Change of the Alpha Amino Nitrogen of Tartary Buckwheat Beer during the Process of Mashing and Its Process Optimization

Jie Chen [1, a], Tingjun Ma[1,b *],Ying Liu[1, c], and Aijing Zhang [2, d]
[1]College of Food Science and Engineering, Beijing University of Agriculture, Beijing, China
[2]University of Science and Technology Beijing, Beijing, China
Email: [a]bbzcj@163.com, [b]tingjunma@hotmail.com,[c]15210926995@163.com, [d]zhangaijing02@sina.com

***Abstract*—To analysis the effect of 4 diastatic technical parameters (the ratio of water and materials, pH, the diastatic temperature of stage Ⅰ and stage Ⅱ) on α-amino nitrogen content of wort, the response surface method is applied and the mathematical model of the four parameters above influencing α-amino nitrogen content was established. The results showed that the optimum technical conditions were as follows: the ratio of water to raw materials was 4:1, the diastatic temperature of stage Ⅰ was 62℃ and the diastatic temperature of stage Ⅱ was 72℃, the pH was 5.35. Under the optimum technical conditions condition, the α-amino nitrogen content of wort in beer was 188.2 mg/L.**

***Index Terms*—tartary buckwheat beer, mashing, α-amino nitrogen, response surface method.**

I. INTRODUCTION

Tartary buckwheat (*Fagopyrum tataricum*) is grown and used in the mountainous regions of southwestern China, northern India and Nepal. In recent years, Tartary buckwheat grain has been important functional food, as it contains proteins with high nutritional value including a balanced amino acid composition and relatively high crude fiber and vitamin B_1, B_2, and B_6 contents [1]. Furthermore, they are found that Tartary buckwheat is a rich source of flavonoids, including quercetin and its glycoside, rutin in its flowers, leaves, stems, and seeds.

An alcoholic beverage that almost certainly predates wine is beer, although in this case there is no attractive legend to account for its discovery. Tartary buckwheat beer is a new kind of beer, which is produced mainly from tartary buckwheat.

The α-amino nitrogen is one of the important index of beer standard. The high content of malt soluble protein and alpha amino nitrogen can provide persistent foam and unique flavor [2]. Therefore in order to improve taste of beer to optimize the content of α-amino nitrogen. This study focuses on optimization of a mashing program by measuring the content of α-amino nitrogen in tartary buckwheat wort by ninhydrin method on different conditions. the buckwheat beer presenting yellow-green has abundant white foam and refreshing taste.

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II. MATERIALS AND METHODS

A. Materials

Tartary buckwheat samples were provided by Shan Xi academy of agricultural sciences. Ninhydrin, fructose, glycin, disodium hydrogen phosphate, potassium dihydrogen phosphate, potassium iodate, pure ethanol were purchased from Beijing Chemical Factory.

B. Method

Mashing

Brewing buckwheat beer of 10 ° P apply infusion mashing. The influence of mashing temperature is important. So we should keep the optimal temperature on resting phase. The infusion mashing have two resting phase: the temperature of the firstly resting phase on mashing (the diastatic temperature of stage I) and the temperature of the second resting phase on mashing (the diastatic temperature of stage II).

Measure α-amino nitrogen by the colorimetric ninhydrin reaction [3].

The following reagents are require

Chromogenic agent

Dissolve 10 g disodium hydrogen phosphate, 6 g potassium dihydrogen phosphate, 0.5 g ninhydrin reagent and 0.3 g fructose in 100 mL water.

Diluent

Dissolve 2 mg potassium iodate in 100 mL water and then add 400 mL 96% ethanol.

Stock standard solution

Dissolve 107.2 mg glycine in 100 mL water. Store at 0 ℃. Per liter of solution contains 200 mg α-amino nitrogen.

Preparation wort diluents

The 1.00 ml wort was added into 100 mL volumetric flask and diluted to the scale, shake well.

Standard glycine diluent

Diluted 1.00 mL of standard solution to 100 mL volumetric flask to the scale.

Procedure

Add malt dilute solution, water and standard of glycine diluent 2.00 mL in the colorimetric tube, respectively (water for ck). Mix 1.00 mL chromogenic agent into

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colorimetric tube, then shake it well and heated in boiling water for 16 min,respectively. Cool in 20 ℃ water bath after 20 min. Then add 5.00 mL potassium iodate diluted solution. The mixture was shaken vigorously and within 30min to measure its absorbance at 517 nm. The α-amino nitrogen content was calculated by using (1)

$$\alpha\text{-amino nitrogen} = \frac{\text{The Average Absorbance of Sample}}{\text{The Average Absorbance of Standard Glycine solution}} \times 2 \times n \quad (1)$$

n: dilution multiple

C. Experimental Design and Statistical Analysis.

The Box - Behnken experimental design [4].

TABLE I LEVEL CODING FACTOR

standard	Index			
	(X_1) the ratio of water to raw materials /(mL/g)	(X_2) diastatic temperature of stage Ⅰ /℃	(X_3) diastatic temperature of stage Ⅱ /℃	(X_4) Mash pH
1	3:1	60	70	5.1
2	4:1	62	72	5.25
3	5:1	64	74	5.4

With single factor experiment method, where four factor(the ratio of water and materials, pH of mash ,the diastatic temperature of stage I and stage II) and three corresponding optimum level of each factor is selected, Box-Behnken experiment has been carried out in this paper(Table I). Optimum diastatic technical conditions is determined by α-amino nitrogen content.

III. RESULT AND ANALYSIS

A. Variance Analysis

Box-Behnken Results is shown in Table II. As show in Table III, the F of the predicted model achieved by experimental data is 17.67 and the P of it is less than 0.0001, indicating that the model has a high level of significance. The degree of lack of fit is 0.3569 and bigger than 0.1, indicating that the model's reliability is high. The regression models fitted by experiment date are predicted as follows (2):

Y=-2.69×106+7439.4X1+18788.2X2+70033.6X3

+3.2×105X4+1.6X1X3-2876.9X1X4-521.3X2X3

+84.7X2X4-7092.3X3X4-1.3X12-3.5X22-486.7X32

-7118.5X42+275.7X1X42+3.61X2X32+49.3X32X4 (2)

R^2=0.9593, the coefficients of the regression models, is similar to R^2adj = 0.9050, the coefficient of determination, indicating that the model was adequate with no significant lack of fit and could predict the experiment results. Sequence of the factors affecting α-amino nitrogen content is the diastatic temperature of stage I>the ratio of water to raw materials> pH of mash > and the diastatic temperature of stage II.

TABLE II BOX-BEHNKEN TEST DESIGN VARIABLE VALUE AND RESULTS

X_1	X_2	X_3	X_4	α-amino nitrogen (mg L^{-1})
-1	-1	0	0	159.3
1	-1	0	0	155.2
-1	1	0	0	148.1
1	1	0	0	119.5
0	0	-1	-1	122.7
0	0	1	-1	103.3
0	0	-1	1	171.7
0	0	1	1	169.8
-1	0	0	-1	101.9
1	0	0	-1	123.7
-1	0	0	1	76.5
1	0	0	1	153.4
0	-1	-1	0	136.9
0	1	-1	0	175.2
0	-1	1	0	140.2
0	1	1	0	164.2
-1	0	-1	0	156.5
1	0	-1	0	115.5
-1	0	1	0	135.5
1	0	1	0	157.9
0	-1	0	-1	180.3
0	1	0	-1	99.6
0	-1	0	1	124.5
0	1	0	1	145.4
0	0	0	0	193.4
0	0	0	0	193.4
0	0	0	0	193.4
0	0	0	0	178.4
0	0	0	0	178.4

TABLE III VARIANCE ANALYSIS FOR REGRESSION EQUATIONS

source of variation	sum of deviation square	df	Mean square	F	P
model	25954.85	16	1622.178	17.67273	<0.0001
X_1	323.4696	1	323.4696	3.524022	0.0850
X_2	1422.444	1	1422.444	15.49674	0.0020
X_3	5.214008	1	5.214008	0.056804	0.8156
X_4	4.197753	1	4.197753	0.045732	0.8343
X_1X_3	1019.525	1	1019.525	11.10716	0.0060
X_1X_4	759.278	1	759.278	8.271913	0.0139
X_2X_3	50.5521	1	50.5521	0.550737	0.4723
X_2X_4	2580.894	1	2580.894	28.11741	0.0002
X_3X_4	76.12563	1	76.12563	0.829347	0.3804
X_1^2	6808.466	1	6808.466	74.17446	<0.0001
X_2^2	1300.347	1	1300.347	14.16656	0.0027
X_3^2	1290.379	1	1290.379	14.05796	0.0028
X_4^2	8445.06	1	8445.06	92.00425	<0.0001
$X_1X_4^2$	2566.181	1	2566.181	27.95712	0.0002
$X_2X_3^2$	2229.443	1	2229.443	24.28854	0.0003
$X_3^2X_4$	2338.302	1	2338.302	25.4745	0.0003
residual error	1101.479	12	91.78989		
lack of fit	831.4787	8	103.9348	1.539775	0.3569
pure error	270	4	67.5		
total error	27056.33	28			
R^2	0.9593				
R^2_{adj}	0.9050				

*P<0.05; **P<0.01; ***P<0.001

B. Analysis of Response Surface

Fig. 1 to Fig. 5 shows the Analysis of Response Surface of quadratic regression equation fitted from data of Table II by Design Expert.

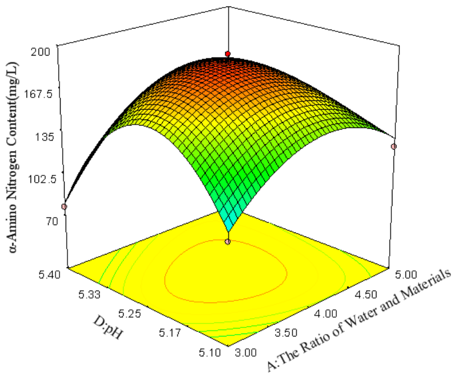

Figure 1. Response surface graphs for the effects of interacting between the ratio of water and materials and the pH of the mash on α-amino nitrogen.

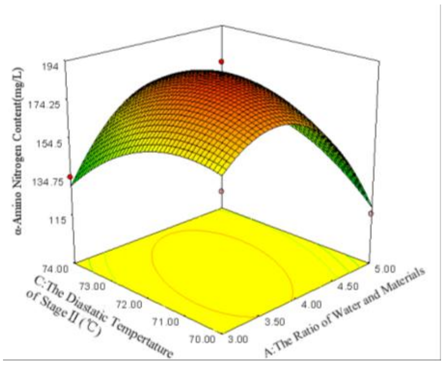

Figure 2. Response surface graphs for the effects of interacting between the ratio of water and materials and the diastatic temperature of stage II on α-amino nitrogen.

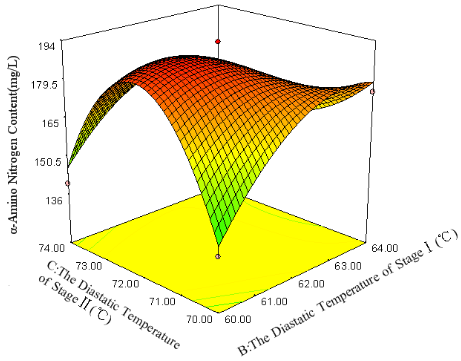

Figure 3. Response surface graphs for the effects of interacting between the diastatic temperature of stage I and the diastatic temperature of stage II on α-amino nitrogen.

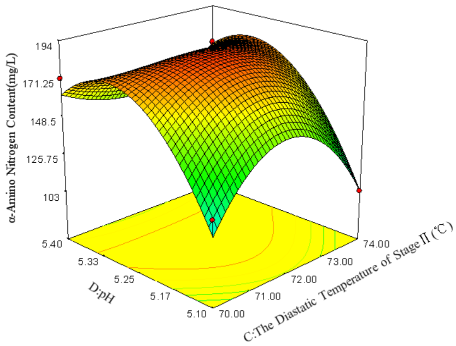

Figure 4. Response surface graphs for the effects of interacting between the diastatic temperature of stage II and the pH on α-amino nitrogen

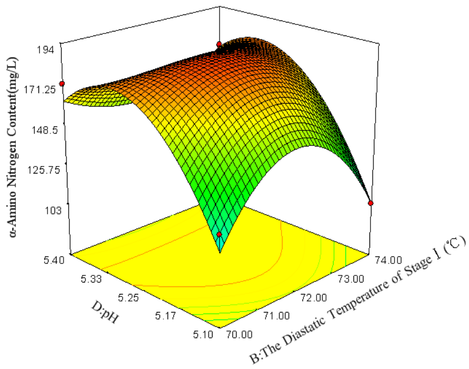

Figure 5. Response surface graphs for the effects of interacting between the diastatic temperature of stage I and the pH on α-amino nitrogen.

Fig. 1 shows that holding the ratio of water to raw materials as the constant, α-amino nitrogen content increases with pH of mash increased. While the pH is greater than 5.25, α-amino nitrogen content declines. Fig. 2 indicates that holding the ratio of water and materials as the constant, α-amino nitrogen content declines with the diastatic temperature of stage II increased, however consistent with the analysis of variance, the change is not obvious ,indicating that the diastatic temperature of stage II has little impact on the diastatic temperature of stage II. While holding the diastatic temperature of stage II as the constant, α-amino nitrogen content increases with the ratio of water and materials. When the ratio of water and materials is greater than 4:1, α-amino nitrogen content declines. Fig. 3 shows that holding the diastatic temperature of stageas II as the constant, α-amino nitrogen content increases with the diastatic temperature of stages I. The α-amino nitrogen content gets maximum when the diastatic temperature of stages I reaches 62℃ and remains unchanged when the diastatic temperature of stages I is greater than 62℃. Holding the diastatic temperature of stages I as the constant, α-amino nitrogen content increases with the diastatic temperature of stages II increased. While the diastatic temperature of stages II is greater than 72℃, α-amino nitrogen content declines. Fig. 4 shows that holding the diastatic temperature of stages II as the constant, α-amino nitrogen content increases with pH of mash increased. While the pH is greater than 5.25, α-amino nitrogen content declines slowly. Holding the pH of mash as the constant, α-amino nitrogen content increases with the diastatic temperature of stages II increased. While the diastatic temperature of stages II is greater than 72℃, α-amino nitrogen content declines. Fig. 5 shows that holding the pH of mash as the constant, α-amino nitrogen content increases with the diastatic temperature of stages I increased. While the pH is greater than 5.25, α-amino nitrogen content declines. Holding the diastatic temperature of stages I as the constant, α-amino nitrogen content increases with the pH of mash increased. While the pH is greater than 5.25, α-amino nitrogen content declines.

C. Verify Experiment

Taking into account the feasibility of the actual operation, the optimum technical parameters of predicted model is corrected as: the ratio of water and materials was 4:1, the diastatic temperature of stage I was 62℃ and the diastatic temperature of stage II was 72℃, the pH was 5.35, on such experiment conditions, α-amino nitrogen content is 188.2mg/L.

D. Discuss

Conclusion α-amino nitrogen an important technical index in beer brewing process, reflects the degree of decomposition of protein of malt and shows the protease activity of the malt. Also, as an important parameter to preview the reduction degree of diacetyl fermentation, α-AN is used to be the only source of synthesis of various enzymes. It is α-amino nitrogen's low content and the slow fermentation speed and large amount of metabolic byproducts which makes beer's bad flavor. In addition, wort with low α-AN content is likely to lack valine, causing high peak of diacetyl in fermentation broth and making low fermentation speed and high content of diacetyl in beer. These changes are adverse to the quality of beer [5].

E. Conclusion

The results showed that the optimum technical conditions were as follows: the ratio of water and materials was 4:1, the diastatic temperature of stage I was 62℃ and the diastatic temperature of stage II was 72℃, the pH was 5.35. Under the optimum technical conditions condition, the α-amino nitrogen content of wort in 10° P buckwheat beer was 188.2 mg/L [6].

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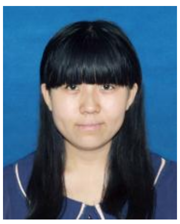

Chen Jie is 23 years old, born in Beijing China. I am a graduate student, studying in Beijing university of agriculture now. My research direction is mainly functional foods and natural products.

She was graduated from Beijing university of agriculture. Her major is Food science and engineering and she got her bachelor degree after her graduation in the year of 2003.