Factor Analysis of the Fermentation Process in Barley Shochu Production

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ABSTRACT

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It was shown that the components of pearled barley, i.e., the enzyme activity of barley koji and amino acid content of koji, were factors affecting final mash components in the fermentation process. The enzyme activities and content of inorganic components of the pearled barley affected alcohol concentration, density of mash and the main aromatic components. In addition, the growth of koji-mould on barley was affected by the nitrogen, phosphorus and potassium content in the pearled barley. The enzyme activities in barley-koji increased in proportion to mycelial growth.

Key words: Aroma, barley koji, barley Shochu, fermentation, pearled barley.

INTRODUCTION

Barley Shochu is a traditional Japanese distilled beverage containing 20~30% alcohol. Because Shochu production involves saccharification of starch using koji and fermentation by yeast at the same time, the process is called a "multiple parallel fermentation". The purpose of this research was to gain a better understanding of the influence of pearled barley components in a barley Shochu fermentation.

In previous research[5], it was reported that barley variety and pearling rate influence the growth of koji mold, koji enzyme activity, the production of acid, and the amino acid content of barley koji. These components were presumed to be very important in barley Shochu production and to the quality of the product. These results suggested that it is possible to change the Shochu fermentation process by changing the pearling rate and variety of pearled barley.

In the Shochu fermentation process, alcohol yield and density of mash are important factors in determining the end of the fermentation process. On the other hand, the quality of flavor in the Shochu is determined by aromatic components. In particular, ethyl acetate, isoamyl acetate and ethyl caproate are considered to be closely associated with Shochu flavor. These aromatic components, known to be produced by yeast from amino acids and organic acids in the mash[4], impart a fruity flavor (banana or apple) to Shochu and the factors that influence the formation of these aromatic components are important.

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Research of these important factors in Shochu-production has been investigated during koji production[17,18] and fermentation by yeast[8]. However, there have been few reports on the influence of ingredient characteristics on these factors. In the case of sake, a traditional Japanese alcoholic beverage, extensive research has examined the influence of rice components on fermentation and aroma production[9,10,23].

This research aims to confirm the differences of fermentation processes by pearling rate and variety and to understand the influence of pearled barley components in the mash during the fermentation process.

MATERIALS AND METHODS

Fermentation experiments

The fermentation experiments are outlined in Table I. Two varieties of Australian barley, *Stirling* and *Schooner* cultivated in 2003/04 and one Japanese variety *Nishinohoshi* cultivated in 2003 were employed.

The barley was pearled using a test mill (TM-05, Satake Co, Japan) and the pearling rate was adjusted to 80%, 70%, 60% and 50% respectively. The pearling rate was calculated as follows:

$$\text{Pearling rate (\%)} = \frac{\text{(weight of 1,000 sound kernels of pearled barley)}}{\text{(weight of 1,000 whole grains)}} \times 100 \quad (1)$$

The barley koji was prepared and the enzyme activity of the koji, mycelial growth, and the amino acid composition of the koji were measured as described previously[5].

Fermentation experiments were conducted on a laboratory scale with the combination of components shown in Table I. The seed mash was prepared with 120 mL of tap water and barley koji was prepared from 100 g of pearled barley. The seed mash was inoculated with 1×10^9 cells of *Saccharomyces cerevisiae BAW-6* (Sanwa Shurui culture collection for Shochu production). The seed mash was fermented for 5 days at 25°C. The main mash was prepared by adding 200 g of steamed barley and 330 mL of tap water to the seed mash. The main mash was fermented for 10 days at 25°C. The amount of carbon dioxide evolution was estimated by the loss of the mash weight.

Analysis methods

Alcohol concentration was analyzed using an ethanol analyzer (AB-1B, BTIC Co., Ltd. Japan), and the density of the mash filtrate was measured using a density meter

TABLE I. Ingredients and composition of mash for fermentation experiments.

1. Varieties of barley

Variety	Harvest area	Source	Harvest year
Stirling	Western Australia	Commercial	2003/04
Schooner	South Australia	Commercial	2003/04
Nishinohoshi	Oita prefecture, Japan	Commercial	2003

2. Ingredients and composition of mash

Fermentation stage	Seed mash (5 days in fermentation period)		Main mash (10 days in fermentation period)	
Ingredient	Barley koji (g)	Water (mL)	Steamed barley (g)	Water (mL)
Composition	100	120	200	330

(DA300, Kyoto Electronics Manufacturing Co., Ltd. Japan). For amino acid analysis, the mash was filtered through gauze, centrifuged for 10 min at 3,000 rpm, and the supernatant was filtered through a 0.8 μm filter. An equal amount of 5% (w/v) trichloroacetic acid was added to 750 μL of filtrate, mixed for 30 sec in a vortex mixer. One mL of supernatant fluid was diluted 20 times with 0.02 N hydrochloric acid. The filtrate was filtered through a 0.45 μm filter and analyzed using an amino acid analyzer (L-8500A, Hitachi Co., Ltd. Japan).

The aromatic components in the mash were measured using a gas chromatograph/FID unit (6890, Agilent Tech. USA) and headspace auto-sampler (7694, Agilent Tec. USA). The GC analysis conditions were as follows: DB-WAX (inner diameter 0.25 mm, length 30 m, film thickness 0.5 μm, J&W Scientific, USA) column; inlet temperature, 200°C, and the column temperature, raised to 55°C (5 min) – 10°C/min – 170°C (7 min); carrier gas, helium; split ratio 1/20; flow rate 1.5 mL/min; detector temperature 200°C.

Phosphorus in pearled barley was analyzed by spectrophotometry (UV-1200, Shimadzu Co., Ltd, Japan) according to Yashui[20]. Calcium was analyzed by ICP luminescence (Optima 2000DV, Perkin-Elmer Co, USA) according to Yashui[20]. Potassium was analyzed by atomic absorption (SOLAAR M6, Japan Jarrelash Co. Japan) with wavelength: 766.5 nm, the frame: air-acetylene according to Yashui[20]. Magnesium was analyzed by atomic absorption (SOLAAR M6, Japan Jarrelash Co. Japan), wavelength: 285.2 nm, the frame: air-acetylene according to Yashui[20]. Nitrogen content of the pearled barley was determined using a nitrogen analyzer (LECO-FP2000, LECO Co. USA). Preparation of the sample was as follows: 4 g of pearled barley was crushed and 0.4 g weighed. This was burnt at 1350°C and measured and the working curve was obtained using EDTA. The total starch content of the pearled barley was determined using an enzyme kit (Megazyme International, Ireland) according to AACC Method 76.13[2].

Analysis of data

Multiple regression analysis (MRA) was carried out using JMP analysis software (SAS Corporation, USA.). The independent variables were selected using stepwise ($P = 0.25$) inclusion of parameters. MRA was conducted with components of mature mash (Table II), pearled barley composition, and barley koji quality characteristics (Table III) as the independent variables.

RESULTS AND DISCUSSION

Results of fermentation experiment

Fig. 1 and Table II show the results of fermentation experiments. Fig. 1 shows the comparison of fermentation time course with different pearling rates and varieties of

TABLE II. Comparison of final mashes with different ingredients.

	(A) *Stirling*				(B) *Schooner*				(C) *Nishinohoshi*			
	Pearling rate (%)				Pearling rate (%)				Pearling rate (%)			
	50	60	70	80	50	60	70	80	50	60	70	80
Alcohol (%)	18.7	18.6	18.5	18.3	18.3	18.1	18.1	18.0	18.1	18.0	17.4	17.1
Density (g/cm^3)	0.992	0.993	0.994	0.997	0.993	0.994	0.996	0.997	0.993	0.993	0.995	0.998
Volume of mash (mL)	722	726	728	731	727	727	718	727	715	717	720	718
*Alcohol yield (L/t)	449	449	448	446	445	438	432	435	431	429	422	410
Acetaldehyde (ppm)	25.0	22.7	22.8	26.9	21.3	24.3	24.3	26.4	28.1	24.6	25.8	25.3
Ethyl acetate (ppm)	41.5	41.8	55.4	65.6	45.4	44.0	50.7	69.9	42.2	54.6	55.6	59.3
n-Propyl alcohol (ppm)	182.5	141.5	88.4	68.9	169.0	129.7	105.7	71.7	152.7	113.9	79.9	63.8
i-Butyl alcohol (ppm)	100.1	94.5	102.8	103.9	95.4	93.4	101.5	100.4	106.5	99.7	105.7	99.8
Isoamyl acetate (ppm)	2.9	2.7	2.8	3.6	2.8	2.5	3.3	2.4	2.4	2.6	2.3	2.1
n-Butyl alcohol (ppm)	12.4	9.0	4.8	2.5	10.7	7.2	5.1	2.9	9.7	6.3	3.1	2.4
i-Amyl alcohol (ppm)	327.4	301.1	303.0	310.5	303.8	286.5	313.6	286.1	317.9	301.9	303.4	281.0
Ethyl caproate (ppm)	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2
Ethyl caprylate (ppm)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Ethyl caprate (ppm)	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
Amino acids in mash (mg/L)	3278	3929	3949	5310	3948	4085	5233	6119	3276	3320	3651	5185

*Alcohol yield (L/t) = (alcohol concentration (%) × mash volume (L))/(weight of pearled barley used (ton)) × 100

TABLE III. Components of pearling barley and barley koji.

1. Pearled barley

Variety	Pearling rate (%)	Total starch (%-dry matter)	Nitrogen (%)	Phosphorus (mg/g-dry matter)	Potassium (mg/g-dry matter)	Magnesium (mg/g-dry matter)	Calcium (mg/g-dry matter)
Stirling	50	76.8	0.88	1.00	1.52	0.18	0.16
	60	76.2	0.99	1.07	1.70	0.24	0.16
	70	72.4	1.14	1.37	1.94	0.36	0.23
	80	65.7	1.30	2.04	2.63	0.64	0.20
Schooner	50	76.6	0.82	1.08	2.41	0.26	0.17
	60	73.3	0.83	1.18	2.20	0.29	0.25
	70	70.4	1.03	1.44	2.56	0.40	0.21
	80	68.3	1.13	1.96	2.99	0.60	0.21
Nishinohoshi	50	77.2	0.84	1.24	2.19	0.16	0.14
	60	76.0	0.96	1.38	2.35	0.22	0.15
	70	72.1	0.99	1.72	2.70	0.35	0.17
	80	67.2	1.26	2.43	3.47	0.61	0.20

2. Barley koji

Variety	Pearling rate (%)	Mycelia of koji (μg/g-dry koji)	α-Amylase (units/g-dry koji)	Glucoamylase (units/g-dry koji)	Acid protease (units/g-dry koji)	Acid carboxy-peptidase (units/g-dry koji)	Amino acid of koji (mg/kg-koji)
Stirling	50	1,493	61	177	7,239	1,099	2,247
	60	1,875	77	207	8,580	3,706	1,994
	70	1,910	120	202	14,217	4,406	2,856
	80	2,199	164	252	23,041	2,265	5,680
Schooner	50	2,042	90	220	6,364	1,500	3,065
	60	2,185	100	214	7,871	3,945	3,625
	70	2,262	111	222	13,743	5,665	3,547
	80	2,159	141	214	16,702	2,420	5,272
Nishinohoshi	50	2,024	94	194	8,786	3,133	3,759
	60	2,401	117	219	9,643	3,715	3,816
	70	2,563	133	288	15,312	5,081	3,697
	80	2,307	152	247	20,174	3,435	5,478

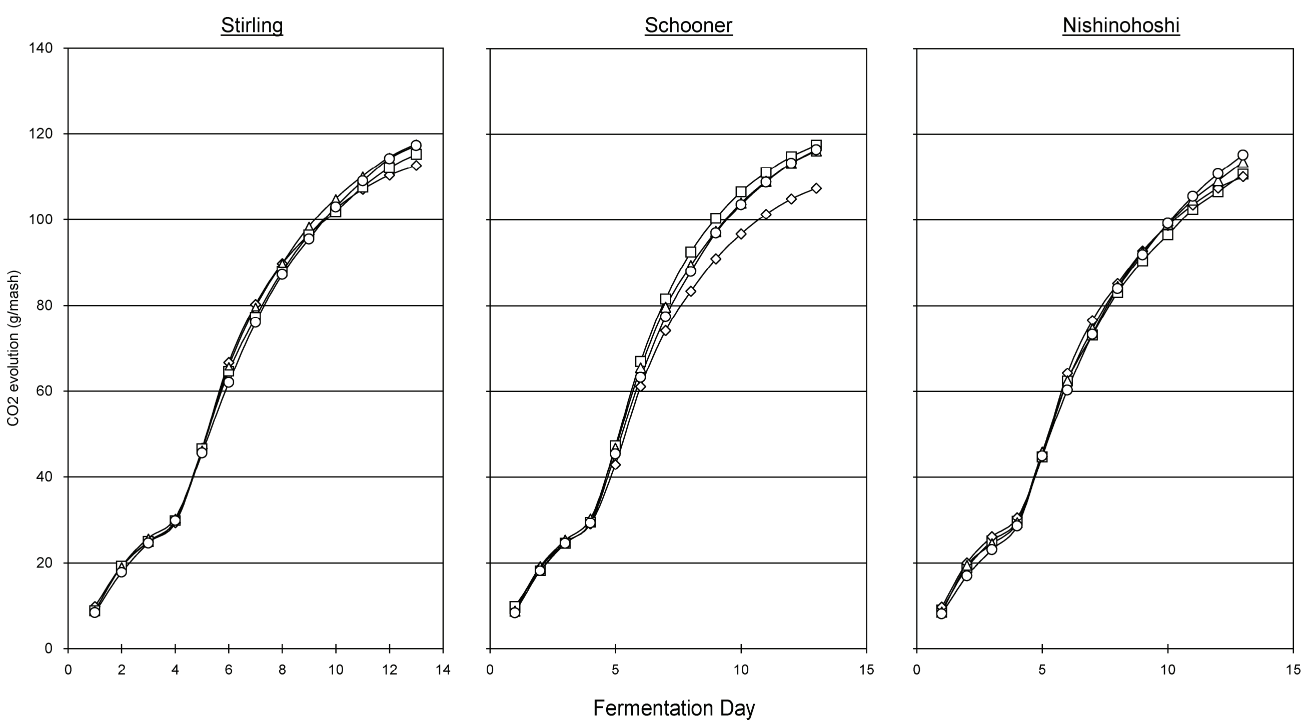

Fig. 1. Comparison of fermentation time course with different ingredients. Symbols: (diamond) Pearling ratio 80%; (square) Pearling ratio 70%; (triangle) Pearling ratio 60%; (circle) Pearling ratio 50%.

barley. Most of the CO_2 evolution profiles were similar, but the 80% pearling rate of *Schooner* gave a different profile. The properties of the final mash were analyzed and are summarized in Table II. Alcohol concentration tended to increase with low rates of pearling. Among varieties, *Nisinohoshi* was lower than other varieties. However, the density of mash tended to decrease with low rates of pearling.

Ethyl acetate tended to increase with high rates of pearling. Isoamyl acetate levels with *Stirling* tended to be higher than with other varieties. Ethyl caproate tended to increase with high rates of pearling. Among varieties, *Nishinohoshi* was higher than other varieties in regard to ethyl caproate levels. Amino acids tended to increase with high rates of pearling. Among varieties, *Schooner* was higher than the other varieties. In Table III, nitrogen and inorganic components in the barley increased in proportion to pearling rate. On the contrary, total starch decreased.

Analysis of factors affecting the fermentation of the mash

The factors affecting important properties of fermentation were tested using multiple regression analysis (MRA). With alcohol concentration as the dependent variable, total starch, phosphorus, magnesium, calcium, glucoamylase activity, and acid carboxypeptidase activity were identified as significant variables, as shown in Table IV. The identification of glucoamylase activity was expected since glucoamylase activity is rate-controlling for glucose production from starch in mash and from the work of Iwano et al.[6], it is known that the supply of glucose controls the rate of alcohol production in Shochu mash.

At the same time, inorganic components are rate-determining for the fermentation speed of yeast in mash. Nutritional factors, such as urea, inorganic components and vitamin B for sake yeast, are known to influence production of high concentrations (20% w/v) of alcohol in sake mash[13,14,19]. As the coefficient of determination and multiple correlation coefficient were 0.984 (R^2) and 0.992 (R) respectively, using these 6 variables as shown in Table IV, alcohol concentration was well explained by these variables.

TABLE IV. Results of multiple regression analysis of alcohol concentration and density.

1) Alcohol concentration

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
TS	1	7.90E-03	2.4	0.1810
P	1	3.20E-01	97.9	0.0002
Mg	1	1.24E-01	37.8	0.0017
Ca	1	2.48E-02	7.6	0.0401
GAase	1	1.25E-01	38.2	0.0016
ACPase	1	2.53E-01	77.5	0.0003

(R^2 = 0.984 RMSE = 0.057)

2) Density of mash

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
TS	1	4.39	139.1	<0.0001
K	1	1.03	32.7	0.0120
AAase	1	0.95	30.1	0.0015
APase	1	0.46	14.5	0.0088
ACPase	1	0.26	8.3	0.0282

(R^2 = 0.996 RMSE = 0.178)

AAase – α-amylase, GAase – glucoamylase, APase – acid protease, ACPase – acid carboxypeptidase

It was considered that the increase of total starch attributed to low rates of pearling resulted in high alcohol concentration levels (Table II). In the case of *Nishinohoshi,* the balance of uptake of fermentable sugar by yeast and supply of fermentable sugar by enzyme was not as good as that of the other varieties.

Total starch content, potassium, α-amylase activity, acid protease activity, and acid carboxypeptidase activity were identified as significant variables affecting the density of the mash as shown in Table IV. Enzyme activity of α-amylase, acid protease and acid carboxypeptidase were identified as significant variables, as these enzymes are important in the degradation of the substrate (starch or protein). At the same time, potassium is a rate-determining factor for glucose uptake and amino acid uptake by yeast in mash.

Regarding the density of mash, the coefficient of determination and multiple correlation coefficient was 0.996 (R^2) and 0.998 (R) respectively using the 5 significant independent variables shown in Table IV. It was considered that the difference between degradation levels of substrate by each enzyme was a result of the increase in density levels due to higher rates of pearling.

Analysis of factors affecting barley koji quality

Table IV confirms that enzyme activity affected alcohol concentration and density of the mash. Factors that influence the enzyme activity of koji were subjected to MRA.

In the case of α-amylase activity, the nitrogen content, phosphorus, and mycelial growth were identified as significant variables as shown in Table V. The high F value for mycelial growth indicated that mycelial growth is an important factor for α-amylase activity. The coefficient of determination and multiple correlation coefficient were 0.920 (R^2) and 0.959 (R) respectively using these 3 variables.

In the case of glucoamylase activity, mycelial growth was identified as the single most significant variable. As the coefficient of determination was high (R^2 = 0.88), glucoamylase activity was shown to be proportional to the growth of the fungus, as was the case with α-amylase.

With acid protease activity as the dependent variable, total starch content, nitrogen, phosphorus, magnesium, calcium and mycelial growth were identified as significant variables. The coefficient of determination and multiple correlation coefficient were 0.953 (R^2) and 0.976 (R) respectively using these 6 variables. Again, fungal growth was an influential factor.

The effect of nitrogen content on acid protease activity confirmed a previous report that showed that protein composition has an influence on acid protease production in rice[7].

Acid carboxypeptidase results were similar to acid protease results. With acid carboxypeptidase activity as the dependent variable, nitrogen content, potassium, and mycelial growth were identified as significant variables.

Table V shows that with mycelial growth as the dependent variable, nitrogen content, phosphorus and potassium were identified as significant variables. In a previous study[5], it was suggested that the mycelia in koji were affected by the phosphorus and potassium in rice. Nitrogen content was identified as a significant variable in barley koji since nitrogen contributed to the growth of koji fungus and the content of crude protein (N) in pearled barley is higher than in pearled rice[16]. Okazaki et al.[15] reported that the specific growth rate and lag time were affected by nitrogen in rice koji. The analysis of mycelial growth data indicated that the nitrogen, phosphorus and potassium content of pearled barley affected the enzyme activity of koji indirectly. On the other hand, with amino acid content in koji as the dependent variable, nitrogen content, phosphorus, potassium, magnesium, acid peptidase activity, and acid carboxypeptidase activity were identified as significant variables as shown in Table V. These results indicate that amino acids in barley koji are the result of enzymatic degradation of endogenous proteins. In conclusion, it was confirmed that mycelial growth affects the activity of all enzymes examined in this study.

TABLE V. Results of multiple regression analyses of barley koji.

1) AAase

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
N	1	8.96E+02	14.3	0.0016
P	1	1.13E+02	1.8	0.1974
Mycelia of koji	1	3.14E+03	50.1	<0.0001

(R^2 = 0.920 RMSE = 7.92)

2) GAase

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
Mycelia of koji	1	4.09E+04	144.5	<0.0001

(R^2 = 0.883 RMSE = 16.83)

3) APase

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
TS	1	9.53E+06	7.8	0.0152
N	1	6.47E+06	5.3	0.0386
P	1	4.48E+06	3.7	0.0778
K	1	5.40E+06	4.4	0.0555
Ca	1	4.00E+06	3.3	0.0935
Mycelia of koji	1	1.60E+07	13.1	0.0031

(R^2 = 0.953 RMSE = 1105.5)

4) ACPase

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
N	1	2.53E+08	21.4	0.0003
K	1	2.33E+07	19.4	0.0004
Mycelia of koji	1	1.28E+08	10.8	0.0046

(R^2 = 0.638 RMSE = 3441.8)

5) Mycelia of koji

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
N	1	1.02E+06	11.4	0.0038
P	1	1.09E+06	12.3	0.003
K	1	1.34E+05	1.5	0.2379

(R^2 = 0.403 RMSE = 298.8)

6) Amino acids in koji

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
N	1	8.38E+05	12.9	0.0033
P	1	3.92E+06	60.5	<0.0001
K	1	4.62E+05	7.1	0.0193
Mg	1	5.33E+06	82.3	<0.0001
APase	1	2.36E+05	3.6	0.0785
ACPase	1	4.47E+05	6.9	0.0209

(R^2 = 0.924 RMSE = 254.6)

AAase – α-amylase, GAase – glucoamylase, APase – acid protease, ACPase – acid carboxypeptidase

Analysis of factors affecting aromatic components in final mash

Regression analysis identified α-amylase activity and glucoamylase activity as the two significant variables related to ethyl acetate levels in the mash (Table VI). The coefficient of determination and multiple correlation coefficient were 0.824 (R^2) and 0.908 (R) using 2 significant independent variables. Yoshioka and Hashimoto[21,22] reported that ethyl acetate and isoamyl acetate are synthesized from ethanol or isoamyl alcohol and acetyl Co-A by alcohol acetyl transferase (AATase) in sake production. The activity of α-amylase and glucoamylase is asso-

TABLE VI. Results of multiple regression analysis of aromatic components.

1) Ethyl acetate

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
AAase	1	600.12	37.2	2.00E-04
GAase	1	33.51	2.1	1.83E-01

(R^2 = 0.824 RMSE = 4.015)

2) Isoamyl acetate

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
ACPase	1	0.96	12.6	6.20E-03
Amino acids of koji	1	0.27	3.5	9.30E-02

(R^2 = 0.554 RMSE = 0.276)

3) Ethyl caproate

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
N	1	3.65E-04	1.8	2.17E-01
Mg	1	1.49E-03	7.3	2.68E-02
AAase	1	2.24E-03	11.0	1.06E-02

(R^2 = 0.572 RMSE = 0.014)

4) Amino acids in mash

Independent variable	Degrees of freedom	Sum of squares	F-value	P-value
APase	1	8.49E+05	7.5	2.32E-02
Mg	1	3.80E+06	33.4	3.00E-04

(R^2 = 0.873 RMSE = 337.6)

AAase – α-amylase, GAase – glucoamylase, APase – acid protease, ACPase – acid carboxypeptidase ciated with the production of ethanol, which is a precursor for ethyl acetate. Yoshizawa and Ishikaw[23] reported that AATase exhibits high activity in the presence of glucose in sake production. Glucoamylase activity was identified as a significant variable in Table VI. In the case of pearling rate, it was considered that an increase in ethyl acetate levels could be explained by increases in α-amylase activity associated with higher pearling rates. Similarly, with isoamyl acetate as the dependent variable, acid carboxypeptidase activity and amino acid content in barley koji were identified as significant variables, and in case of ethyl caproate, nitrogen content, magnesium, and α-amylase activity were identified. With isoamyl acetate, the two variables identified, carboxypeptidase activity and amino acid content, were related to amino acids in the mash supply. Given that leucine is precursor of isoamyl alcohol, this is not surprising.

Akita et al.[1] reported that there is an optimum concentration of amino acids for maximum isoamyl acetate formation. In addition, an excess of amino acids decreases isoamyl acetate formation. Regarding variety, it was considered that the amino acid level of *Stirling* associated with carboxypeptidase activity and the amino acid content of koji was more suitable for isoamyl acetate formation than with the other varieties.

Ethyl caproate is produced by AATase and esterase using ethanol and caproic acid as substrates[4,11,12]. Magnesium and α-amylase are related to ethanol production for ethyl caproate. Magnesium accelerates ethanol production by yeast. In the case of pearling rate, it was considered that the increase in ethyl caproate levels could be explained by the increases in α-amylase activity associated with the higher pearling rates and ethanol production by yeast relating to inorganic components.

The concentration of the amino acids in the mash is thought to affect the aroma of barley Shochu because amino acids are involved in Maillard reactions with reducing sugars that result in the development of a distinctive aroma[3]. Acid protease activity and magnesium were identified as significant variables associated with the amino acid concentration in the mash. Acid protease activity and magnesium are closely related to amino acid production and uptake by yeast. It was considered that acid protease activity would affect the supply of amino acids and therefore the concentration of amino acid levels in the mash.

These results suggest that the components of pearled barley, enzyme activity in barley koji, and amino acid concentration, are all factors that affect the development of aromatic components during the fermentation process. This work confirms that Shochu fermentation and aroma development are affected by the complex relationship between koji enzymes and pearled barley components. Inorganic components are also important factors that affect yeast fermentation in Shochu mash.

CONCLUSIONS

It was shown that the components of pearled barley, enzyme activity of barley koji and amino acid content of koji are all factors affecting final mash components in the fermentation process. Enzyme activity and content of inorganic components of pearled barley affected alcohol concentration, density of mash and some main aromatic components. In addition, the results of the analysis of mycelial growth indicated that the nitrogen, phosphorus and potassium content in pearled barley affected the enzymes of koji indirectly. It was suggested that the content of nitrogen and inorganic components in pearled barley are important factors in Shochu production. These results are useful information for ingredient selection in barley Shochu production.

REFERENCES

1. Akita, O., Hasuo, T., Ohba, T. and Miyano, N., Effects of amino acid composition on higher alcohols and isoamyl acetate formation by a yeast. *Hakkokogaku.,* 1987, **65**, 19–26.
2. American Association of Cereal Chemists, Total starch. Method 76.13. In: Approved Methods of the American Association of Cereal Chemists, 10th ed. The Society: St Paul, MN, 2000.
3. Higashi, K., Fermentation and Brewing II, Korin Publishing Co., Ltd: Tokyo, 2004, pp. 328.
4. Ishikawa, Y., The Element of Brewing, Brewing Society of Japan, 1999, pp. 119–123.
5. Iwami, A., Kajiwara, Y. and Omori, T., Effect of the variety of barley and pearling rate on the quality of Shochu koji. *J. Inst. Brew.,* 2005, **111**(**3**), 309–315.
6. Iwano, K., Mikami, S., Fukuda, K., Shiinoki, S. and Simada, T., Difference in fermentation efficiency between rice-shochu and barley-shochu. *J. Brew. Soc. Japan.,* 1987, **82**, 661–664.
7. Iwano, K., Nakazawa, N., Ito, T., Takahashi, H., Uehara, Y. and Matsunaga, R., The influence of protein components in raw material rice. *J. Brew. Soc. Japan.,* 2001, **96**, 857–862.
8. Kajiwara, K., Ogawa, K., Takashita, H., Omori, T., Simoda, M. and Wada, H., Intracellular fatty acid formation and alcohol acetyl transferase gene expression in brewing yeast (*Saccharomyces cerevisiae*) treated with heat shock. *J. Ferment. Bioeng.,* 1987, **84**, 594–598.
9. Kitamoto, K., Miyake, M. and Watanabe, S., Reduction of amino acid content in the sake by rice germ addition to moromi mash. *J. Brew. Soc. Japan,* 1985, **80**, 53–58.
10. Kitamoto, K., Miyake, M., Kohno, M. and Watanabe, S., Inorganic components in rice grain affecting reduction of amino acid content in sake moromi mash. *J. Brew. Soc. Japan,* 1985, **80**, 59–63.
11. Kuriyama, K., Ashida, S., Saito, Y., Hata, Y., Suginami, K. and Imayasu, S., Ethyl caproate synthesis and hydrolysis activity of sake yeast. *Hakkokogaku.,* 1986, **64**, 175–180.
12. Kuriyama, K., Ashida, S., Saito, Y., Hata, Y., Suginami, K. and Imayasu, S., The role of yeast and koji in the formation of ethyl caproate in moromi. *Hakkokogaku,* 1986, **64**, 253–259.
13. Nakazato, A., Hosaka, Y., Takeda, T., and Tsukahara, T., Influence of polished rice on production of high concentration of alcohol in the mash with enzymatic preparations. Examination of production of high concentration of alcohol in the mash with enzymatic preparation (Part 1). *J. Brew. Soc. Japan,* 1984, **79**, 581–582.
14. Nakazato, A., Hosaka, Y., Takeda, T., and Tsukahara, T., Influence of polished rice on production of high concentration of alcohol in the mash with enzymatic preparations. Examination of production of high concentration of alcohol in the mash with enzymatic preparation (Part 2). *J. Brew. Soc. Japan,* 1984, **79**, 583–585.
15. Okazaki, N., Fukaya, I., Sugama, S., and Tanaka, T., Influence of rice grain components on koji-mold growth and enzyme production. *J. Ferment. Bioeng.,* 1981, **59**, 491–500.
16. Omori, T., Nagano, S., Teshima, N. and Simoda, M., Effects of polishing ratio on general components and water-absorbing rate of two row barley. *J. Brew. Soc. Japan,* 1992, **87**, 527–532.

17. Saigusa, T., Harada, M. and Shinohara, T., Control of flavour formation by changing the time of koji culture during all-koji shochu fermentation. *J. Biosci. Bioeng.,* 1993, **71**, 105–109.
18. Saigusa, T., Harada, M., Okamura, S. and Shinohara, T., Factors affecting the formation of isoamyl acetate during all-koji shochu fermentation. *J. Biosci. Bioeng.,* 1993, **71**, 383–388.
19. Takeda, T., Nakazato, A. and Tsukahara, T., Difference in formation of a high concentration of alcohol between *Saccharomyces sake* and *Saccharomyces cerevisiae* in sake mash. *Hakkokogaku,* 1982, **60**, 137–144.
20. Yasui, A., Trace inorganic analysis. Analytical methods for inorganic elements in food. *Bunseki,* 1990, **9**, 664–674.
21. Yoshioka, K. and Hashimoto, N., Cellular fatty acid and ester formation by brewer's yeast. *Agric. Biol. Chem.,* 1983, **47**, 2287–2294.
22. Yoshioka, K. and Hashimoto, N., Ester formation by alcohol acetyl transferase from brewer's yeast. *Agric. Biol. Chem.,* 1981, **45**, 2183–2190.
23. Yoshizawa, K and Ishikaw, T., Changes of lipids during sake brewing and their contribution to ester formation by yeast. *Hakkokogaku.,* 1985, **63**, 161–173.

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