196

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Elucidation of the ester formation mechanism in top fermenting yeast

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Abstract

We have developed a Weizen beer with both "refreshing finish" and overwhelming "banana-like sweet and fruity aroma". Isoamyl acetate contributes to the "banana-like, sweet, and fruity aroma", and its normal concentration in Weizen beer is 2.5-3.5 ppm. To achieve the overwhelming "banana-like, sweet, and fruity aroma", we hypothesized that a higher concentration of isoamyl acetate was required. Therefore, we aimed to elucidate the mechanisms of ester formation during the beer production process, in order to regulate the final isoamyl acetate concentration in beer. According to previous research, isoamyl acetate is synthesized from acetyl-CoA and isoamyl alcohol via catalysis by alcohol acetyl transferase (AATase) in yeast. The formation of isoamyl acetate is influenced by various factors, such as wort composition, including unsaturated fatty acids, amino acids, and fermentation conditions. The aim of this study is to elucidate the effect of the following factors on isoamyl acetate production during mashing and fermentation: substrate concentration, fermentation temperature, and aeration rate. These were investigated by a 2-L scale fermentation test. Concentration of substrates, such as valine, leucine, in wort has more influence than fermentation conditions among the investigated conditions. Based on the results obtained from the 2-L scale test, we optimized the brewing conditions to produce beer with a higher concentration of isoamyl acetate at a 50 h-L scale, and evaluated the effectiveness of the various factors investigated. Through this study, we developed a Weizen beer with 5-ppm isoamyl acetate concentration and improved the "banana-like, sweet, and fruity aroma" produced by top fermenting yeast by controlling specific brewing conditions.

Objective

The aim of our research is to achieve the overwhelming "banana-like, sweet, and fruity aroma". Isoamyl acetate contributes to the typical banana-like aroma; thus, we focused on the isoamyl acetate formed during the brewing process.

To achieve our objective,

Step1: We elucidated the mechanisms of isoamyl acetate formation during beer production process.

Step2: We identified the optimal conditions to increase the "banana-like, sweet, and fruity aroma" produced by top fermenting yeast by controlling specific brewing conditions.

Materials and Methods <Step1>

Isoamyl acetate is synthesized from acetyl-CoA and isoamyl alcohol via catalysis by alcohol acetyl transferase (AATase) in yeast cells (Fig. 1). The formation of isoamyl acetate is influenced by various factors, such as wort composition including unsaturated fatty acids, amount of precursors such as amino acids, and fermentation conditions such as aeration rate. To elucidate the effect of the following factors on isoamyl acetate production during mashing and fermentation, we performed 2-L scale brewing trials. The effects of ① substrate (of precursor) concentration, ② fermentation temperature, and ③ aeration rate were investigated. Only the effect of leucine as substrate of isoamyl alcohol was investigated, as valine blocks the uptake of leucine.

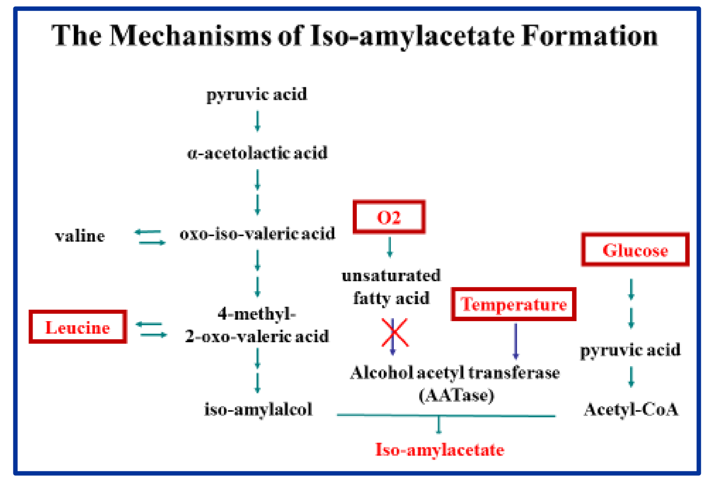


Fig.1 The mechanism of isoamyl acetate formation

Reference
Kodama Y, Omura F, Miyajima K, and Ashikari T (2001) Control of higher alcohol productions by manipulation of BAP2 gene in brewing yeast. Journal of American Society of Brewing Chemists 59(4):157-162

<Brewing condition>
Size：2L scale
yeast：top fermenting yeast
INSY：10×10⁶ cells/ml
Others：shown in Table 1

Table1. Brewing condition (No.1~14)

Brew No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14
fermentation condition	temperature(℃)	21					19			17			21		
	DO(ppm)	16											12		
the concentration in the wort	leucine	L	M	M	H	H	M	M	H	M	M	H	M	M	H
	glucose	L	M	M	H	H	M	M	H	M	M	H	M	M	H

L :low
M :medium
H :high

For each experiment, the original extract of wort was 16.5 w/w% and the concentration of FAN (free amino acids) was adjusted to 30 mg/100mL. The concentrations of glucose and leucine were set up according to Table 1. In order to make the same original extract and FAN level in each trial, maltose and arginine were used, respectively.

Results & Discussion <Step1>

Figure 2 and Table 2 show the concentration of isoamyl acetate in beer. The concentration of isoamyl acetate brewed in each trial was different. To elucidate the mechanisms of isoamyl acetate formation, we calculated the effect of each factor using PLS regression analysis (Table 2). The measured value of the concentration of isoamyl acetate brewed in each trial corresponded approximately to the expectancy by PLS regression analysis (r^2=0.823287).

Results of PLS regression analysis suggested that the substrate concentration in wort such as leucine and glucose is more effective to increase isoamyl acetate than fermentation conditions in the investigated conditions (Figure 3). It has been reported that the addition of glucose to the medium causes ①an increase of acetyl-CoA pool, and ②an induction of the expression of the AATase gene ATF1 (not in case of addition of maltose). It has been also reported that the addition of leucine to the medium causes ①an increase of formation of isoamyl alcohol, and ②activate the expression of ATF genes coding AATase.

Based on the results obtained by 2L scale test, we designed the brewing conditions to produce beer with higher concentration of isoamyl acetate at larger scale, and evaluated the effectiveness of various factors (Table 3).

In order to increase the concentration of glucose in wort, we investigated the effect of the saccharification temperature and the maltose degradation rest (45℃) after maltose generation. In order to increase the concentration of leucine in wort, we investigated the effect of peptidase rest.

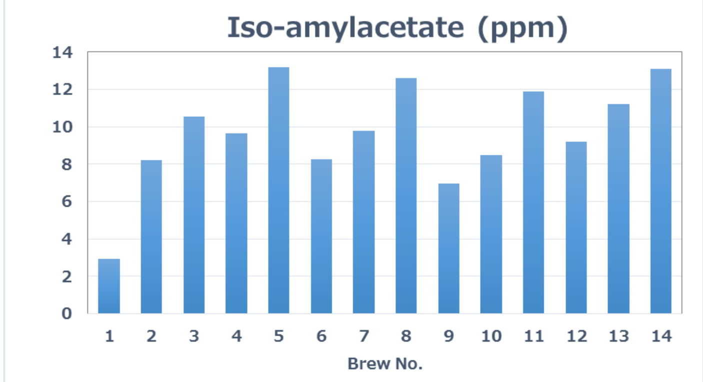


Fig 2.
The concentration of isoamyl acetate in each brewed beer

Table 2. Calculation the effect of each factor by using PLS regression analysis

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
y dependent variable	iso-amylacetate (ppm)	2.92	8.21	10.57	9.66	13.20	8.25	9.79	12.63	6.98	8.49	11.91	9.18	11.20	13.12
x explanatory variable	fermentation temperature (℃)	21	21	21	21	21	19	19	19	17	17	17	21	21	21
	DO (ppm)	16	16	16	16	16	16	16	16	16	16	16	12	12	12
	leucine(w/v%)	0.017	0.021	0.022	0.030	0.030	0.021	0.022	0.030	0.021	0.022	0.030	0.021	0.022	0.030
	glucose(w/v%)	1.29	1.55	4.57	1.60	4.65	1.55	4.57	4.65	1.55	4.57	4.65	1.55	4.57	4.65

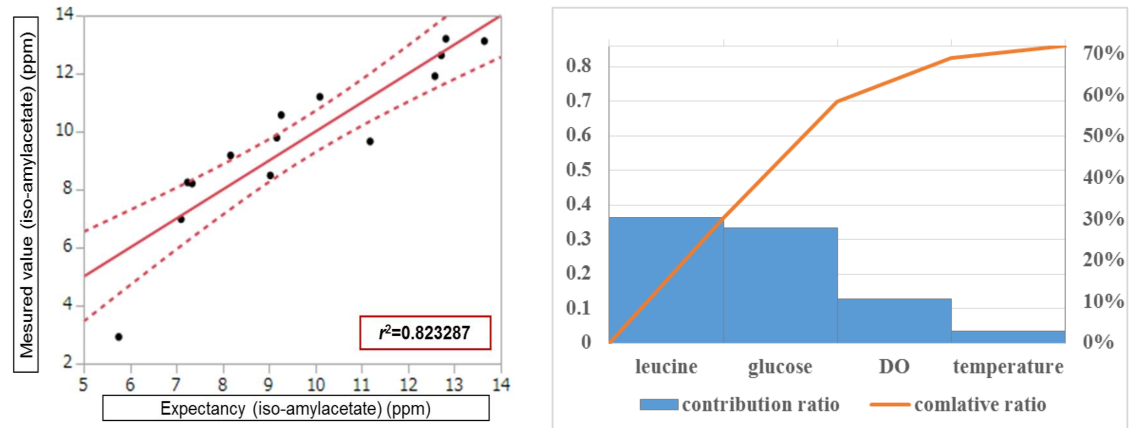


Fig 3. Results of PLS regression analysis (left: predictive model; right: contribution ratio of each factor)

Table 3. Strategy for increasing the concentration of glucose or leucine in the wort

Objective	Strategy: Mashing porocedure rest	Temperature	Trial	
The concentration of glucose in the wort ↑	The saccharification rest	63℃	Pilot-scale trial I	Microbrewery trial
	The maltose degradation rest after maltose generation	45℃	Pilot-scale trial II	
The concentration of leucine in the wort ↑	The peptidase rest	45℃		

Materials and Methods <Step2>

Pilot-scale trial I Saccharification temperature (Fig. 5)

In order to investigate the effect of the saccharification temperature on glucose and maltose formation, brewing trials were performed with two different temperatures of 63°C and the 65°C.

Test 1 (Blue Line)
A decoction mashing procedure, in which the saccharification temperature was 65°C.

Test 2 (Red Line)
A decoction mashing procedure, in which the saccharification temperature was 63°C.

<Brewing condition>
Brewing scale: 100-liter pilot brew
Malt : wheat malt 50%, barley malt 50%

<Analysis>
-wort : composition of sugar compounds

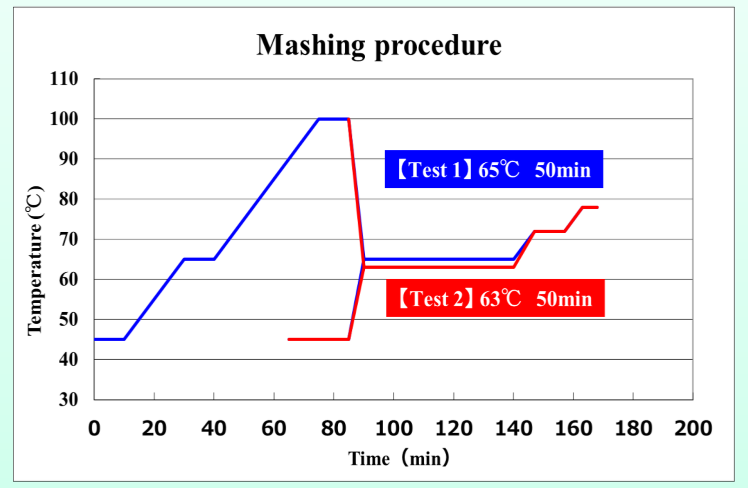


Fig 5. Mashing procedure for evaluation of the effect of saccharification temperature

Pilot-scale trial II Maltose degradation rest (Fig. 6)

We investigated the effect of the maltose degradation rest on glucose production.

Test 3 (Blue Line)
A decoction mashing procedure where there is no maltose degradation rest.

Test 4 (Red Line)
A decoction mashing procedure where there is a maltose degradation rest.

<Brewing condition>
Brewing scale: 100-liter pilot brew
Malt : wheat malt 50%, barley malt 50%

<Analysis>
-wort : composition of sugar compounds

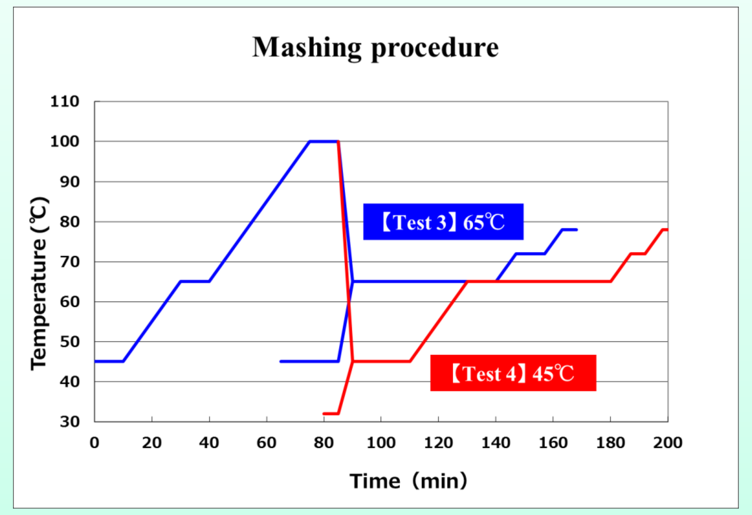


Fig. 6 Mashing procedure for evaluation of the effect of the maltose degradation rest

Microbrewery trial Best Brewing condition (Fig.7)

In previous trials, the effectiveness of the saccharification temperature of 63℃ and the maltose degradation rest to increase glucose in wort were confirmed. Then we investigated the effects of the saccharification temperature and maltose degradation rest on beer characteristics by 50-hL scale brew.

Test 5 (Blue Line)
A decoction mashing procedure, in which the saccharification temperature was 65℃ and there was no maltose degradation rest.

Test 6 (Red Line)
A decoction mashing procedure, in which the saccharification temperature was 63℃ and there was a maltose degradation rest.

<Brewing condition>
・Brewing scale : 50 HL scale
・Malts : wheat malt 50%, barley malt 50%

<Analysis>
wort : composition of sugar compounds
composition of amino acid compounds
beer : sensory evaluation
isoamyl acetate

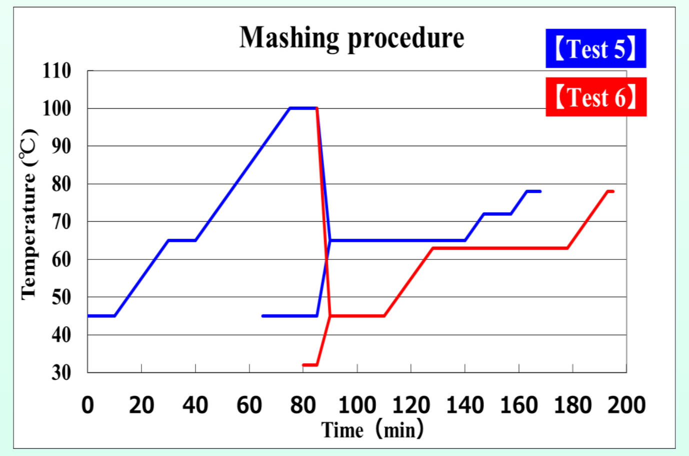


Fig. 7 Mashing procedure for evaluation of the effects of both saccharification temperature and degradation rest on beer characteristics by 50-hL scale brew

Results & Discussion <Step2>

【Pilot-scale trial】

I, Saccharification temperature

First, the effect of the saccharification temperature is shown in Figure 8. The concentration of glucose in wort of test 2 (63℃) was higher than that in the wort of test 1 (65℃) (Fig. 8). Therefore, we found this condition important to control the saccharification temperature properly during mashing in order to produce glucose in wort.

II, Maltose degradation rest

Second, the effect of the maltose degradation rest is shown in Figure 9.The concentration of glucose in wort of test 4 (the mashing procedure with maltose degradation rest) was higher than that in the wort of test 3 (without rest) (Fig. 9). Therefore, to increase the concentration of the glucose in wort, we found this condition important to set the maltose degradation rest after generating maltose.

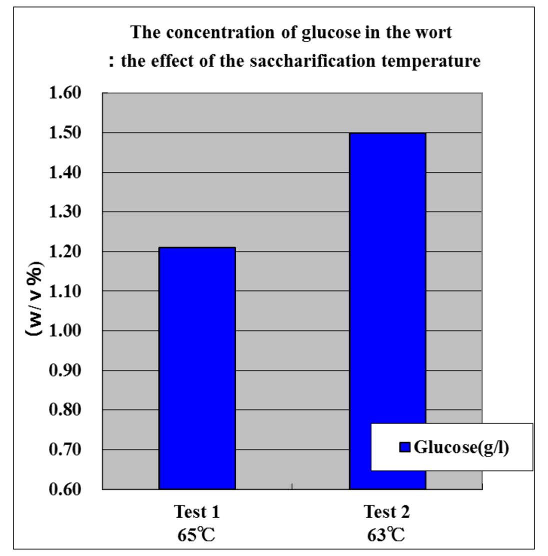


Fig. 8 The concentration of glucose in the wort: the effect of the saccharification temperature

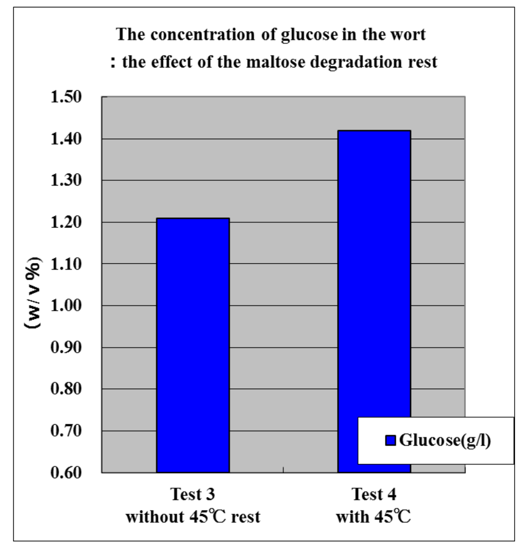
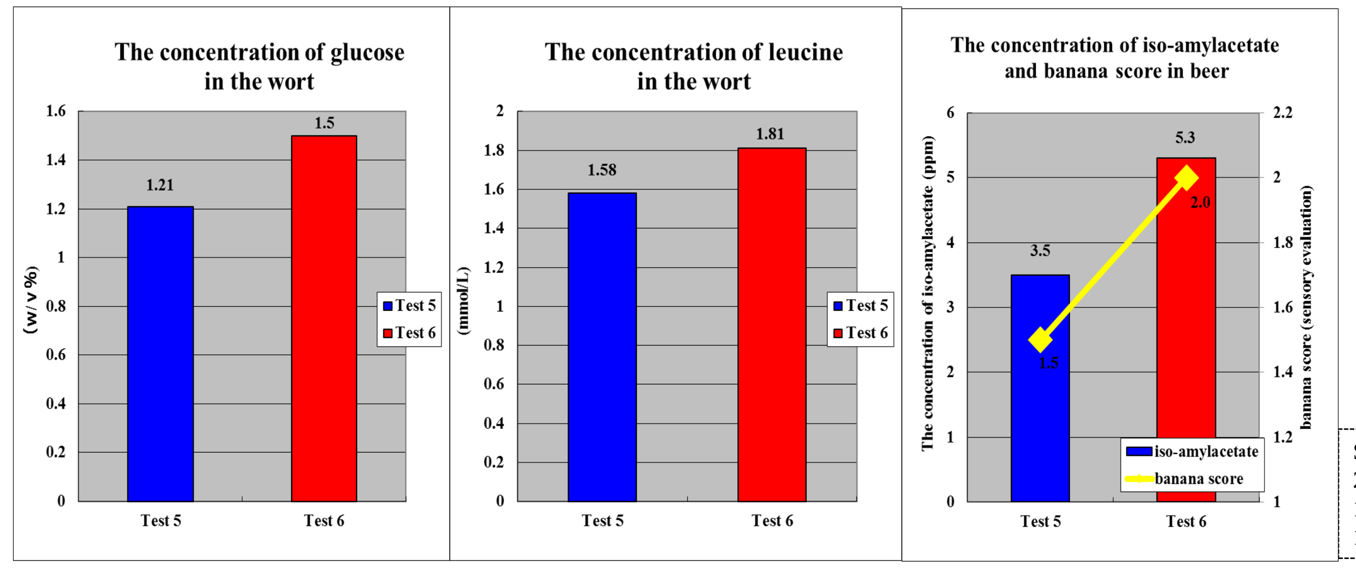


Fig. 9 The concentration of glucose in the wort: the effect of the maltose degradation rest

【Microbrewery trial】 Optimal brewing condition

Finally, the effects of both the saccharification temperature and the maltose degradation rest (peptidase rest) are shown in Figure 10. Not only the concentration of glucose but also the concentration of leucine in wort of test 6 was higher than that in the wort of test 5 (Fig. 10). As a result, the concentration of isoamyl acetate in beer of test 6 was higher and achieved an overwhelming "banana-like, sweet, and fruity aroma"



Sensory evaluation score
2.0 : high
1.5 : medium
1.0 : low

Fig.10 The results of trail 5 and 6 (Microbrewery trial)

Conclusion

Step1

The effects of various factors on isoamyl acetate production during mashing and fermentation were investigated by a 2-L scale trials. To elucidate the mechanisms of isoamyl acetate formation, we calculated the effect of each factor by using PLS regression analysis. Results of PLS regression analysis suggested that the concentration of substrates such as leucine and glucose in wort was more effective in increasing isoamyl acetate production.

Step2

Based on the results obtained from 2-L scale trials, we designed the brewing conditions to produce beer with a higher concentration of isoamyl acetate. As a result, a decoction mashing procedure, in which the saccharification temperature was 63°C and where there was a maltose degradation rest (peptidase rest), increased the concentration of glucose and leucine in the wort. Therefore, this is conducive to the formation of isoamyl acetate in the top fermentation yeast. It was found that the overwhelming "banana-like, sweet, and fruity aroma" can be achieved by controlling these brewing conditions.