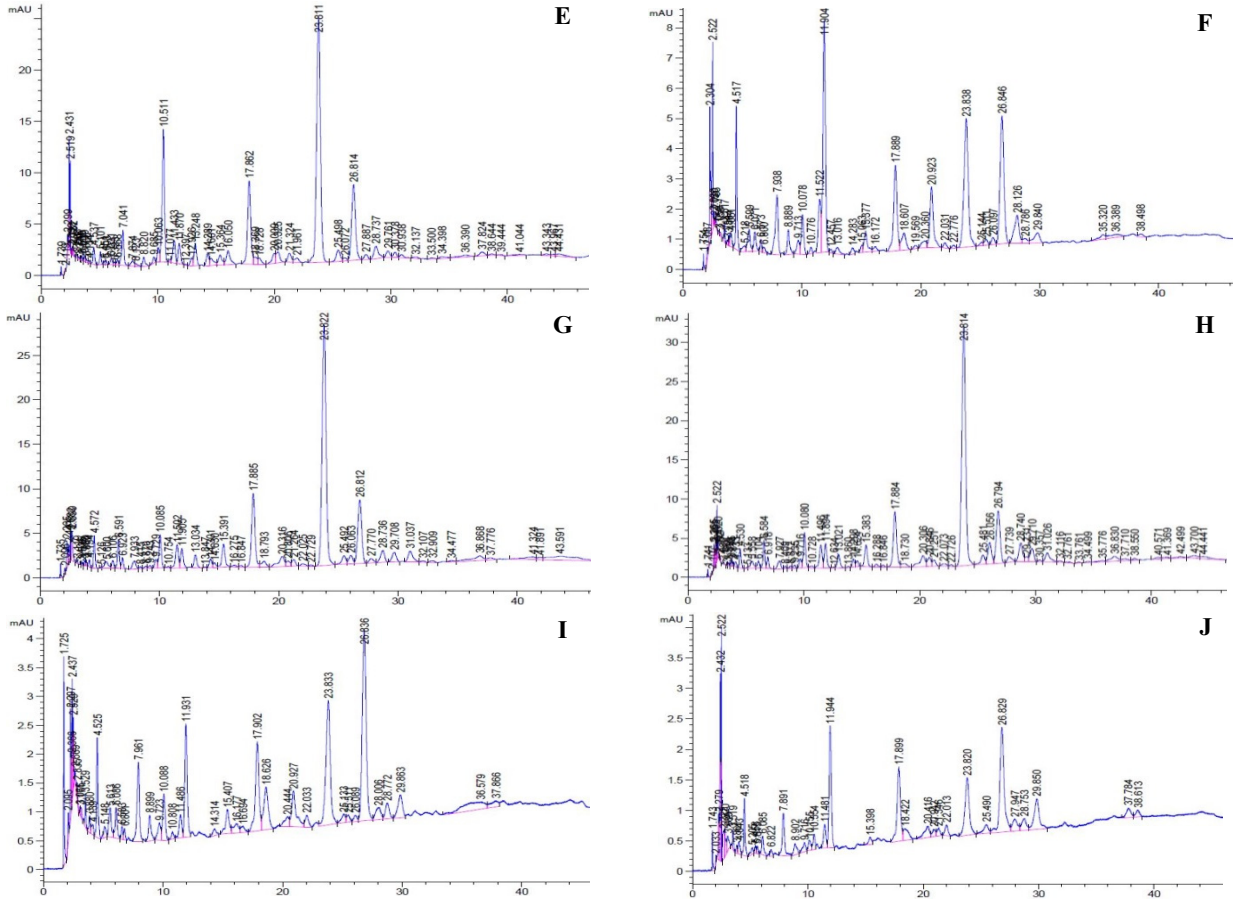

Fig. 4. HPLC-chromatogram of phenolic compounds in enzyme-treated samples. (E) 24 M and (F) 72 M in α-amylase treatment, (G) 24 M and (H) 72 M in β-amylase treatment and (I) 24 M and (J) 72 M in Gluco-amylase treatment.

that the content and type of phenolic acid varied dependent on the enzyme treatment and malting period. Eight compounds were identified in this study as follows: 3-hydroxybenzoic acid, vanillic acid, o-cumaric acid, syringic acid, ferullic acid, sinapic acid, p-cumaric acid, and protocatechic acid. In the treatment of 72 hr malting, all of eight compounds were increased in enzyme-treated samples while both o-cumaric acid and sinapic acid were decreased in enzyme-treated samples in 24 hr malting, compared to the control (Fig. 3 & 4). Therefore, the increase of total polyphenol content in an application if enzymes barley malts are seemed to be attributed to the increase of those phenolic acids under the condition of 72 hr malting at latest in this study.

DPPH radical scavenging activity

The DPPH radical scavenging activity of enzyme treated malt liquid samples (EMLS) at different malting periods were investigated. DPPH inhibition capacity was found to be concentration dependent (Fig. 5). All treated samples showed good potential of DPPH radical scavenging activities after both 24hr and 72hr of malting periods. Amongst EMLS after different malting periods, Gluco-24M exhibited highest inhibition percent (75.84%), followed by Beta-24M (75.33%) and Alpha-72M (71.02%) respectively. The radical scavenging activity of Gluco-24M was significantly higher than Gluco-72M and Beta-72M treatment ($p < 0.05$). Though the EMLS at 24M showed higher DPPH scavenging activity in between treatments. However, the significant difference was not found between Glucose-24M and Beta-24M treatment.

The antioxidant activity of MLS in 24 M in various treatments were ranged from 53.78% to 75.84%. The lowest DPPH radical scavenging activity was obtained in Gluco-72M and Beta-72M. Beta *et al.* (2005) reported that the interior part of wheat kernel had low TPC and DPPH activity as compared to the outer fractions. In addition, Quingming *et al.* (2010) mentioned that malt barley extract prevented the deceasing in antioxidant enzyme activity and carbornyl content, but the total antioxidant capability was improved in D-lactose treated mice.

Generally, the high antioxidant activity was proportional to the increasing of phenolics content in barley (Attila-Levente Fogarasi, 2015). Phenolic compounds were considered as a major group which contributed to the antioxidant activity of cereals (Zielinshi & Kozlowska, 2000). Cook and Summan (2006) reported that, phenolic compounds act as natural antioxidants, which may exhibit one or more of the following roles: free radical scavenging, reducing agent, potential producer.

Hydroxyl radical scavenging activity

All enzyme treated malt liquid samples (EMLS) samples at different malting periods exhibited high hydroxyl radical scavenging ability with 60%~75% (Fig. 6). Gluco-amylase treated MLS obtained from 24 M (Gluco-24M) showed highest hydroxyl radical scavenging activity with 76.841%, followed by Beta-amylase treated MLS obtained from 24 M (Beta-24M) with 70.64%. For the EMLS obtained from 72 M, Alpha-amylase treated (Alpha-72M) sample showed the highest value with

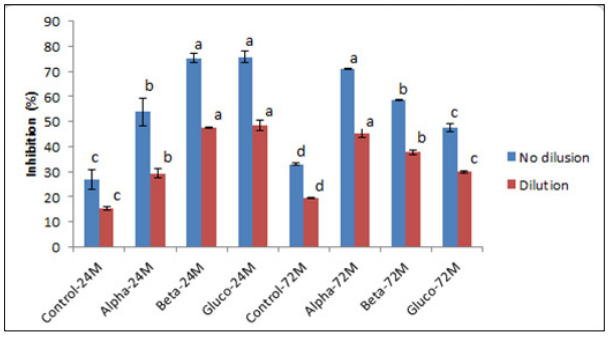

Fig. 5. The DPPH radical scavenging activity of enzyme treated malted liquid samples (no diluted and diluted samples) at the different malting periods. Means with same letter in column are not significantly different at $p < 0.05$ level by Duncan's multiple range test. The bars represent the standard error.

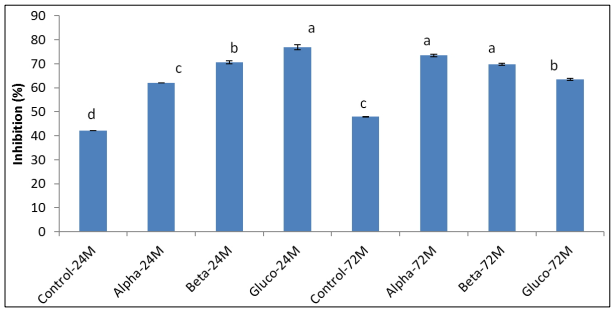

Fig. 6. The Hydroxyl radical activity of enzyme treated malted liquid samples (EMLS) at the different malting periods. Means with same letter in column are not significantly different at $p < 0.05$ level by Duncan's multiple range test. The bars represent the standard error.

72.3%. For the overall aspect, hydroxyl radical scavenging activity showed similar trends to DPPH free-radical scavenging ability and TPC. Significant difference in Antioxidant activities of different malting periods, different enzymatic treatment was found to be significantly different with each. Especially, hydroxyl radical scavenging ability showed remarkable antioxidative activity to process the enzyme.

The results of this study indicated that total phenolics, and antioxidant activity properties were affected by enzyme application to barley malts within 72 hr at latest. However, we did not compare these results to the any positive control in this study. Thus, comparisons in phenolic contents and antioxidant activity between EMLS and a positive control would be needed to clarify more finely the difference between both in further study.

In conclusion, enzyme treatments such as alpha-, beta-, and glucose-amylase not only affected total phenolic content, but also affected their biological benefit of barley malt. This finding has contributed to the understanding of under utilized cereals and proper processing methods to enrich their biological activity. These results have provided useful information on the improved barley malt as fermentor or ingredients of beaverage and powder for functional food.

Acknowledgement

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References

Beta, T., S. Nam, J.F. Dexter and H.D. Sapirstein. 2005. Phenolic content and antioxidant activity of pearled wheat fraction. Cereal Chemistry 82(4):390-393.

Braca, A.G., I. Morelli, F. DeiSimone and N.D. Tommasi. 2003. Antioxidant and free radical scavenging activity of flavonol glycosides from different Aconitum species. J. Ethnopharmacol. 86:63-67.

Bryjak, J. 2003. Gluco-amylase, alpha-amylase and beta-amylase immobilisation on acrylic carriers. Biochemical Engineering Journal 16:347-355.

Bu, S.Y., W.B. Jeon, D.S. Kim, H.Y. Hoe and Y.W. Seo. 2002. Antioxidant activity and total phenolic compounds in grain extracts of wheat, barley, and oat. Korean J. Crop Sci. 47(2):201-207.

Chon, S.U., T.S. Kim, J.S. Shin and H.O. Boo. 2008. Antioxidant activity of methanol extracts from root part of Korean salad plants. Korean J. Plant Res.12(6):413-419.

Chon, S.U and Y.M. Kim. 2006. Phytotoxicity and DPPH radical scavenging activity of barley seedling extracts. Korean J. Crop Sci. 51(4):322-328.

Cook, N.C. and S. Samman. 2006. Flavonoids-chemistry, metabolism, cardio protective effect, and dietary sources. Nutr. Biochem. 7:66-76.

Evers, A.D. and E.E. McDermot. 1970. Scanning electron microscopy of wheat starch. II. Structure of granules modified by α-amylolysis-preliminary report. Staerke 22:23.

Fogarasi, A.L. 2015. A comparative assessment of antioxidant properties, total phenolic content of einkorn, wheat, barley and their malts. Food Chem. 167:1-6.

Jang, H.J., H.J. Bu and S. Lee. 2015. Screening for antioxidative activity of Jeju native plants. Korean J. Plant Res. 28(2):158-167.

Halliwell, B., J.M.C.Guttridge and O.I. Aruoma. 1987. Analytical Biochemistry 165:215-219.

Laseken, O.O. 1999. Effect of *Vernonai amygdalina* (bitter leaf) extract on brewing qualities and amino acid profiles of stout drinks from sorhum and barley malts. Food Chem. 64:507-510.

Lee,Y.T., S.J. Seo and H.G. Chang. 1999. Quality characteristics of barley varieties related to enzymatic activity in malt. Korean J. Food Sci. Technol. 31(6):1421-1426 (in Korean).

Lee, N.Y., Y.K. Kim, I. Choi, S.K. Cho, H.J. Kweon, K.W. Lee, J.N. Hyun, J.S. Choi, K.H. Park, K.J. Kim and M.J. Lee. 2010. Antioxidant activity and biological activity of specialty barley (*Horduem vulgare* L.) extracts. Korean J. Crop Sci. 55(2):159-164.

Maillard, M.N., M.H. Soum, P. Boivin and C. Berset. 1996. Antioxidant activity of barley and malt: Relationship with phenolic content. Lebensmittel Wissenschaft and Technologie. 29:238-244.

Qingming, Y., P. Xianhui, K. Weiboa,Y. Hong and S.Yidan. 2010. Antioxidant activity of malt extract from barley (*Hordeum vulgare* L.) toward various oxidative stress *in vitro* and *in vivo*. Food Chem. 188:84-89.

Ryu, S.N., S.Z. Park and H.Y. Kim. 2002. Antioxidant activity in rice cultivar, wild rice, and barley. Korean J. Crop Sci. 47(1):54-61.

Seog, H.M., M.S. Seo, S.R. Kim., Y.K. Park and Y.T. Lee. 2002. Characteristics of barley polyphenol extract (BPE) separated from pearling by-products. Korean J. Food Sci. Technol. 34(5):775-779 (in Korean).

Singleton, V.L. and J.A. Rossi. 1965. Calorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American J. of Enology and Viticulture 16:144-158.

Zielinski, H. and H. Kozlowska. 2000. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fraction. J. Agric. Food Chem. 48:2008-2016.

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