Extraction of Trehalose from Thermally-treated Bakers’ Yeast

Yumi Yoshikawa, Kanji Matsumoto, Kazuhisa Nagata, and Toshio Satô*

Department of Material Science and Chemical Engineering, Yokohama National University, Hodogaya-ku, Yokohama 240, Japan

* Laboratory of Geoenvironmental Ecotechnology, Faculty of Agriculture, Shimane University, Matsue 690, Japan

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Trehalose is a disaccharide of glucose mainly found in bakers’ yeast. We investigated the extraction conditions for trehalose from bakers’ yeast with an ethanol–water solution to increase the yield of trehalose from the yeast cells. A high extraction ratio of trehalose was obtained from thermally treated yeast, that is, from yeast cells treated by drying and heating, when compared with that from untreated raw yeast. This thermal had the effect of inactivating trehalase, which is a hydrolytic enzyme of trehalose, and accelerated the disruption of the yeast cells. These effects facilitated the extraction of trehalose within a short time at 25°C. When an ethanol–water solution of high concentration was used as the extraction solvent, the concentration of residual protein in the yeast extract was quite small. Especially in the case of dried yeast, the removal of protein from the yeast extract was not necessary for isolating and purifying trehalose after it had been extracted.

Trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) is a non-reducing disaccharide of glucose and an isomer of maltose. It is found in various organisms such as yeast, fungi, insects, bacteria, and plants. In these organisms, trehalose is consumed as the energy source and has a variety of functions. For example, trehalose protects biological membrane structures against damage by freezing and desiccation. Although it has a sweet taste, it doesn’t decay the teeth. From these features, trehalose can be used as a material for foods and toiletries and it is also anticipated as a material for anticancer drugs.

Trehalose is commonly found in bakers’ yeast in a high concentration, so it is mainly obtained from yeast. Several studies on the isolation of trehalose from bakers’ yeast have been reported since Koch’s early works. Based upon the result obtained by these studies, the method for isolating trehalose from bakers’ yeast has been generally established by extracting with a 70-95% hot ethanol–water solution from active dried bakers’ yeast. This method, however, needs complicated procedures for deproteinization and concentration of the ethanol in the extract. Moreover, the extraction yield of trehalose is low due to its low solubility in a solution with high ethanol concentration.

The authors have reported that the extraction yield of trehalose from raw bakers’ yeast was affected by the activity of trehalase, a hydrolytic enzyme of trehalose in yeast cells. It was also reported that suppressing the trehalase activity was necessary to increase the extraction yield of trehalose by adjusting the ethanol concentration, pH and extraction temperature. However, it was impossible to completely eliminate the hydrolysis of trehalose during extraction by controlling the pH value or ethanol concentration, although it was by thermal treatment.

In this report, the inactivation of trehalase was attempted by heating or drying the raw yeast before extraction to prevent the hydrolysis of trehalose. The influence of thermally pretreating the raw yeast and of the extraction conditions on the yield of trehalose was investigated.

Materials and Methods

Pretreatment of the raw yeast. A packed bakers’ yeast (Oriental Yeast Co.) stored in a refrigerator at 4°C was used, the content of dry solid in the packed bakers’ yeast being about 30 wt% when it was dried at 105°C for 12 h. Two types of pretreatment for the raw yeast were carried out. 30 g of raw bakers’ yeast was heated in an open beaker or in a closed bottle. These pretreatments are called the drying treatment and heating treatment, respectively, both being done at 40-105°C for 0.5-40 h in an electric drying oven. The drying ratio is defined as the percentage loss in weight of the yeast under defined drying conditions to that by drying at 105°C for 12 h.

Extraction of trehalose. Trehalose was extracted from raw yeast or thermally-treated yeast by putting the sample of yeast (10 g dry weight) and an extraction solvent (150 ml) into a 500-ml flask with a reflux condenser, and stirring the mixture. An ethanol–water solution was used as the extraction solvent, the ethanol concentration being adjusted in the range of 0 to 85 wt% by mixing a 95 wt% ethanol solution with pure water that had been filtered with a reverse-osmosis membrane. The extraction time was varied from 0 to 3.0 h, and the extraction temperature from 25 to 100°C. After being extracted, the yeast suspension was passed through a 0.45-μm membrane filter (Fuji Photo Film Co.), the filtrate being called the yeast extract.

Assay of trehalose and protein. The amount of trehalose in the yeast extract was measured by high-performance liquid chromatography with a Shimadzu LC-6A chromatograph and a Bio Rad HPX-87C column. The amount of extracted trehalose was evaluated by the relative extraction ratio, which is defined as the amount of trehalose extracted from the untreated or treated yeast under defined conditions to that extracted from untreated raw yeast under standard conditions, i.e., with pure water at 100°C for 1 h. The amount of trehalose extracted from the raw yeast under the standard conditions was 0.15–0.20 g/g of dried yeast. The total soluble protein concentration was measured by the Coomassie Brilliant Blue G250 (Fukuh Chem.) method, using bovine serum albumin as the standard protein for calibration.

Evaluation of trehalase activity. The activity of trehalase contained in the raw yeast was determined for a yeast suspension that had been prepared by adding 120 g of packed raw yeast to 100 ml of pure water. The yeast cells in the suspension were disrupted with an ultrasonic homogenizer (Branson Co., SONIFIRE 250) at 26°C for 10 min, and then centrifuged at 12,000 rpm and 4°C for 20 min. Trehalase was contained in the supernatant. 0.5 ml of 0.15% trehalose was added to 1.5 ml of the supernatant. Trehalose-trehalase mixtures were incubated for 1.5 h at temperatures of 40 to 80°C and the trehalase was then inactivated by placing the mixture at 4°C.
in a boiling water bath for 1 min. The mixtures were filtrated with a 0.22 μm membrane filter, and the trehalase activity was determined by measuring the amount of remaining trehalose. Trehalase contained in 1.5 ml of supernatant hydrolyzed the 0.033 g of trehalose under the reaction condition mentioned above. That is, trehalase contained in one gram of packed raw yeast hydrolyzed the 0.1 g of trehalose.

Results and Discussion

Effect of pretreatment temperature on the raw yeast

Packed raw yeast stored in a refrigerator at 4°C was heated or dried for 12 h at different temperatures. Trehalose was extracted from these thermally-treated yeast samples with a 45 wt% ethanol-water solution for 1.5 h at 80°C (i.e., at boiling point). These extraction conditions are the optimum ones obtained in our previous report. The dependence of trehalase activity and relative extraction ratio of trehalose on the heating temperature is shown in Fig. 1. The maximum trehalase activity was when heated at 45°C and was lost at 70°C. The relative extraction ratio increased with increasing heating temperature with both the heating and drying treatments. These results suggest that the relative extraction ratio of trehalose was related to the trehalase activity of the yeast. The relative extraction ratio with the heating treatment was slightly higher than that with the drying treatment at the same heating temperature, except at 100°C.

Effect of pretreatment time

The thermal treatments inactivated the yeast trehalase and resulted in an increased relative extraction ratio as shown in Fig. 1. The dependence of the relative extraction ratio of trehalose on the heating or drying time for raw yeast was then studied. The heating or drying time was varied in the range of 1 to 12 h at 105°C, at which with the maximum relative extraction ratio was obtained with both treatments. The results are shown in Fig. 2, together with the change in drying ratio of the yeast. It took about 2 h of the heating treatment and 5 h of the drying treatment, respectively, to reach the equilibrium value for relative extraction ratio. The maximum relative extraction ratio shown in Fig. 2 does not agree with that in Fig. 1. This disagreement would have been due to the different condition of the raw yeast samples used in the extraction experiments, that is, lot number and storage period. From the results shown in Fig. 2, it was expected that 2 h of heating or 5 h of drying would be enough for a good extraction of trehalose. As it took about 30 min to raise the temperature of 30 g of yeast to 100°C when it was heated at 105°C, trehalase was presumed to be inactivated within one hour. If the relative extraction ratio would be restricted by the trehalase activity, the maximum relative extraction ratio should result after 1 h of treatment time. However, it required more than 2 h with both treatments to achieve the maximum relative extraction ratio. This suggests that the trehalase activity was not the sole factor for determining the relative extraction ratio. From the result in Fig. 2, for the case of the drying treatment, it was presumed that the relative extraction ratio would be influenced by the drying ratio.

Effect of drying ratio

The effect of drying ratio on the relative extraction ratio of trehalose by the drying treatment was studied. In Fig. 3, as shown the relationship between the drying ratio and the relative extraction ratio of trehalose when raw yeast was dried at 45 and 105°C under the same extraction conditions as those described in Fig. 1. It required more than 40 h to dry the raw yeast completely at 45°C. When the yeast was dried at 105°C, the relative extraction ratio increased with increasing drying ratio. A similar result was obtained when raw yeast was dried at 80°C (data not shown). It took about 5 h to achieve a 100% drying ratio, and this time coincides with the time required to give the maximum relative extraction ratio as shown in Fig. 2. When the raw yeast was
dried at 105°C, the effect of drying ratio on the relative extraction ratio was less than that obtained when the yeast was dried at 45°C. A similar curve was obtained with drying at 60°C (data not shown). The reason why the relative extraction ratio of trehalose decreased with increasing drying ratio when drying at 45°C and 60°C can be explained by the action of trehalase contained in the yeast cells during drying. This result agrees with results shown in Fig. 1.

**Observation of the yeast cells**

When packed raw yeast was subjected to the drying treatment, it shrank with time, and the formation of numerous cracks on the surface of the yeast cells like those on charcoal were observed. On the other hand, when the packed yeast was heated, it became soft and finally assumed the state of a fluid which was obtained by autolysis. From these observations, it is presumed that the yeast cells were deformed or disintegrated by the heating or drying treatment. SEM photos of yeast cells treated by heating and drying are shown in Fig. 4. Photos. (a), (b), and (c) show raw yeast, yeast heat-treated at 105°C for 12 h, and yeast dried at 105°C for 12 h, respectively. Deformation of the cell wall of the heat-treated yeast like the appearance of a pickled plum and a depression in the germ cavein are apparent. The shape of the cells treated by drying was completely deformed, and numerous cracks are apparent on the cell walls. After extracting trehalose from the raw and thermally-treated yeast, the cells were disrupted by the ultrasonic homogenizer, and the concentration of trehalose in a suspension containing cell debris was measured. There was no change in trehalose concentration, meaning that almost all the trehalose had been extracted by the hot ethanol-water solution.

**Effect of ethanol concentration on extraction**

The relative extraction ratio of trehalose from raw bakers' yeast was influenced by the concentration of ethanol used as the extraction solvent in the previous experiments. The most appropriate ethanol concentration was about 30-50 wt%, because at a lower concentration, the trehalase activity became high, and at a higher concentration, the yeast cells tended to contract. It was expected, on the other hand, that the extraction characteristics of thermally-treated yeast would be different from those of raw yeast, because the trehalase activity in the thermally-treated yeast had already been lost. The effect of ethanol concentration on the relative extraction ratio of trehalose in raw and thermally-treated yeast was therefore investigated.

The results are shown in Fig. 5. Throughout the range of ethanol concentration, the relative extraction ratio of trehalose from the thermally-treated yeast was higher than that from the raw yeast. However, the relative extraction ratio of trehalose decreased with increasing concentration of ethanol above 50 wt% regardless of the yeast samples. The reason why the relative extraction ratio decreased at the higher ethanol concentrations is not known at this stage. The higher relative extraction ratio of trehalose with the thermally treated yeast would have been due to easier extraction from the partially disrupted and lysed cell walls of the treated yeast as mentioned in the previous section.

Protein was also extracted together with trehalose. It is desirable to extract as little protein as possible from yeast

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**Fig. 3.** Relationship between the Drying Ratio of Yeast and Relative Extraction Ratio of Trehalose.

The relative extraction ratio of yeast dried at 105°C (□) and at 45°C (△) was obtained when trehalose was extracted at 80°C for 1 h with a 45 wt% ethanol-water solution.

**Fig. 4.** SEM Photographs of the Yeast Samples before Extraction.

(a) raw yeast; (b) heated yeast; (c) dried yeast. Treated yeasts were prepared at 105°C for 12 h.
to purity trehalose in a yeast extract. In general, as the solubility of protein is low in a high-concentration ethanol–water solution, it could be expected that the amount of protein in the yeast extract can be decreased by using a high-concentration ethanol–water solution as the extraction solvent. Therefore, the concentration of protein released in each yeast extract was measured, the results being shown in Fig. 6. The protein concentration in the extract obtained from the heated yeast was the highest, and that from the dried yeast was the lowest at the same ethanol concentration. The protein concentration decreased with increasing ethanol concentration in each yeast sample. As conventional deproteinizing agents, mercuric sulfate, zinc sulfate or trichloroacetic acid have been used. However, it is said that an ethanol–water solution can be used as a deproteinizer as well as an extraction solvent.

From the results already mentioned, it was proved that trehalose could be extracted with an ethanol–water solution of high ethanol concentration as well as with pure water, and that the relative extraction ratio from the treated yeast was higher than that from raw yeast. Accordingly, the following experiments were carried out with pure water and a 45 wt% ethanol–water solution as the extraction solvents.

Effect of extraction temperature

When trehalose was extracted from raw yeast, the relative extraction ratio was influenced by the treatment temperature of the yeast (see Fig. 1). As the activity of trehalase was lost in the treated yeast, the influence of extraction temperature on the relative extraction ratio was expected to be little. In Fig. 7 is shown the relationship between the extraction temperature and relative extraction ratio for each yeast sample, using pure water or a 45 wt% ethanol–water solution. At all extraction temperatures, the relative extraction ratio of trehalose from the treated yeast is higher than that from the raw yeast, and doesn’t depend on the temperature of either extraction solvent, although the relative extraction ratio from raw yeast depended on the temperature. It was found from the results in Fig. 7 that the trehalase was inactivated by thermal treatment and could be extracted with the solvent at room temperature.

Effect of extraction time

The time taken to reach equilibrium for trehalose extraction would depend on the treatment conditions of the yeast. The effect of the time of trehalose extraction from raw and treated yeast was studied, and the results are shown in Fig. 8. When trehalose was extracted from raw yeast, it took about one hour to reach equilibrium for extraction with either extraction solvent. However, with the treated
Fig. 8. Relationship between the Extraction Time and Relative Extraction Ratio of Trehalose.

A 45 wt% ethanol-water solution at 80°C and pure water at 100°C were each used as the extraction solvent in Figs. 8(a) and (b), respectively. The relative extraction ratio of trehalose was obtained from raw yeast (□), dried yeast (○), and heated yeast (●) after 1 h of extraction. These treated yeast samples were prepared at 105°C for 12 h.

yeast, the time required to extract enough trehalose was much shorter than that with raw yeast. With heated yeast, almost 100% of the trehalose was extracted as soon as the heated yeast had been dispersed in the solvent. Trehalose was also extracted rapidly from the dried yeast; however, the relative extraction ratio increased with increasing time. This rapid release of trehalose from thermally treated yeast indicates that the cells of the heated yeast had been considerably disrupted when compared with raw yeast.

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