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Effects of Chemical, Biochemical and Physical Treatments on the Kinetics and on the Role of Some Endogenous Enzymes Action of Baker's Yeast Lysis for Food-Grade Yeast Extract Production

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ABSTRACT

The effects of chemical, biochemical and physical treatments on lysis of baker's yeast cells were studied at pH 5.2 and temperature 54 °C. An unstructured kinetic model of first order was developed to understand the mechanism of yeast lysis. The autolysis rate constant (k), yield factor for protein (β_P), total carbohydrate (β_C) and autolysate (α_A) were sensitive to disruption methods. Since there were endogenous enzymes in the yeast cells such as proteinases, glucanases and carboxypeptidases, these enzymes were responsible for synergistic of cell disruption and their activity profiles were affected by different treating methods. From the determined results of amino acids composition in yeast autolysate, there were differences in amino acid content. The biochemical treated sample using 0.1% papain gave the highest total amino acid content among all analysed samples.

Key words : baker's yeast, yeast extract, yeast autolysate, kinetics, endogenous enzyme

INTRODUCTION

Yeast extracts are used widely in food with two main purposes, i.e., to improve the flavor of food to satisfy consumer and to increase the nutritional value of food (Moresi *et al.*, 1995). The consumption of yeast extract in the world market in 1994 was 35,000 metric ton or 200 millions U.S. dollar and it is expected that by the year 2,000 the consumption will increase to 70,000 metric ton (Keeman and Snick, 1994).

The normal method to produce yeast extract is by adjusting the environmental conditions to be suitable for yeast autolysis and the product is called yeast autolysate. Other methods have been done by the chemical, biochemical and physical conditions to increase the autolysis reaction (Kelly, 1983). The previous studies indicated that the suitable chemical method was done by mixing 40 g. of fresh yeast in 100 ml solution containing 5% ethanol (95% pure) by volume and 5% NaCl by weight. This processing method causes cell plasmolysis resulting in the release of enzymes and other material inside the cell (Reed and Nagodawithana, 1991). Sugimoto (1974) found that using 1-9 percent ethanol by volume mixing with 2-10 percent of NaCl by weight of fresh yeast will help increasing the autolysis rate. The biochemical method using 0.1 percent papain enzyme by wieght of fresh yeast. The physical method was done by breaking

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the yeast cell using homogenizer twice at 8,000 lb/in^2 pressure. All three methods were done at pH 5.2, 54°C (Foo-trakul *el al.,* 1998). There are many enzymes released from yeast autolysis such as protease, β(1-3), β(1-6) glucanase, mannase and kitanase (Breddam and Beenfelt, 1991). The mechanism of these enzymes are complex and controlling of the enzyme reactions are rather difficult. Studying the kinetic of yeast autolysis is one way to explain the phenomena and help to understand the process better. The purpose of this experiment is to construct the unstructured models of kinetic of yeast autolysis by studying the effects of using chemical, biochemical and physical methods and the role of important enzymes in yeast autolysis. The basic information obtained will be used to develop the structured models later. The amino acid contents in yeast extract samples were analyzed for further improvement.

MATERIALS AND METHODS

1. Studying of kinetic of Baker's yeast autolysis and the role of related enzymes

Prepared cells of fresh Baker's yeast at 40 percent concentration by weight for chemical and biochemical treatments and 30 percent concentration by weight for homogenization, adjusted pH to 4.5 for treatments. The chemical method used about 5 percent by volume at 95 percent pure ethanol or 5 percent by weight of sodium chloride and 40 gram of fresh yeast in 100 ml. solution. The biochemical used 0.1 percent by weight of papain. The physical method was done by feeding the yeast samples into the homogenizer at 8,000 lb/in^2 pressure and incubated at 54°C for 24 hours. The samples were centrifuged to separate the yeast cells and discarded the cells. The clear supernatant samples were analyzed for protein content using Lowry's method (Herbert *et al.,* 1971) and the content of yeast autolysate using Hill's method (1981), tasting proteinase enzyme activity using Walker's method (1981), testing carboxy peptidase enzyme activity by Sara *et al.*'s method (1988) and testing glucanase enzyme activity using Rechielt and Fleet's method (1981).

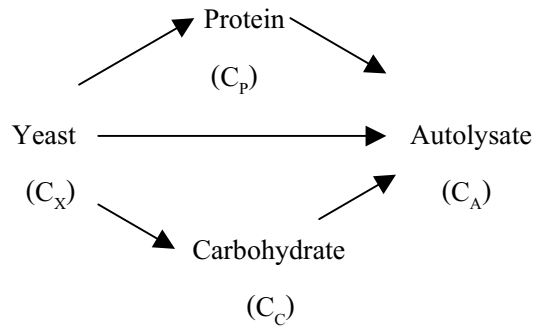
2. Amino acids analysis

Amino acids analysis were made for samples of yeast autolysate obtained from treatments such as control samples left under autolysis at pH 5.2 and 54°C, chemical treated samples using 5 percent sodium chloride by weight mixed with 40 gram of fresh Baker's yeast in 100 ml solution and the biochemical method using 0.1 percent by weight of papain enzyme. The samples obtained from physical treatment was not analyzed for amino acid content because the yeast autolysate obtained was too low (Foo-trakul *et al.,* 1998). All samples were centrifuged to separate the cell debris and concentrated in vacuum evaporator using amino acid analyzer of Shimadzu Co. Ltd. at Thailand Institute of Scientific and Technological Research.

RESULTS AND DISCUSSION

1. The kinetic of Baker's yeast autolysis

The kinetic of Baker's yeast autolysis and the effects of chemical and physical treatments in this study was a simple unstructured model modified from Moresi *et al.* (1995). It was proposed that the yeast cell is composed of cell wall tissues, cytosol and various organnels. All these are combined to become biomass of yeast cell (C_x). Hunter and Asenjo (1987b) made a hypothesis that yeast cell is composed of protein (C_p), carbohydrate (C_c) and other components that can be released from the cell and are in soluble forms. All these components are grouped together in the form of "autolysate" (C_A) (Hill, 1981). From the above hypothesis we could write the following



From the above simple model we could form the mathematical relationships as the following

$$\frac{dC_X}{dt} = -k\,(C_X - C_X^{\bullet}) \quad (1)$$

$$\frac{dC_P}{dt} = -\alpha_P\,(dC_X/dt) \quad (2)$$

$$\frac{dC_C}{dt} = -\alpha_C\,(dC_X/dt) \quad (3)$$

$$\frac{dC_A}{dt} = -\alpha_A\,(dC_X/dt) \quad (4)$$

Considering the relationships of the mathematical models, the amount of protein and carbohydrate is a part of obtained autolysate. Thus, from equation (2) and (3) a new relationship could be written as :

$$\frac{dC_P}{dt} = -\beta_P\,(dC_A/dt) \quad (5)$$

$$\frac{dC_C}{dt} = -\beta_C\,(dC_A/dt) \quad (6)$$

when $C_X = C_{X0}$, $C_P = C_{P0}$, $C_C = C_{C0}$, $C_A = C_{A0}$ and t =0 integrate the equations (1), (4), (5) and (6) will get

$$\frac{\ln(C_X - C_{X\infty})}{(C_{X0} - C_X)} = -kt \quad (7)$$

As :
$$C_A = C_{X0} - \alpha_A\,(C_X - C_{X0}) \quad (8)$$
$$C_P = C_{P0} - \beta_P\,(C_A - C_{A0}) \quad (9)$$
$$C_C = C_{C0} - \beta_C\,(C_A - C_{A0}) \quad (10)$$

The relationship between the amount of yeast (C_x) that was autolyzed and the time was shown in Figure1. When substituted all the data into equation (7) and used non linear regression method, the parameter $C_{x\alpha}$ and constant value of yeast autolysis (k) could be found from samples under different treatments. The relationship between the increasing amount of autolysate and the decreasing amount of yeast was shown in Figure 2. The same results were obtained from the relationships of protein, carbohydrate and the increasing amount of autolysate as shown in Figure 3 and Figure 4, respectively.

After substituting the data obtained from the experiment into equations (8), (9) and (10) and using the least square method, the constant values of α_A, β_P and β_c would be obtained as shown in Table1. It was found that the constant values α_A, β_P and β_c of the samples treated under different conditions would have regression coefficient (r^2) more than 0.90 but some samples had lower (r^2) than 0.90. This was due to the fact that the proposed model was a simple unstructured model, so it could not well explain the complex reactions. If it is changed to structured model the value of more variables are needed, such as protein which will be classified according to the source obtained from various part of the cell. They would be used to develop and modified to obtain basic information needed to forecast some of the yeast autolysis process that could not be measured directly (Hunter and Asenjo, 1987a).

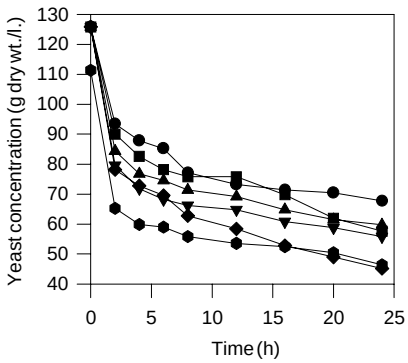


Figure 1 The residual of yeast after time course of autolytic process.

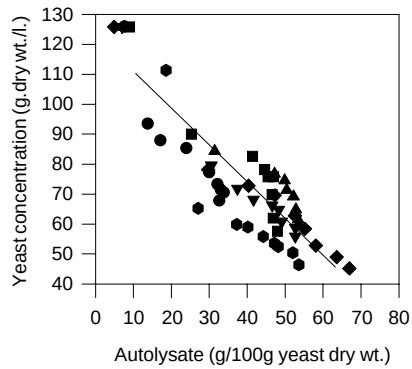


Figure 2 Correlation of yeast concentration and autolysate.

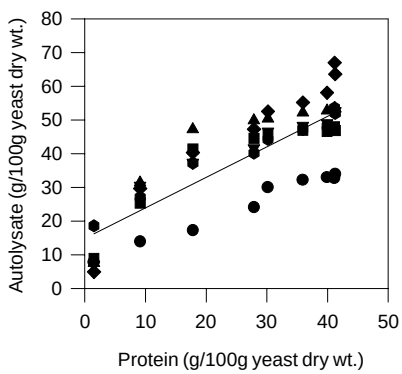


Figure 3 Correlation of protein and autolysate.

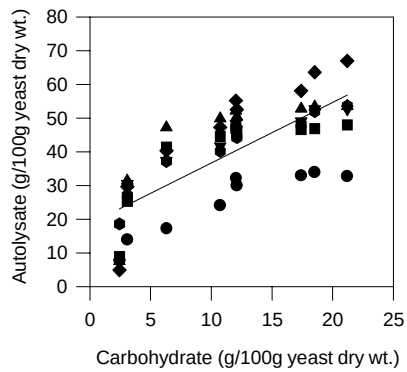


Figure 4 Correlation of carbohydrate and autolysate.

Symbol ● Control ■ 5% ethanol ▲ 5%NaCl ▼ 5% ethanol+5%NaCl
◆ 0.1%papain ⬢ Homogenization

Table 1 Kinetic parameters and stoichiometric constant of Baker's yeast lysis as performed at pH 5.2, temperature 54 °C under different treatments.

Treatments	Parameters							
	$C_{X\infty}$ (g/l)	k (h^{-1})	α_A	(r^2)	β_P	(r^2)	β_C	(r^2)
Control	70.79	0.239	0.503	(0.87)	0.689	(0.93)	0.416	(0.94)
5% ethanol	66.47	0.306	0.625	(0.88)	0.833	(0.97)	0.257	(0.95)
5% NaCl	65.52	0.449	0.747	(0.95)	0.861	(0.98)	0.262	(0.78)
5% ethanol+5% NaCl	61.78	0.537	0.672	(0.96)	0.894	(0.97)	0.238	(0.92)
0.1%papain	52.38	0.320	0.784	(0.96)	0.731	(0.94)	0.311	(0.80)
Homogenization (8,000 psi)	52.80	0.667	0.529	(0.78)	0.839	(0.95)	0.539	(0.88)

2. Activities of enzymes related to Baker's yeast autolysis

There were many enzymes working during autolysis of Baker's yeast. The enzymes that played important roles were proteinase, glucanase and carboxy peptidase (Asenjo and Andrew, 1990). The enzymes activities in the yeast samples under different treatments were shown in Figure 5. The activities of enzymes were reported as percent of the highest value in each sample. It was found that the activity of proteinase from all samples except the one under homogenization would show their activities starting from the second hour and had the highest activity after 6 hours comparing to the control sample in Figure 5-A which showed the highest activity after 8 hours. Neklyudov *et al.*

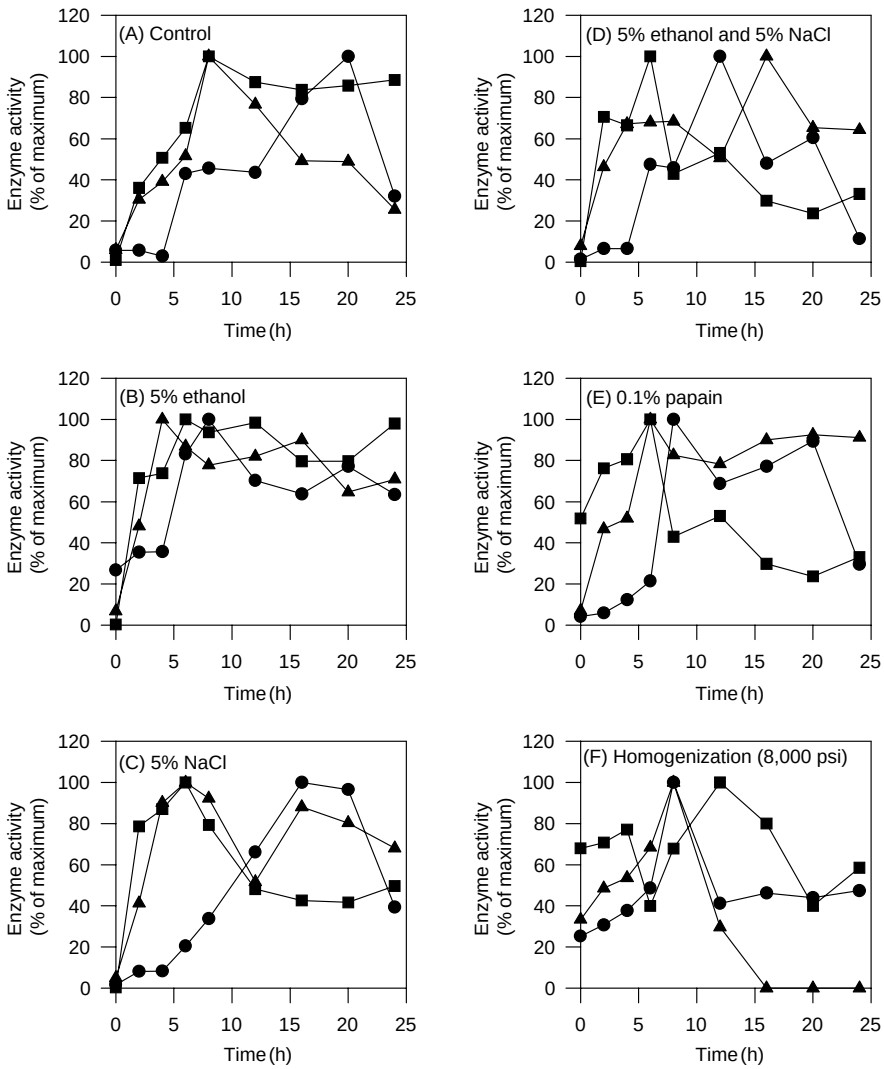


Figure 5 Endogenous enzyme activities during autolysis process of yeast at pH 5.2 and temperature 54°C.

(1993) had studied the activities of these enzymes during yeast autolysis using toluene and chloroform as plasmolyzer and found that the enzymes could work well after the yeast autolysis had been done for 4 and 6 hours respectively at 50-55ºC.

It was found that in the homogenized samples the proteinase had about 65 percent of its highest activity starting from the first hour. This showed that homogenization could break the yeast cell causing the release of enzymes to digest the protein. Anyway the enzyme activity would reduce after 6 hours but increasing again after 12 hours (as shown in Figure 5-F). It was possible that there were different kinds of proteinase and the ones that play important roles were proteinase YSC A and proteinase YSC B. During the first 4 hours enzyme YSC B could start working first (Reed and Nagodowithana, 1991). Hayashi *et al.* (1967) had separated 3 kinds of proteinase (A, B and C) from Baker's yeast autolysate. After testing their activities at pH 5.0 it was found that the activities of enzymes would be highest starting with proteinase YSC B after 6 hour of incubation and proteinase YSC C which worked at about the same time as proteinase YSC A at the last hour of the experiment or at the 30^{th} hour.

The activities of glucanase enzyme from every sample except the one treated with 5% ethanol mixed with 5% NaCl (Figure 5-D) showed the highest activity at about the same time as proteinase. The glucanase was located near the cell wall (Fleet, 1991) and its main position was located at periplasmic space (Reichelt and Fleet, 1981). Thus when the cell wall was destroyed it could readily start working.

The carboxy peptidase had the highest activity during the last period comparing to the other two enzymes in the case of control, ethanol and NaCl treated samples and the sample treated with papain. Breddam and Beenfeldt (1991) used 0.25 ml ether per gram of fresh yeash and left it for autolysis at pH 7.4 and found that the activity of carboxy peptidase would be highest after 18 and 20 hours. These results were close to the control sample and NaCl treated sample which showed highest enzyme activity after 20 and 16 hours respectively. Empter and Wolf, 1984 reported that the carboxy peptidase would work faster due to the dertruction of vacuoles which are the collector of enzymes such as carboxypeptidase Y and S. This would cause fast release of enzymes. Considering the activity of enzymes related to the Baker's yeast autolysis, it was noted that proteinase and glucanase started working first by digesting the yeast cell wall and glucan, respectively. The carboxy peptidase enzyme would work during the last period by digesting protein from different parts of the cell. The work of enzymes observed in this study was similar to the yeast autolysis as reported by Hunter and Asenjo (1988b).

3. Amino acid analysis in yeast autolysate

The results of amino acid analysis of the control, NaCl treated and papain treated samples are shown in Table 2. It could be seen that the total amino acid content were listed from the most to the least for papain treated, control and NaCl treated samples. The papain treated samples gave higher essential amino acid contents. They were threonine (Thr), valine (Val), methionine (Met) isoleucine (Ile), leucine (Leu), phenylalanine (Phe), and lysine (Lys) which was much higher than other samples. Glazer and Smith (1971) reported that the peptide bond from lysine was easily digested by papain, Table 2 showed that papain treated samples had lower alanine (Ala) content than control and NaCl treated samples, this might caused by complex yeast protein molecules which were digested to peptides and would have the amino acid sequences as following. The phenylalanine was at the position 2 next to the carboxyl group (C-terminal) such as -Ala-Ala-Phe-Lys-COO^- or -Ala-Ala-Phe-Ala-

Table 2 Amino acids composition in yeast autolysate.

Amino acids (g/100 g dry extract)	Yeast autolysate			
	Control	5%NaCl	0.1% papain	Maxazome[1/] (g/100 g product)
Aspartic acid	5.09	4.02	6.34	4.2
Threonine	1.98	1.66	2.78	1.9
Serine	2.13	1.63	2.64	1.9
Glutamic acid	7.41	7.64	7.35	6.0
Proline	1.75	1.46	2.18	1.5
Glycine	2.94	2.20	2.59	1.9
Alanine	4.56	3.43	2.91	2.7
Cystine	1.31	0.54	1.10	no data
Valine	2.50	2.19	3.38	2.9
Methionine	0.43	0.42	0.69	0.5
Isoleucine	1.87	1.87	3.00	1.7
Leucine	2.83	2.70	4.23	2.6
Tyrosine	1.23	1.18	1.30	1.3
Phenylalanine	1.71	1.54	2.36	1.5
Histidine	0.95	0.85	1.30	0.8
Lysine	3.63	3.13	4.78	2.9
Arginine	1.93	2.04	2.91	2.0
Tryptophan	0.33	0.36	0.56	no data
Total amino acids (except tryptophan and cystine)	42.94	37.96	50.74	36.30

1/ Data from Keeman and Snick (1994) and product has 80% dry solid

COO^- . This type of peptide chain would inhibit papain from digesting the bond of Ala (Glazer and Smith, 1971). When comparing 16 amino acids except tryptophan and cystine of yeast autolysate in this study with the commercial sample or Maxarom which is the yeast autolysat produced by Gis-brocades company of Netherlands. It was found that the papain treated smples had more amino acid content. The Mexarome had 80% total dry weight. After converting to 100 gram dry sample. The total 16 amino acids would be 45.38 g comparing to 42.94 g of control sample and 50.74 g of papain treated sample.

CONCLUSION

After studying the kinetic of yeast autolysis under chemical, biochemical and physical treatments. It was found that the developed mathematic models could explain the process to a

certain extent. The enzymes which play important role were proteinase, glucanase and carboxy peptidase. During the first period of digestion proteinase and glucanase would start working first, after that carboxy peptidase would digest the protein to make it smaller and became amino acid. The result of amino acid analysis showed that the samples treated with papain had higher amino acid content than other samples. It was concluded that production of yeast autolysate was best done by papain treatment. It was recommended that the feasibility study of using yeast autolysate should be done as well as increasing the production capacity and testing for consumer acceptance.

Symbols

C_A : amount of autolysate (g/100 g yeast dry wt.)
C_C : amount of carbohydrate (g/100 g yeast dry wt.)
C_P : amount of protein (g/100 g yeast dry wt.)
C_X : amount of yeast (g dry wt./l)
k : constant for yeast digestion rate (h^{-1})
α_A : coefficient of autolysate yield
β_C : coefficient of carbohydrate yield
β_P : oefficient of protein yield
0 : original point
∞ : last point or equilibrium point

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