

CHAPTER VIII

AUTOLYSIS

8.1 Autolysing Condition

8.1.1 Apparatus

- 8.1.1.1 Centrifuge (MSE Centaur 2)
- 8.1.1.2 Electric oven (Kottermann)
- 8.1.1.3 Digestion unit (BUCHI 425)
- 8.1.1.4 Distillation unit (BUCHI 322)
- 8.1.1.5 Fume cupboard
- 8.1.1.6 Thermocouple and temperature recorder (CHINO DR 015)

8.1.2 Reagents

- 8.1.2.1 Lactic acid 80%
- 8.1.2.2 Sodium chloride, analytical grade
- 8.1.2.3 Ethanol 95%
- 8.1.2.4 Papain (see appendix III)

8.1.3 Procedure

8.1.3.1 Incubation time

- i) Added distilled water to yeast cells at ratio 1:1
(based on dry weight)
- ii) Adjusted yeast slurries to pH 6.0 by lactic acid
- iii) Incubated yeast slurries at 40° C for different period
of time ranging from 24 to 72 hours
- iv) Separated yeast autolysate from cell debris by
centrifuge (8.1.1.1) at 3000 rpm, 15 minutes

v) Analysed the data by comparing percentage yield of nitrogen (see 7.2.1),

Percentage yield of nitrogen = $\frac{\text{gm nitrogen in supernatant after centrifuge}}{\text{gm nitrogen in homogenate before centrifuge}}$

8.1.3.2 Concentration of sodium chloride and ethanol

- i) Added combination of sodium chloride and ethanol in 4 levels ranging from 0% to 10% (based on dry weight) to yeast cells
- ii) Adjusted yeast slurries to pH 6.0 by lactic acid
- iii) Incubated yeast slurries at 50° C for 48 hours
- iv) Did the same process as 8.1.3.1 iv to v

8.1.3.3 Concentration of papain

- i) Added combination of papain at the level of 0.1% and 1.0% (based on dry weight of yeast protein) to yeast cells
- ii) Did the same process as 8.1.3.2 ii to iv

8.1.3.4 pH and temperature

- i) Adjusted yeast slurries to pH 4, 5, 6 by lactic acid
- ii) Incubated yeast slurries at 40°, 50°, 60° C
- iii) Did the same process as 8.1.3.1 iv and analysed the data according to the algorithm for 3² factorial design of experiments with pH and temperature as variables (Cochran, 1957).

8.1.3.5 Freezing and thawing cycle

- i) Froze yeast cells from 17° C to -6° C and thawed to 20° C
- ii) Froze and thawed at the same temperature for 4 times
- iii) Determined percentage yield of nitrogen between freezing and thawing cycle
- iv) After 4 cycles of freezing and thawing, did the same process as 8.1.3.2 ii to iv

8.2 Optimum condition for autolysis of yeast cells using 2^3 factorial design of experiments

The variables and corresponding levels of the variables in the experiments are :

1. Preservative agents, using combination of sodium chloride and ethanol at the level of 0% and 5% (based on dry weight of yeast cells)
2. Addition of papain at the level of 0% and 0.1% (based on dry weight of yeast protein)
3. Autolysing temperature of 40° C and 55° C

After incubation for 48 hours, determined percentage yield of nitrogen in each sample, and analysed the data by Yate's method (Cochran, 1957) to obtain contrast means. Plotted ranked contrast means against probability.



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8.3 Results of autolysis conditions

8.3.1 Incubation time

Results on profiles of percentage yields of nitrogen contents with respect to incubation time are shown in Table 8-1. The percentage yields of nitrogen content in control samples incubated for 72 hours were not determined because it was badly contaminated and putrid.

Table 8-1 Percentage yield of nitrogen content in duplicate samples of yeast extract obtained by autolysis* at different incubation time

	24 hours	48 hours	72 hours
Control	37.90 ± 0.39	59.50 ± 1.07	contaminated
Sodium chloride 5%, ethanol 5%	55.64 ± 0.99	68.50 ± 1.81	72.10 ± 0.70
Sodium chloride 5%, ethanol 10%	43.42 ± 0.83	50.49 ± 0.83	54.10 ± 1.23

* The autolysing temperature and initial pH were 40° C and 6, respectively.

8.3.2 Concentration of sodium chloride and ethanol

Results on percentage yields of nitrogen contents with respect to concentration of sodium chloride and ethanol are shown in Table 8-2.

Table 8-2 Percentage yield of nitrogen content in duplicate samples of yeast extract obtained by autolysis* with addition of sodium chloride and ethanol after 48 hours of incubation

	Percentage yield of nitrogen
Sodium chloride 0%, ethanol 5%	63.67 \pm 1.44
Sodium chloride 5%, ethanol 0%	57.82 \pm 1.30
Sodium chloride 5%, ethanol 5%	65.03 \pm 1.85
Sodium chloride 10%, ethanol 10%	55.22 \pm 0.40

* The autolysing temperature and initial pH were 50°C and 6, respectively.

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8.3.3 Concentration of papain

Results on percentage yield of nitrogen contents with respect to concentration of papain are shown in Table 8-3.

Table 8-3 Percentage yield of nitrogen content in duplicate samples of yeast extract obtained by autolysis* with addition of papain after 48 hours of incubation

	Percentage yield of nitrogen
Control	56.66 \pm 0.40
Papain 0.1%	75.47 \pm 0.77
Papain 1.0%	83.97 \pm 1.01

* The autolysing temperature and initial pH were 50°C and 6, respectively.

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8.3.4 pH and temperature

Results on profiles of percentage yield of nitrogen contents with respect to three different pH and three incubating temperatures are shown in Table 8-4.

Table 8-4 Percentage yield of nitrogen content in duplicate samples of yeast extract obtained by autolysis at different pH and incubating temperatures following 3^2 factorial design experiments with 48 hours of incubation

pH	Temperature ($^{\circ}$ C)		
	40	50	60
4	77.92 \pm 0.19	79.96 \pm 0.68	67.76 \pm 1.20
5	75.90 \pm 1.12	79.61 \pm 0.67	59.98 \pm 1.40
6	78.60 \pm 1.02	81.26 \pm 1.27	61.36 \pm 0.89

Analysis of variance for 3^2 factorial design experiment (see appendix I)

Both pH and temperature affect percentage yield of nitrogen in yeast autolysate at 95% confidential level.

8.3.5 Freezing and thawing

8.3.5.1 Temperature time profiles

Temperature and time profiles of freezing and thawing cycles of yeast cells are shown in Figure 8-1.



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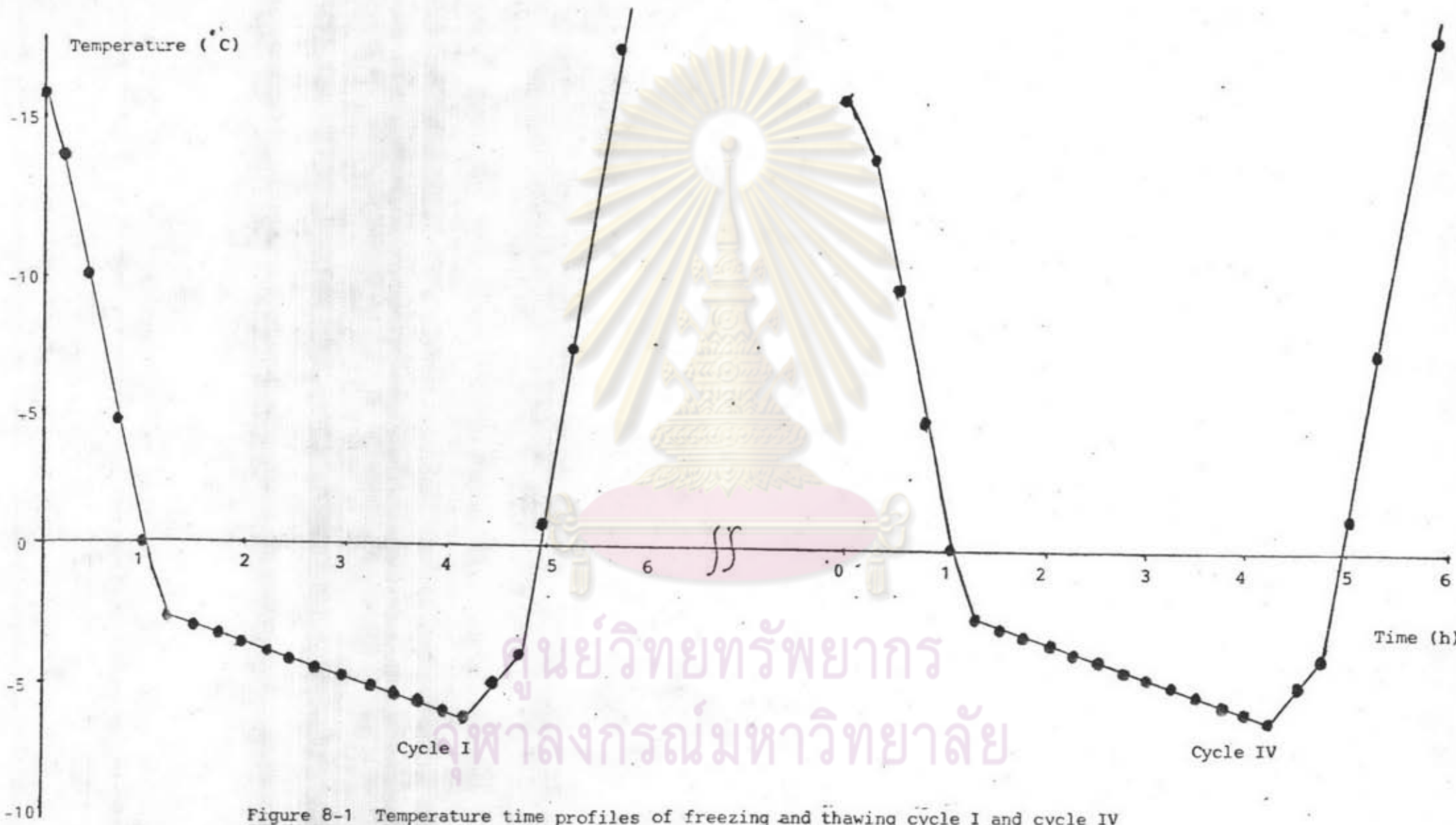


Figure 8-1 Temperature time profiles of freezing and thawing cycle I and cycle IV

8.3.5.2 Freezing and thawing cycles

Results on percentage yield of nitrogen contents with respect to various cycles of freezing-thawing are shown in Table 8-5.

Table 8-5 Percentage yield of nitrogen content in duplicate samples of yeast extract obtained by rupturing yeast cells after various cycles of freezing-thawing

	Percentage yield of nitrogen in extract
Control	38.44 \pm 0.62
Cycle I of freezing and thawing	42.30 \pm 0.20
Cycle II of freezing and thawing	43.97 \pm 0.12
Cycle III of freezing and thawing	46.48 \pm 0.76
Cycle IV of freezing and thawing	46.70 \pm 0.07

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8.3.6 Optimum condition for autolysis of yeast cell using 2^3 factorial design

Results on percentage yield of nitrogen contents with respect to treatment combinations of 2^3 factorial design of experiment with preservative agents, namely, sodium chloride and ethanol, addition of papain and autolysing temperature are shown in Table 8-6. Contrast means of the factors of 2^3 factorial design experiment are shown in Table 8-7 and half normal plot of the contrast means are shown in Figure 8-1.

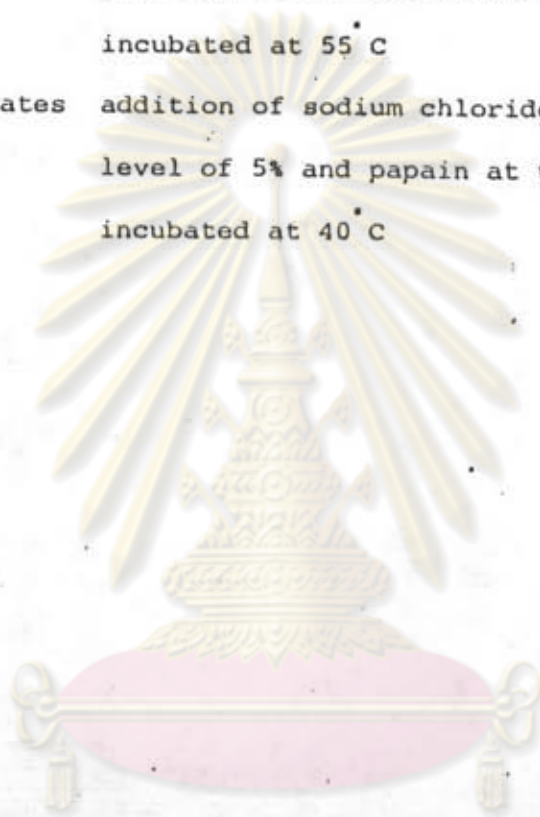
Table 8-6 Result of 2^3 factorial design with preservative agents, addition of papain and autolysing temperature as variables

Treatment combination *	Observation in percentage yield nitrogen
1	56.95 ± 1.84
a	86.31 ± 3.03
b	57.19 ± 0.01
ab	84.54 ± 2.51
c	66.70 ± 1.05
ac	86.96 ± 0.01
bc	65.66 ± 3.80
abc	86.82 ± 1.44

- * 1 indicates control incubated at 55° C
 a indicates control incubated at 40° C
 b indicates addition of sodium chloride and ethanol at the level of 5% incubated at 55° C
 ab indicates addition of sodium chloride and ethanol at the level of 5% incubated at 40° C
 c indicates addition of papain at the level of 0.1% and incubated at 55° C

Table 8-6 (continue)

- * ac indicates addition of papain at the level of 0.1% and incubated at 40 C
- bc indicates addition of sodium chloride and ethanol at the level of 5% and papain at the level of 0.1%, incubated at 55 C
- abc indicates addition of sodium chloride and ethanol at the level of 5% and papain at the level of 0.1%, incubated at 40 C



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Table 8-7 Contrast Table of 2^3 factorial design with preservative agents, addition of papain and autolysing temperature as variables

Treatment Combination	Contrast mean *
a	+23.91
b	-0.05
ab	-0.91
c	+5.91
ac	-4.45
bc	+0.71
abc	+0.102

* Contrast mean is specific comparison among various treatment means of an experiment with ≥ 3 fixed treatments with assumption that the contrasts are independent.

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Figure 8-2 Half normal plot of ranked contrast means in 2³ factorial design

8.4 Discussion on autolysis conditions

8.4.1 Incubation time

Percentage yield of nitrogen increased with incubation time, the rate of increase, however, decreased with respect to time as shown in Table 8-1. Percentage yield of nitrogen content in control samples incubated for 72 hours was contaminated.

8.4.2 Concentration of sodium chloride and ethanol

Experimental results from Table 8-2 show that increasing the concentration of sodium chloride and ethanol at 10% each decreased percentage yield of nitrogen. Without addition of sodium chloride and ethanol, the autolysis may run the risk of putrefaction.

8.4.3 Concentration of papain

Experimental results from Table 8-3 show that, addition of papain to yeast autolysis gave a significantly increased amount of percentage yield of nitrogen. These results confirm the importance of adding exogenous enzyme to enhance yeast autolysis (Chao et al, 1980). Increasing papain concentration increased percentage yield of nitrogen, however, further increasing dose of enzyme may impose higher cost on the process.

8.4.4 pH and temperature

Experimental results from Table 8-4 show that pH and temperature had great effect on the percentage yield of nitrogen. High temperature gave a significantly decreased amount of percentage yield of nitrogen. These results confirm the importance of incubating temperature in the yeast autolysis (Peppler, 1982). Incubating temperature of 40-50 C at pH 6 gave more percentage yield of nitrogen than those at pH 4 and 5.

8.4.5 Freezing and thawing

Experimental results from Table 8-5 show that freezing and thawing did not appreciably affect percentage yield of nitrogen on rupturing yeast cells. From cycle I to cycle IV of freezing and thawing percentage yield of nitrogen of freezing and thawing product was slightly increased.

8.4.6 Optimum condition for autolysis of yeast using 2³ factorial design of experiments

Experimental results from Table 8-2 to 8-5 show that three factors may assist rupture of yeast cells. These factors are preservative agents such as sodium chloride and ethanol, enzyme papain and autolysing temperature. Effects of these three factors will be assessed for interactive effects by using 2³ factorial design of experiments (Cochran, 1957).

Experimental results from Table 8-6 and Figure 8-1 show that contrast mean of incubating temperature was the highest (+23.71). It indicates that at higher incubating temperature the yield of nitrogen in yeast autolysate would be lower. Contrast mean of papain was +5.71. It indicates that papain at 0.1% of dry weight of yeast protein would increase yield of nitrogen in the yeast autolysate. These results conformed with the results of preliminary analysis shown in Table 8-3 and 8-4.