J. Serb. Chem. Soc. 72 (5) 451–457 (2007) UDC 582.282.23:664.642:66.094.941+577.15
JSCS–3576 *Original scientific paper*

Utilization of baker's yeast (*Saccharomyces cerevisiae)* for the production of yeast extract: effects of different enzymatic treatments on solid, protein and carbohydrate recovery

TATJANA VUKAŠINOVIĆ MILIĆ*, MARICA RAKIN and SLAVICA ŠILER-MARINKOVIĆ#

Faculty of Technology and Metallurgy, Karnegijeva 4, Belgrade, Serbia
(e-mail: tanjavmilic@yahoo.com)

(Received 5 May, revised 4 December 2006)

Abstract: Yeast extract (YE) was produced from commercial pressed baker's yeast (active and inactivated) using two enzymes: papain and lyticase. The effects of enzyme concentration and hydrolysis time on the recovery of solid, protein and carbohydrate were investigated. Autolysis, as a basic method for cell lysis was also used and the results compared. The optimal extraction conditions were investigated. The optimal concentrations of papain and lyticase were found to be 2.5 % and 0.025 %, respectively.

Keywords: baker's yeast, yeast extract, enzymatic hydrolysis, papain, lyticase.

INTRODUCTION

Yeast cells contain a lot of protein, carbohydrates, lipid, vitamins, and minerals. Yeast extract (YE) comprises the water-soluble components of the yeast cell, the composition of which is primarily amino acids, peptides, carbohydrates and salts. Nitrogen components and vitamins are the value of yeast extract because of their nutritional characteristics. Hence, YE has been mainly used in the food industry, as a flavoring agent in soup, sauces, gravies, stews, snack food and canned food,[1] as well as in pet foods and cosmetic materials, and as a plant nutrient. Other applications include vitamin and protein supplements in health foods and as a source of nutrients in microbiological media.[2]

The carbohydrates are not such an important nutritional component of YE but it is important to know their recovery kinetics if cell walls are used for carbohydrate isolation (glucans, mannans) after yeast extract production.

YE is manufactured by breaking down the cells using endogenous or exogenous enzymes. There have been many reports on the manufacturing processes.[3,4]

* Author for correspondence.

Serbian Chemical Society active member.

doi: 10.2298/JSC0705451V

Autolysis by endogenous enzymes occurs naturally in yeasts when they complete the cell growth cycle and enter the death phase. Autolysis is a traditional disruption method in YE production but it has some disadvantages: low yield, difficulty in solid–liquid separation due to the high content of residue in the autolysate, poor taste characteristics as a flavor enhancer and risk of deterioration due to microbial contamination.

Plasmolysis is a modified autolysis process in the presence of a so-called accelerator, such as an inorganic salt (sodium chloride) or organic solvent (toluene, ethanol, ethyl acetate).[5,6] Despite its simplicity, yeast extract manufactured by plasmolysis may have limited use, since there is a growing demand for low-salt processed foods.

Mechanical discruption is process that includes homogenization, sonication, bead milling or high pressure treatment.[7–9]

Hydrolysis which is performed by acid or exogenous enyzmes is the most efficient method of solubilizing yeast. Despite a high production yield, acid hydrolysis is less attractive to manufacturers because of the relatively high capital investment cost, high salt content and high probability of containing carcinogenic compounds, such as monochloropropanol and dichloropropanol.[10]

The enzymatic degradation of the yeast is carried out by means of suitable enzyme preparations of bacterial, vegetable, yeast, or animal origin.[8,11–13] If part of the enzymatic degradation is done before the yeast is inactivated, the own internal enzyme activity of the yeast may also contribute to the process (active yeast). The employed enyzme preparations have one or more of the following activities: proteolytic activity, cell wall degradation activity, amylase or glycogen degrading activity, RNA degrading activity leading to 5'-ribonucleotides, lipolytic activity or deaminase activity.

In this study, yeast extract from commercial pressed baker's yeast cells by autolysis and enzymatic treatment was produced. The strategy was to hydrolyze both active and inactivated yeast cells using different concentrations of these enzymes and different incubation time and to develop a process for the production of high-protein yeast extracts.

EXPERIMENTAL

Materials

The fresh yeast ("Vrenje" – Spirit and Yeast Factory, Belgrade, Serbia) used in this study was purchased at a green market. The yeast was stored at 8 °C and was taken out just before running the experiment to avoid contamination and the reduction of its enzyme activities. Two types of enzyme were used in the yeast hydrolysis: papain (Sigma, Switzerland) from papaya latex (3.2 units/mg solid) and lyticase (Sigma, Switzerland) from *Artrobacter luteus* (983 units/mg solid).

Yeast degradation

Autolysis. Fresh baker's yeast was suspended in 10 ml distilled water to achieve a concentration of 50 % (w/v). The yeast suspensions were placed in 100 ml glass vessels immersed in a temperature-controlled water bath with shaker. The initial pH was adjusted to 5.2 with NaOH (10 M). The autolysis was carried out at 52 °C for 72 h. The reaction mixture was shaken at a speed of 120 rpm.

To determine the solid, protein and carbohydrate contents of the autolysate, the reaction mixture was heated in a water bath at 100 °C for 10 min to deactivate the enzyme and centrifuged (3500 rpm) for 10 min.

Enzyme hydrolysis. Fresh baker's yeast was suspended in 10 ml distilled water to achieve a concentration of 50% (w/v). The yeast suspensions were placed in 100 ml glass vessels immersed in a temperature-controlled water bath with shaker. The initial pH was adjusted to 6.0 with NaOH (10 M) and the solution was heated in a water bath at 100 °C for 10 min to deactivate the yeast's endoenzymes. When active yeast was used, the hydrolysis is performed without this heat treatment. Papain (0.5, 1.0, 2.5 and 5.0 %) or lyticase (0.005, 0.010, 0.020 and 0.025 %) were added. The hydrolysis was performed at 60 °C. The time of hydrolysis and the shaking speed were as for the autolysis. After all the enzymatic treatments, the hydrolysate was heated (boiling water bath, 10 min) and centrifuged.

Analytical methods

The total solids in the fresh yeast and YE were determined by the dry weights following drying at 105 °C until constant mass was achieved. The total solids content was expressed as a % of solids recuperated in the YE with respect to the total solids present in the fresh yeast.

The total crude protein content was determined by the Lowry method.[14]

For the determination of the carbohydrate content, the "basic filtrate" was used. It was obtained from the "basic solution" by precipitation with a saturated plumbous acetate solution.[15] Both the standard and the samples were analyzed by the anthrone–sulphuric acid method.[16]

RESULTS AND DISCUSSION

Effects of papain treatment on the recovery of solid, protein and carbohydrate content

Inactivated yeast. Chao *et al*. reported that the most effective enzymes in aiding yeast autolysis belong to the group of sulfhydryl-proteases, which includes papain, ficin, and bromelain.[17] Papain was the most effective among these three proteases. In the present study, the yeast cells were treated with different concentrations of papain and the influence of different enzyme dosages on the recovery of solid and protein are shown in Fig. 1. Each result represents an average value from triplicate measurements. The results obtained from the process of autolysis are also shown.

The release of solid and protein recovery increased with increasing incubation time and papain dosage. When a concentration of 0.5 % papain was used, there was no significant difference between the hydrolysis and autolysis. Lower concentrations of intracellular components were obtained using this papain concentration, compared to the process of autolysis. Therefore, yeast endoenzymes show more efficiency in the proteolysis than 0.5 % of papain. The optimum dosage of papain was found to be 2.5 %. The maximum solid, protein, and carbohydrate contents, obtained after 72 h of hydrolysis, with this concentration of papain were: 59.84 %, 56.75 mg/ml and 9.83 mg/ml, respectively. A further increase in the papain dosage to 5.0 % resulted in a negligible increase in the recovery, which was not proportional to the increase in the enzyme dosage. This indicated that the active centers of the enzyme were saturated at a concentration of 2.5 %. Therefore, a further increase in the enzyme dosage had no significant influence on the release of protein.

The maximum carbohydrate concentration obtained by papain hydrolysis of yeast cells was lower than 10 mg/ml. Approximately the same content was ob-

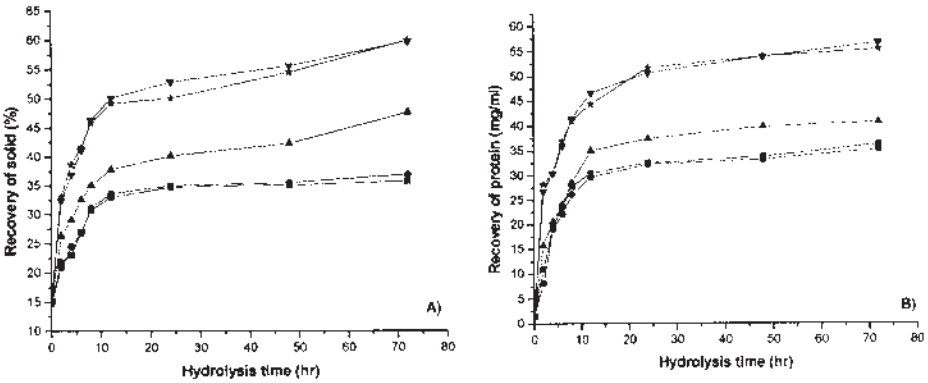

Fig. 1. Time dependence of the recovery of solid (A) and of protein (B) using different papain dosages: ■ – ■, without papain (autolysis); ● – ●, 0.5 %; ▲ – ▲, 1.0 %; ▼ – ▼, 2.5 %; ★ – ★, 5.0 %.

tained after only 2 h of autolysis. Papain is proteolytic enzyme and the yeast endoenzymes were inactivated; hence this low carbohydrate concentration must have resulted from the dissolution of monosaccharides and disaccharides.

Extraction of carbohydrates during autolysis is a two-phase release. During the first eight hours, most of the reserve carbohydrates (glycogen and trehalose) were hydrolyzed. Then, the yeast degradation passed through a stationary phase, which lasted approximately 24 h. There was not important change in carbohydrate concentration during the stationary phase. The content of carbohydrates increased during the last 48 h of treatment. This was due to the degradation of structural carbohydrate (glucans and mannans). This is very important when cell walls after the production of yeast extract are employed for the isolation of glucan or mannan. The walls from the stationary phase should be used because their degradaton had not yet commenced and all of the structural carbohydrates are intact.

Considering total yeast degradation, including the disruption of the yeast cell walls, the optimal hydrolysis time is 24 h. A solid recovery of 52.83 % of the crude dry matter and 50.68 mg/ml of proteins were attained after treatment for 24 h with the optimal papain dosage of 2.5 %.

Active yeast. When active yeast cells were used, a synergistic activity of yeast endoenzymes and papain was achieved. In the previous case, the solid and protein recovery strongly depended on the enzyme dosage and 2.5 % of papain was found to be optimal. However, the combined effects of yeast endoenzymes and papain are not equal to the sum of their individual activity. This can be explained by the lower autolytic activity of the endoenzymes, the cause of which were the conditions of papain hydrolysis (t = 60 °C and pH 6.0), which were not optimal for the activity of the endoenzymes.

As shown in Table I, a high solid recovery of 61.95 % was obtained by papain treatment of native baker's yeast. This solid recovery was higher than that (52.5 %) reported previously and obtained using a combined treatment of proteolytic enzymes.

TABLE I. Comparison of the yield of yeast extracts derived from different proteolytic processes

Yeast strain	Solid recovery/%	Reference
Baker's yeast	61.95	This work
	50.0	8
Brewer's yeast	55.1	12
	42.0	18

The carbohydrate content was higher than that obtained with inactivated yeast and it was not affected by the papain dosage.

Effect of lyticase treatment on recovery

Inactivated yeast. Lyticase hydrolyzes poly-(β-1,3-glucose), such as yeast cell-wall glucan. Therefore, an increased carbohydrate content in the YE, resulting from the release of cell-wall carbohydrates, is to be expected.

The recovery of solid and carbohydrate with different concentrations of lyticase, added at concentration of 0.005 %, 0.01 %, 0.02 %, and 0.025 %, is illustrated in Fig. 2. The results are compared with those achieved by autolysis.

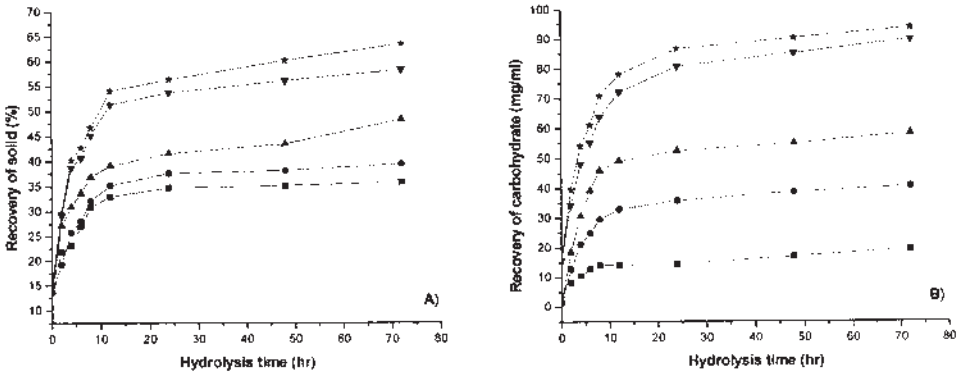

Fig. 2. Time dependence of the recovery of solid (A) and of carbohydrate (B) using different lyticase dosages: ■ – ■, without luticase (autolysis); ● – ●, 0.005 %; ▲ – ▲, 0.01 %; ▼ – ▼, 0.02 %; ★ – ★, 0.025 %.

The release of solid and carbohydrate recovery increased with increasing incubation time and lyticase dosage and as shown in Fig. 2, no matter which dosage of the enzyme was employed, the values attained were higher than those obtained by the autolysis process. This increase is approximately proportional to the enzyme dosage until a dosage of 0.02 %, but decreased with a dosage of 0.025 %. Further increasing of enzyme dosage would increase the cost of the process which would not be proportional to the achieved effects of hydrolysis. Therefore, a concentration of 0.025 % of lyticase was found to be optimal. After 72 h, the solid, protein and carbohydrate recovery reached 63.52 %, 10.14 mg/ml and 93.71 mg/ml, respectively.

The low content of protein resulted from the deactivation of the proteolytic enzymes of the yeast. The high temperature and protein–carbohydrate complex (glucomannoproteins of the yeast cell wall) degradation cause this low protein release. The release of carbohydrates, in contrast to autolysis, was not a two-phase process because the yeast structural carbohydrates were degraded from the beginning of the process.

Active yeast. Higher contents of the yeast constituents were obtained in the extracts with active than than with inactive yeast. The maximal contents of 69.15 % of crude solid, 37.38 mg/ml of protein and 98.39 mg/ml of carbohydrates were obtained with 0.025 % of lyticase after 72 h of hydrolysis. As explained previously for papain, the degradation of the native yeast was achieved by the synergistic activity of yeast endoenzymes and lyticase. The protein recovery strongly depended on such activity.

CONCLUSIONS

In the enzymatic hydrolysis of yeast cells, the enzyme dosage strongly affected the recovery of the cell constituent. The optimum dosages of papain and lyticase were found to be 2.5 % and 0.025 %, respectively. The use of active yeast resulted in maximum degradation. Papain treatment under optimized conditions produced yeast extract which contained a maximum protein content of 56.38 mg/ml with a high solid recovery of 61.95 %. Lyticase treatment under optimized conditions produced yeast extract which contained a maximum carbohydrate content of 98.39 mg/ml. Consequently, a process using active yeast and papain was developed for the production of high-protein yeast extract from baker's yeast.

ИЗВОД

ПРИМЕНА ПЕКАРСКОГ КВАСЦА (*Saccharomyces cerevisiae*) ЗА ДОБИЈАЊЕ КВАШЧЕВИХ ЕКСТРАКАТА: УТИЦАЈ РАЗЛИЧИТИХ ПАРАМЕТАРА ЕНЗИМСКЕ ХИДРОЛИЗЕ НА САДРЖАЈ СУВЕ МАТЕРИЈЕ, ПРОТЕИНА И УГЉЕНИХ ХИДРАТА

ТАТЈАНА ВУКАШИНОВИЋ МИЛИЋ, МАРИЦА РАКИН и СЛАВИЦА ШИЛЕР-МАРИНКОВИЋ

Технолошко-металуршки факултет, Карнегијева 4, 11000 Београд

За добијање квашчевих екстраката је коришћен комерцијални пресовани пекарски квасац (нативни и инактивисани). Екстракција је вршена поступком ензимске хидролизе папаином и литиказом. Испитиван је утицај различитих концентрација ензима и времена трајања хидролизе на садржај суве материје, протеина и угљених хидрата у екстрактима. Резултати су поређени са резултатима добијеним аутолизом, као основним поступком за добијање екстраката. Утврђени су оптимални услови екстракције. Оптимална концентрација папаина била је 2,5 %, а литиказе 0,025 % у односу на суву материју квасца.

(Примљено 5. маја, ревидирано 4. децембра 2006)

REFERENCES

1. R. Sommer, *9th International Symposium on Yeasts,* Sydney, 1996, www.abfingredients.com/files/upload/Yeast%2-Extract1.pdf
2. S. Saksinchai, M. Suphantharika, C. Verduyn, *World J. Microbiol. Biotecnol.* **17** (2001) 307
3. K. Breddam, B. H. Chung, *Appl. Microbiol. Biotechnol.* **35** (1991) 323
4. S. J. Choi, H. J. Chung, *Kor. J. Biotechnol. Bioeng.* **13** (1998) 308
5. K. J. Shetty, J. E. Kinsella, *Biotechnol. Bioeng.* **20** (1978) 755
6. C. P. Champagne, J. Barrete, J. Goulet, *Food Res. Int.* **32** (1999) 575
7. M. G. Lindblom, H. L. Mogren, USP 3996104, 1976
8. C. Verduyn, A. Suksomcheep, M. Suphantharika, *World J. Microbiol. Biotechnol.* **15** (1999) 57
9. K. A. J. Borthwick, *J. Microbiol. Methods* **60** (2004) 207
10. T. Nagodawithana, *Food Technol.* **46** (1992) 138
11. R. P. Potman, J. Wesdorp, USP 5288509, 1994
12. H. J. Chae, H. Joo, M.-J. In, *Bioresour. Technol.* **76** (2001) 253
13. J. Conway, H. Gaudreau, C. P. Champagne, *Can. J. Microbiol.* 47 (2001) 18
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193** (1951) 265
15. J. Trajković, J. Baras, M. Mirić, S. Šiler, *Analiza životnih namirnica,* Tehnološko-metalurški fakultet Univerziteta u Beogradu, 1983 (in Serbian)
16. M. Dubois, K. A. Giles, J. K. Hamilton, P. A. Rebus, F. Smith, *Anal. Chem.* **248** (1956) 3441
17. K. C. Chao, E. F. McCarthy, G. A. McConaghy, USP 4218481, 1980
18. S.-K. Lee, K.-H. Park, U.-H. Pek, J.-H. Yu, *Kor. J. Appl. Microbiol. Biotechnol.* **21** (1993) 276.