M. Yano, W. Back and M. Krottenthaler

The Impact of Liquid Adjunct and Barley on Wort and Beer Quality.

The use of adjunct is one of the most important factors in the beer industry, since adjunct not only influences production variables such as quality and cost, but also has an influence on market sales strategy, such as pricing strategy in Japan where liquor tax is assessed according to malt usage. Although a great deal of knowledge has been accumulated regarding adjuncts such as liquid adjunct (L. A.) and barley, not many reports have been published regarding special quality characteristics derived from these adjuncts, such as beer flavor stability. In this study, we have performed exhaustive research to elucidate the impact of L. A. and barley on process and quality, specifically with regard to taste and flavor stability, by comparing wort and beer made from 100 % malt, 75 % malt with 25 % adjunct, and 60 % malt with 40 % adjunct, using a pilot plant capable of making 60 L of cold wort. No exogenous enzymes were used in the brews. As a result of our experiments, we discovered a tendency toward improved flavor stability when 40% of malt was substituted for L. A. We also found a tendency that flavor stability decreased when 40 % of malt was substituted for barley. Other process and quality characteristics of adjunct beers are also discussed as well.

Descriptors: adjunct, barley, brewing, flavor stability, foam, liquid adjunct

Abbreviations: AAL, apparent attenuation limit; B, barley; Coag.N, coagulable nitrogen; DLG, Deutsche Landwirtschafts Gesellschaft e. V.; d.m., dry matter; EBC, European Brewery Convention; FAN, free amino nitrogen; FID, flame ionization detector; GC, gas chromatography; 5-HMF, 5-hydroxymethyl furfural; JAS, Japanese Agricultural Standard; L.A., liquid adjunct (syrup); LTP1, lipid transfer protein 1; M, malt; MEBAK, Mitteleuropäische Brautechnische Analysenkommission e. V.; SN, soluble nitrogen; TBN, thiobarbituric acid number; TN, total nitrogen; TP, total polyphenols

1 Introduction

1.1 Outline of adjunct usage

The use of adjunct is one of the most important factors in the beer industry, since adjunct impacts both production and sales. In terms of production, adjunct is used mainly to improve quality and reduce costs. For example, many breweries in African countries, where malt is particularly expensive [1], use local adjunct, which is less expensive than malt, to attain acceptable quality standards and reduce production costs at the same time. With respect to sales, adjunct can influence pricing strategy. For example, in countries such as Japan, the liquor tax rate depends on malt ratio [9, 49]. As a result, product pricing will change according to the quantity of adjunct. It is important to have sufficient information on the process and qualities of adjunct beers in order to make the optimal decisions related to production and sales.

Adjunct is divided into solids, which should be saccharified in mash tun, and liquids, for which no saccharification is necessary. Primary solid adjuncts include maize, wheat, rice, barley, sorghum, millet, oats [1, 3, 12, 26, 60, 68]. Primary liquid adjuncts include refined maize-starch hydrolysates [1, 3, 12, 60]. In this report, we focus on barley as a solid.

1.2 The influence of L.A. on the production process

In terms of the production process, yeast variety and high concentratoins of glucose in L.A. [14, 18] used to cause a problem with slow sugar consumption by yeast during fermentation. At present, however, the carbohydrate composition can be controlled so as to be equivalent to that of all-malt wort produced by multi step exogenous enzyme reaction and mixture [38, 60]. Accordingly, there are no problems with slow sugar consumption when using liquid adjuncts at low levels with appropriate yeast types. On the other hand, since L.A. is refined [20] and generally has only a dilutive effect on malt wort, the usage of liquid adjunct at high levels may result in a deficiency of amino acids [32], fats [46], trace elements (eg. zinc) [46], vitamins (eg. biotin) [24], potentially leading to yeast vitality and viability problems. Regarding beer filtration, there are differences between L.A. beer and all-malt beer on the adsorption characteristic of sensitive protein to small pore silica gel [42].

1.3 The influence of barley on the production process

When barley is used in the brewing process, it is pre-gelatinized before mashing in some cases. The major pre-gelatinized products are flaked barley [53, 60], torrified barley [48, 53], micronised barley [53] and extruded barley [53]. Before milling, barley is

Authors: Masaaki Yano[1,2], W. Back[3], M. Krottenthaler[3];
[1] M. Agric. Masaaki Yano, Planning section, Technology Development Department, Production Division, Kirin Brewery Co., Ltd., 2–10, Shinkawa, Chuo-ku, Tokyo, 104-0033, Japan;
[2] Corresponding author. Tel.: +81 3 5540 3461; Fax: +81 3 5540 3541. E-mail: myano@kirin.co.jp
[3] Univ. Prof. Dr.-Ing. Werner Back, PD Dr.-Ing. habil. Martin Krottenthaler, Technische Universität München, Lehrstuhl für Technologie der Brauerei I, Weihenstephaner Steig 20, 85354 Freising, Germany

Tables and Figures see Appendix

sometimes washed [11], steeped [65, 66] or dehusked [60]. Though these procedures are performed mainly for hygenic reasons, steeping facilitates endosperm grinding and prevents husk breakage, while dehusking increases extract. Barley is either milled finely by dry mill [29] or comparatively coarsely by wet mill [53]. The advantage of dry mill is that extract recovery is higher [29], but wort β-glucan will also be higher [12, 35] with lautering tending to be slower [57] in comparison to wet milling. On the other hand, wet milling offers advantages of ease of milling [53], shorter saccharification time [17], less husk breakage [16], and potentially shorter lauter time compared to dry milling. Since the gelatinization temperature of barley is below saccharification rest temperature [12, 40, 53, 57], the barley can be processed directly in the mash [57]. In some cases, particularly when used in high ratios exceeding 20 % [11], 30 % [16] or 50 % [53], barley will be saccharified with commercial enzymes [51, 63, 66], mainly in order to improve extract yield or lautering performance. Use of barley led to low apparent fermentability [27, 29, 51]. When barley beer is filtered, the high β-glucan content has been reported as potentially impairing the process [8, 31, 46, 50, 58].

1.4 The influence of L.A. on wort and beer quality

In terms of wort, FAN is generally diluted through the use of L. A. [38]. Regarding beer, the use of L. A. can be detrimental to foam formation [4], since proteinaceous materials are diluted. The colloidal stability of L. A. beer is a controversial area. According to Canales [13], L. A. does not improve colloidal stability, and there are other reports showing some deterioration compared to all-malt beer [54]. On the other hand, some reports indicate that L. A. provides improved resistance to haze formation resulting from the dilution of nitrogen compounds [33]. Regarding beer taste, syrup generally neutralize beer taste, and the principal effect is to dilute the malt character [38, 53]. We are aware of only a few reports indicating effects on flavor stability. One report indicated the concentration of aging indicator substances of ale beers with L. A. after aging were lower compared to all-malt ale beer. The stale flavor of L. A. ale beer was weaker than all-malt ale beer, which corresponds with the chemical results discussed above [6].

1.5 The influence of barley on wort and beer quality

While some show the additive influence of barley on SN [16] and amino nitrogen [34] of cold wort, it appears that the majority of research indicates only a minor influence on amino nitrogen [57, 1, 67]. One report indicates that barley adds anthocyanogen [29] to wort.

The use of barley leads to improved head retention in beer [2, 4, 7, 29, 55, 56, 57, 60]. This is particularly the case with higher nitrogen barley [52]. The permanent haze formation of barley beer is a controversial subject. Some reports show that the barley beer has a slightly weaker haze stability than all-malt beer [46]. Other reports show that barley beer has almost the same haze stability as all-malt beer [54]. Still other reports indicate that barley beer improves haze stability [55]. However, it may have been the exogenous enzymes used in this last investigation that improved haze stability. When barley is used, beer could have an influence of raw-grain flavors [12], strong harsh flavor [60], stronger body and undesirable changes in flavor and taste profile (eg. estery, rougher bitterness, astringent, sharp biting taste) [22]. These taste defects, particularly harshness, have been reported to decrease by lowering the pH of the wort to 4.9 prior to boiling [51], by heat treatment of barley [39], by kieselguhr treatment at the copper [51], by peeling off the husk [15] or by wet milling [17]. Still, however, there are few reports on barley beer flavor stability. One report does indicate a worse flavor stability [54] when using barley flakes. Perhaps one reason for this is the use of a longer lautering time and low wort clarity [57].

1.6 Objective of this research

As shown above, the effects of L. A. and barley on the production process and quality are varied, with some influences being significant. On the other hand, some characteristics have yet to be fully investigated. Therefore, the purpose of this investigation is to provide a total picture of the impact of using L. A. or barley on the production process and on wort and beer quality, particularly with respect to the influence on beer taste and flavor stability, by using a 60 L pilot plant with no exogenous enzymes.

2 Material and methods

2.1 Material

We used the same malt, L. A., and barley throughout the study.

2.1.1 Malt

Since we wanted to maximize the adjunct ratio in order to investigate the effect of those adjuncts and have a proper fermentation at the same time, we needed to use high SN malt.

Moisture; 3.9 %

Extract; 80.0 % (d.m.)

Apparent attenuation limit; 80.3 %

Gelatinization temperature; 65.6 °C

Color; 5.1 EBC

Total protein; 12.4 % (d.m.)

SN; 867 mg/100 g malt

Kolbach index; 43.7 %

FAN; 186 mg/100 g malt

Supplier; Ireks GmbH (Kulmbach, Germany)

2.1.2 Liquid adjunct

Since we did not want to change the fermentation performance caused by the carbohydrate composition, we used refined L. A.

having a comparable carbohydrate composition compared to all-malt wort. The major specifications are shown below.

Solid content; 76.1 %

Glucose; 1.4 % (w/w)

Maltose; 52.1 % (w/w)

Maltotetraose and above; 26.1 % (w/w)

pH; 4.3

Supplier; Nihon Shokuhin Kako Co, Ltd. (Tokyo, Japan)

2.1.3 Barley

Since we wanted to investigate the influence of total barley grain on wort and beer quality, we used the entire grain without any further treatment.

Variety; Scarlett crop 2006

Gelatinization temperature; 67.6 °C.

Moisture; 11.5 %

Extract; 76.8 % (d.m.)

Total protein; 11.6 % (d.m.)

Supplier; Mich. Weyermann GmbH & Co. KG (Bamberg, Germany)

2.1.4 Hop

Since a thin taste matrix was expected from using high amount of adjuncts, only aroma hops were used in order not to give too much hoppy flavor, and/or harsh bitterness. The major specifications are shown below.

Variety: Hallertauer Hallertauer Tradition, Hallertauer Perle

Type; pellets type 90

α-acid; 6.3–10.1 %

Supplier; Simon H. Steiner, Hopfen, GmbH. (Mainburg, Germany)

2.2 Analysis methods

2.2.1 Malt, barley, hop

The method of MEBAK [43] was used for all the malt, barley and hop analysis.

2.2.2 L. A.

The Japanese Agricultural Standard (JAS) [30] method was used for all the L. A. analysis.

2.2.3 Wort

The MEBAK [44, 45] method was used for all analyses except buffer capacity and wort aroma substances.

In order to calculate the buffer capacity, 3 mL of 0.1 N HCl was added to 50 mL wort and the pH before (pH before) and after (pH after) adding HCl was measured. The calculation was done by the formula shown below. The first formula shows the added proton concentration divided by the change of proton concentration resulting from adding the HCl. The second formula gives the buffer capacity in 11.0 plato.

$$K \text{ (buffer capacity)} = (0.1*3/(50+3)*10^{6})/(10^{(6-\text{pH after})}-10^{(6-\text{pH before})})$$

K11 (buffer capacity in 11 plato)

$$= (\text{square root}((K-0.9259)/0.9921)-((\text{wort plato})-11))^{2}+0.9259$$

The wort aroma substances were concentrated by steam distillation and extracted by dichloromethane. Analysis was done by using gas chromatography with a flame ionization detector (GC-FID) according to the procedure of Lehrstuhl für Technologie der Brauerei I.

2.2.4 Beer chemical analysis

The MEBAK [44, 45] method was used for all the beer analysis except for aging indicators.

The aging indicators were concentrated by steam distillation and extracted by dichloromethane. Analysis was done by using gas chromatography with a GC-FID according to the procedure of Lehrstuhl für Technologie der Brauerei I.

2.2.5 Beer tasting analysis

Flavor intensity and quality analysis was carried out by a panel of 9–12 trained tasters.

2.2.5.1 Fresh beer

For the flavor intensity tasting, the strength was given between 0 (none) and 5 (extremely strong) for each condition. Quality analysis was done according to the method of Deutsche Landwirtschaftliche Gesellschaft e.V. (DLG) [19]. For each item, the score was decided between 1 and 5. Flavor: 1 (strong flavor failure) to 5 (pure); Taste: 1 (strong taste failure) to 5 (pure); Body: 1 (untypical) to 5 (very typical); Liveliness: 1 (very insipid) to 5 (pleasant liveliness); Bitterness: 1 (strong lingering bitterness) to 5 (very fine).

2.2.5.2 Forced beer

Beers were shaken for a day and kept at 40 °C for 4 days to produce forced beer samples.

For the flavour intensity tasting the strength was assessed between 0 (none) to 5 (extremely strong) for each condition.

2.3 Production methods

2.3.1 Raw material

The raw materials used are shown below (Table 1). The figure given in condition row is the ratio in extract.

2.3.2 Brewing

We used a brewing pilot plant (G. Feistlbauer GmbH, Lengdorf, Germany) capable of producing 60 L of wort. The brewing conditions are shown below.

2.3.1.1 Milling

Malt was milled by using a two roller mill (Heger GmbH, Herrenberg-Oberjesingen, Germany) in 0.6 mm gap. Barley was milled by using hammer mill (Werkhuizen Schepens N.V., Dendermonde, Belgium) in 2.0 mm sieve.

2.3.2.2 Mashing

Brewing liquor was heated to 62 °C in a mash tun and malt (and barley if necessary) was added. Mash was maintained at 62 °C for 30 minutes; heated from 62 °C to 66 °C over 4 minutes; maintained at 66 °C for 20 minutes; heated from 66 °C to 72 °C over 6 minutes; maintained at 72 °C for 30 minutes; increased heat to 78 °C over 6 minutes; maintained at 78 °C for 5 minutes.

2.3.2.3 Lautering

Mash was transferred to a lauter tun, and the mash tun was washed with 2 L of brewing water to wash out the remaining mash. After the mash was kept for 15 minutes at 78 °C, the initial part of the 2 L of first wort was kept in a reserve tank to lower the turbidity of the running wort. Next, the first wort was collected to a wort kettle. The 2 L of wort in the reserve tank was returned to the lauter tun. When the surface reached a level a few cm above the spent grain, we conducted the 1st sparging. When the surface level reached a few cm above the spent grain, we conducted the 2nd sparging. Soon after the 2nd sparging, we performed a deep cut, which was raked for 2 minutes continuously. All the processes beginning from the collecting of 1st wort to the wort kettle, to the end of the 2nd sparging, was done in approximately 2 hours. When the flow rate of the wort was slow, additional deep cut and raking was done in order to meet the total wort collection time, which leads to the same wort heat load. The temperature of the lauter tun and the wort kettle was kept in 78 °C, 75 °C respectively during lautering.

2.3.2.4 Boiling

Wort was heated from 75 °C to 90 °C over 10 minutes; maintained at 90 °C for 15 minutes; and then the heat increased to boiling temperature over 10 minutes. In the case of the L. A. brew, syrup was added to the wort kettle, and the wort was diluted by brewing water to reach 11.4 plato extract during the heating procedure described above. All of the hops were added when boiling started. The wort was cooked for 60 minutes to reach an approximate 10 % evaporation rate.

2.3.2.5 Whirlpool and wort cooling

The wort was transferred to whirlpool, and was kept at 90 °C for 15 minutes. The wort cooling was done over approximately 25 minutes.

2.3.3 Fermentation and lagering

Yeast (W34, bottom fermenting) was propagated in 13 plato wort at room temperature for 2 days. The yeast was cropped and pitched to the fermentation tank to give approximately 13 million cells/ml in the wort. Soon after, approximately 30 L of the cold wort was added with aeration. The fermentation tank was kept at 12 °C for 7–8 days. The green beer was transferred to a storage vessel, after the yeast in the bottom of fermentation tank was discharged. The vessel was kept at 16 °C for 7 days, and the total diacetyl was analyzed. When the total diacetyl was lower than 0.1 mg/L, the tank was kept at 4 °C for 14 days for lagering. However, when the total diacetyl was 0.1 mg/L or higher, the green beer was kept at 16 °C, and the total diacetyl was analyzed until the concentration gets under 0.1 mg/L.

Table 1 Amount of raw materials and brewing water

Condition			Amount of raw materials and brewing water					
Malt	L.A.	Barley	Malt	L.A.	Barley	Brewing liquor	1st sparging	2nd sparging
%	%	%	Kg	Kg	kg	L	L	L
100	0	0	10	0	0	40	15	15
75	25	0	7.5	2.5	0	30	11	11
60	40	0	6.0	4.0	0	24	9	9
75	0	25	7.5	0	3.0	40	15	15
60	0	40	6.0	0	4.9	44	13	13

2.3.4 Filtration and bottling

The maturated beer was filtered by using a sheet filter (Housing; A20Z, Seitz Werke GmbH, Bad-Kreuznach, Germany, Filter; Seitz K 100, Pall Filtersystems GmbH, Wardstetten, Germany). The filtered beer was diluted to the same original extract as other beers by degassed brewing water. After dilution, beer was gassed in 1500 hPa CO_2, bottled by using a hand filler and closer. The bottled beer was stored in a dark chamber at 4 °C.

3 Results and discussion

3.1 The influence of L.A. and barley on beer production process

Only processes for which problems were experienced will be discussed here.

3.1.1 Mashing in

In our research, barley was casted to the mashing vessel from a manhole. However, the finely milled barley generated by the hammer mill would not dissolve easily. Therefore, we used a stick in order to break the cakes. It may be useful to use an mashing in screw or mashing in tank to have a good dissolution, otherwise it may lead to less wort gravity.

3.1.2 Lautering

According to the lautering method we used, all of the L. A. brew and barley brew for which 25 % of malt was replaced had almost the same filterability. A deep cut was done only once. However, when 40 % of malt was replaced by barley, deep cuts were performed 2 to 4 times, indicating that 40 % or less is a maximum barley usage for lautering performance. We used a hammer mill in order to produce a higher extract. However, when steeping before milling [65, 66], wet milling [53] or even when commercial enzymes [51, 63, 66] were used, the wort filtration may improve and maximum barley usage may increase. The causes for the long run off times were probably the β-glucan [37, 57, 59], gelprotein [47], undigested small starch granules and granule surface protein [5].

3.1.3 Fermentation

Figure 1 shows the sugar consumption of yeast during fermentation. When malt was substituted by L. A. or barley for 25 %, there were no such influences on the sugar consumption pattern. However, when the substitution ratio is 40 %, the sugar consumption especially during early fermentation is slow. Though in 40 % L. A. wort, the apparent extract of 5 days after pitching was almost as same as that of other adjunct beers, in 40 % barley wort, it was higher than other adjunct beers. The concentration of FAN of L. A. and barley cold wort is more than the figure which is reported to be necessary for proper fermentation (150 mg/L) [44], therefore there is a possibility that other factors for propagation (e.g. biotin, myo-inositol and pantothenic acid [10]) are less in L. A. and barley wort compared to all-malt wort.

3.2 Influence of L. A. and barley on quality

Each chemical analysis data of adjunct cold wort was divided by that of all-malt wort to give a relative value, so that it is easier to understand the influence of the replacement of malt to L. A. and barely.

3.2.1 Cold wort quality

3.2.1.1 pH, color, bitter unit (BU)

PH of wort was slightly higher (3–4 %) in 40 % adjunct wort than that of all-malt (data not shown). The buffer capacity of kettle full wort (Fig.2) showed that in tendency the more L.A. used, the less buffer capacity will be. Therefore wort with high L. A. ratio was easier to be influenced by the factors involving the change of pH.

Wort color decreased as malt was being replaced by L. A. and barley (Fig.3). The color of barley wort did not decrease as much as that of L. A. This would be due to the protein load and poyphenols (Fig.9) which is more abundant in barley compared to L. A.

Though wort BU of barley was almost the same compared to that of all-malt, L. A. had higher concentration (9 % higher) compared to all-malt wort (data not shown). One of the reason may be that the L. A. cold wort had a less protein precipitation which precipitates bitter substances (e.g. isohumulone) as well [36] .

3.2.1.2 Carbohydrate composition, apparent attenuation limit (AAL)

L. A. wort had lower glucose (Fig. 4), fructose and higher maltotriose compared to all-malt wort (in case of 40 % substitution, 22 % less, 51 % less, 36 % more respectively), but the AAL of L.A. wort was almost the same as that of all-malt wort (data not shown) because of the sugar composition of the L. A. Barley wort had lower glucose (Fig.4), fructose, and slightly lower maltose (Fig.5) compared to all-malt wort (in case of 40 % substitution, 24 % less, 50 % less, 8 % less respectively), and had a slightly lower AAL compared to all-malt wort (in case of 40 % substitution, 10 % less. data not shown) [27, 29, 51]. There may be two reasons for this phenomenon. First, because α-amylase activity of barley is very low [23], when malt was substituted for 40 % to barley, the total amylolytic activity during mashing will be less. However, though it is less, there is still an amylolytic degradation of barley starch. Therefore, the barley wort had higher wort maltose and glucose concentration than that expected from simple wort dilution. Second, since the gelatinization temperature of the barley which we used was 67.6 °C, and we used the infusion process, the β-amylase may have lost some of its activities before the gelatinization of the barley started.

3.2.1.3 Total nitrogen (TN), Free amino nitrogen (FAN), Coagulable nitrogen (Coag. N), amino acids

The concentration of barley wort TN and FAN was slightly higher, or almost the same of that of L. A. wort (Fig. 6, 7). This is in accordance with the result of several reports showing that raw barley has a limited additive effect of FAN [1, 67] to the wort.

Although the concentration of wort Coag. N of L. A. got lower than that of all-malt wort, that of barley got higher than that of all-malt wort (Fig.8). This phenomenon will be further discussed in the beer foam analysis.

Barely wort had a slightly higher or similar concentration of each amino acid analysed compared to L. A. wort (data not shown), which shows that amino acid pattern of those coming from barley is similar to malt.

3.2.1.4 Total polyphenols (TP), anthocyanogens, tannoids

Barley wort had lower concentration of anthocyanogens (Fig. 10) and about the same of that of TP (Fig.9) and tannnoids (Fig.11) compared to all-malt wort. Since anthocyanogen concentration of barley wort is higher than that of L. A. wort, polyphenols are being extracted from the barley husks and aleurone layers [36] to wort. On the other hand, L. A. wort TP (Fig. 9) and anthocyanogen (Fig.10) concentration got lower in conformity with the usage of L. A., which shows that these substances were simply diluted.

3.2.1.5 Thiobarbituric acid number (TBN)

The wort TBN of L. A. and barley was almost the same (Fig.12). There are two possible reasons for this phenomenon. TBN has a correlation with 5-hydroxymethyl furfural (5-HMF) [41], and one of its producing pathways requires amino nitrogen [59]. Therefore, since there was no significant difference between L. A. and barley wort, it may have had the similar formation of 5-HMF and TBN figure. Second, since the lautering time of each brew was controlled to approximately 2 hours, and the boiling time was 1 hour, thermal load was almost the same in each brew.

3.2.1.6 Aromatic compounds

Though there are no statistically significant differences, sum of concentration of strecker aldehydes (Sum of 3-methyl butanal, 2-methyl butanal, 3-methylthio propanal, benzaldehyde, 2-phenyl ethanal. Fig.13) and sum of concentration of lipid oxidation products (sum of hexanal, pentanal, heptanal, γ-nonalacton. data not shown) had a tendency that barley wort had a higher value compared to that of L.A. wort, regardless of adjunct usage ratio. Since, in one of the pathways, strecker aldehydes are produced from dicarbonyl compounds and amino acids, it is assumed that, the barley wort, which in tendency has slightly higher FAN compared to L. A. wort (Fig. 7), apt to have higher strecker aldehydes.

3.2.1.7 Dimethyl sulphide (DMS), Dimethyl sulphide precursor (DMS-P)

The more L. A. and Barley are used, the less wort DMS and DMS-P concentration became (in case of 40 % L.A. and 40 % barley, the wort DMS was 45 % less and 40 % less respectively and the wort DMS-P was 49 % less, 50 % less respectively to those of all malt wort. data not shown). Since DMS-P is produced during steeping and germination, DMS-P is absent in raw barley [64]. Therefore, wort DMS-P of barley wort is as low as L. A. wort.

3.2.2 Beer quality

3.2.2.1 Chemical analysis

3.2.2.1.1. pH

PH of L. A. and Barley beer has almost the same pH value as the all-malt beer (data not shown).

3.2.2.1.2 Color, BU

Color of L. A. and barley beer was lower than that of all-malt beer (in case of 40 % barley, 31 % less, data not shown), and that of L.A. was slightly lower than barley beer (in case of 40 % L. A. 9 % less, data not shown), which shows the same trend as wort.

BU of L. A. and barley beer was almost the same as that of all-malt beer (data not shown).

3.2.2.1.3 TN, delta-TN

Concentration of TN of L. A. and barley beer was lower than that of all-malt beer, and that of barley beer was slightly higher than L. A. beer (Fig.14).

The decrease ratio of TN during fermentation (consumption ratio), which concentration of TN decreased during fermentation was divided by the cold wort TN, was slightly higher in 40 % barley beer than that of all malt beer (Fig.14). This is probably because barley wort had more protein (eg. protein which was dissolved to wort form spent grains during additional deep cut in 40 % barley brew) which precipitated during fermentation.

3.2.2.1.4 TP, anthocyanogens

Concentration of TP and anthocyanogens of L. A. and barley beer was lower than those of all-malt beer, and those of barley beer were higher than L. A. beer (Fig. 15, 16).

3.2.2.1.5 Foam

The foam stability of barley beer was higher than that of all-malt beer, and that of L. A. beer was as same as that of all-malt beer (Fig.17). Even if it is only a tendency, this result maches the Coag. N result (Fig. 8) [36]. Since proteins such as lipid transfer protein 1 (LTP1) [21] are considered to act as foam positive substances and lipid [4, 28], fatty acid [4, 28], mono-, di-glycerides [4] as foam negative substances in barley, in this case, the former substances must have more influence than the latter.

3.2.2.1.6 Higher alcohol, acid, ester, aldehyde

We noted several basic tendencies. First, when malt was being replaced by L. A., many of the higher alcohols and esters of L. A. beer, namely 1-propanol, 2-methyl-1-propanol, 2-methyl butanol, ethyl acetate, 3-methylpropyl acetate, 3-methylbutyl acetate, ethyl butyrate had a tendency to have similar concentration compared to those of all-malt beer (e.g. concentration of ethyl acetate is shown in Fig.18).

Second, when malt was being replaced by barley, many of the higher alcohols, acids and esters of barley beer namely, 2-methyl propanol, 3-methyl butanol, 2-methyl butanol, decanoic acid, ethyl acetate, 3-methylbutyl acetate, 2-methylbutyl acetate, ethyl octanoate, i-butyl acetate, 2-phenyl acetate apt to have lower concentration than those of all-malt beer (As an example concentration of ethyl acetate is shown in Fig.18). The reason for the low esters might be that the additional unsaturated fatty acids from barley suppressed the production of esters in yeast by inhibiting the membrane bond ester synthesizing enzymes [25, 61, 62].

3.2.2.1.7 Total diacetyl

There were no significant differences between all-malt, L. A. and barley beers (data not shown).

3.2.2.1.8 Aging indicators

There was a tendency that the sum of aging indicators during forcing of L. A. and barley beer decreased compared to all-malt beer (Fig. 19). Though the increasing ratio did not change significantly for L. A. beer, that of barley beer slightly increased compared to the all-malt beer (Fig.21). This phenomenon will be further discussed in the forced beer tasting analysis.

3.2.2.2 Tasting analysis

3.2.2.2.1 Fresh beer

When malt was replaced by L. A. by 40 %, there was a tendency for an intensely fruity, flowery taste compared to all-malt beer (Fig. 20), but other conditions, namely, sweetness, maltiness, grassiness, astringency, bitterness and body did not show any particular tendencies (data not shown) in two different tasting analysis with different brews. In terms of DLG evaluation, which shows the quality compared to general pale beers in this case, there was a trend that qualities of flavor, liveliness and bitterness improved (Fig. 21) in 40 % L.A. beer compared to all-malt beer. However, taste and body did not have particular tendencies (data not shown) in two different tasting analysis with different brews. On the other hand, the DLG evaluation of barley beer indicated a tendency for inferior qualities in terms of flavor, liveliness and bitterness compared to all-malt beer (Fig. 21). No particular tendencies were noted for taste and body (data not shown).

Though the concentration of many of the esters was as same as that of all-malt beer, one of the explanations for the stronger intensity of fruity and flowery is that the flavor matrix of L. A. beer was less complex than that of all-malt beer, and the ester flavor was more pronounced. The reason for the low score of flavor and bitterness in barley brews in DLG analysis might stem from the fact that the beer has raw-grain flavors and harsh bitterness, something that some of the panelists pointed out in the tasting analysis, and also reported by some authors [12, 22, 60].

3.2.2.2.2 Forced aged beer

When malt was replaced by L. A. for 40 %, there was a trend toward weaker oxidized flavor, oxidized taste and bitterness (Fig. 22), and the acceptance of the beer, indicating how close the overall perception of the sample was to fresh beer, was higher (closer to fresh beer) than all-malt beer in two different tasting evaluations with different brews.

On the other hand, when malt was replaced by barley by 40 %, there was a tendency for a stronger oxidized flavor, taste and bitterness (Fig. 22), and the acceptance of the beer decreased compared to all-malt beer in single tasting evaluation.

There are two possible reasons for the high flavor stability tasting results of L. A. beer compared to all-malt beer. First, the diluted precursors of aging substance, which can be estimated from the less aging indicators after forced aging compared to that of all-malt beer (Fig. 19), had an effect on improved flavor stability. Second, the high sweet and flowery flavor intensity which was observed in the fresh beer (Fig. 20) may have masked the deteriorated flavor and improved the flavor stability.

On the other hand, there are two possible reasons for the low flavor stability tasting results of barley beer compared to all-malt beer. First, other oxidizing mechanism which comes particularly from barely and cannot be detected by the aging indicators we used may have influenced the flavor. Second, since there were some characteristics of flavors of fresh barley beers (e.g. raw-grain flavors, harsh bitterness), this might have influenced the deteriorated flavor perception of forced aged beers by the panelists.

4 Conclusions

As shown above, the use of L. A. will be beneficial in many aspects of wort and beer quality. Therefore, under certain marketing strategies, it might be very useful to use L. A. to produce a high-quality, low-cost product. On the other hand, barley produced a somewhat low quality in several points of wort and beer quality. However, we conducted our experiments using a 40 % substitution of barley without the usage of exogenious enzymes. This was an extreme condition. Accordingly, it might be useful to utilize the character of barley to give an improved beer taste and flavor profile by carefully adjusting the barley ratio.

5 Acknowledgement

We thank the reviewer for their constructive comments, all of our colleagues in Technische Universität München Lehrstuhl für Technologie der Brauerei I for offering useful suggestions and technical assistance. We also thank Kirin Brewery Co., Ltd. for letting us present this research, Dr. Graf of Ireks GmbH and Mr. Richter of Mich. Weyermann GmbH & Co. KG for providing us malt and barley.

6 References

1. Agu, R. C.: A comparison of maize, sorghum and barley as brewing adjuncts. J. Inst. Brew. **108** (2002), pp. 19-22.
2. Anderson, F. B.: Further studies on the foam-stabilizeing substances in beer. J. Inst. Brew. **72** (1966), pp. 384-387.
3. Back, W.: Bierbereitung mit vermälzten und unvermälzten Zerealien und Pseudozerealien. in: Ausgewählte Kapitel der Brauereitechnologie, 1. Aufl., Fachverlag Hans Carl, Nürnberg, Germany (2005), pp. 331-357.
4. Bamforth, C. W.: The foaming properties of beer. J. Inst. Brew. **91** (1985), pp. 370-383.
5. Barrett, J.; Clapperton, J. F.; Divers, D. M. and Rennie; H.: Factors affecting wort separation. J. Inst. Brew. **79** (1973), pp. 407-413.
6. Barret, J.; Halsey, S. A. and Peppard, T. L.: Flavour stability of ales brewed using hydrolysed maize syrup-sensory and analytical aspects.

J. Inst. Brew. **89** (1983), pp. 356-360.

7. Bateson, J. B. and Leach, A. A.: Nitrogen studies of worts in relationship to beer quality. in: Proc. Congr. Eur. Brew. Conv. Interlarken **12** (1969), pp. 161-171, Elsevier Scientific Publishing, Amsterdam, Holland.
8. Bathgate, G. N: Complex polysaccharides in top fermenting worts. In: Wort symposium, Eur. Brew. Conv. Monogr. **1** (1974), pp. 198-207, Fachverlag Hans Carl, Nürnberg, Germany.
9. Brewers association of Japan home page. http://www.brewers.or.jp (accessed Nov. 2007)
10. Briggs, D. E.; Hough, J. S.; Stevens, R.; Young, T. W.: 4. The biochemistry of malting grain: In Malting and brewing science Vol.1, Chapman and Hall Ltd., London, UK (1981), pp. 57-109.
11. Briggs, D. E.: 12. Competitors for malt in: Malts and malting, Blackie Academic & Profesional, London (1998), pp. 533-578.
12. Briggs, D. E.; Boulton, C. A.; Brooles, Pp. A. and Stevens, R.: 2. Malts, adjuncts and supplementary enzymes in: Brewing science and practice, Wood head Publishing Limited, Cambridge, UK (2004), pp. 34-45.
13. Canales, A. M.: 3. Unmalted grains in brewing in: Brewing science, vol.1, Ed. Pollock, J. R. A., Academic Press, London , UK (1979), pp. 233-278.
14. Chantler, J.: Third generation brewers adjunct and beyond. Tech. Q. Master Brew. Assoc. Am. **27** (1990), pp. 78-82.
15. Collier, J. A.: Recent advances in brewing raw materials. Brewers Guild J. **56** (667)May (1970), pp. 242-253.
16. Collier J.A.: Barley conversions in the brewhouse. Brewers' guardian June (1972), pp. 35-40.
17. Cuřín, J.; Černohorský, V. and Štichauer, J.: Verarbeitung ungemälzter Gerste mittels Nassschrotung. Monatsschr. Brauwiss. **26** (3) (1973), pp. 76-82.
18. D'Amore, T.; Russell, I. and Stewart, G. G.: The effect of carbohydrate adjuncts on brewer's wort fermentation by Saccaromyces Uvarum (Carlsbergensis), J. Inst. Brew. 95 (1989), pp. 333-336.
19. Deutsche Landwirtschaftliche-Gesellschaft e.V. home page. http://www.dlg.org/en/food/beer/index.html (accessed Nov. 2007)
20. Dutton, I: Corn syrup brewing adjuncts – their manufacture and use. Tech. Q. Master Brew. Assoc. Am. **33** (1) (1996), pp. 47-53.
21. Evans, D.E. and Hejgaard, J.: The impact of malt derived protein on beer foam quality. Part I. The effect of germination and kilning on the level of protein Z4, protein Z7 and LTP1, J. Inst. Brew. **105** (3) (1999), pp. 159-169.
22. Ferkl, Pp. and Cuřín, J: Change of organoleptic parameters of beer in relation to the use of adjuncts and shortening of maturating times. Tech. Q. Master Brew. Assoc. Am. **16** (4) (1979), pp. 214-217.
23. Goode, D. L.; Wijngaard, H. H.; Arendt, E. K.: Mashing with unmalted barley – Impact of malted barley and commercial enzyme (Bacillus sppp.) additions, Tech. Q. Master Brew. Assoc. Am. **42** (3) (2005), pp. 184-198.
24. Graham, R. K.; Skurray, G. R. and Caiger, Pp. J.: Nutritional studies on yeasts during batch and continuous fermentation. I. Changes in vitamin concentrations, J. Inst. Brew. **76** (1970), pp. 366-371.
25. Hammond J.: The contribution of yeast to beer flavour, Brewers' guardian September (1986), pp. 27-33.
26. Hanke, S.; Zarnkow, M.; Kreisz, S. and Back, W.: The use of oats in brewing. Monatsschr. Brauwiss. **58** (2005), pp. 11-17.
27. Harris, G. and MacWilliam, I. C.: Charbohydrates in malting and brewing XII. Recovery and properties of worts prepared using cereal adjuncts. J. Inst. Brew. **67** (1961), pp. 154-158.
28. Hollemans, M.; Tonies T. R. J. M.; Bisperink, G. J. and Ronteltap, A. D.: The role of malt lipids in beer foam, Tech. Q. Master Brew. Assoc. Am. **28** (1991), pp. 168-173.
29. Hudson, J. R.; MacWilliam, I. C. and Birtwistle, S. E.: Beer from unmalted barley. J. Inst. Brew. **69** (1963), pp. 308-309.
30. Japanese Agricultural Standard home page. http://www.matt.go.jp/soshiki/syokuhin/hinshitu/e_label/index.htm (accessed Nov. 2007)
31. Jin, Y.-L.; Speers, R. A.; Paulson, A. T. and Stewart, R. J.: Barley β-blucans and their degradation during malting and brewing. Tech. Q. Master Brew. Assoc. Am. **41** (2004), pp. 231-240.
32. Kirsop, B. H. and Brown, M. L.: Some aspects of wort composition on the rate and extent of fermentation by brewing yeasts. J. Inst. Brew. **78** (1972), pp. 51-57.
33. Klopper, W. J.: An investigation of the technical brewing merits of various farinaceous raw materials. The brewer **59** (706) August (1973), pp. 395-297.
34. Koszyk, P. F. and Lewis, M. J.: Unmalted grains as malsters' adjuvant and brewers' adjunct. , J. Am. Soc. Brew. Chem. **35** (2) (1977), pp. 77-80.
35. Kühbeck, F.; Dickel, T.; Krottenthaler, M.; Back, W.; Mitzscherling, M.; Delgado, A. and Becker, T.: Effects of mashing parameters on mash β-glucan, FAN and soluble extract levels, J. Inst. Brew. **111** (3) (2005), pp. 316-327.
36. Kunze, W.: Technology brewing and Malting, 3rd Int. Edition, VLB Berlin, Berlin, Germany (2005).
37. Lee, R.; Allan, G.; Greig, R.; Dunn, C. and Stuart, M.: The effect of (1–>3, 1–>4)-β-glucan and arabinoxylan on malt quality and brewhouse performance. In: Proc. Conv. Inst. Brew. Asia Pacific Sect. **25** (1998), pp. 33-37, Institute of Brewing, London, UK.
38. Lloyd, W. J. W.: Centenary review adjuncts. J. Inst. Brew. **92** (1986), pp. 336-345.
39. Macey, A.; Stowell, K. C. and White, H. B.: Experimental brewing procedures using unmalted cereals and enzymes. in: Proc. Congr. Eur. Brew. Conv. Madrid **11** (1967), pp. 283-294, Elsevier Scientific Publishing, Amsterdam, Holland.
40. Marchbanks, C.: A review of carbohydrate sources for brewing. Brew. Distill. Int. January (1987), pp. 16-18.
41. McDougall, J.; Shada, J. D. and Dakin, Pp. E.: Studies on furfural and 5-hydroxymethyl-furfural in beer. Am. Soc. Brew. Chem. Proc. (1963), pp. 48-51.
42. McKeown, I; Earl, G.; Stewart, G. and Leiper, K.: The influence of brewing adjuncts and silica structural properties on selective protein adsorption from lager beers. Tech. Q. Master Brew. Assoc. Am. **37** (1) (2000), pp. 119-124.
43. MEBAK: Brautechnische Analysenmethoden Rohstoffe. Selbstverlag der MEBAK, Freising-Weihenstephan, Germany, 2006.
44. MEBAK: Brautechnische Analysenmethoden Band II (4 Aufl.). Selbstverlag der MEBAK, Freising-Weihenstephan, Germany, 2002.
45. MEBAK: Brautechnische Analysenmethoden Band III (2 Aufl.). Selbstverlag der MEBAK, Freising-Weihenstephan, Germany, 1996.
46. Meilgaard, M. C.: Wort composition: with special reference to the use of adjuncts. Tech. Q. Master Brew. Assoc. Am. **13** (2) (1976), pp. 78-90.
47. Moonen, J. H. E.; Graveland, A. and Muts, G. C. J.: The molecular structure of gelprotein from barley, its behavior in wort filtration and analysis. J. Inst. Brew. **93** (1987), pp. 125-130.
48. Morgan, K.: Heat lability of barley β-glucan. J. Inst. Brew. **77** (1971), p. 509.

49. Nakatani, K.: Beer in Japan – present and future trends. The story of Happoshu and the "Third way", 2007, Brew. Distill. Int. **3** (5)May (2007), pp. 54-57.

50. Narziß, L.; Reicheneder, E. and Edney, M.: Studying beer filtration with an accurate β-glucan assay. Monatsschr. Brauwiss. **42** (1989), pp. 277-285.

51. Nielsen, E. B.: Brewing with barley and enzymes-a review. in: Proc. Congr. Eur. Brew. Conv. Estoril, **13** (1971), pp. 149-170, Elsevier Scientific Publishing, Amsterdam, Holland.

52. O'Donnell, D. C.: Raw material quality control of barley as a brewing adjunct. In: Proc. Conv. Inst. Brew. Australian and New Zealand Sect. **17** (1982), pp. 47-55, Institute of Brewing, London, UK.

53. O'Rourke, T.: Adjuncts and their use in the brewing process. Brewers' guardian March (1999), pp. 32-36.

54. Peppard, T. L.; Buckee, G. K. and Halsey, S. A.: Relating flavour stability to different raw materials and brewing processes. In: Proc. Congr. Eur. Brew. Conv. London **19** (1983), pp. 159-162, IRL Press Ltd., Oxford, UK.

55. Pfenninger, H. B.; Schur, F. and Wieg, A. J.: Zur Technologie der Rohfruchtverarbeitung mit industriellen Enzympräparaten. In: Proc. Congr. Eur. Brew. Conv. Estoril **13** (1971), pp. 171-184, Elsevier Scientific Publishing, Amsterdam, Holland.

56. Pierce, J. S.: The role of positive and negative factors in head retention. In: Proc. Conv. Inst. Brew. Australian and New Zealand Sect. **15** (1978), pp. 51-65, Institute of Brewing, London, UK.

57. Pierce, J. S.: Adjuncts and their effect on beer quality. In: Proc. Congr. Eur. Brew. Conv. Madrid, **21** (1987), pp. 49-60, IRL Press Ltd., Oxford, UK.

58. Sadosky, P.; Schwarz, P. B. and Horsley, R. D.: Effect of arabinoxylans, β-glucans, and dextrins on the viscosity and membrane filterablility of a beer model solution. J. Am. Soc. Brew. Chem. **60** (2002), pp. 153-162.

59. Shimizu, C.; Nakamura, Y.; Miyai, K.; Araki, S.; Takashio, M. and Shinotsuka, K.: Factors affecting 5-Hydrozymethyl furfural formation and stale flavor formation in beer, J. Am. Soc. Brew. Chem. **59** (2) (2001), pp. 51-58.

60. Stewart, G. G.: 6. Adjuncts in: Handbook of brewing second edition, Ed. Priest, F. G. and Stewart , CRC Press, FL, USA (2006), pp. 161-175.

61. Thurston, P. A.; Taylor, R. and Ahvenainen, J.: Effects of linoleic acid and supplements on the synthesis by yeast of lipids and acetate esters, J. Inst. Brew. **87** (2) (1981), pp. 92-95.

62. Thurston, P. A.; Quain, D. E. and Tubb, R. S.: Lipid metabolism and the regulation of volatile ester synthesis in Saccharomyces cerevisie, J. Inst. Brew. **88** (2) (1982), pp. 90-94.

63. Wainwright, T: Enzymes in brewing, Ferment **12** (1999), pp. 30-34.

64. White, F. H. and Parsons, R.: The development of dimethyl sulphide in malting brewing and fermentation. in: Proc. Congr. Eur. Brew. Conv. Nice **15** (1975), pp. 721-735, Elsevier Scientific Publishing, Amsterdam, Holland.

65. Wieg, A. J.: Brewing adjuncts and industrial enzymes, Tech. Q. Master Brew. Assoc. Am. **10** (2) (1973), pp. 79-86.

66. Wieg, A. J.: 5. Barley brewing in: Brewing science, vol.3, Ed. Pollock, J. R. A., Academic Press, London, UK (1987), pp. 533-571.

67. Yano, M.; Tsuda, H.; Imai, T.; Ogawa, Y. and Ohkochi, M.: The effect of barley adjuncts on free amino nitrogen contents in wort. Manuscript submitted.

68. Zarnkow, M. and Back, W.: Probleme bei der Herstellung von Rohfruchtbieren mit hohen Reisgaben. Brauwelt **144** (13/14) (2004), pp. 391-396.

Received 11 December, 2007, accepted 08 January, 2008

Appendix

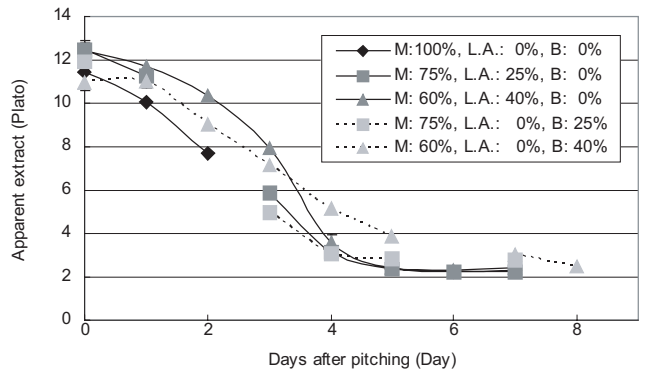

Figure 1 **Influence of L. A. and barley on sugar consumption of yeast**

M: malt ratio, L. A.: L. A. ratio, B: barley ratio

Each data are average of n=2 (M: 75 %, L. A.: 0 %, B: 25 % is n=1). Upper bar shows maximum value and lower bar shows the minimum value.

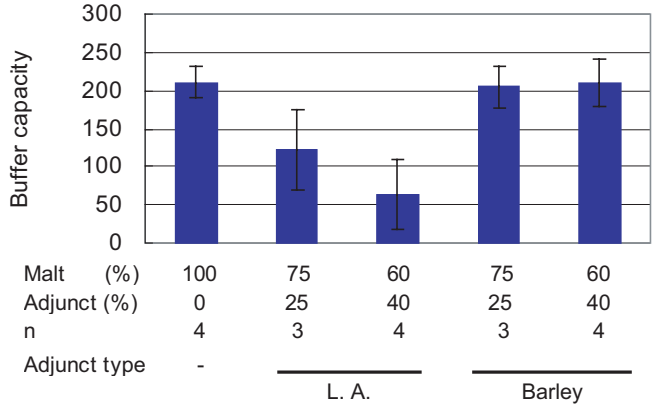

Figure 2 **Influence of L.A. and barley on kettle full wort buffer capacity**

Error bar shows the range of confidence (95 %).

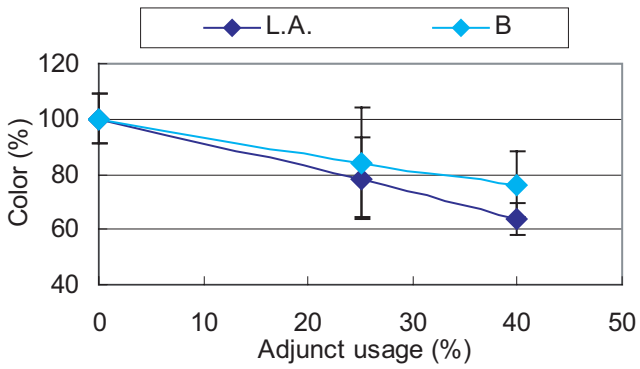

Figure 3 **Influence of L. A. and barley on cold wort color**

B: barley. All the data are average of n=3. Error bar shows the range of confidence (95 %). Each adjunct cold wort data are re-calculated to 12 plato and divided by that of all-malt data.

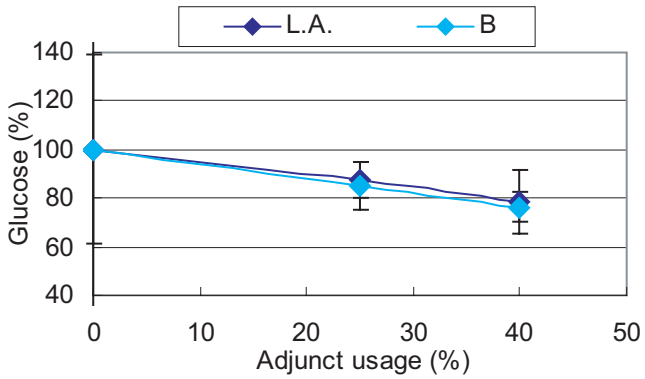

Figure 4 **Influence of L. A. and barley on cold wort glucose**

B: barley. All the data are average of n=3. Error bar shows the range of confidence (95 %). Each adjunct cold wort data are re-calculated to 12 plato and divided by that of all-malt data.

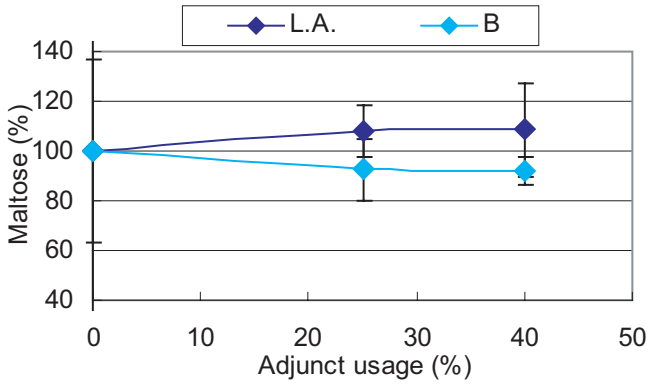

Figure 5 **Influence of L. A. and barley on cold wort maltose**

B: barley. All the data are average of n=3. Error bar shows the range of confidence (95 %). Each adjunct cold wort data are re-calculated to 12 plato and divided by that of all-malt data.

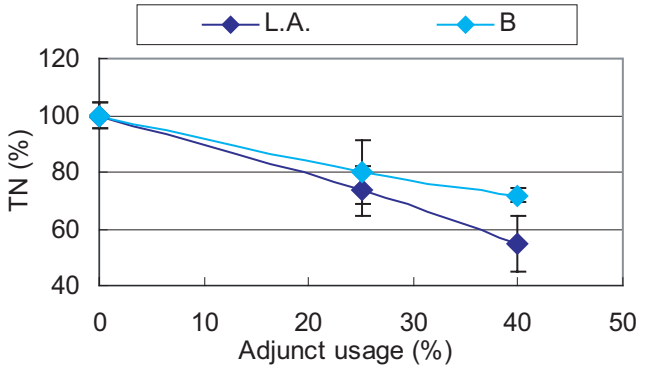

Figure 6 **Influence of L. A. and barley on cold wort TN**

B: barley. All the data are average of n=3. Error bar shows the range of confidence (95 %). Each adjunct cold wort data are re-calculated to 12 plato and divided by that of all-malt data.

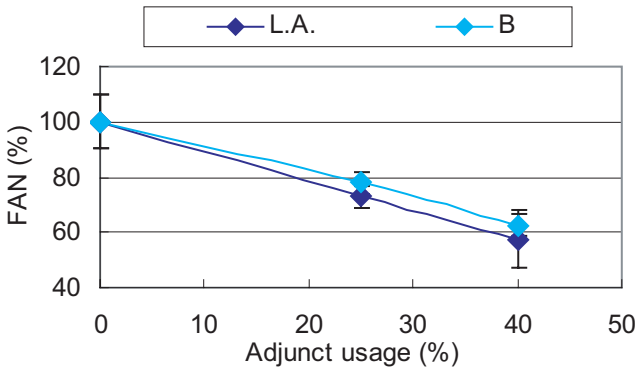

Figure 7 **Influence of L. A. and barley on cold wort FAN**

B: barley. All the data are average of n=3. Error bar shows the range of confidence (95 %). Each adjunct cold wort data are re-calculated to 12 plato and divided by that of all-malt data.

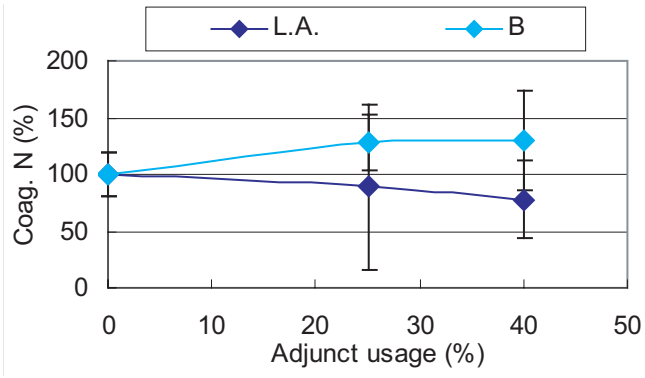

Figure 8 **Influence of L. A. and barley on cold wort Coag. N**

B: barley. All the data are average of n=3. Error bar shows the range of confidence (95 %). Each adjunct cold wort data are re-calculated to 12 plato and divided by that of all-malt data.

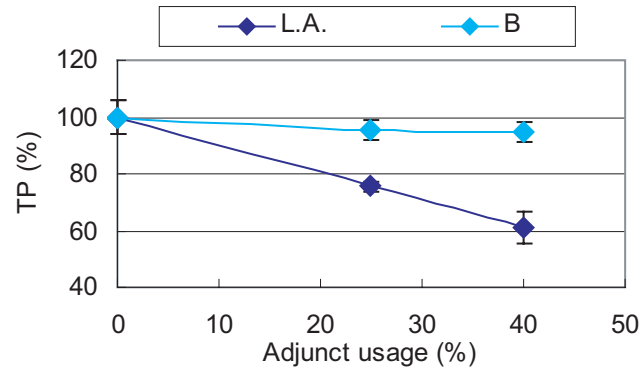

Figure 9 **Influence of L. A. and barley on cold wort TP**

B: barley. All the data are average of n=3. Error bar shows the range of confidence (95 %). Each adjunct cold wort data are re-calculated to 12 plato and divided by that of all-malt data.

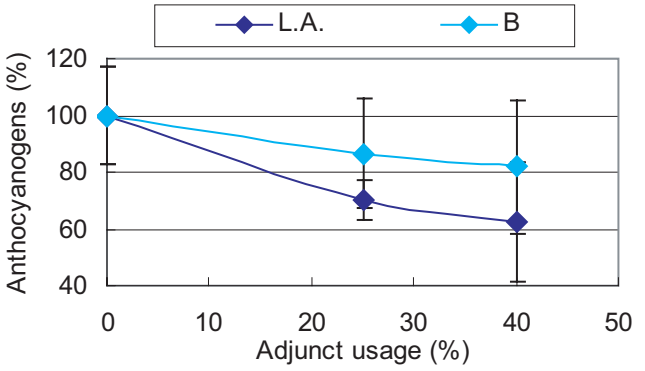

Figure 10 **Influence of L. A. and barley on cold wort anthocyanogens**

B: barley. All the data are average of n=3. Error bar shows the range of (95 %). Each adjunct cold wort data are re-calculated to 12 plato and divided by that of all-malt data.

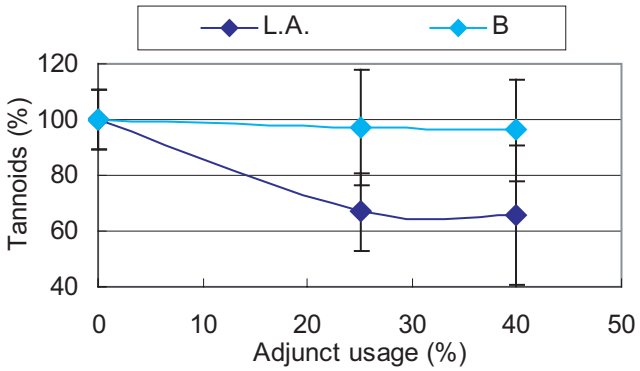

Figure 11 **Influence of L. A. and barley on cold wort tannoids**

B: barley. All the data are average of n=3. Error bar shows the range of confidence (95 %). Each adjunct cold wort data are re-calculated to 12 plato and divided by that of all-malt data.

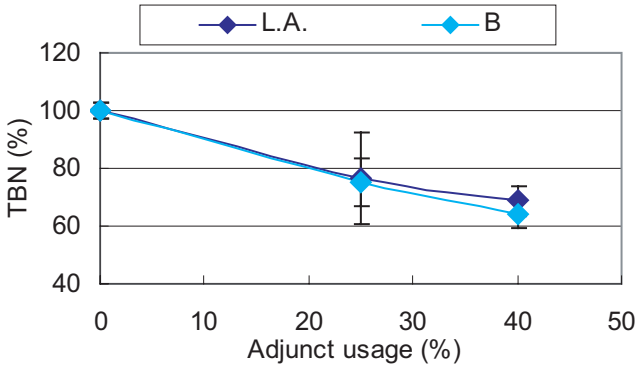

Figure 12 **Influence of L. A. and barley on cold wort TBN**

B: barley. All the data are average of n=3. Error bar shows the range of confidence (95 %). Each adjunct cold wort data are re-calculated to 12 plato and divided by that of all-malt data.

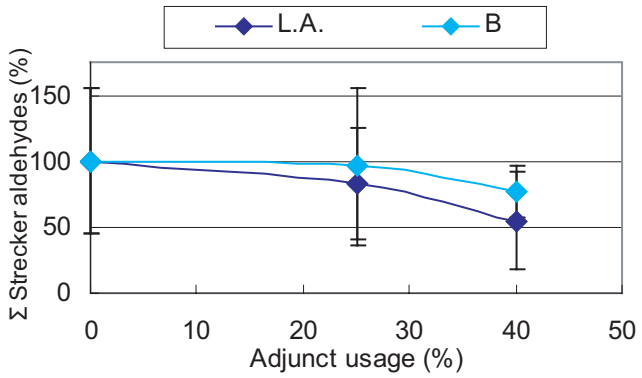

Figure 13 **Influence of L. A. and barley on sum of concentration of strecker aldehydes of cold wort.**

B: barley. All the data are average of n=3. Error bar shows the range of confidence (95 %). Each adjunct cold wort data are re-calculated to 12 plato and divided by that of all-malt data.

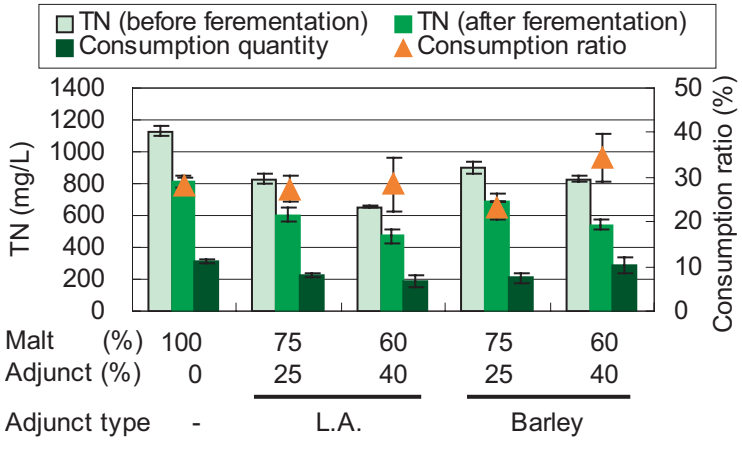

Figure 14 **Influence of L. A. and barley on consumption of TN during fermentation**

All the data are average of n=2. Error bar shows the maximum and minimum value. Each adjunct beer data are re-calculated to 12 plato.

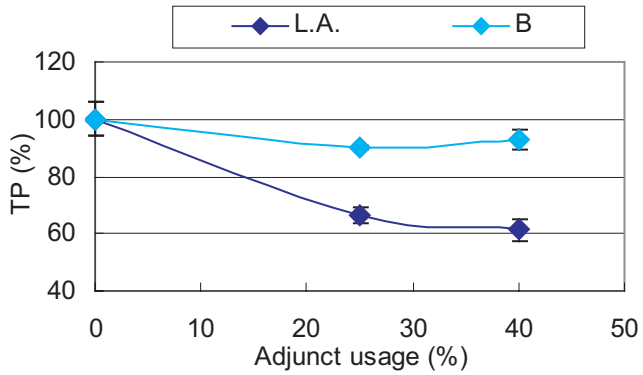

Figure 15 Influence of L. A. and barley on beer TP

B: barley. All the data are average of n=2. Error bar shows the maximum and minimum value. Figures are re-calculated to 12 plato.

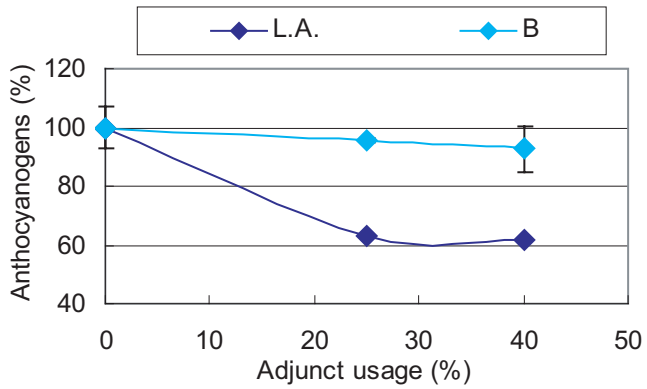

Figure 16 Influence of L. A. and barley on beer anthocyanogens

B: barley. All the data are average of n=2. Error bar shows the maximum and minimum value. Figures are re-calculated to 12 plato.

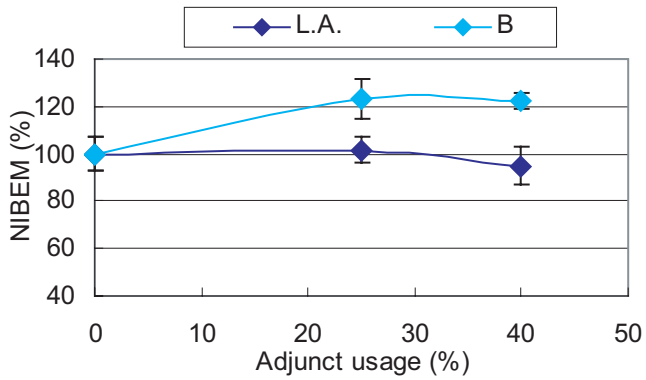

Figure 17 Influence of L. A. and barley on beer foam stability

B: barley. All the data are average of n=2. Error bar shows the maximum and minimum value.

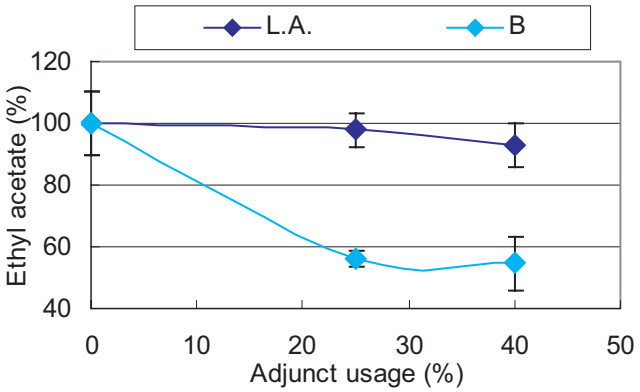

Figure 18 Influence of L. A. and barley on beer ethyl acetate

B: barley. All the data are average of n=2. Error bar shows the maximum and minimum value. Figures are re-calculated to 12 plato.

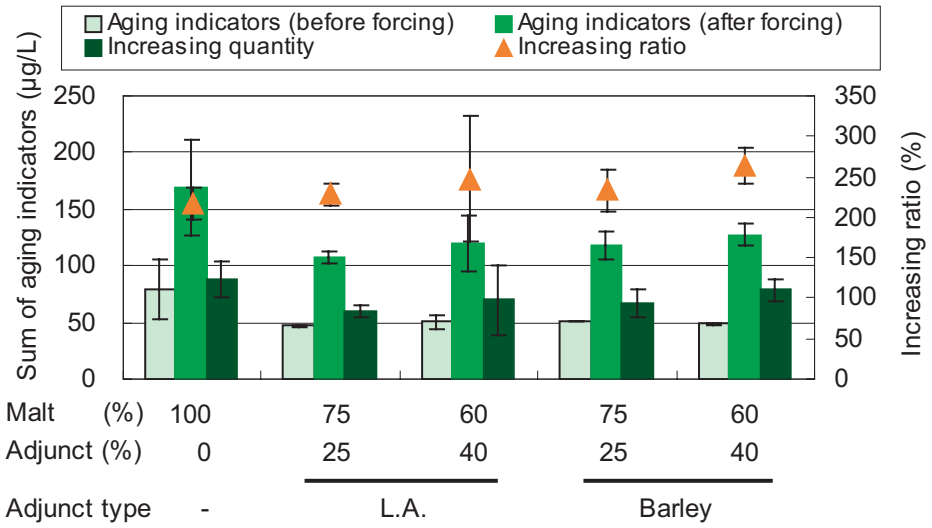

Figure 19 Influence of L. A. and barley on the beer aging indicators

All the data are average of n=2. Error bar shows the maximum and minimum value. Figures are re-calculated to 12 plato. The sum of is given in the figure.

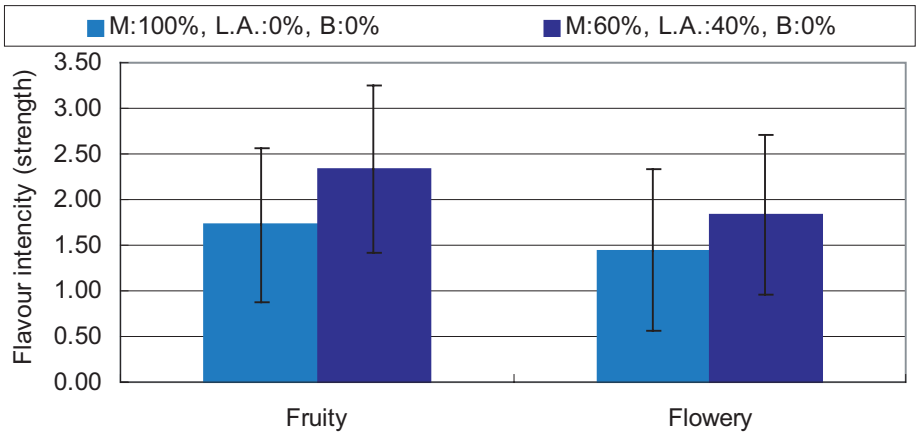

Figure 20 Influence of L. A. and barley on flavor intensity of fresh beer

M: malt ratio, L. A.: L. A. ratio, B: Barley ratio, 0: no such flavor or taste, 1: weak, 2: moderate, 3: strong, 4: very strong, 5: extremely strong. Average value of 9 panelists. Error bar shows the range of confidence (95 %)

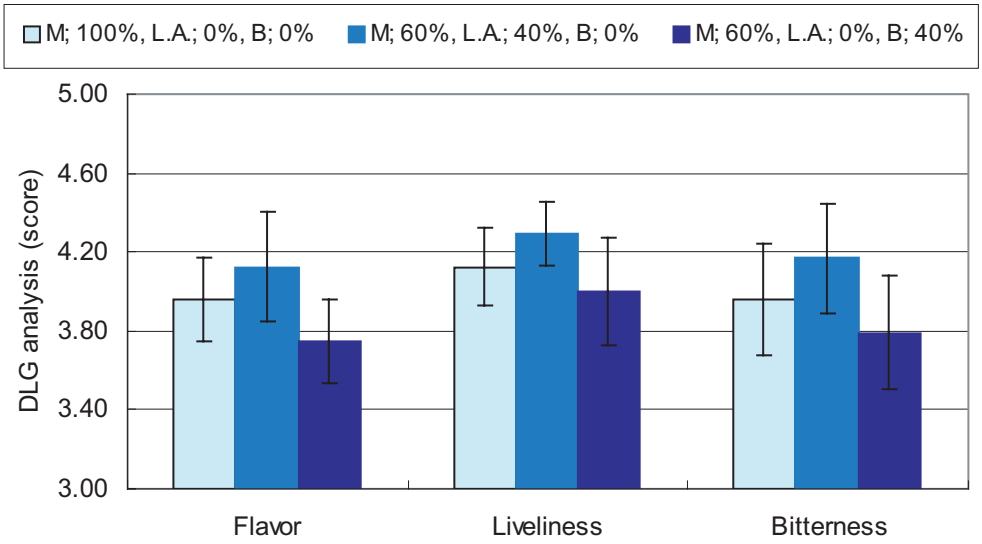

Figure 21 Influence of L. A. and barley on flavor intensity of fresh beer DLG evaluation

M: malt ratio, L. A.: L. A. ratio, B: Barley ratio, Flavor: 1 (strong flavor failure) – 5 (pure), Liveliness: 1 (very insipid) – 5 (pleasant liveliness), Bitterness: 1 (strong lingering bitterness) – 5 (very fine). Average value of 12 panelists. Error bar shows the range of confidence (95%)

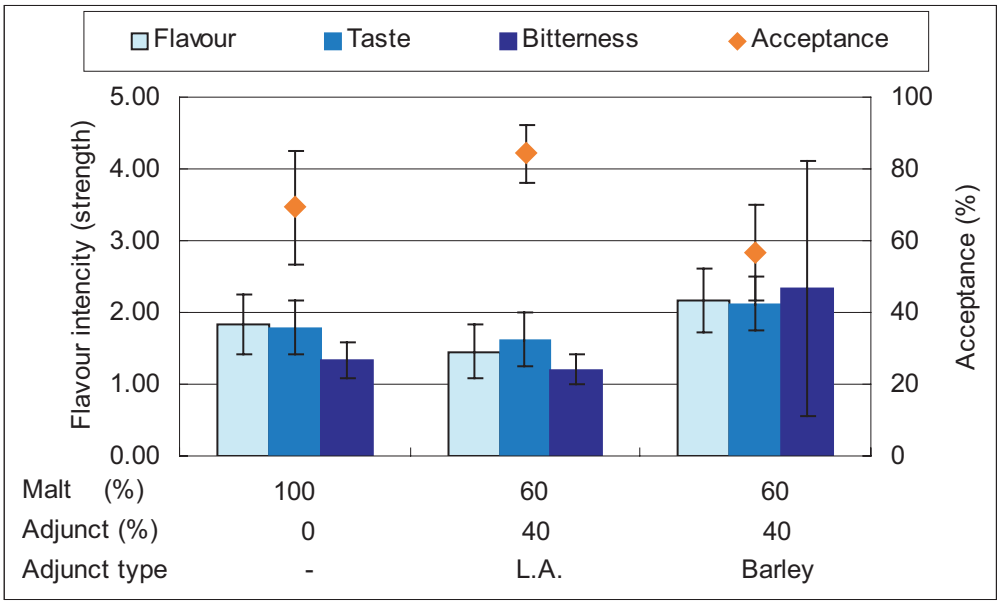

Figure 22 Influence of L. A. and barley on flavor intensity of oxidized flavor in forced aged beer

1: fresh, 2: weak oxidized , 3: strong oxidized, 4: extremely oxidized. Average value of 12 panelists. Error bar shows the range of confidence (95 %)