

Optimization of a Fast Condiment Production Process Using the Distillers' Grains of Shaoxing Rice Wine

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Abstract:

The main components of the distillers' grains of Shaoxing rice wine were determined. A fast production process of a condiment, rice wine sauce, was then established using the distillers' grains. The process mainly depends on the flavourzyme's hydrolysis, supplemented with acidolysis pretreatment. Orthogonal experiments were conducted to optimize such steps as the pre-fermentation, acidolysis pretreatment, enzymolysis, decolorization/debittering, rice wine sauce formula. Enzymolysis, the most important step, was optimized as 'flavourzyme 4%+ enzymolysis temperature 55°C+ enzymolysis time 6h'. The influence capability of the 3 factors is enzyme dosage, reaction time and reaction temperature in descending order. Sensory evaluation showed that the condiment got similar scores with that made using traditional method.

Keywords: Shaoxing rice wine, Distillers' grains, Flavor, Enzymatic hydrolysis, Condiment.

I. INTRODUCTION

Chinese Rice Wine (Huangjiu) is one of the three oldest wines in the world. Distillers' grains, the waste produced in rice wine brewing, are rich in residual nutrients and therefore are valuable for resource development. According to statistics, there are more than 900 QS certified rice wine factories in China currently. The annual output of rice wine in China was 3,348 thousand kiloliter in 2018. According to the density of rice wine (about 1.0), rice wine yield ratio (about 190%) and distillers' grains yield ratio (20%-30%), the current annual output of distillers' grains in China is estimated to be 352,400 to 528,630 tons. The distillers' grains of Chinese rice wine are rich in starch, protein, fat and cellulose, among which the protein content commonly reaches 29.58-41.29% (dry base) [1, 2, 3]. Although the prospect of utilization of distillers' grains is attractive, most factories are paying little attention to the deep processing of it currently. In most cases, rice wine distillers' grains are only treated as feed for animals or as waste. The abandonment or poor utilization of distillers' grains not only wastes resources, but also brings tremendous amount of solid waste pollutants.

Because of its unique flavor, Chinese rice wine is popular, especially in East Asia. As the by-products of fermentation, the distillers' grains contain also a lot of flavor substances. For example, flavor amino

acids, esters, acids, alcohols and so on. In addition, starch can hydrolyze to produce dextrin and glucose, which present flavor too [4, 5, 6]. In China, rice wine distillers' grains have been used for pickling food for many years [7]. At present, many factories also produce commercialized rice wine sauce as condiment. However, the production process of them is time consuming (usually six months to a year of natural fermentation) and space-consuming (to be stored separately in the jars). The reaction is susceptible to environmental impact (temperature, humidity, etc.). Therefore, it is necessary to simplify the process, shorten the time and unify the reaction conditions. This study intends to establish a rapid production process of aromatic marinade based on enzymatic hydrolysis. The production process is expected to be efficient, low cost and easy to popularize.

II. METHODS

2.1. Main Components Analysis of the Distillers' Grains

The content of water, ash, protein, starch and fat in the distillers' grains of Shaoxing rice wine was detected according to national standard of China *GB/T 5009.3-2016*, *GB/T 5009.4-2016*, *GB/T 5009.5-2016*, *GB/T 5009.9-2016* and *GB/T 5009.6-2016*, respectively. Total esters were detected using saponification method. Total acid was determined by titration [8].

2.2 Preparation Process of Rice Wine Sauce

Preparation process of rice wine sauce using distillers' grains could be described in order as pre-fermentation (Step 1), acidolysis pretreatment (Step 2), enzymatic hydrolysis (Step 3), debittering/decolorization (Step 4), Spice preparation (Step 5), preparation of rice wine sauce (step 6) and evaluation of rice wine sauce (Step 7). Five independent orthogonal experiments were then designed for optimization of Step 1, Step 2, Step 3, step4 and Step 6, respectively. As to Step 5, *Syzygium aromaticum*, *Foeniculum vulgare*, *Zanthoxylum bungeanum*, *pericarpium citri reticulatae*, and *Cinnamomum cassia* were crashed and mixed according to a ratio of 1.5/3/6/3/6 for 2 h of extraction at 80 °C. [4]

2.3. Optimization of Pre-Fermentation of Distillers' Grains

For pre-fermentation, an orthogonal experiment of was designed as follow: koji content(A) of 0.5%, 1% and 5%, fermentation time(B) of 2D, 4D and 6D, and loading capacity(C) of 70%, 85% and 100%. Distillers' grains and koji were put into 250 mL-conical bottle for fermentation at 25°C. Finally, the starch residue was detected according to the A_{620} value after iodine reaction to optimize the fermentation reaction.

2.4. Optimization of Acidolysis Pretreatment of Distillers' Grains

For acidolysis pretreatment, an orthogonal experiment of was designed as follow: HCl concentration(A) of 1.5% 10%, and 15%, acidolysis time(B) of 1.5h, 3h and 6h, and feed/liquid ratio(C) of 1/8, 1/4 and 1/2. The reaction system was 10 g distillers' grains with 50 mL HCl at 100°C. Finally, the protein residue was

detected using Coomassie brilliant blue method for optimization.

2.5. Optimization of Protease Hydrolysis Reaction

For enzymatic hydrolysis, an orthogonal experiment of was designed as follow: flavourzyme dosage(A) of 1%, 2% and 4%, enzymolysis time(B) of 2h, 6h and 18h, and enzymolysis temperature(C) of 45°C, 55°C and 65°C. Finally, the protein residue was detected using Coomassie brilliant blue method for optimization.

2.6. Optimization of Debittering/Decolorization of Enzymatic Hydrolysate

Activated carbon adsorption method was used to for debittering and decolorization. An orthogonal experiment of was designed as follow: Activated carbon content(A) of 0.5%, 1% and 5%, reaction time(B) of 20 h, 40 h and 60 h, and reaction temperature(C) of 30°C, 45°C and 60°C. Finally, transparency was evaluated for optimization.

2.7. Formula Optimization for Rice Wine Sauce Preparation

Rice wine sauce was prepared by adding 5% rice wine and other 3 additives into the enzymatic hydrolysate above. An orthogonal experiment was designed for optimization of the 3 additives: spice extractive content(B) of 1%, 2% and 3%, aginomoto content(C) of 1%, 2% and 3%, sucrose content of 1%, 2% and 3%. Finally, aroma, sweetness, freshness and aftertaste were scored respectively and were then summed for total scores.

2.8. Trial Production and Evaluation of Rice Wine Sauce

The self-made rice wine sauce was compared with the commercial products (made by traditional preparation methods). Comparing was based on the scores of color, aroma, taste and viscosity.

2.9 Statistical Analysis

For each experimental group, 3 replicates were conducted. Duncan multiple comparison method was for statistical analysis with SPASS (version 20.0). The visual tables were analyzed with Orthogonal Design Assistant II (version 3.1).

III. RESULTS

3.1. Main Components of the Distillers' Grains of Shaoxing Rice Wine

As shown in Fig. 1, the main components of the distillers' grains of Shaoxing rice wine are 48.55% moisture, 33.03% starch, 14.3% protein, 1.51% fat. The ash, esters and acids are all less than 1%. Among

these ingredients, starch, protein, esters and acids, or their degradation products, can all present various flavors.

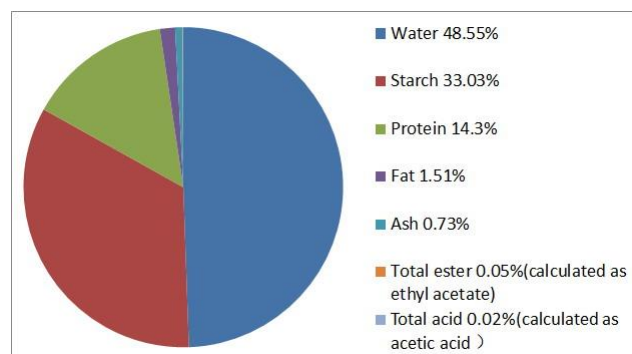


Fig. 1. The main components in the distillers' grains of Shaoxing rice wine

3.2. Pre-fermentation of the Distillers' Grains

As shown in TABLE I, the lowest starch residue (0.328) occurred to group $A_1B_3C_3$. Range analysis showed that $A_1B_3C_1$ is the best pre-fermentation condition of distillers' grains, and the influence capability is $B>A>C$. Variance Analysis showed that koji content(A) and fermentation time(B) affect starch residue significantly. Variance analysis shows that the influence capability is $B>A>C$ too, which is consistent with range analysis. Duncan analysis found no significant difference between level 1 and level 3 of loading volume(C). Considering production efficiency, the optimal condition is $A_1B_3C_3$ (koji content 0.5%+fermentation time 6d + loading volume 100%).

TABLE I. Visual analysis of pre-fermentation optimization orthogonal experiment

	level combination	A(koji)	B(fermentation time)	C(loading volume)	starch residue(A580)
1	$A_1B_1C_1$	1	1	1	1.195
2	$A_1B_2C_2$	1	2	2	0.567
3	$A_1B_3C_3$	1	3	3	0.328
4	$A_2B_1C_2$	2	1	2	1.878
5	$A_2B_2C_3$	2	2	3	0.690
6	$A_2B_3C_1$	2	3	1	0.582
7	$A_3B_1C_3$	3	1	3	1.828
8	$A_3B_2C_1$	3	2	1	0.803
9	$A_3B_3C_2$	3	3	2	0.414
K1		0.697	1.634	0.860	

K2	1.050	0.687	0.953	
K3	1.015	0.441	0.949	
R	0.353	1.193	0.093	
optimum	A1	B3	C1	influence: B>A>C

3.3. Acidolysis Pretreatment of Distillers' Grains

As shown in TABLE II, the lowest protein residue (0.430) occurred to group A₃B₂C₁. Range analysis showed that A₃B₃C₁ is the best acidolysis pretreatment condition for distiller's grains, and the influence capability is A>C>B. Variance analysis confirmed that HCl concentration (A) and solid-liquid ratio(C) affect protein residue significantly. Variance analysis shows that the influence capability is A>C>B, which is consistent with range analysis. Duncan analysis found no significant difference between level 2 and level 3 of acidolysis time (B). Considering the time cost and the protection of certain amino acids, the optimal condition is A₃B₂C₁ (HCl concentration 15% + acidolysis time 3h + feed/liquid ratio 1/8).

TABLE II. Visual analysis of acidolysis pretreatment optimization orthogonal experiment

	level combination	A(HCl concentration)	B(acidolysis time)	C(feed/liquid ratio)	Protein residue(A595)
1	A1B1C1	1	1	1	0.681
2	A ₁ B ₁ C ₁	1	2	2	0.786
3	A ₁ B ₂ C ₂	1	3	3	0.712
4	A ₁ B ₃ C ₃	2	1	2	0.740
5	A ₂ B ₁ C ₂	2	2	3	0.763
6	A ₂ B ₂ C ₃	2	3	1	0.490
7	A ₂ B ₃ C ₁	3	1	3	0.493
8	A ₃ B ₁ C ₃	3	2	1	0.430
9	A ₃ B ₂ C ₁	3	3	2	0.557
K1	A ₃ B ₃ C ₂	0.726	0.638	0.534	
K2		0.664	0.660	0.694	
K3		0.493	0.586	0.656	
R		0.233	0.074	0.160	
optimum		A3	B3	C1	Influence: A>C>B

3.4. Enzymatic Hydrolysis with Flavor Protease

As shown in TABLE III, the lowest protein residue (0.399) occurred to A₃B₃C₂. Range analysis showed that A₃B₃C₂ was the best enzymolysis condition, and the influence capability is A>B>C. Variance analysis shows that flavourzyme dosage (A) and enzymolysis time (B) affect protein residue significantly. Variance analysis shows that the influence capability is A>B>C too, which is consistent with range analysis. Therefore, the optimal condition is A₃B₃C₂ (flavourzyme dosage 4% + enzymolysis time 18h +

enzymolysis temperature 55°C).

TABLE III. Visual analysis of enzymolysis optimization orthogonal experiment

	level combination	A(flavourzyme dosage)	B(enzymolysis time)	C(enzymolysis temperature)	Protein residue(A595)
1	A ₁ B ₁ C ₁	1	1	1	0.756
2	A ₁ B ₂ C ₂	1	2	2	0.613
3	A ₁ B ₃ C ₃	1	3	3	0.610
4	A ₂ B ₁ C ₂	2	1	2	0.600
5	A ₂ B ₂ C ₃	2	2	3	0.605
6	A ₂ B ₃ C ₁	2	3	1	0.483
7	A ₃ B ₁ C ₃	3	1	3	0.524
8	A ₃ B ₂ C ₁	3	2	1	0.435
9	A ₃ B ₃ C ₂	3	3	2	0.399
K1		0.660	0.627	0.558	
K2		0.563	0.551	0.537	
K3		0.453	0.497	0.580	
R		0.207	0.130	0.043	
optimum		A3	B3	C2	influence: A>B>C

3.5. Debittering and Decolorization of the Enzymatic Hydrolysate

As shown in TABLE IV, the highest clarity (chroma 3) occurred to A₃B₂C₁. Range analysis showed that A₃B₁C₂ and A₃B₂C₂ are both the best reaction condition, and the influence capability is A>C>B. Variance analysis shows that the activated carbon concentration (A) and reaction temperature (C) affects the clarity significantly. Variance analysis shows that the influence capability is A>C>B, which is consistent with range analysis. Duncan analysis shows that there is no significant difference between level 1 and level 2 of reaction temperature(C). Therefore, the optimal process is A₃B₂C₁ (activated carbon dosage 1.5% + reaction time 40h + reaction temperature 30°C).

TABLE IV. Visual analysis of decolorization/debittering optimization orthogonal experiment

	level combination	A(activated carbon content)	B(reaction time)	C(reaction temperature)	chroma
1	A ₁ B ₁ C ₁	1	1	1	4
2	A ₁ B ₂ C ₂	1	2	2	4
3	A ₁ B ₃ C ₃	1	3	3	5
4	A ₂ B ₁ C ₂	2	1	2	5

5	A ₂ B ₂ C ₃	2	2	3	7
6	A ₂ B ₃ C ₁	2	3	1	7
7	A ₃ B ₁ C ₃	3	1	3	5
8	A ₃ B ₂ C ₁	3	2	1	3
9	A ₃ B ₃ C ₂	3	3	2	4
K1		4.333	4.667	4.667	
K2		6.333	4.667	4.333	
K3		4.000	5.333	5.667	
R		2.333	0.666	1.334	
optimum		A3	B1 或 B2	C2	influence: A>C>B

3.6. Formula Optimization for Rice Wine Sauce Preparation

As shown in TABLE V, the highest score (34) occurred to A₃B₃C₂. Range analysis showed that A₃B₃C₂ is the best combination condition too, and the influence capability is A>B>C. Variance analysis shows that spice extractive(A) and aginomoto (B) affects the score significantly. Variance analysis shows that the influence capability is A>B>C, which is consistent with range analysis. Therefore, the optimal process is A₃B₃C₂ (spice extractive 3% + aginomoto 3% + sucrose 2%).

TABLE V. Visual analysis of formula optimization experiment

	level combination	A(spice extractive)	B(aginomoto)	C(sucrose)	Total scores
1	A ₁ B ₁ C ₁	1	1	1	22
2	A ₁ B ₂ C ₂	1	2	2	23
3	A ₁ B ₃ C ₃	1	3	3	25
4	A ₂ B ₁ C ₂	2	1	2	27
5	A ₂ B ₂ C ₃	2	2	3	29
6	A ₂ B ₃ C ₁	2	3	1	29
7	A ₃ B ₁ C ₃	3	1	3	28
8	A ₃ B ₂ C ₁	3	2	1	31
9	A ₃ B ₃ C ₂	3	3	2	34
K1		23.333	25.666	27.333	
K2		28.333	27.666	28.000	
K3		31.000	29.333	27.333	
R		7.667	3.667	0.667	
optimum		A3	B3	C2	influence: A>B>C

3.7. Trial Production and Evaluation of Rice Wine Sauce

As shown in Fig. 2, the rice wine sauce made using the above method and that using traditional method showed similar scores. Detailed compare showed that the former presented a bit darker color, lower freshness, lower aftertaste, higher fragrance and higher sweetness.

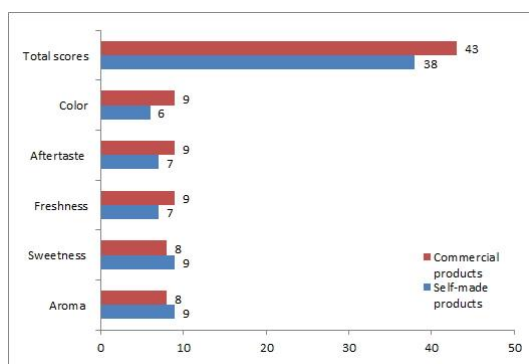


Fig.2. Sensory evaluation of self-made rice wine sauce and commercial products

IV. DISCUSSION

The brewing technology of rice wine varies greatly from region to region in China. In this study, the distillers' grains of Shaoxing rice wine are determined to contain mainly water, starch, protein and fat. Compared with that in other regions in China, distillers' grains of Shaoxing rice wine has significantly higher starch content (33.0% vs 11.0-14.0%). While the content of protein(14.3% vs. 12.4-15.2%), fat (1.5% vs. 2.3%) and ash (0.73% vs. 1.1%) showed little difference.[2] The reason of the high starch content for Shaoxing rice wine distillers' grains might be that the fermentation is conducted using not ripe wheat starch as other regions but raw wheat starch, which leads to poor starch utilization.[9] The flavor components in the distillers' grains include flavor amino acids(including degradable protein), carbohydrates(including degradable starch), esters, alcohols, acids, phenols, aldehydes, ketones, etc. According to a detection of the distillers' grains in Jiangsu, China, there are more than 40 esters and 20 alcohols. It should be noted, some of these flavor components are only found in Chinese rice wine related products and are potential valuable flavor resource for food industry [4, 10, 11]. Although there were a few studies on the utilization of Chinese rice wine distillers' grains, large-scale applications have not yet been realized. Currently, the distillers' grains are mostly treated as animal feed or culture medium for fungi or plant [12, 13]. Sometime, the distillers' grains are also used for making of starter or liquor, protein extracting, sugar extracting, etc. [14, 15, 16, 17] The another way is to produce condiments, as done in this study.[18, 19] Traditional methods of condiment preparation often take a long time. In this study, enzymatic hydrolysis of the distillers' grains, supplemented with temperate acidolysis, can transform and decompose protein, starch and other macromolecules rapidly and efficiently to produce a large number of flavor components [20, 21, 22]. Natural fermentation for rice wine sauce production usually takes about half to one year, while the process established in this study costs time less than 1 week.

The key step in this study is to establish the enzymatic hydrolysis process of macromolecule, supplemented with a short-term fermentation and temperate acidolysis. In addition to flavor protease, trypsin was also evaluated for enzymatic hydrolysis, but trypsin performed worse than flavourzyme. Therefore, flavourzyme was chosen for further optimization [23, 24]. Trypsin is a serine proteolytic enzyme extracted from the pancreas of cattle, sheep or pigs. In vertebrates, it acts as a digestive enzyme. Trypsinogen was produced in pancreas and secreted as a component of pancreatic juice. It is a highly specific endopeptidase which can cut off the carboxyl side of lysine and arginine residues in the polypeptide chain. However, the site specificity of trypsinase makes it incapable to decompose protein completely, on the other hand. Different from trypsin, flavourzyme is prepared via fermentation of *Aspergillus oryzae* strains, purification and formulation. Therefore, flavourzyme is mixed enzymes, which have two kinds of activities, endo-protease and exopeptidase. Flavourzyme has been confirmed to be one of the most effective proteases for proteolysis. Tests using bovine milk protein showed that the hydrolysis was almost complete. Flavourzyme can decrease the bitter peptide in hydrolysates, and can also be used to thoroughly hydrolyze proteins and improve the flavor of hydrolysates. Therefore, flavourzyme is usually added to the animal/plant-derived hydrolysis products in food processing, which can remove bitterness, improve taste and improve the effective utilization of protein. Of course, different enzymolysis conditions can affect the reaction significantly. Considering that flavourzyme can not decompose those non-protein macromolecules (even difficult to decompose protein completely), auxiliary pre-fermentation and acidolysis pretreatment were also designed in this study. The short-term pre-fermentation aimed to improve the decomposition of organic substances and the accumulation of flavor substances via the complex metabolism of microorganisms. Acidolysis pretreatment means a mild hydrolysis of proteins, starches and other macromolecules. Selection of the most suitable indicator which can reflect the differences between experimental groups significantly was expected to reduce the interference of contingency errors. As a supplement, sensory evaluation was also taken into account in practical operation, bad smell or bad taste is not acceptable. For example, as to acidolysis pretreatment, excessive hydrolysis might lead to isomer production, destruction of some amino acids, and production of toxic and odorous substances. Such amino acids as tryptophan, asparagine and glutamine are especially vulnerable to acidolysis. Therefore, it is necessary to control the hydrolysis to reduce the adverse effects.

The enzymatic hydrolysate of distillers' grains mainly provides flavor substances such as amino acids, esters and alcohols, but some other spices are also necessary for rice wine sauce production. In fact, for many manufacturers, the spice formulations are regarded as commercial secrets, because spices are a key factor for flavor. This study also showed that spices affect the flavor of condiment greatly. The spice usage varies greatly for different manufacturers, some manufacturer even use over ten kinds of spice. In this study, *Syzygium aromaticum*, *Foeniculum vulgare*, *Zanthoxylum bungeanum*, *Pericarpium citri reticulatae*, and *Cinnamomum cassia* are chosen, which are frequently used in condiment industry. Spices can be used either in extract form or in powder form directly. The latter might lead to sediments in the final production, showing poor clarity but stronger fragrance. In the organoleptic evaluation, the rice wine sauce got scores similar to the marketed products, showing that the method established in this study is feasible. The products by us lost scores mainly in color clarity. But darker colors may not be a problem for condiments since the pigment in normal distillers' grains is harmless to human [25, 26, 27]. More and more studies

show that the flavor substances in rice wine distillers' grains are worthy of development and utilization [28, 29, 30, 31, 32].

V. CONCLUSIONS

The distillers' grains of Shaoxing rice wine are confirmed to contain mainly water, starch, protein and fat. The starch content in the distillers' grains of Shaoxing rice wine is much higher than those in other regions in China. By optimized acidolysis pretreatment and flavourzyme's hydrolysis, the flavor components could be extracted quickly for production of condiment. The established process for condiment production costs only about 1 week, which saves a lot of time and space compared with traditional method. The condiment produced by this method presents similar quality with that by traditional method.

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