Simple Process for the Reduction in the Nucleic Acid Content in Yeast

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A simple one-step process for the nucleic acid reduction in Rhodotorula glutinis is described. The process consists of submitting the yeast cells to a heat treatment in an acidic (pH 2) spent medium. The optimal temperature for pH 2 medium is 90°C and the final nucleic acid content in treated yeasts was 1.2%. Heat treatment at acidic pH is preferred to that at alkaline pH because it offers a better protection for amino acids and crude protein, while being more efficient in lowering the nucleic acid level. The new process is economic and rapid and could be easily used for industrial application.

Biological removal by yeast of elements which are responsible for water pollution yields an economically valuable biomass. Our work is a continuation of a research program having a major concern for protein shortage and pollution abatement.

Simard and Blackwood (11, 12) observed that 50% of yeast isolates in the St. Lawrence River belonged to the Rhodotorula spp. Particularly, Rhodotorula glutinis demonstrated the highest efficiency in converting domestic sewage and waste water into high protein biomass (13, 16). in another series of experiments, Goulet (Masters thesis, Laval University, Laval, Québec) evaluated the digestibility of R. glutinis and found that an increase of 30% in the digestibility of the lyophilized cells was obtained when cells were suspended in water and heated in an autoclave. These experiments led us to believe that R. glutinis could be an excellent protein source if its nucleic acid content could be reduced and its digestibility increased.

Reduction of yeast nucleic acid content has been studied extensively for the past few years. Various methods have been proposed and may be summarized as follows: (i) heat-shock process, (ii) heat-shock and enzymatic treatment, and (iii) cell disintegration and precipitation of protein.

Nucleic acid content in yeasts varies from 6 to 8% (dry weight). The heat-shock process, which consists of heat shock and incubation, has been used with success by Imada et al. (7a) for the reduction in nucleic acid content in Candida lipolytica; also Maul et al. (8) and Ohta et al. (9) have used this method to lower nucleic acid content in Candida utilis to less than 2%. Canepa et al. (1) used a similar heat treatment followed by dialysis. The enzymatic treatment (method b) with bovine pancreatic ribonuclease was described by Castro et al. (2). The method consists of treating heat-shocked cells by the ribonuclease, yielding a product containing a final nucleic acid content of 1.5%. The cell disintegration method was used by Hendenskog et al. (5–7) for the extraction of protein. Protein concentrates containing cell walls were produced by heating at alkaline pH directly after the mechanical disintegration of cells; protein was then precipitated by the addition of an acid. The final ribonucleic acid content in the precipitate was about 2%.

It has been observed that the described methods are time consuming and tedious. The hypothesis put forward is that the reduction of nucleic acids could be obtained by heating yeast cells directly in an acid or alkaline medium, since it is well known that these acids are easily hydrolyzed by acids or bases (3, 10, 14, 17). Consequently, heating could increase the digestibility of these cells.

MATERIALS AND METHODS

Cell growth. R. glutinis Y917 (collection of Laval University, Food Sciences Department) was grown batchwise in 150 ml of Bacto-mycological yeast broth (Difco Laboratories, Detroit, Mich.) at 20°C, and was shaken in 500-ml baffled flasks on a gyrotary shaker (model G-10, New Brunswick Scientific Co., New Brunswick, N.J.) at 150 rpm for 72 h. The flasks were then stored at 4°C until used for the experiments.

Dry matters. Five milliliters of the suspension was centrifuged at 2000 × g for 20 min using a universal centrifuge UV (International Centrifuge Co., Mass.). The supernatant was decanted, and the cells were washed twice with cold water and then transferred to
aluminum plates for drying under vacuum at 60°C for 12 h by means of a vacuum oven.

**Quantitative analyses.** Absorbanies of nucleic acids (ribonucleic acid from Nutritional Biochemicals Corp., Cleveland) were measured at 260 nm with the use of an ultraviolet spectrophotometer (Unicam). Supernatants were diluted to measurable absorbancies in ultraviolet light at 260 nm.

**pH.** pH was measured with a Fischer Accumet pH meter, model 310.

**Amino acids.** Yeast protein was hydrolyzed in 6 N HCl at 110°C in a sealed tube for 16 h. The liquid was evaporated under vacuum and the residue was taken up in 10 ml of buffer and then re-evaporated. The final neutral residue (amino acids) was again dissolved in 10 ml of buffer. Analysis was done by means of an automatic amino acid analyzer (ionic-exchange chromatography) (15).

**Total nitrogen.** Total nitrogen was measured by the Kjeldahl method (4). Percentage of protein was obtained by multiplying the percentage of the total nitrogen by 6.25.

**Heat treatment.** Yeast cells in spent medium were heated in a water bath at temperatures varying from 50 to 100°C at different pH values. Initially, the pH was adjusted by adding either 1 N hydrochloric acid or 1 N sodium hydroxide. At different intervals of time (every 2 min for the first 16 min and then every 10 min until the end of the experiment), 5 ml of the suspension was pipetted into 50-ml graduated centrifuge tubes, cooled immediately in ice, and centrifuged at 2000 × g for 15 min.

The supernatant was decanted. The cells were washed twice with cold water and resuspended in 5 ml of ice cold 0.5 N perchloric acid. The centrifuge tube was shaken on a Burrell wrist-action shaker (Burrell Corp., Pittsburgh, Pa.) for 15 min at 4°C. After centrifugation at 2000 × g, the supernatant (cold perchloric acid fraction; any residual acid-soluble small molecules in the cells) was pooled. The residual cells were resuspended in 5 ml of 0.5 N perchloric acid and heated at 100°C for 15 min for the determination of remaining nucleic acid content in the cells. After centrifugation at 2000 × g for 15 min, the hot perchloric acid fraction was analyzed (9).

**RESULTS AND DISCUSSIONS**

**pH-time-temperature relationships.** (All these experiments were done in duplicates and the curves are based on the mean of these results.) Figure 1 shows that the nucleic acid content of yeast cells was lowered either at high or at low pH. In the range studied, maximal reduction was observed at pH 2. Total nucleic acids of the cells was decreased from 6.5 to 1.2% as the pH approached 2 by the addition of 1 N HCl and 1.5% as the pH was raised to 11. Experiments were not conducted at higher pH values because the rate of protein hydrolysis is high and also on account of the large volumes of alkali required. These constraints would lessen the industrial feasibility of the process.

The influence of temperature on the reduction of nucleic acids in the cells upon heat treatment at pH 2 is shown in Fig. 2. Nucleic acids decreased rapidly at the beginning of the treatment, but came to a plateau between 60 and 80°C. Then another decrease took place until the temperature reached 90°C, where only 1.2% of nucleic acids remained in the cells.

The lowering of nucleic acid level in yeast cells was proportional to the time of heating. However, nucleic acid content varies only slightly when the treatment continues further than 120 min, and heating at 90°C was slightly more efficient in this regard than heating at 100°C.

When the yeast cells were heated at pH 11, the percentage of nucleic acids in the cells decreased as the temperature increased and
reached a minimum of 1.5% at 90°C (Fig. 3). This is also illustrated in Fig. 4, which shows in addition that the major portion of the decrease took place in the first 20 min. From this period up to 2 h, the rate of decrease is low. The lowering of nucleic acid content of cells at pH 11 was equally efficient at 90 and 100°C and less at 80°C.

The variation in protein content in acid or alkali heat-treated cells is shown in Table 1. A slight decrease of protein was observed. The percentage of total nitrogen, which was initially 42.60%, decreased to 40.40% in the case of the acidic treatment and to 39.20% in the case of alkaline treatment. (These values are corrected for dry matter loss after the heat treatments.)

The total amino acid distribution in the yeast cells is presented in Table 2. Among the 18 amino acids analyzed, cystine was not detected. Except for histidine, proline, valine, tyrosine, and phenylalanine, acid treatment gave higher values in all amino acids than by alkali treatment. When these values were compared to those in nontreated cells, it was found that lysine had decreased from 2.77 to 1.90, histidine from 1.23 to 0.66, and arginine from 2.47 to 1.97 mg/100 mg of dry cells, giving a loss of 30 to 50% of these amino acids. However, an increase in other amino acids was observed. (These values are corrected for dry matter loss after the heat treatments.)

From the above results, it can be concluded that reduction of nucleic acid content by alkali treatment is less effective than by acid treatment, since it yields lower amino acid and protein values and also a smaller reduction in the nucleic acid content. Part of the amino acids and hydrolyzed protein could have been excrated into the reaction medium and could be recuperated by drying. The feasibility of recovering solubilized amino acids and proteins will be studied in a further step.

Also, it can be seen from Table 3 that our heat treatment process reduces nucleic acids to a level equivalent to that obtained by previous workers using more complicated processes. Moreover, this process for producing a yeast product with low nucleic acid content is inexpensive and suitable for industrial technology. Chromatographic studies on characterization of
degradation products resulting from the hydrolysis of nucleic acids in the cells after heat treatment are under study. This will enable us to determine the mechanism of this hydrolysis. Also, protein efficiency utilization of this yeast product will be evaluated as well as long term studies as to its digestibility in animals.

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