

INFLUENCES OF TECHNOLOGICAL HYDROLYSIS CONDITION ON NUCLEIC ACID CONTENT OF SPENT BREWER'S YEAST HYDROLYSATE

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ABSTRACT

Currently, with the strong increasing of the brewing industry output, the consequencing amount of yeast residue is very large. Utilizing a large source of protein from brewers yeast to produce hydrolysed products using protease as food and food additives has a high real-life benefit. However, one limitation in the use of yeast and hydrolysis products is that the amount of nucleic acid in the yeast in particular and in the microbial cells is generally high. Nucleic acid is abundant in food that causes gout in humans and animals. There are many methods for reducing or separating nucleic acids in hydrolysed products such as extracellular ribonuclease enzymes, chemical agents, thermal shock and autolysis. Use extracellular ribonuclease enzyme for hydrolysis of nucleic acid gives good efficiency, but with high production cost. Chemical agents affect the quality of the hydrolysed products used in the food industry. There have been many good-efficiency studies using heat shock and autolysis to reduce the amount of nucleic acid in the hydrolysate. However, no research has been conducted to reduce the amount of nucleic acid by hydrolysis techniques. In this paper, we investigated the effects of heat shock, autolysis and hydrolysis techniques (batch, continuous overflow and continuous circulation) of brewery yeast protein to nucleic acid content in yeast hydrolysate. The results showed that the content of nucleic acid in the hydrolysate (with a concentration of 55 % dry matter) was the smallest. Under normal hydrolysis conditions, the nucleic acid content was 8.7 g / kg and when there was a heat shock+ autolysis, it decreased to 6.34 g/kg. After optimizing the hydrolysis conditions, the nucleic acid content of the hydrolysate was reduced to 5.41g/kg on continuous hydrolysis system.

Keywords: heat shock, autolysis, continuous circulation hydrolysis, nucleic acid, spent brewer's yeast.

1. INTRODUCTION

In recent years much attention has been directed toward the development of new sources of protein for human consumption. There exists a need for protein material which can be

incorporated in foods or which is usable as a basic proteinaceous substance for human consumption. One possible solution to the problem of supplying the ever increasing world-wide need for food protein is provided by processes for the bio-synthetic manufacture of protein through the growth of microorganisms on various substrates. It is known, for example, that microorganisms such as bacteria and yeast, which are grown by single-cell reproduction, contain high proportions of proteins. In which, spent brewer's yeast (from the brewing industry) contains high protein content (about 50 – 55 %) with essential amino acids and it is a very cheap protein source [1]. The main application of spent brewer's yeast (SBY) is the production of hydrolysed and yeast extract. Spent brewer's yeast hydrolysis (SBYH) has been widely used as the nitrogen source for the integral feeding of individuals who have specific nutritional and physiological needs. These include patients with impaired gastrointestinal function (e.g., Crohn's disease), short bowel syndrome or specific organ disease, e.g., pancreatitis, renal and hepatic [2]. SBYH have also found application in sports nutrition, weight control diets and nutritional supplements [3]. More recently, protein hydrolysates have been used in dietetic products designed for the nutritional management of cancer patients [4] and individuals with acquired immune deficiency syndrome [5].

However, a limiting factor in utilization of SBY biomass as a protein source for human consumption is its high nucleic acid content (about 12 to 15 grams of nucleic acid per 100 grams of crude protein), primarily ribonucleic acid (RNA), which may account for one third of the total cell protein. In 1972, the Recommended Daily Allowance of The Food and Nutrition Board, National Research Council in protein was 65 grams per day for an adult male with weight of 70 kilogram. The Protein Advisory Group of the United Nations System recommended that the amount of nucleic acid ingested per day from microbial protein should be less than 2 grams. Therefore, the nucleic acid content of the protein should be less than 6%, if microbial protein supplied 50% of dietary protein [2, 6]. The nucleic acid content should be below about 3%, if microbial protein is the sole source of protein in the diet. The human metabolic system produces uric acid as the result of the metabolism of materials such as ribonucleic acid. Since man does not have an uricase enzyme system, uric acid is not broken down and excreted with urine. However, if produced in larger quantities than the body can excrete, the body stores uric acid leading to the condition known as gout. In order for SBYH to compete with vegetable proteins and to share the protein market, it is necessary that it be processed to remove nucleic acid, primarily ribonucleic acid (RNA) [4].

The reduction of the nucleic acid content can be accomplished by the hydrolysis of the nucleic acid within the cell to fragments of such size that the fragments can be diffused from the cell away from the protein [2]. It is known that the enzyme, nuclease, is present in certain yeast cells and that nuclease hydrolyzes or breaks up nucleic acid molecules to smaller fragments. It also is known in the art that the hydrolysis of nucleic acids within the cell can be accomplished by a multi-step heating process to activate the self-contained or endogenous nuclease to produce cells containing two to three grams of nucleic acid per 100 grams of protein [7]. Nucleic acid also can be hydrolyzed by exposing the cell to an external nuclease. In either of these procedures, two fractions are obtained. One fraction is the cell containing a reduced content of nucleic acid [8]. The other fraction is the surrounding medium containing nucleic acid fragments and other diffusible material. One disadvantage of these processes is that the protein remains within the cell in a non-functional form for food use [4]. Another disadvantage is that the processes by which the cell wall is made permeable to the nucleic acid fragments also severely decrease the ability of the cell to be ruptured to allow the protein to be harvested [5]. A further disadvantage is the difficulty in controlling the endogenous protease. Hydrolysis of the nucleic

acids by enzymatic methods allows the use of much milder conditions than those necessary for the chemical methods of hydrolysis. As previously mentioned, the enzyme, nuclease, is known to hydrolyze nucleic acids. Several sources of nuclease have been described in the literature [7]. However, the nuclease preparations must meet certain criteria, namely, the preparation must be free of secondary enzyme systems (such as protease, which would cause a decrease in protein recovery), and the preparation must not contribute an undesirable flavor to the products. Furthermore, to be of commercial interest, the nuclease preparation must be readily available at a reasonable cost and the nuclease must be of food grade acceptability. None of the known nuclease preparations meet these criteria [7, 9].

Besides, heat shock method used to reduce nucleic acid content has been studied extensively for the past few years [10]. At heat shock conditions have the effect of denaturing the ribosome and then the RNA hydrolyzed by enzyme ribonuclease, lead to reducing nucleic acid content in the SBYH with the efficiency of 55-75%. Autolysis process can be induced by exposing the cells to elevated temperatures (40-60°C), salts, or organic solvents [2]. Through an autolysis process, the yeast's own enzymes break down the RNA into mononucleotides, polynucleotides, and nucleosides. Both heat shock method and autolysis process have many advantages in terms of ease of use on an industrial scale, reasonable production costs and quality of SBYH that assure food safety [6, 10]. Most studies for reducing nucleic acid content have stopped in physical, chemical and enzymatic methods. No studies have yet approached to the effect of hydrolysis technique on the nucleic acid content in the SBYH. Thus, it is a principal object of this invention to provide a SBYH relatively free of nucleic acid, but still having good nutritional value and of acceptable eating quality [3, 4, 5]. Therefore, in this paper, it is necessary to study the effect of heat shock and autolysis process, hydrolysis techniques on the nucleic acid content in SBYH.

2. MATERIALS AND METHODS

Materials: The spent brewer's yeast *Saccharomyces* used as a substrate was donated by Sai Gon Ha Noi beer's company. The suspension of SBY was washed with cold water 2 times and cold 0.1N NaOH for debittering (bitter acids of houblon) and removing beer solids. Centrifuged SBY slurry was frozen before running the hydrolysis process. Two types of enzyme were used for hydrolysis are Flavourzyme and Alcalase (Novozymes, Denmark).

Heat shock process: The SBY suspension (20 % w/w) after washing and centrifugal process was heated at three different temperatures for various times. Initially a heat shock at 68 °C for 1 - 3 minutes is performed followed by incubation for 1 hour at 45 – 50 °C and for a 2nd at 52 – 55 °C.

Autolysis process: After heat shock process, the hydrolysis of SBY suspensions (20% w/w) was carried out at 50 °C, pH 5,5 for 24 hours with the agitation speed 250 rpm.

Enzymatic hydrolysis (before optimization): After heat shock and autolysis process. The SBY (20 % w/w) was hydrolyzed by mixture Flavourzyme (7.5 U/g) and Alcalase (7.5 U/g), with the agitation speed 250 rpm, at 52.5 °C, pH 7.5 for 12 hours under three technological hydrolysis conditions: batch, continuous overflow and continuous circulation.

Enzymatic hydrolysis (after optimization): After heat shock and autolysis process. The SBY (20 % w/w) was hydrolyzed under optimal conditions for 3 hydrolysis techniques (according to Table 1).

Table 1. Optimal conditions for different hydrolysis techniques.

Hydrolysis techniques	Temperature (°C)	pH	Ratio E/S (U/g)	Time (hour)	% inverter of pump
Batch	52	7.5	8.5	9	
Continuous overflow	51	7.5	10	9	
Continuous circulation	51	7.5	8.0	8	65

Hydrolysis systems: Batch hydrolysis process was performed in a big tank (total volume 30 liters). At the top of the tank is a stirrer, stirring speed is controlled by inverter. Heating bars are used to heat of the SBY suspensions. The thermal sensor, which is connected to receive signals from the heating bar, was set to regulate the temperature during protein hydrolysis. Continuous overflow hydrolysis process was performed in the six-equipment system (include 1 tank 30 liters and 5 tanks 5 liters) using agitator with agitation speed 250 rpm. Hydrolysate was continuously overflowed from big tank to five small tanks in turn. Sludge yeast in tanks was heated by heating bars. Hydrolysate was circulated by pump if it is necessary. Continuous hydrolysis process was performed on continuous circulation system using agitator with agitation speed 250 rpm under different conditions. Autolysate was continuously circulated through tank and tube heat exchanger by pump.

Concentration process: After centrifugation, hydrolysate (about 20 liters) was concentrated in a vacuum condenser at 70 °C (~ 1bar), stirring speed 150 rpm and obtained 2 liters SBYH concentrate with a dry matter content of 55 % (w / w) after 6 hours.

Analytical methods: After all the hydrolysis, it was inactivated by 0.5 M TCA and removed the sludge by using centrifuge (6000 rpm, 4 °C for 10 min), hydrolysate was haversted in order to determine amino acid content by HPLC. SBYH was concentrated to 55 %, determined nucleic acid content (by spectrophotometric method at 260 nm) and calculated according to the following formula [5]:

$$\text{Nucleic acid content (mg/l)} = (\text{OD}_{260}) \times (\text{dilution factor}) \times (50 \mu\text{g DNA /ml}).$$

in which: $\text{OD}_{260\text{nm}} = 1$ corresponding with 50 $\mu\text{g/ml}$ for a double-stranded nucleic acid solution and 40 $\mu\text{g/ml}$ for a single nucleic acid solution.

3. RESULTS AND DISCUSSION

3.1. Influence of heat shock and autolysis on nucleic acid and amino acid content in SBYH.

Prior to hydrolysis, SBY was treated by thermal shock (sample M1), autolysis (M2) and combined method including heat shock and then autolysis (sample M3) to evaluate variation in nucleic acid content. The nucleic acid content of M1, M2 and M3- samples was 7.24, 6.79 and 7.31 g/kg, respectively. The difference is small when compared to the value of 8.95 g/kg of the

sample without treatment SBY (M). So that, under the action of thermal shock or autolysis process, the nucleic acid was penetrated through the cell membrane from inside to outside. Meanwhile, the content of nucleic acid is reduced to 70 % when using thermal shock [7, 10] and decreased to 55 % when using autolysis process [7, 9].

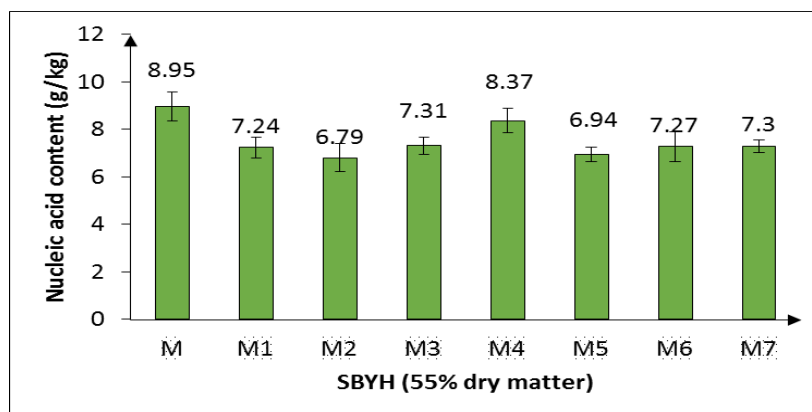


Figure 1. Nucleic acid content in samples.

M. SBY, M1. Heat shock, M2. Autolysis, M3. Heat shock and then autolysis, M4. Hydrolysis, M5. Heat shock and then hydrolysis, M6. Autolysis and then hydrolysis, M7. Heat shock, autolysis and then hydrolysis.

According to Figure 1, the nucleic acid content is 8.37 g/kg when SBY is hydrolyzed with Flavourzyme + Alcalase for 12 hours (M4). It is higher than thermal shock or autolysis process. Moreover, the decrease in the content of nucleic acid is not clear when experiments are carried out by the combination of two above methods, the achieved values were 6.94 (M5), 7.27 (M6) and 7.3 (M7).

Table 2. Amino acid composition in SBYH (1% dry matter).

Amino acid composition	M4	M5	M6	M7
Glycine	0.33	0.1	0.24	0.24
Alanine	0.32	0.2	0.27	0.31
Valine + Methionine	0.22	0.32	0.35	0.44
Phenylalanine	0.22	0.24	0.26	0.23
Leucine	0.28	0.43	0.21	0.25
Isoleucine	0.17	0.22	0.25	0.27
Aspartic acid	0.11	0.17	0.15	0.22
Glutamic acid	0.15	0.14	0.16	0.17
Serine	0.04	0.16	0.13	0.27
Histidine	0.07	0.2	0.28	0.39
Arginine	0.4	0.44	0.31	0.25
Threonine	0.19	0.16	0.25	0.23
Tyrosine	0.5	0.47	0.53	0.2
Cysteine	0.3	0.24	0.21	0.28
Lysine	0.28	0.27	0.29	0.32
Acid amin composition total (mg/ml)	3.58	3.76	3.89	4.07

However, according to Table 2, the total amino acid content of SBYH was the highest when using combination treatment method (thermal shock, autolysis, then hydrolysis), and reached value of 4.07 mg/ml. Therefore, the combination of thermal shock and autolysis before hydrolysing was selected for further studies.

3.2. Influence of hydrolysis techniques on nucleic acid and amino acid content in SBYH.

SBY is pretreated by heat shock and autolysis and then hydrolysed by Flavourzyme + Alcalase according to 3 hydrolysis techniques: Batch, continuous overflow and continuous circulation. The nucleic acid content of the obtained SBYH is shown in Fig. 2 and Fig. 3.

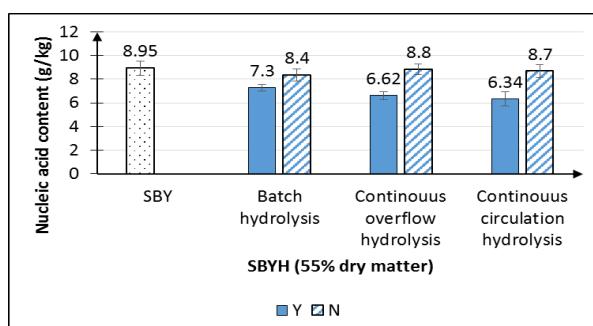


Figure 2. Nucleic acid content in SBYH by different hydrolysis techniques (Y and N-With and without heat shock and autolysis)

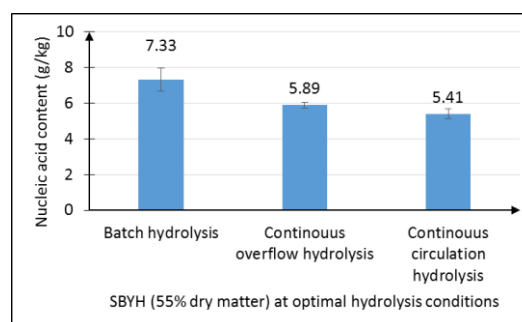


Figure 3. Nucleic acid content in SBYH by three hydrolysis techniques under optimal conditions

Table 3. Amino acid composition in SBYH (1% dry matter) by three different hydrolysis techniques

Amino acid composition	acid	Batch		Continuous overflow		Continuous circulation	
		N	Y	N	Y	N	Y
Glycine		0.37	0.24	0.53	0.24	0.56	0.42
Alanine		0.32	0.31	0.36	0.89	0.21	0.36
Valine + Methionine		0.22	0.44	0.21	0.26	0.27	0.34
Phenylalanine		0.27	0.23	0.37	0.21	0.43	0.35
Leucine		0.28	0.25	0.19	0.25	0.45	0.59
Isoleucine		0.19	0.27	0.33	0.49	0.37	0.51
Aspartic acid		0.11	0.22	0.37	0.3	0.38	0.4
Glutamic acid		0.15	0.17	0.25	0.44	0.43	0.59
Serine		0.04	0.27	0.3	0.29	0.31	0.61
Histidine		0.07	0.39	0.43	0.22	0.2	0.44
Arginine		0.38	0.25	0.26	0.49	0.61	0.41
Threonine		0.15	0.23	0.25	0.24	0.33	0.29
Tyrosine		0.45	0.2	0.29	0.43	0.29	0.37
Cysteine		0.3	0.28	0.35	0.57	0.32	0.62
Lysine		0.28	0.32	0.23	0.38	0.34	0.53
Amino acid composition (mg/ml)	acid total	3.58	4.07	4.72	5.7	5.5	6.83

(Y): with heat shock and autolysis; and (N): without heat shock and autolysis.

From the results in Figure 3, the content of nucleic acid in hydrolysate depends on the hydrolysis technique and treatment before hydrolyzing process. Comparing with the pretreatment, in the absence of thermal shock and autolysis before hydrolyzing, nucleic acid content in hydrolysates is higher in all three cases of hydrolysis techniques. Among three hydrolysis techniques, nucleic acid content is the highest in SBYH using continuous overflow technique, valued 8.84 g/kg. Using continuous circulation system, nucleic acid content of hydrolysate is the lowest, reached 6.34 g/kg. It is again confirmed that the process of heat shock and autolysis have greatly affects of reducing nucleic acid. Besides, hydrolysis techniques have a great influence on hydrolysis of nucleic acid. In fact, the thermal shock process makes ribosomalisation. Temperature suitable for autolysis is good condition for the activity of the ribonuclease enzyme in yeast cells. Therefore, in order to minimize nucleic acid content of hydrolysate, the choice of heat shock, autolysis and hydrolysis is required.

In addition, according to Figure 3, the hydrolysis of SBY under optimum conditions had a significant reduction of nucleic acid content, in 15 % (reduced from 6.34 to 5.41 g/kg) when using continuous circulation system and 11 % (from 6.62 to 5.89 g/kg) when using continuous overflow system. It is demonstrated that, under optimum conditions of hydrolysis, temperature, pH, E/S ratios, hydrolysis times are more appropriate conditions for the intracellular ribonuclease activity. The composition of some amino acids in SBYH by different hydrolysis techniques, with and without thermal shock and autolysis before hydrolyzing is shown in the Table 3. The results showed that total composition of some amino acids in SBYH (1 % dry matter) varies depending on hydrolysis technique (including with and without shock + autolysis). Continuous circulation hydrolysis process with heat shock + autolysis gives the highest total amino acid content in SBYH (1 %), reached the value of 6.83 mg/ml.

Table 4. Amino acid composition in SBYH obtained by using different hydrolysis techniques under optimal conditions

Amino acid composition	mg/ml SBYH (1% dry matter)			g/100g SBYH (55% dry matter)		
	Batch	Continuous overflow	Continuous circulation	Batch	Continuous overflow	Continuous circulation
Glycine	0.37	0.18	0.26	1.34	0.65	0.94
Alanine	0.42	0.48	0.87	1.52	1.73	3.14
Valine + Methionine	0.24	0.51	0.36	0.87	1.84	1.3
Phenylalanine	0.26	0.45	0.31	0.94	1.62	1.12
Leucine	0.39	0.62	0.49	1.41	2.24	1.77
Isoleucine	0.22	0.41	0.58	0.79	1.48	2.09
Aspartic acid	0.19	0.38	0.48	0.69	1.37	1.73
Glutamic acid	0.18	0.87	0.87	0.65	3.14	3.14
Serine	0.25	0.39	0.44	0.9	1.41	1.59
Histidine	0.17	0.43	0.51	0.61	1.55	1.84
Arginine	0.51	0.62	0.99	1.84	2.24	3.57
Threonine	0.22	0.44	0.25	0.79	1.59	0.9
Tyrosine	0.35	0.45	0.57	1.26	1.62	2.06
Cysteine	0.42	0.84	0.95	1.52	3.03	3.43
Lysine	0.3	0.77	1.02	1.08	2.78	3.68
Amino acid composition total	4.49	7.84	8.95	16.21	28.29	32.3

Under optimal hydrolysis conditions, the total some amino acids content continue to increase in SBYH by other hydrolysis techniques (with thermal shock + autolysis) (Table 4). The result of the total amino acid composition was 8.95 mg/ml (1 % dry matter) by continuous circulation hydrolysis, correspondingly achieved 32.3 g/100g SBYH (55 % dry matter).

4. CONCLUSIONS

The pretreatment of thermal shock and autolysis, as well as hydrolysis techniques, greatly affects on the nucleic acid composition. Nucleic acid content in the hydrolysed product (dry matter concentration 55 %) by continuous hydrolysis is minimal. Under the normal hydrolysis conditions, the nucleic acid content was 8.7 g / kg and, when applying the thermal shock and autolysis, reduced to 6.34 g / kg. After optimizing hydrolysis conditions, the nucleic acid content of the hydrolysed product was reduced to 5.41 g/ kg in continuous circulation hydrolysis. Meanwhile, in the condition of batch hydrolysis and continuously overflow hydrolysis, the nucleic acid content reached values of 7.33 g/kg and 5.89 g / kg, respectively. Besides, the amino acid content of the hydrolysed product (55 % dry matter) was 16.21 g/100 g, 28.29 g / 100 g and 32.3 g/100g in batch, continuously overflow and continuous circulation hydrolysis, respectively.

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TÓM TẮT

ẢNH HƯỞNG CỦA ĐIỀU KIỆN CÔNG NGHỆ THỦY PHÂN ĐẾN HÀM LƯỢNG ACID NUCLEIC TRONG SẢN PHẨM THỦY PHÂN BÃ NẤM MEN BIA

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Hiện nay, với sự tăng mạnh về sản lượng của ngành công nghiệp sản xuất bia, kéo theo lượng bã nấm men bia thải ra rất lớn. Tận dụng nguồn protein lớn từ bã nấm men bia để sản xuất sản phẩm thủy phân bằng chế phẩm protease làm thực phẩm và chất phụ gia thực phẩm có ý nghĩa thực tiễn cao. Tuy nhiên, một hạn chế ở việc sử dụng nấm men và sản phẩm thủy phân là lượng acid nucleic trong nấm men nói riêng và trong tế bào vi sinh vật nói chung thường cao. Axit nucleic có nhiều trong thức ăn là nguyên nhân gây bệnh gút ở người và động vật. Có nhiều phương pháp để giảm hoặc tách các acid nucleic trong các sản phẩm thủy phân như sử dụng enzyme ribonuclease ngoại bào, các tác nhân hóa học, sốc nhiệt và tự phân. Sử dụng enzyme ribonuclease ngoại bào để thủy phân acid nucleic cho hiệu quả cao, nhưng chi phí sản xuất lớn. Tác nhân hóa học ảnh hưởng đến chất lượng sản phẩm thủy phân ứng dụng trong công nghiệp thực phẩm. Đã có nhiều nghiên cứu đạt hiệu quả tốt khi sử dụng phương pháp sốc nhiệt và tự phân để làm giảm hàm lượng acid nucleic trong sản phẩm thủy phân. Tuy nhiên, chưa có nghiên cứu nào tiếp cận đến việc làm giảm hàm lượng acid nucleic bằng các kỹ thuật thủy phân. Trong bài báo này, chúng tôi thực hiện nghiên cứu ảnh hưởng của quá trình sốc nhiệt, tự phân và các kỹ thuật thủy phân (gián đoạn, chảy tràn liên tục và tuần hoàn liên tục) protein bã nấm men bia đến hàm lượng acid nucleic trong sản phẩm thủy phân nấm men. Kết quả nghiên cứu cho thấy hàm lượng acid nucleic trong sản phẩm thủy phân (có nồng độ chất khô 55 %) bằng kỹ thuật thủy phân tuần hoàn liên tục là nhỏ nhất. Ở điều kiện thủy phân thường hàm lượng axit nucleic là 8,7 g/kg và khi có giai đoạn sốc nhiệt + tự phân, giảm còn 6,34 g/kg. Sau khi tối ưu hóa các điều kiện thủy phân, trên hệ thống thủy phân tuần hoàn liên tục, hàm lượng acid nucleic trong sản phẩm thủy phân giảm xuống còn 5,41 g/kg.

Từ khóa: sốc nhiệt, tự phân, thủy phân tuần hoàn liên tục, acid nucleic, bã nấm men bia.