

JASSA



*Journal of Applied Science in Southern Africa
The Journal of the University of Zimbabwe*

Volume 6 • Number 2 • 2000

ISSN 1019-7788

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Published by University of Zimbabwe Publications
P.O. Box MP203, Mount Pleasant, Harare, Zimbabwe

Typeset by University of Zimbabwe Publications
Printed by Mazongororo Paper Converters

Susceptibility of selected commercial yeasts to autolysis for yeast extract production

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Ten yeasts comprising nine *Saccharomyces cerevisiae* and one *S. carlsbergensis* were assessed for their susceptibility to autolysis for yeast extract production. The work was done in the period April to June, 2000. Each strain was autolysed in distilled water (pH 6.5) at 45°C for 6 hours and dry weight losses, release of proteins into the medium and pH monitored. Dry weight losses appeared to be the most discriminatory evidence of autolysis. *Saccharomyces cerevisiae* Y1045 and Y58 showed 25.0 percent and 23.9 percent dry weight losses respectively; the other strains autolysed less rapidly. *Lactobacillus bulgaricus* grew comparably well in a basal medium supplemented with 5 percent (v/v) of *S. cerevisiae* Y1045 autolyzate, and in the same basal medium supplemented with 0.5 percent (w/v) commercial yeast extract. The study showed that *S. cerevisiae* Y1045 has the potential for use in yeast extract production

Keywords: *commercial yeasts, autolysis, yeast extract.*

Introduction

Autolysis is a phenomenon by which a microbial cell destroys itself by means of endogenous enzymes. It occurs naturally in a culture after the stationary phase and invariably leads to cell death. During the process, the cell's normal metabolic control of important enzymes is lost: intracellular and cell wall degradative enzymes attack specific substrates uncontrollably, leading to production of soluble amino acids, nucleotides and other materials which leak out through the weakened walls into the extracellular environment.

Since autolysis coincides with the death phase of a culture, it has important economic consequences in industrial processes where microbial cells constitute the final product or are used as agents of production. For example in the fermentation industry where yeast is traditionally used, autolysis is undesirable in brewing, bakers' yeast production and in bread-making. In these processes, it can lead to development of unwanted flavours, to low biomass production and to loss in leavening power, as appropriate. However, autolysis is profitably applied to yeast

extract/autolyzate production by yeast extract manufacturers: fully-grown yeast slurries are incubated at suitable temperatures (45 to 55°C) for up to three days. Soluble extracts which are processed into powders are widely used as flavouring agents and as additives to foods. Additionally, yeast extract is used as a rich source of vitamins and other growth factors in culture media used in microbiology laboratories and in fermentation industries. In biochemical research yeast autolysis is also applied to the extraction of some enzymes. The various aspects of yeast autolysis and yeast extract manufacture have been discussed in detail by Hough and Maddox (1970) and by Phaff *et al.*, (1978).

Commercial production of yeast extract constitutes an important aspect of biotechnology and it is estimated that world wide annual production amounts to 35 000 tons (Reed and Nagodawithama, 1991). In Zimbabwe, yeast extract is readily available, as an imported product for use in food preparation and in microbiology work. In an earlier article in which microbial biotechnology in Zimbabwe was reviewed (Okagbue, 1995), it was suggested that yeast extract can be produced as an ancillary product of existing yeast biomass production facilities, to conserve the country's foreign exchange. Unfortunately, despite the apparent simplicity of the process of autolysis, yeast strains differ considerably in their susceptibility to the phenomenon (Okagbue and Lewis, 1984). In this study, some commercial yeasts have been subjected to an autolytic screening process in order to identify any or those suitable for use in yeast extract production.

Materials and Methods

A total of 10 strains were studied. Eight of them comprising seven *S. cerevisiae* (Y48, Y51, Y53, Y58, Y107, VIN7, Y1045) and one *S. carlsbergensis* (Y2) were obtained from a brewery at Harare, Zimbabwe. The remaining two strains (Br9 and Br10) were obtained by plating out on malt extract agar (HiMedia) adjusted to pH 4.6, suspensions prepared with imported bakery yeasts contained in sachets purchased from a supermarket. Inoculated plates were incubated at 28°C and resulting colonies were purified by repeated streaking on the same type of agar medium. All the 10 strains were maintained on malt extract agar slants at 5°C and subcultured every two weeks.

Inoculum used for propagating each strain was produced by introducing a loopful of the organism from the slant into a test tube containing 5 ml 0.82 percent w/v sterile saline. The contents of each tube were then used to inoculate four 500 ml Erlenmeyer flasks, each containing 100 ml of malt extract broth (HiMedia). The flasks were incubated at room temperature (26 to 28°C) on an orbital shaker (Precision Scientific, Chicago) at 200 rpm for 24 hours. Cell mass was harvested by centrifugation at 10 000 rpm for 15 minutes and washed twice with sterile distilled water.

Autolysis was carried out using the method described by Hough and Maddox (1970) in which the biomass was suspended in distilled water (pH 6.5) at 45°C, with

occasional stirring. Samples were withdrawn immediately after the suspension and subsequently at 2 hour intervals for a total period of 6 hours and assessed for some indicators of autolysis.

Parameters used for assessing suspended yeast biomass for autolysis were: dry weight of biomass and protein concentration in the extracellular fluid (Hough and Maddox, 1970).

Exactly 5 ml of autolyzate taken as described above was put into duplicate pre-weighed aluminum pans. The pans and the contents were then dried in an oven at 105°C for 18 hours. They were cooled in a desiccator for 30 minutes and then weighed. The losses in dry weights determined for the samples were calculated as percentages of the dry weight of the unautolysed biomass.

Change in protein concentration during incubation was determined using the Bradford method (Lewis *et al.*, 1980). A volume of the autolyzate was centrifuged and 0.1 ml of the supernatant pipetted into duplicate test tubes. Exactly 5 ml of Coomassie dye reagent was added and the tubes shaken well. They were then left standing for 10 minutes before reading the absorbance at 595 nm against a blank containing 0.1 ml distilled water instead of yeast extract. Actual protein concentration was determined using a protein standard curve prepared with bovine serum albumin as the standard.

The yeast strain that showed the greatest susceptibility to autolysis was propagated further and the resultant biomass autolysed as before. Exactly 5, 10, 15, and 20 ml volumes of the liquid extract obtained after centrifugation of the autolysed yeast were incorporated into a basal broth medium consisting of (as final concentration (w/v) tryptone (4.6 percent), gelatin (5.7 percent) glucose (11.5 percent), lactose (11.5 percent), sucrose (11.5 percent), sodium chloride (9.2 percent), sodium acetate (3.4 percent) and ascorbic acid (1.15 percent). The basal medium was essentially a modification of conventional Elliker's medium (Lactic agar) except that agar and yeast extract were omitted. The complete medium (Elliker's medium) is well known to promote growth of lactic acid bacteria (Elliker *et al.*, 1956). A total of 100 ml of the experimental medium were prepared in each 250 ml Erlenmeyer flask with the stated volumes of the yeast autolyzate and distilled water being used as appropriate to make the final volume. Exactly 100 ml of complete Elliker's (Lactic) broth containing commercial yeast extract was also prepared. All the flasks were incubated with equal volumes (5 ml) of a pure culture of *Lactobacillus bulgaricus* obtained from Chibuku Breweries at Harare. Lactic broth containing yeast extract was used as the blank. Growth of *Lactobacillus* was spectrophotometrically determined at 600 nm after 24 hours.

Results

Tables 1 and 2 show the responses of the 10 yeast strains to autolysis when they were assessed for specific autolysis indicators *viz*: dry weight losses and protein concentration in the extract.

All the strains recorded losses in dry weights, to various extents, when they were autolysed in distilled water (pH 6.5) at 45°C for 6 hours (Table 1). *S. cerevisiae* Y1045 was the most susceptible to autolysis, followed by Y58 (25.0 percent and 23.9 percent losses in dry weights respectively). All other strains recorded less than 20 percent losses in dry weight with Br10 being the least (2.9 percent loss in dry weight).

Table 1: Changes in dry weights of yeast strains during autolysis in distilled water at 45°C.

Strain	Dry weight change (g/ml) at			
	0 hour	2 hours	4 hours	6 hours
Y1045	(0.068)* 100**	(0.056) 82.4	(0.054) 79.4	(0.051) 75.0 (1)***
Y51	(0.078) 100	(0.067) 85.9	(0.066) 84.6	(0.065) 83.3 (5)
Y58	(0.046) 100	(0.044) 95.7	(0.035) 76.1	(0.035) 76.1 (2)
Y53	(0.037) 100	(0.033) 89.2	(0.030) 81.1	(0.03) 81.1 (3)
Y52	(0.038) 100	(0.038) 100	(0.036) 94.7	(0.036) 94.7 (2)
Y107	(0.045) 100	(0.045) 100	(0.042) 93.3	(0.042) 93.3 (7)
VIN7	(0.063) 100	(0.062) 98.4	(0.059) 93.7	(0.059) 93.7 (8)
Y48	(0.089) 100	(0.080) 89.9	(0.079) 88.8	(0.073) 82.0 (4)
Br9	(0.090) 100	(0.089) 98.9	(0.079) 87.8	(0.076) 84.4 (6)
Br10	(0.069) 100	(0.068) 98.6	(0.067) 97.1	(0.067) 97.1 (10)

Data given as means of duplicate determinations.

* Data in brackets indicate actual dry masses of biomass (g/ml) from the yeast suspension in distilled water at various times.

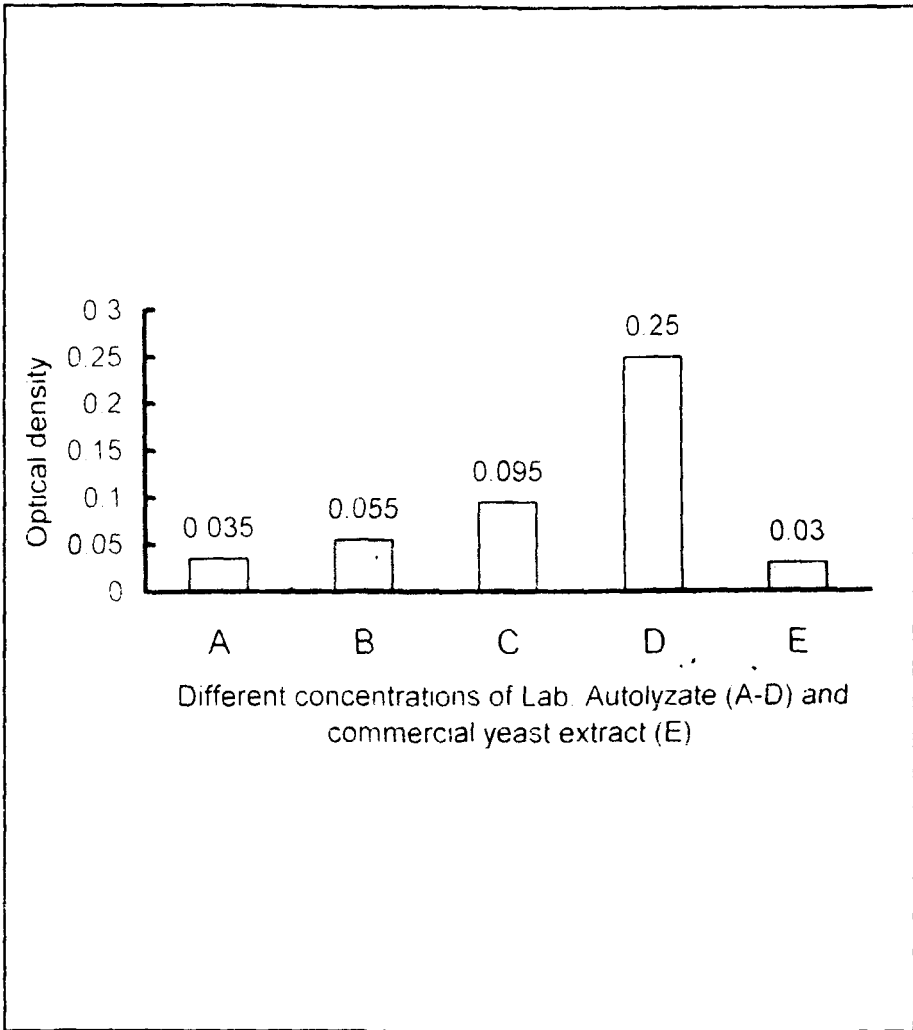
** Unbracketed data refer to percent dry weights of biomass at various times.

*** Figures indicate the order in which the strains responded to the treatment.

Table 2: Change in protein concentration of yeast extracts during autolysis.

Strain	Change in protein concentration (mg/ml)			
	0 hour	2 hours	4 hours	6 hours
Y1045	0.04	0.44	0.51	0.56
Y51	0.07	0.17	0.17	0.21
Y58	0.02	0.39	0.42	0.48
Y53	0.05	0.51	0.51	0.52
Y2	0.04	0.43	0.51	0.54
VIN7	0.03	0.34	0.40	0.42
Y48	0.02	0.25	0.26	0.26
Y107	0.05	0.51	0.51	0.52
Br9	0.05	0.52	0.52	0.53
Br10	0.02	0.26	0.26	0.27

Data given as means of duplicate determinations.



Medium

- A Basal medium + 5 percent (v/v) experimental extract.
- B Basal medium + 10 percent (v/v) experimental extract.
- C Basal medium + 15 percent (v/v) experimental extract.
- D Basal medium + 20 percent (v/v) experimental extract.
- E Complete Elliker's broth (containing commercial yeast extract (0.5 percent w/v)).

Figure 1: Growth of *L. bulgaricus* in a basal medium supplemented with different amounts of the *S. cerevisiae* Y1045 autolyzate and growth in complete Elliker's medium containing commercial yeast extract.

Saccharomyces species Y1045 and Y2 released the greatest amount of protein (0.52, and 0.50 mg/ml respectively) into the extracellular fluid during the 6 hours autolysis period (Table 2). Br9, Y53 and Y58 had fair abilities to release protein and the remaining strains released relatively little amounts of protein into the extracellular fluid.

Media containing yeast extract produced from *S. cerevisiae* Y1045 promoted the growth of *L. bulgaricus* better than complete Ellikers medium containing commercial yeast extract (5 percent w/v) (Figure 1). Growth in basal medium containing different amounts of experimental yeast extract increased with increasing amounts of yeast extract (medium containing 5 ml yeast extract had optical density 0.035 as compared to 0.25 for medium containing 20 ml of yeast extract). Optical density for medium containing commercial yeast extract was 0.03 after 24 hours of incubation.

Discussion

Yeasts which readily autolyse in relatively simple systems are potentially useful for industrial production of yeast extract. Such systems should be employed in developing countries where use of unsophisticated equipment is desirable (Okagbue, 1995).

Most of the yeast strains studied in this work exhibited appreciable levels of autolysis in distilled water at 45°C (Table 1). Thus they are potentially useful for large scale production of yeast extract using distilled water as the autolysing medium. Recovery of yeast extract from water would be relatively cheap since the purification steps usually needed when other systems such as toluene (used in preparation of yeast invertase) would be precluded. The differences in the autolytic responses of all the yeast strains are obviously due to strain differences since all of them were propagated in the same manner.

Although release of proteins and amino acids is generally considered to be the most important aspect of yeast autolysis (Hough and Maddox, 1970), protein release into the extracellular medium, in this study, showed no direct relationship to the level of autolysis (Table 2). *S. carlsbergensis* Y2, which recorded only a 5.3 percent decrease in dry weight was second to Y1045 in the amount of protein released into the extracellular medium after 6 hours. Y58 was second to Y1045 in loss of dry weight but released only a fair amount of protein into the extra cellular medium (0.46 mg/ml). Again the observed trend may have also been due to strain differences. Activities of the different proteolytic enzymes involved in cell wall break down may have been different for various stains resulting in the differences of protein release to the extracellular fluid.

S. cerevisiae Y1045 appears to have considerable potential for industrial production of yeast extract. It responded very favourably to the attributes tested for in this study, through mainly its high susceptibility to autolysis at 45°C and the high protein content in the resulting yeast extract. Although Y58 was highly autolysable, protein content of the resulting yeast extract was not high enough, thus reducing

its suitability as a protein supplement for foods. The yeast extract produced using *S.cerevisiae* Y1045 compared favourably with commercial yeast extract.

While consistent increases in levels of growth of *L. bulgaricus* were observed in media using different concentrations of the yeast extract, only the growth in the 5 percent w/v appeared to be comparable to the growth achieved in the complete medium containing 5 percent w/v commercial yeast extract. This suggests that the 5 ml of the autolyzate from Y1045 was comparable to 0.5 g of the commercial yeast extract.

In general this study has helped to identify a yeast strain that could be developed further for commercial production of yeast extract. *S. cerevisiae* Y1045 can be autolysed relatively easily in simple medium comprising distilled water (pH 6.5) at 45°C and also showed indications of good nutritional composition.

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