

Influence of Incubation Conditions on Hydrolysis Efficiency and Iodine Enrichment in Baker's Yeast

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Abstract The influence of incubation conditions, enzyme type, hydrolysis time, and potassium iodide concentration on hydrolysis and iodine enrichment were studied in supernatant and pellets of *Saccharomyces cerevisiae* hydrolysates. The type of enzyme used and incubation time significantly influence hydrolysis efficiency and protein concentration in supernatant and pellet. The highest protein hydrolysis efficiency was obtained by 24-h incubation with papain. Significantly lower values were observed for pepsin and autolysis. The potassium iodide concentration influences the iodine content of supernatant and pellet, but not hydrolysis. Iodide enrichment of supernatant and pellet depends on the concentration of iodide using during incubation. High concentration of iodide and long incubation times were the conditions for optimal iodide enrichment and high-protein hydrolysates. The optimal hydrolysis efficiency and iodine enrichment were obtained during 24-h incubation with papain in a 4.5-mM potassium iodide medium. The efficiency reached 98.22% with iodine concentrations of 2,664.91 and 9,200.67 µg/g iodine in pellet and supernatant, respectively.

Keywords Baker's yeast · Incubation conditions · Hydrolysis efficiency · Hydrolysates · Iodine enrichment

Introduction

Yeast autolysis is used in the food industry as a method for production of yeast hydrolysates. This process occurs when the yeast is entering the death phase, after consuming the available nutrients. Autolysis is a slow process, so it is necessary to seek the optimal conditions to make it faster [1, 2].

Different conditions have been tried to obtain yeast autolysate. The process temperature, pH, degree of homogenization, vitamins, and use of autolysis promoters and solvents are some of the factors that have been studied [1–3]. These factors were studied as independent variables, but in some cases, interactions between the factors were observed [4].

Saccharomyces cerevisiae has over 30 proteolytic enzymes that are active in a wide pH range [3]. In intact cells, the enzymes remain as inactive precursors within intracellular vacuoles. Activation begins when intracellular energy sources have been used and the cells' structure has been disrupted. This slow process can be sped up by mechanical disintegration of the cells and increases in temperature. Disintegration leads to rupture of lysosomes and release of digestive enzymes. Common methods of mechanical disintegration are bead milling, sonication, high pressure, and homogenization [1, 5, 6].

There are three important enzymes in *S. cerevisiae*, denominated proteases A, B, and C. Protease A is an activator of the other two. It is an acidic protease with optimal activity in the 2–3 pH range. Protease B shows maximum activity at pH 9, while protease C, known as carboxypeptidase, digests optimally at pH 5–6. Some amount of protease

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B is necessary for the activation of protease A. These enzymes work efficiently at temperatures $>40^{\circ}\text{C}$, but are inhibited at 50°C , except carboxypeptidase, which resists higher temperatures and is more active at 50°C . At 40°C , proteolysis occurs mostly due to the activity of protease B. Carboxypeptidase acts during the last phase of autolysis producing free amino acids [4, 5].

Several substances are used to enhance autolysis, including organic solvents such as ethanol and ethyl acetate, sodium chloride, and chitosan. At 40°C , NaCl inhibits autolysis, probably by stabilizing the complex of protease B with its specific inhibitor, but at 50°C , it activates autolysis by releasing carboxypeptidase from its inactive inhibitor-bound form [5].

Exogenous digestive enzymes are used in production of yeast hydrolysates. Sulfhydryl proteases of plant origin are quite effective, especially papain [1, 6]. Papain can be used in concentrations that go from 0.1% to 2.5% at temperatures in the $50\text{--}60^{\circ}\text{C}$ range and slightly acidic pH ~ 6 . The addition of exogenous enzymes disintegrates cells, but their intracellular digestive enzymes remain active [1, 4, 6], thus digestion of intact yeast cells with papain yields higher amounts of proteins than digestion by endogenous enzymes alone.

Yeast hydrolysates obtained using papain yield higher concentrations of valine, methionine, leucine, threonine, and phenylalanine [4]. The composition of the product depends on other process conditions, resulting in differences in protein content, total and amino acid nitrogen, and turbidity [1–3, 6]. It is desirable that the product has a higher concentration of soluble proteins.

The problem of iodine deficiency is real in both developing and developed countries. The World Health Organization recommends the use of new iodine supplements as a partial substitute for iodized salt. Iodine-enriched yeast hydrolysates can be used in production of dietary supplements and feed additives. A method for production of iodine-enriched yeast in anaerobic liquid culture medium has been already reported [7]. The aim of this work was to evaluate the influence of incubation conditions on hydrolysis efficiency and iodine enrichment of obtained hydrolysates.

Materials and Methods

Chemicals

Fresh *S. cerevisiae* or brewer's yeast was purchased from Lessafre, Wolczyn, Poland. The yeast contained 33% dry matter and had a soluble protein content of 26 mg/g wet matter and 78 mg/g dry matter. The pH of a 1% suspension was 5.19. The viability of the yeast was determined with the

methylene blue test. Whey powder was purchased from OSM Kosów Lacki, Poland. Whey had a dry matter content of 94%, 318 mg/g soluble protein in dry matter, and the pH of a 1% suspension was 6.4. Potassium iodide p.p.a. grade was purchased from POCh, Gliwice, Poland. Papain with 1.8 u/mg activity and pepsin with 800–2,500 u/mg activity were obtained from Sigma-Aldrich. A Hydrolab water purification system was used to obtain the water used in all the experiments.

Experimental Procedure

Fresh yeast (15 g of fresh, 5 g of dry mass) was placed in a 100-ml flask to which 30 ml of water and 0.5 g of whey powder were added. The yeast was mixed for 10 min using a magnetic stirrer. At that point, 0.5 g of papain (or pepsin) was added. Enzymes were not added for controls. After 10 min mixing, 5 mL of potassium iodide solution was added for a final concentration of 1.5 or 4.5 mM iodide. The concentration of iodide in the controls was maintained at 3 mM. The cultures were again mixed for 10 min, pH was adjusted to 6.0 using 0.1 M NaOH and 0.1 M HCl, and then placed in an incubation oven at 50°C for 12 or 24 h. The incubation time for the controls was 18 h. After incubation, the yeast was cooled to 20°C and centrifuged for 15 min at 3,000 rpm, producing a clear supernatant and cell pellet. Pellets were added with 80 mL of water and then stirred until homogenous suspensions were obtained. The suspensions were centrifuged for 15 min at 3,000 rpm. The new supernatant was freeze-dried and the remaining pellet was suspended in 10 mL of water and also freeze-dried. Table 1 presents the experimental conditions of our experiments for producing iodine-enriched yeast hydrolysates.

Table 1 Plan of experiment

Variant	X1	X2	X3	Enzyme	Hydrolysis time (h)	Potassium iodide concentration (mM/l)
1	-1	-1	-1	Papain	12	1.5
2	-1	-1	+1	Papain	12	4.5
3	-1	+1	-1	Papain	24	1.5
4	-1	+1	+1	Papain	24	4.5
5	+1	-1	-1	Pepsin	12	1.5
6	+1	-1	+1	Pepsin	12	4.5
7	+1	+1	-1	Pepsin	24	1.5
8	+1	+1	+1	Pepsin	24	4.5
9	0	0	0	0	18	3.0

Analytical Procedures

All the experiments were conducted in triplicate.

Iodide Concentration

The iodine concentration was measured using an ion-specific iodide electrode type EI-01 from Hydromet, Poland. The electrode was calibrated using a series of iodide solutions of known concentrations. The dependence between the molar (M) iodide concentration and the value in the display was given by a regression equation:

$$y = 4E - 13e^{2.303x}$$

where x is the value in the display and y is the concentration of iodide (in mole per liter). The fit of the equation to the experimental points was close to unity, $R^2=0.99999$.

Supernatant Protein Concentration

The concentration of protein in yeast supernatant was measured using a Marcel Media UV–VIS Spectrophotometer (France) with $10 \times 10 \times 45$ mm quartz cuvettes (Sarsted, Germany). Supernatant samples were diluted with purified water and the absorbance was measured at 280 nm. The absorbance was linear in the 0.2–0.8 range. One absorbance unit was equivalent to 0.333 mg/mL protein.

The protein hydrolysis efficiency was calculated using following equation:

$$E = 100x \frac{M_{HP}}{M_{IUP}}$$

where M_{HP} is the mass of hydrolyzed protein and M_{IUP} is the initial mass of non-hydrolyzed protein, in grams.

Statistical Treatment of the Results

The results were calculated as mean values ($x \pm SD$) of three replicates. The statistical analysis was carried out using the Excel (Microsoft) and Statistica (StatSoft Inc.) software. Applied methodology was based upon the Central Composite Design method (three-level full-factorial design); Design of Experiment software options were used. The significance level was set at $p < 0.05$.

Results and Discussion

Table 2 presents the influence of incubation conditions on the production of iodine-enriched yeast hydrolysate. Figure 1 shows the influence of incubation conditions on hydrolysis and iodine enrichment. Nonsignificant variable relations are marked with gray surrounding.

The addition of enzymes results in higher efficiency of proteolysis. The type of enzyme used significantly influenced all of measured parameters: protein hydrolysis efficiency, iodine concentration in supernatant, and pellet ($p < 0.05$). Papain proved to be the most effective of the enzymes tested, yielding the highest proteolysis efficiency (98.22%), the highest supernatant protein mass (2.13 g), and pellet iodine concentration (2,664.9 $\mu\text{g/g}$).

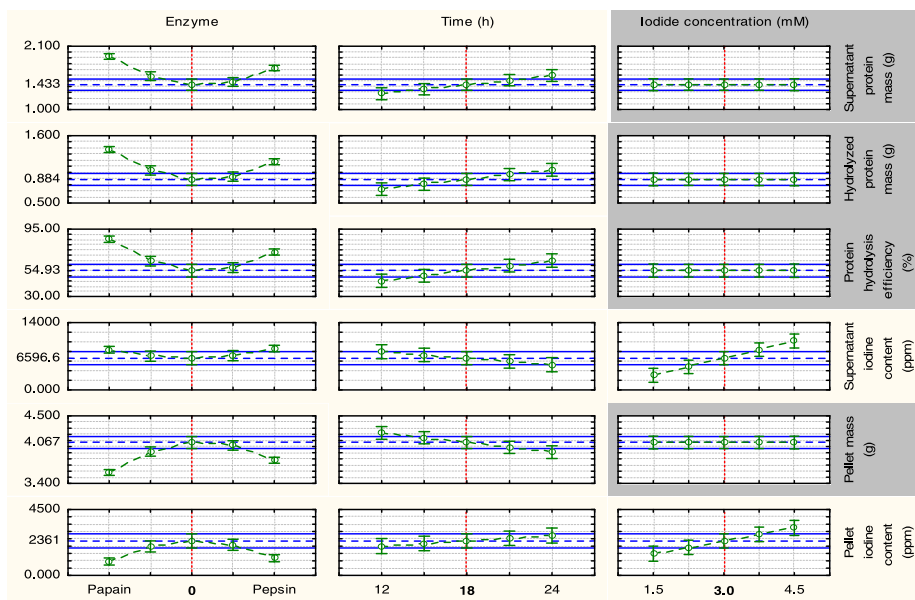
The presented results support published data on the use of papain for hydrolysis of yeast [1, 4, 6]. Pepsin is less efficient than papain and autolysis is the least efficient of the processes tested.

The addition of enzymes decreases the incorporation of iodine into the cell pellet, with higher amounts of iodine remaining in the supernatant in comparison to controls. The data from Table 2 allow estimating how much of the initial 1.61 g of non-soluble protein was hydrolyzed and released into the supernatant as soluble protein. The best results using papain were 96.4% and 98.2%, depending on the

Table 2 The influence of incubation conditions on the process of iodine-enriched yeast hydrolysate production

Variant	Total supernatant protein mass (g)	Hydrolyzed protein mass (g)	Hydrolysis efficiency (%)	Supernatant iodine concentration ($\mu\text{g/g}$)	Pellet dry mass (g)	Pellet iodine concentration ($\mu\text{g/g}$)
1	1.702 \pm 0.021	1.153 \pm 0.021	71.63 \pm 1.33	5,433.89 \pm 53.20	3.797 \pm 0.021	73.73 \pm 10.14
2	1.753 \pm 0.117	1.204 \pm 0.117	74.83 \pm 7.27	14,465.90 \pm 1733.19	3.746 \pm 0.117	856.86 \pm 422.40
3	2.102 \pm 0.061	1.550 \pm 0.061	96.46 \pm 3.80	4,210.12 \pm 147.75	3.397 \pm 0.061	200.12 \pm 18.97
4	2.130 \pm 0.076	1.581 \pm 0.076	98.22 \pm 4.77	9,200.67 \pm 582.3	3.369 \pm 0.077	2,664.91 \pm 229.29
5	1.646 \pm 0.041	1.097 \pm 0.041	68.14 \pm 2.57	5,315.66 \pm 154.67	3.854 \pm 0.041	202.39 \pm 33.68
6	1.557 \pm 0.012	1.008 \pm 0.012	62.63 \pm 0.76	14,062.89 \pm 182.69	3.942 \pm 0.012	1,694.27 \pm 62.9
7	1.841 \pm 0.102	1.292 \pm 0.102	80.27 \pm 6.35	4,659.78 \pm 268.00	3.658 \pm 0.102	259.65 \pm 18.27
8	1.847 \pm 0.089	1.298 \pm 0.089	80.67 \pm 5.56	10,406.63 \pm 449.64	3.652 \pm 0.089	2,560.14 \pm 102.63
9	1.433 \pm 0.067	0.884 \pm 0.067	54.93 \pm 4.16	6,593.18 \pm 173.99	4.066 \pm 0.067	2,360.64 \pm 20.14

Fig. 1 The influence of incubation conditions on hydrolysis and iodine enrichment. Profiles for predicted values



concentration of potassium iodide. Autolysis yields only 54.9%, which is significantly lower than the results obtained for both enzymes.

Incubation time significantly influences the efficiency of protein hydrolysis and iodine concentration in supernatant and pellet ($p < 0.05$). Increasing incubation time yields higher supernatant protein mass and lower pellet mass. Within the studied time range, the highest protein hydrolysis efficiency was obtained using papain and 24-h incubation time (98.2%). The significance of incubation times is significant: 18-h incubation without enzymes yields comparable protein content as with 12-h incubation with enzymes.

Also, increasing incubation time positively influences iodine incorporation. At higher incubation times, the iodine content in pellet increases but decreases in the supernatant. The highest concentration of pellet iodine was seen after 24-h incubation with papain (2,664.9 $\mu\text{g/g}$).

The concentration of iodine in the incubation medium results in increased iodine content in supernatant and pellet ($p < 0.05$). At 4.5 mM iodide, the highest enrichment can be achieved: 2,665 $\mu\text{g/g}$ compared to 259.56 $\mu\text{g/g}$ in 1.5 mM medium and 2,360.64 $\mu\text{g/g}$ in 3 mM/L medium. It can be concluded that 4.5 mM iodide medium gives the highest iodine enrichment in comparison to 1.5 and 3.0 mM iodide medium. Potassium iodide concentration does not significantly influence hydrolysis efficiency, pellet mass, or hydrolyzed protein mass.

Taking the results into consideration, long incubation time and higher iodide concentrations with the use of papain should be recommended for optimal enrichment and high-soluble protein yield. Within the studied range of variables, 24-h incubation time, iodide concentration of 4.5 mM/L and papain yielded the best results. Hydrolysis efficiency reached 98.22%, pellet iodine content—2,664.91 $\mu\text{g/g}$, and

supernatant iodine concentration—9,200.67 $\mu\text{g/g}$. Variant 4 ensures effective hydrolysis and efficient iodine incorporation.

Fresh yeast was used in the experiment, what gave some proteolytic activity of yeast enzymes, besides the activity of added proteases. Both temperature and pH were appropriate for the activation of yeast proteases. Based on the obtained results and literature cited, it can be concluded that the proteolysis occurred due to the activity of both yeast and added proteases [1, 6].

Iodine-enriched yeast, containing hydrolysed protein, is a proper component for the production of dietary supplements for humans and feed additives for animal nutrition. Due to proceeding decrease in salt consumption, recommended by WHO/FAO, new iodine carriers in the diet are implemented, like milk and vegetable oils. Iodized salt is also vulnerable to iodine loss during transport, storage, and cooking procedures using high temperatures [8, 9]. Iodine-enriched yeast emerges as a novel source of iodine and is deemed appropriate for human nutrition. Yeast produced according to presented model contains no salt; therefore, it is proper for use in low-salt diets. It should be also stated that *S. cerevisiae* belongs to the GRAS group and is safe for use in humans and animals [10].

Whey used in the model is a by-product of the dairy industry, which can cause serious damage to the environment when contamination occurs. The use of whey presents a significant ecological issue. Application of various yeast strains offers an ability to use different by-products of the food and fat industry [11]. The production process can be modified to fit local conditions, such as technological issues and raw materials' availability. It is an important advantage, since iodine supplementation is vital for the developing countries. Presented process can be carried out using simple

technology, what creates a possibility for global-scale application. The process can be modified by adding a mechanical disintegration procedure to enhance yeast hydrolysis [4, 12]. Presented results indicate that iodine enrichment in yeast is possible to achieve. Papain addition gave the highest concentration of easily digestible protein, but significantly lower efficiency of iodine incorporation in comparison to the control experiment. It has, however, no contraindication against further application of the product. Iodine concentration in obtained product makes it proper for use in dietary supplements, for example, tablets. Since iodine deficiency is a major worldwide healthcare problem, creating a technology of iodine-enriched yeast production is of strong necessity and could be widely applied in both human and animal nutrition [1, 13].

Conclusions

1. The type of enzyme used significantly influences protein hydrolysis efficiency and iodine concentration in both pellet and supernatant.
2. Papain assures high efficiency of protein hydrolysis. The highest efficiency was observed using papain during 24-h incubation in comparison to both pepsin and controls.
3. The concentration of iodide in the medium significantly influences iodine enrichment of supernatant and cell pellet. The highest enrichment was observed in 4.5 mM iodide medium.

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