African Journal of Biotechnology Vol. 9(53), pp. 9018-9025, 27 December, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.370
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Effects of malting conditions on the amino acid compositions of final malt

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Accepted 10 December, 2010

Barley has been widely used for the production of malt in the brewing industry. Malt was the main raw material indispensable for beer brewing. The fermentability of malt wort is dependent on an adequate supply of the essential nutrients required by yeast. The amino acid content is an important malt parameter to the yeast growth and metabolism in malt wort. To increase brewing fermentability and efficiency, malts with high levels of free amino nitrogen and amino acids are essential. However, the barley variety and malting conditions affecting this supply of nutrients are not well understood. The aims of this study were to determine the amino acid and malt quality parameters of barley varieties (Harrington, GanIll, Esterel and Kendall) changed during germination and final malt and investigate the amino acid composition of barley copeland in three malting programs. The contents of twenty amino acids during germination and in the final malt stage were determined using a reverse-phase high-performance liquid chromatography (RP-HPLC) method. Total amino acid had high positive correlation with free amino nitrogen (0.9354), Kolbach index (0.9719) and soluble protein (0.8316) in final malt. This study identified various important malting conditions that may lead to improvements in malt quality and thus enhancements in beer flavor. The optimal malting program of barley is a malting process that can provide desirable amino acid contents.

Key words: Malting program, amino acid, barley variety.

INTRODUCTION

Malt has been widely used for brewing, in which the first process is malting. Malting of barley is a complex process depending on numerous factors, such as the variety of barley, steeping, germination and kilning stages (Bamforth and Barclay, 1993). These steps influence the malt quality parameters such as Kolbach index, wort viscosity, fine-coarse extract difference and free amino nitrogen (Wentz et al., 2004). Total soluble nitrogen, Kolbach index and free amino nitrogen values of malts increase with increased germination time due to more extensive protein hydrolysis.

Hydrolyzed by proteases, barley proteins are broken down into low molecular weight peptides and amino acids, which serve as nutrients for embryo secretion and new protein synthesis (Klose et al., 2008). Because of the equilibrium between decomposition and dynamic germi-

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Abbreviations: Lys, Lysine; **His,** histidine; **Arg,** arginine; **Leu,** leucine; **Ile,** isoleucine; **Val,** valine; **Phe,** phenylalanine; **Gly,** glycine; **Ala,** alanine; **Tyr,** tyrosine; **Asp**, aspartic acid; **Asn**, asparagine; **Glu**, glutamic acid; **Gln**, glutamine; **Thr,** threonine; **Ser,** serine; **Met,** methionine; **Pro,** proline; **Trp,** tryptophan; **GABA,** γ-amino butyric acid; **TP,** total protein; **SP,** soluble protein; **KI,** kolbach index; **FAN,** free amino nitrogen; **TAA,** total amino acid.

nation, the level of barely protein is maintained.

Table 1. Malting programs used to steep, germinate, kiln barley samples.

Malting program	Steeping	Germination	Kilning
I	10 h wet, 5 h dry, 6 h wet, 5 h dry, 6 h wet (total 32 h), 15℃	17℃ 96 h	45℃ 6 h, 50℃ 4 h, 55℃ 8 h, 70℃ 1 h, 84℃ 3 h (total 22 h)
II	5 h wet, 9 h dry, 6 h wet, 12 h dry, 6 h wet (total 38 h), 14℃	16℃ 120 h	45℃ 6 h, 50℃ 4 h, 55℃ 8 h, 70℃ 1 h, 84℃ 3 h (total 22 h)
III	6 h wet, 14 h dry, 8 h wet, 10 h dry, 2 h wet (total 40 h), 15℃	14℃ 24 h, 15℃ 48 h, 17℃ 24 h (total 96 h)	60℃ 8 h, 65℃ 8 h, 70℃ 2 h, 75℃ 2 h, 80℃ 1 h, 83℃ 3 h (total 24 h)

During kilning, Maillard reaction occurs between amino acids and sugars, generating melanoid that gives beer color and flavor (Samaras et al., 2005).

Amino acid content plays a crucial role in yeast nutrition (Clapperton, 1971). The fact that the increase in amino acid level is mainly due to the respiration of barley during malting is generally recognized. It has been speculated that different malts produced by different malting programs or barley varieties, contain various amino acids at different levels. Many different types of low malt beer were brewed around the world. In China, the malt adjuncts of rice, sugar syrups and maize starch are used in most of the breweries. To increase the amino acid contents of barely is absolutely necessary. The amino acid levels will affect the fermentability because they are essential yeast nutrients.

The difference in amino acid contents will affect beer flavors (Jones and Pierce, 1964; Perpète et al., 2005); therefore, the determination of amino acids during barley germination is of great importance. Modern methods for determination of free amino acids either before or after protein hydrolysis include ion-exchange chromatography (IEC), high-performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (Erbe, 2000; Kutlán and Molnár 2003). In recent years, many novel methods without derivatization have been published. Özcan and Senyuva (2006) worked on the quantification of underivatized amino acids of biological interest, highlighting numerous interferences. Recently, γ-amino butyric acid (Gaba) has attracted attention due to its anti-hypertensive and anti-stress effects on human health (Ichimura et al., 2006; Vaiva et al., 2004). Therefore, the level of Gaba in malt was also investigated in this study.

The amino acid analysis was carried out by reverse-phase high-performance liquid chromatography (RP-HPLC) method. Results of the RP-HPLC analysis revealed that amino acid contents varied during malting. To better understand the amino acid content changes during malting, we analyzed many varieties of malt and compared their amino acid content to that of barley from different countries. The purpose of this study was to evaluate the changes in amino acid contents of five kinds of barley varieties through the process of germination to the final malt. This study also investigated the effects of barley variety in different malting conditions on amino acid contents in final malts.

MATERIALS AND METHODS

Barley varieties

Five barley varieties were selected and used in this study. Gan III is a Chinese native barley which is harvested in 2008 in Gan Su Province of China. Three Canadian barley varieties are Harrington, Kendall and Copeland which were obtained from CWB (Canadian Wheat Bureau). Esterel is a French barley variety offered by France Export Cereal Peking.

Micromalting and mashing

The malting trials were carried out in duplicate (n = 2). In each trial, 1 kg portion of barley was malted in a micromalting machine (Joe White Malting Systems, Perth, Australia). Steeping, germination and kilning conditions of three malting programs used in this malting experiment are listed in Table 1. Malt (50 g) was milled using an EBC standard mill (Buhler-Miag, Braunschweig, Germany); then wort was prepared in a mash bath (LB-8, Electronic LOCHNER, Germany), in which mashing temperature and time was controlled automatically according to EBC congress mash procedure 4.5.1 malt section (European Brewing Convention, 1998). In each trial, duplicate samples were collected from germination 24, 48, 72 and 96 h, respectively of green malt and final malt. The wort was produced by congress mash. The amino acids and free amino nitrogen were analyzed as described below.

Malt quality analysis

The malt protein content and extract yield were analyzed using a grain analyzer (Infratec 1229, Foss Tecator AB, Höganäs, Sweden). Total soluble protein (TSP) and free amino nitrogen (FAN) were determined by EBC methods Wort (European Brewing Convention, 1998). Kolbach index (KI) can be calculated with the formula: KI = (TSN/TN) ×100%.

Determination of amino acids

Table 2. Elution gradient for HPLC analysis of derivatized amino acids[a].

Time (min)	0	0. 5	18	19	29. 5	35	38	48
% A	100	99	95	91	82	0	100	100
% B	0	0	0	0	0	40	0	0
% C	0	1	5	9	18	60	0	0

[a]A = 100 µmol/L sodium acetate, pH 5.70, with 5.6 µmol/L triethylamine; B = ultrapure water; C = acetonitrile.

The concentration of amino acids in wort was determined using the AccQ-Tag amino acid analysis method, based on derivatization by treatment with 6-Aminoquinoly-N-hydroxysuccinimidyl carbamate and separation by AccQ-Tag amino acid analysis column with gradient elution. Spectrophotometric measurement was set at 248 nm.

Apparatus

A Waters Alliance system consisting of a Waters 2695 separation module and a Waters 2996 photodiode array detector was used (Milford, MA, USA).

Reagents

Waters AccQ-Fluor kit contains borate buffer, reagent powder (2A), and reagent diluent (2B). From Waters Company, we bought 17 amino acids (2.5 mmol/l): lysine, histidine, arginine, leucine, isoleucine, valine, phenylalanine, glycine, alanine, tyrosine, aspartic acid, glutamic acid, glutamine, threonine, serine, methionine, proline. Other amino acids of asparagine, γ-amino butyric acid, tryptophan, sodium acetate trihydrate, triethylamine of analytical grade were obtained from Fluka (Neu-Ulm, Germany). Acetonitrile was of HPLC grade.

Reagent preparation

1 ml of acetonitrile was added to the Waters AccQ-Fluor kit. The kit was sealed up and mixed for 10 s. The vial was heated for 10 min at 55°C in a water bath until the powder was dissolved completely.

Sample preparation

Wort samples were diluted with distilled water from 2 to 5 times in its original volume before use; they were then filtered through a polytetraethylene filter (PTEE) with a 0.45 µm pore diameter.

AQC precolumn derivatization reaction

10 µl sample and 70 µl borate buffer were transferred into a derivatization vial and mixed. Within 15 s, 20 µl 6-aminoquinoly-N-hydroxysuccinimidyl carbamate was transferred into the same derivatization vial.

Chromatographic condition and analysis

An AccQ-Tag amino acid analysis column (3.9 mm × 150 mm ID, 4 µm) was prepared. The column temperature was kept at 39°C. An aliquot of 20 µl of the derivative solution was injected into the column.

The UV detection wavelength was set at 248 nm. Excitation and emission wavelengths of fluorescence detector were 250 nm and 395 nm, respectively. The eluent phase consisted of triethylamine solution (pH 5.64), acetonitrile and water. We used the gradient profile of elution shown in Table 2 (Phase A, 100 µmol/l sodium acetate, pH 5.70, with 5.6 µmol/l triethylamine; phase B, ultrapure water; phase C, acetonitrile). The flow rate was 1 ml per min. The sample injection volume was 10 µl.

Statistical analysis

All analyses were performed in duplicate. The associations among individual traits were determined with simple linear correlation. Correlation analysis was performed using the SPSS statistical software (version 16.0 for Windows, SPSS Inc., Chicago).

RESULTS AND DISCUSSION

Changes in amino acid contents during malting

During malting process, changes in the amino acid contents of the three imported barley (Harrington, Esterel, Kendall) and the Chinese native barley (GanIII) were assessed in duplicate under malting program III (Table 1). Malt modification depends on malting conditions; the enzy- mes affect the structure of barley because of structural and compositional differences between the barley varieties. Malting conditions should be adapted accordingly. The Kolbach index (KI) is an indicator of proteolytic modi- fication of the malt and reflects protein solubilized during malting and preparation of the congress mash. Free amino nitrogen, amino acid composition and KI can fully show the degradation of protein. The optimum steeping and germination time and temperature are the key factors influencing the protein modification. In the germination phase, most of the amino acid contents carried out increased. Nevertheless, the contents of them showed significant difference after kilning.

Based on the variation trend, four groups of amino acids were divided. Glycine, histidine, alanine, proline, tyrosine, methionine, leucine, phenylalaine and tryptophan belong

to group A, whose concentrations significantly increased. However, the pattern of change for each amino acid was remarkably different from one another. The concentrations of amino acids in group B, including aspartic acid, serine, asparagine, glutamine, γ-amino butyric acid and

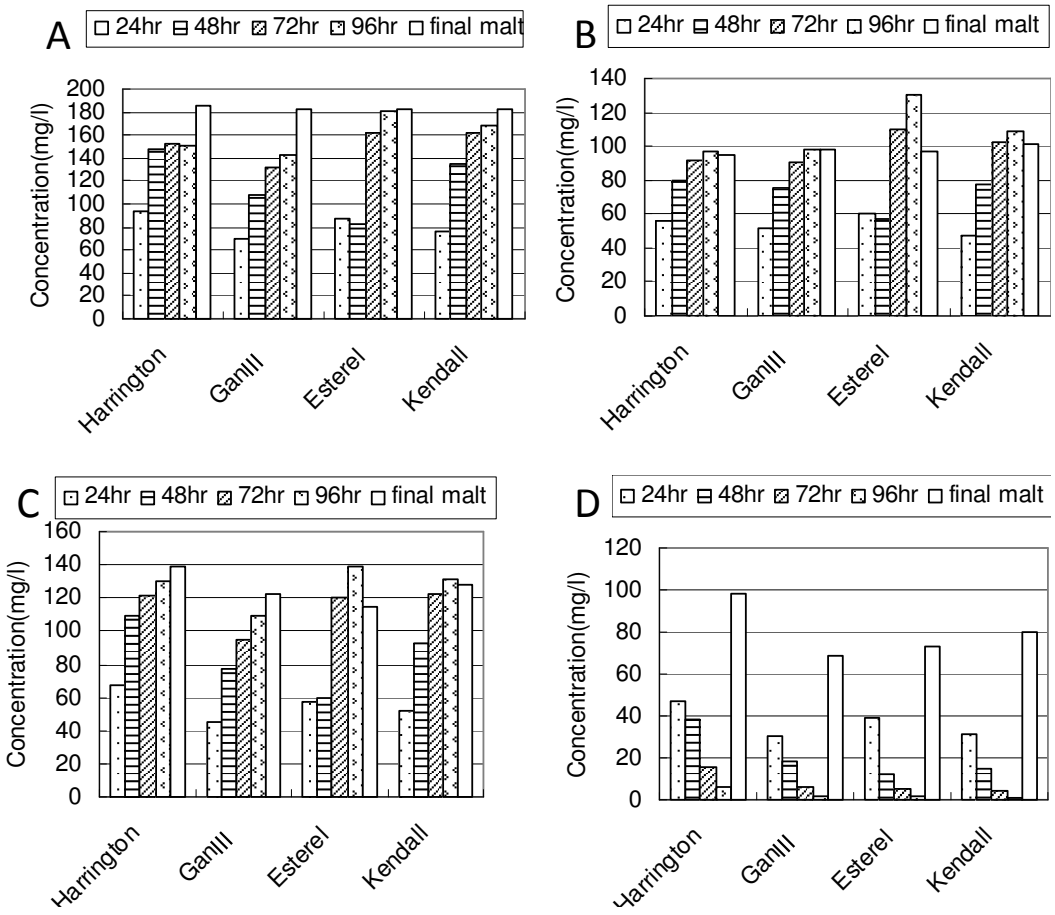

Figure 1. Amino acid contents changed in malting program III; A, leucine belong to group A (Gly, His, Ala, Pro, Tyr, Met, Leu, Phe and Trp); B, lysine belong to group B (Asp, Ser, Asn, Glu, Gaba and Lys); C, valine belong to group C (Glu, Arg, Val and Ile); D, arginine belong to group D.

lysine, showed slight decrease after kilning. Glutamic acid, threonine, valine, and isoleuine belong to group C, which has an irregular variation trend. They showed a decrease in Esterel and Kendall but an increase in Gan III and Harrington after kilning which may be caused by different characteristics of barley varieties. Arginine belongs to group D showing significant changes. Figure 1 described the details of leucine, lysine, valine and arginine representing different groups which showed the changes of amino acid contents in malting program III.

Figure 1 showed that the content of leucine increased during malting. However at 96 h, the leucine level in the Harrington sample was not different from the other three kinds of barley (Figure 1A). For Kendall, GanⅢ and Harrington samples, lysine accumulated gradually and reached a peak during the 96 h of malting but then decreased slightly towards the end; whereas, the Esterel sample has a significant decrease in lysine throughout the process (Figure 1B). Valine reached a peak at different times in the four kinds of barley and has a rapid increase from 48 to 72 h in the Esterel sample, which differed from other barley samples (Figure 1C). On the contrary, arginine levels decreased slightly in the first 96 h, whereas has a significant increase during kilning process (Figure 1D). There is a close relationship between arginine, gluta- mic acid, glutamine, and proline in metabolism. Glutamic acid is the precursor to synthesized proline. Firstly glutamic acid synthesizes ornithine, which synthesizes citrulline and then forms arginine. Arginine can be broken down to ornithine and urea (NH_3 and CO_2), so there forms a urea cycle between ornithine, citrulline and arginine (Pierce and Margaret, 1982; Mckee and Mckee, 1999).

During germination, arginine decreased because of the generation of NH_3 and CO_2. So if the content of proline increased, the content of arginine would decrease. During

kilning, aspartic acid and citrulline synthesize arginine. The decrease in aspartic acid induces the increase of arginine. Glutamate and glutamine can synthesize citrulline which is the arginine precursor. Glutamate, glutamine and aspartic acid decreased, part of them forming arginine. On the other hand, lysine and arginine are two basic amino acids that certainly have antagonistic effect. The reduction

Table 3. Malt Parameters of Harrington, GanIII, Esterel, Kendall[a].

Parameters	Harrington	GanIII	Esterel	Kendall
Total protein (%)	11.2	12.1	11.4	11.8
Soluble protein (%)	5.2	4.8	4.7	4.9
Kolbach index (%)	46.4	39.7	41.2	41.5
Free amino nitrogen (mg/L)	209.1	180.8	191.8	206.4
Total amino acid (mg/L)	2347.4	2030.7	1974.2	2143.1

[a] Values are given as mean of duplicate experiments

Table 4. Correlation coefficients among malt quality parameters and total amino acid[a].

	TP	SP	KI	FAN	TAA
TP	1.000				
SP	0.8312*	1.000			
KI	0.5304	0.9117**	1.000		
FAN	0.6102	0.9411**	0.9803**	1.000	
TAA	0.4003	0.8306*	0.9719**	0.9354**	1.000

[a] TP = total protein; SP = soluble protein; KI = Kolbach index; FAN = free amino nitrogen; TAA = total amino acid; * and ** = significant at $P < 0.05$ and $P < 0.01$ levels of probability, respectively.

of lysine will lead to the increase of arginine. From the above we draw a conclusion that kilning is a crucial procedure influencing amino acid composition in the malt.

Relationship between malt quality parameters and total amino acid content of malt samples

During malting program III, protein modification and free amino acid contents are gradually increased. The malt quality parameters for four malts (Table 3) varied but in a normal range, which reflected the better quality of the malts. The relationship between the quality parameters and the corresponding total amino acid contents of the malts were investigated. Correlation coefficients among malt quality parameters and total amino acid are presented in Table 4. Total amino acid (TAA) had a high positive correlation (0.9354) with free amino nitrogen (FAN). The correlation of total amino acid between Kolbach index (KI) and soluble protein (SP) were 0.9719 and 0.8316, respectively.

Effect of barley variety

Barley variety has a great influence not only on agronomic properties such as yield, fertilization and resistance to diseases, but also on quality characteristics, such as suitability for malting and brewing (Kattein and Hartmann, 2008). The difference in total nitrogen content of the individual barley grains is influenced by the variety of the barley and the topography of the field in which the barley was grown (Agu and Palmer, 2003). Four varieties of barely were used in malting program III (Table 1). The results showed that the total amino acid contents were significantly different between the four malts (Figure 2). Total amino acids also showed obvious differences among the four malts (Figure 3). Esterel samples reached the peak level of total amino acids in 96 h, whereas, Harrington and Kendall samples achieved the maximum level of total amino acids in final malt (Figure 2). Proline was higher than any other amino acid in final malt. The levels of leucine, lysine, glycine and aspartic acid showed slight differences between Kendall, GanIII, Harrington and Esterel in final malt. Levels of histidine, arginine, valine, phenylalanine, glutamine, asparagine, glutamine, threonine, proline and tryptophan in the Harrington sample were higher than those of the other malts (Figure 2). These results indicated that barley variety is indeed an important factor for the amino acid content in final malt.

Effect of barley variety in different malting programs

According to Jones and Pierce (1969), amino acids can be classified on the basis of their “essential characters” in yeast metabolism during fermentation. Amino acids belonging to class I (Asp, Asn, Glu, Gln, Thr, Ser, Met, Pro) are not important. However, the concentration of class II amino acids (Ile, Val, Phe, Gly, Ala, Tyr) are critical, and the concentrations of class III amino acids (Lys, His, Arg, Leu) are essential. Deficiencies of class III amino acids

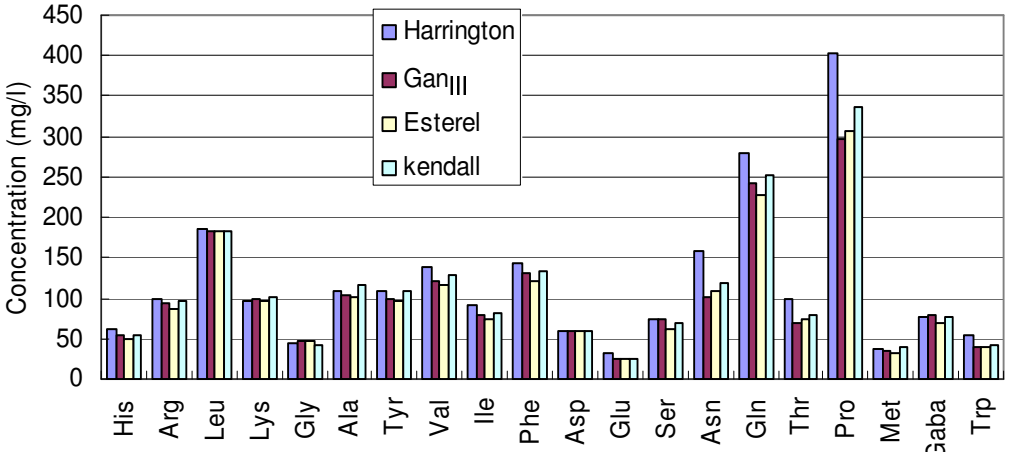

Figure 2. Twenty kinds of amino acid content in final malt of Harrington, GanIII, Esterel and Kendall.

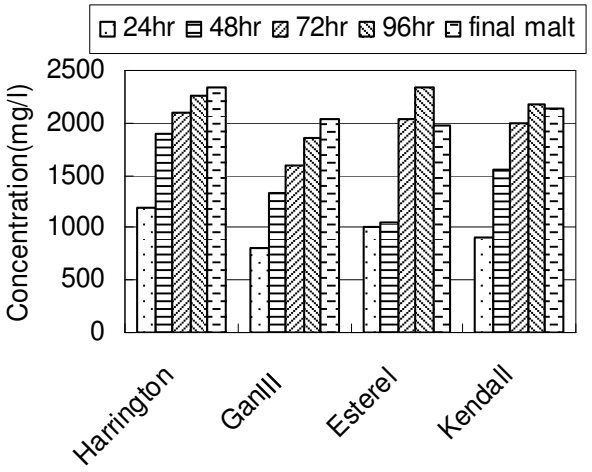

Figure 3. Amino acid content changed during mating and in final malt of Harrington, GanIII, Esterel and Kendall.

can cause major changes in beer quality of flavor (Jones and Pierce, 1969; Romkess and Lewis, 1971). The levels of these amino acids in malt Copeland are depicted in Figures 4 and 5.

The levels of amino acids varied when using Copeland barely under three malting programs (Table 1). The content of glutamine in program I and II is higher than that of program III. Levels of classes I and II seem to have a similar variation trend, as well as the total amino acid content. Classes I and II reached a peak under malting program III but obtained a minimum value under malting program I (Figure 5). The most important category is class III. The content of amino acid in program II is the highest among the three programs, although the content of amino acid in program III did not reach a peak, it is higher than that of others in classes II and III. The results indicated that the program is an important factor which affected the final content of amino acids.

The previous study showed that the levels of amino acid in wort influenced the yeast activity, fermentation time and beer flavor stability (Lekkas et al., 2007). Sawada et al. (2008) also found that methionine is confirmed to be a key compound of deterioration precursor in 100% malt beer. Methionine, phenylalanine, valine, leucine, and isoleucine will be degraded to Strecker aldehydes from the amino- carbonyl reaction. In program III those amino acids such as phenylalanine, valine, leucine, and isoleucine have a higher content than those in program I and II (Figure 4). We have tried to control the malting condition in order to reduce the undesirable amino acids content of wort, and at the same time the improvement of beer flavor stability could be achieved. The high proportion of proline in the malt is undesirable, because the yeast could not assi- milate proline. Usually, the more proline the malt has, the lower is the proportion of other amino acids. The content of Gaba in different barely varieties shows little changes during malting (Figures 2 and 4).

Conclusion

We investigated the conditions that affect the amino acid

contents during germination and in final malt, and found that the levels of amino acid showed significant changes in germinating stages. Total amino acids had high positive correlation with free amino nitrogen (0.9354), Kolbach index (0.9719) and soluble protein (0.8316) in final malt. Our study confirmed that barley variety and the malting process were mainly responsible for the variations in amino acid content in final malts. This study identified various important malting conditions that may lead to improvements in malt quality and thus enhancement in beer flavor. The optimal malting program of barley in this study is malting program II, which can provide desirable amino acid contents. However, the complex relationship between amino acid and malting conditions needs to be investigated further, especially in the area of arginine.

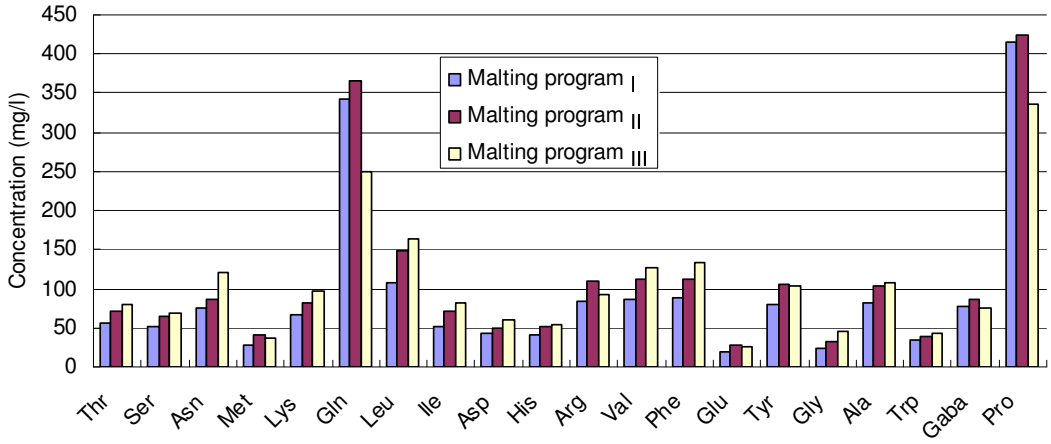

Figure 4. Effect of malting program on amino acid contents in final malt (Copeland)

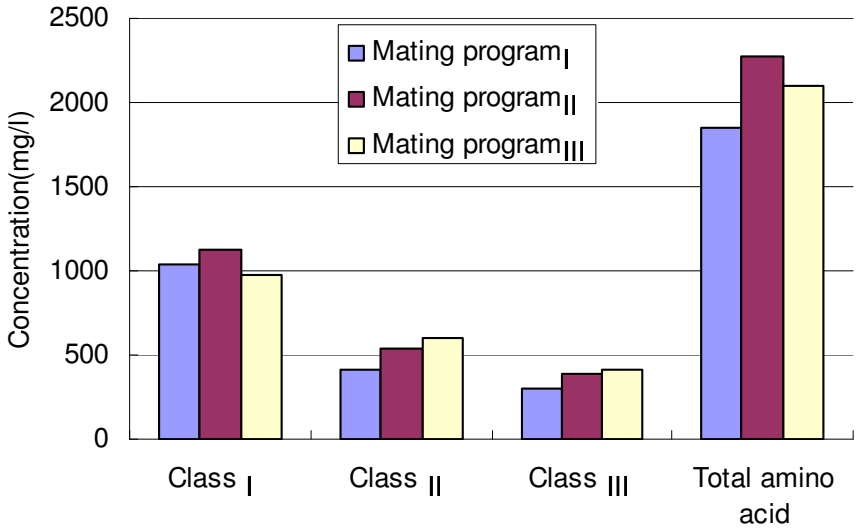

Figure 5. Content of amino acids in malt of wort derived from Copeland in final malt. The amino acids are divided into three classes according to Jones and Pierce (1969). Class I include (Asp, Asn, Glu, Gln, Thr, Ser, Met and Pro); class II include (Ile, Val, Phe, Gly, Ala, Tyr); class III include (Lys, His, Arg, Leu).

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