CHAPTER X

CHARACTERISATION OF THE YEAST EXTRACT

10.1 Preparation of yeast extract

10.1.1 Apparatus

10.1.1.1 Rotary vacuum evaporator (BUCHI RE 120)

10.1.1.2 Centrifuge (MSE Centuar 2)

10.1.1.3 Spray drier (Niro Spray Drier, Mobile Minor)

10.1.2 Reagent

10.1.2.1 Lactic acid 80%

10.1.2.2 Papain

10.1.3 Procedure

i) Aqueous yeast extract from alkaline washed and distilled water washed yeast cells were prepared by addition of papain at the level of 0.1% (based on dry weight of yeast protein), adjusted yeast slurries to pH 6.0 by lactic acid and incubated at 50° C for 48 hours.

ii) Separated aqueous extract from cell debris and concentrated by vaccum evaporator (10.1.1.1) at 40° C to paste form (about 65% solid contents).

iii) Sprayed slurry of 30° brix through atomizing nozzle by air pressure at 3 kg/cm^2, temperature of incoming air was 180° C and that of out going air was 100° C.

10.2 Product characterization

10.2.1 Hop substance in yeast extract

10.2.1.1 Apparatus

10.2.1.1.1 Shimadzu L. C. 3A HPLC was used. The instrument was equipped with a U. V. visible spectophotometer and a 0.46 cm. x 25 cm. column packed with Zorbax ODS

10.2.1.1.2 Shaker (GFL)

10.2.1.1.3 Centrifuge (MSE CENTAUR 2)

10.2.1.2 Reagents

10.2.1.2.1 Urea, 6 M (analytical grade)

10.2.1.2.2 Isooctane (spectograde)

10.2.1.2.3 Hydrochloric acid, 6 N (analytical grade)

10.2.1.2.4 Methanol (HPLC grade)

10.2.1.3 Chromatography

The following chromatographic condition were used :

Detection wave length of absorption photometer at 314 nm

Injection volume of 10 microlitre

Flow rate of eluent at 0.8 ml/minutes

Column temperature at 25° C

Pressure of pump at 100 kg/cm^2

Operated in isocratic mode

Mobile phase methanol : distilled water : phosphoric acid 85% = 85 : 17 : 0.25 (v/v/v)

10.2.1.4 Procedure

10.2.1.4.1 In yeast extract

i) Yeast extract (61% dry weight) was diluted to 1:10 with distilled water

ii) Weigh 8.9 gm of diluted yeast extract into a centrifuge tube

iii) Add 0.5 ml of 6 N. hydrochloric acid (10.2.1.2.3) and 20 ml of isooctane (10.2.1.2.2), respectively and place two or three glass beads into the centrifuge tube

iv) Screw a cap on the centrifuge tube

v) Shake for 15 minutes at 130 $\pm$ 5 rpm

vi) Centrifuge the tube for 3 minutes at 3000 rpm

vii) Collect the isooctane layer and then evaporate to dryness

viii) Dissolve the residue with methanol and inject to HPLC

10.2.1.4.2 In beer

Did the same process as 10.2.1.4.1 except pipette 10 ml of beer instead of weighing 8.89 gm of diluted yeast extract.

10.2.2 Composition of yeast extract

10.2.2.1 Total solid (Clerck, 1958)

Did the same process as 7.2.3

10.2.2.2 Total protein (European brewery convention, 1975)

Did the same process as 7.2.1

10.2.2.3 α-Amino acid nitrogen (Indian standard, 1973)

10.2.2.3.1 Reagent

10.2.2.3.1.1 Standard sodium hydroxide solution 0.7143 N.

10.2.2.3.1.2 Phenol red

10.2.2.3.1.3 Phophate buffer solution pH 8.0

10.2.2.3.1.4 Formaldehyde solution, 40% of formaldehyde in water

10.2.2.3.2 Procedure

i) Shake 10 gm of sample in a conical flask with 150 ml distilled water

ii) Stand for one hour, shake occasionally

iii) Centrifuge or filter

iv) Add a few drops of phenol red (10.2.2.3.1.2)

v) Titrate 10 ml of the filtrate with sodium hydroxide solution (10.2.2.3.1.1) to pH 8.0

vi) Use the phosphate buffer solution, together with a few drops of phenol red as a colour standard to indicate the correct end point

vii) Allow to stand for 15 minutes and titrate again to pH 8.0, let this reading be A ml

viii) Now take 8 ml of formaldehyde solution (10.2.2.3.1.4) and add to 10 ml of water

ix) Titrate this mixture to pH 8.0 in the presence of phenol red as before, let this reading be B ml

10.2.2.3.3 Calculation

The percentage of amino acid nitrogen

$$= \frac{A-B}{10M} \times 100$$

M = Mass in gm dry weight of the material taken for the test.

10.2.2.4 Riboflavin (AOAC, 1984)

Riboflavin in the yeast extract obtained was determined by chemical method according to AOAC, 1984 in 43.049-43.042 at Division of Nutrition, Department of Health, Ministry of Public Health.

The analytical work was carried out by official of Ministry of Public Health.

10.2.2.5 Niacin

Niacin in yeast extract was determined by microbiological method according to AOAC, 1984 in 43.126 at Division of Nutrition, Department of Health, Ministry of Public Health. The Analytical work was carried out by Official of Ministry of Public Health.

10.2.2.6 Amino acid

Amino acid in yeast extract was determined by amino acid analyzer (Hitachi KLA 38) at Division of Nutrition, Department of Health, Ministry of Public Health. The analytical work was carried out by Official at Ministry of Public Health.

10.2.2.7 Solubility

i) Dissolved 0.5 gm of the spray dried yeast extract obtained (94% solid content) to 100 ml distilled water

ii) Checked the solution whether it could be dissolved or not

10.2.2.8 Application trial of the yeast extract obtained

Yeast extract is an essential component of cultivating medium for Streptomyces sp 190-1 to produce glucose isomerase. The yeast extract produced in this investigation was applied as the substitute of normally used yeast extract in the medium. Glucose isomerase activity and cell mass of Streptomyces sp 190-1 were determined (Teeradakorn, 1985) for comparison with results using commercial yeast extract.

10.3 Result on characterization of yeast extract

10.3.1 Hop substances

HPLC chromatogram of hop substances in beer and in the yeast extract obtained from alkaline and distilled water washed cells are shown in Figure 10-1 to 10-3.

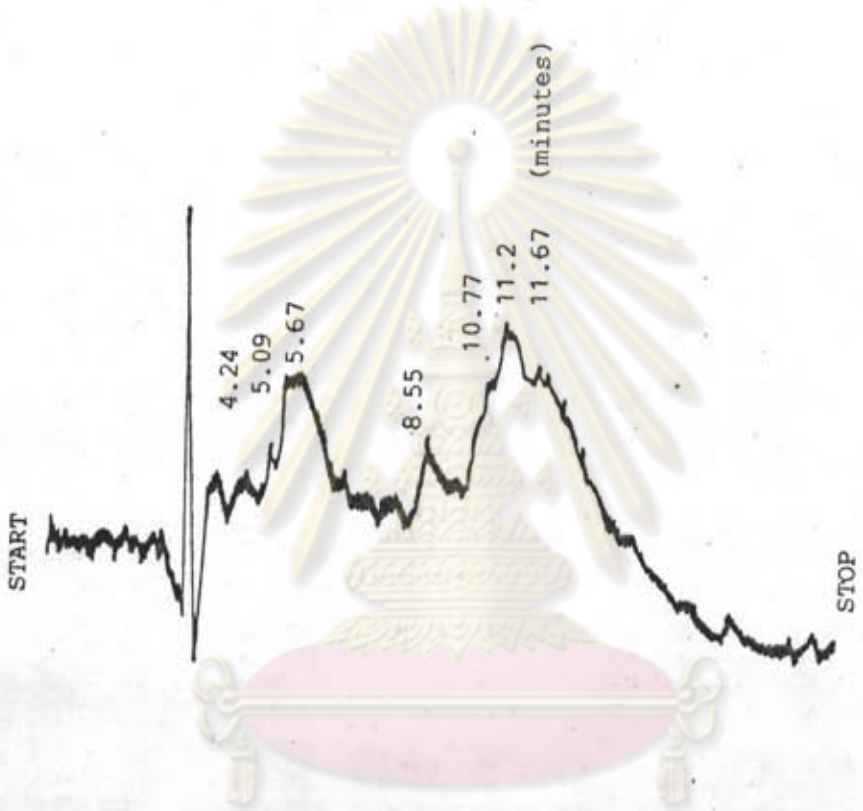

Figure 10-1 HPLC chromatogram of hop substance in beer

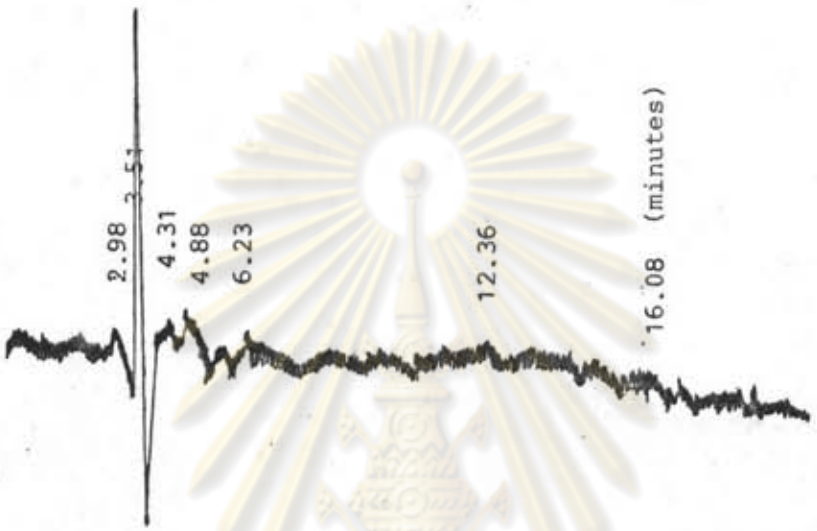

Figure 10-2 HPLC chromatogram of hop substances in the yeast extract obtained from alkaline washed yeast cells

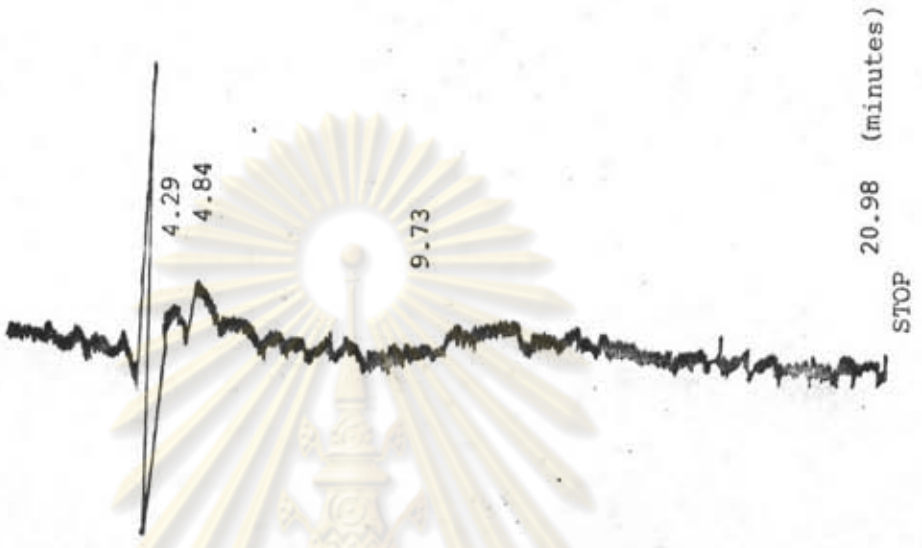

Figure 10-3 HPLC chromatogram of hop substances in the yeast extract obtained from distilled water washed yeast cells

10.3.2 Composition of yeast extract

Results on content of total solid, total nitrogen, amino acid nitrogen, riboflavin, niacin of commercial autolysed yeast extract and the yeast extract obtained are shown in Table 10-1.

Table 10-1 Chemical composition of yeast extract (expressed in percentage)

Components	Obtained yeast extract	Commercial yeast extract
Total solid	65.30 ± 0.55	94.64 ± 0.49
Total nitrogen (based on dry weight)	10.86 ± 0.02	11.28 ± 0.04
Total protein (Nx6.25) (based on dry weight)	67.90 ± 0.13	70.47 ± 0.30
Amino acid nitrogen (based on dry weight)	3.18 ± 0.14	3.33 ± 0.05
Riboflavin (mg/100 g)	11.06	7.5*
Niacin (mg/100 g)	11.24	12.5*

* Peppler, 1982.

10.3.3 Amino acids composition

Results on contents of amino acids of commercial autolysed yeast extract and the yeast extract obtained are shown in Table 10-2.

Table 10-2 Proximate amino acid compositions of five commercial autolysed yeast extracts (Peppler, 1982) and the yeast extract obtained

Amino acids	Composition (g per 100 g of extract)					
	A	B	C	D	E	The yeast extract obtained
Arginine	2.9	1.5	3.7	2.8	0.9	3.6
Cystine	NR	NR	NR	NR	0.6	0.5
Histidine	1.3	0.8	1.4	1.1	1.6	1.7
Isoleucine	2.4	2.2	2.6	2.7	3.4	2.4
Leucine	3.7	3.4	4.0	3.9	4.6	3.2
Lysine	4.2	3.8	4.3	4.0	5.0	3.7
Methionine	0.8	0.7	0.9	0.8	1.1	0.6
Phenylalanine	2.1	1.9	2.4	2.2	2.7	1.7
Threonine	2.4	2.2	2.6	2.5	2.5	2.2
Tryptophan	0.5	0.4	0.5	0.6	1.1	0.5
Tyrosine	1.8	1.5	2.0	1.3	1.8	1.4
Valine	2.8	2.6	3.2	3.2	3.8	2.9
Alanine	3.5	3.3	4.1	4.1	5.8	2.7
Aspartic acid	5.5	4.7	5.9	5.2	7.1	3.8
Glutamic acid	7.0	5.6	8.0	9.9	9.1	6.1
Glycine	2.7	2.3	2.8	2.3	3.3	1.7
Proline	2.4	2.4	2.8	2.1	2.5	1.6
Serine	2.6	2.3	2.8	2.6	3.0	1.9
Total amino acids	49.5	42.5	55.0	52.3	61.0	42.2

NR indicates that the analysis was not reported.

10.3.4 Solubility test

A 0.5% aqueous solution of the spray dried yeast extract was clear with a pH 6.0.

10.3.5 Application trial

The results of assay for glucose isomerase activity and cell mass of Streptomyces sp 190-1 are shown in Table 10-3.

Table 10-3 Glucose isomerase activity produced by Streptomyces sp 190-1 and cell mass of Streptomyces sp 190-1 cultivated with commercial yeast extract and the yeast extract obtained

	Glucose isomerase activity (unit/gm dry cell)	Cell mass $\left(\frac{\text{gm dry basis}}{\text{litre}}\right)$
Commercial yeast extract	334	2.58
Alkaline washed yeast extract	320	2.40
Distilled water washed yeast extract	282	2.11

10.4 Discussion

10.4.1 Hop substances

Experimental results show in Figure 10-1 demonstrated that there were two major groups of peak on HPLC chromatogram of beer. Chromatogram of hop substance in beer could not be identified into isolated components because HPLC analysis was not carried out in gradient mode with adequate chromatographic resolution.

Experimental results show in Figure 10-2 to 10-3 demonstrated that the hop substance in yeast extract from alkaline and distilled water washed cell were not detectable when compared with that of beer with similar soluble solid content. Components of hop extract adsorbed on the yeast cell wall (Dixon, 1968) might be removed together with the insoluble cellular components during aqueous extraction of the autolysed yeast cells.

10.4.2 Composition of yeast extract

Except for the total solid and riboflavin contents, the analytical composition of the yeast extract obtained as shown in Table 10-1 , Table 10-2 conformed with standard commercial yeast extract (The Oxoid Mannual, 1968).

The yeast extract obtained gave significantly different amounts of total solids when compared with commercial yeast extract because the yeast extract obtained was in paste form, and the commercial yeast extract was in powder form. Riboflavin in the yeast extract obtained was more than twice of that in commercial yeast extract. It was probably due to the more riboflavin rich spent yeast from the local recipe of beer processing.

The solubility test of the spray dried yeast extract

obtained (94% solid content) conformed with standard commercial yeast extract (The Oxoid Mannual, 1968).

10.4.3 Application trial

Glucose isomerase activity produced by Streptomyces sp 190-1 and corresponding yield of cell mass cultivated from the yeast extract of alkaline washed cells and distilled water washed cells was not appreciably lower than those cultivated from commercial yeast extract.