Alpha-Galactosidase Production and Use in a Hollow-Fiber Reactor

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Received for publication 3 November 1975

Soybean milk serves as a base for a variety of beverages designed for consumption in developing countries. Soybean flour contains raffinose and stachyose considered to be responsible for flatulence often associated with these products (J. J. Rackis, D. H. Honig, D. J. Sessa, and F. R. Steggerda, 1970). \( \alpha \)-Galactosidase, produced on wheat bran, hydrolyzes the galactooligosaccharides of soybean milk.

Aspergillus awamori NRRL 4869 was supplied by the Agricultural Research Service Culture Collection which is maintained at the Northern Laboratory. From 0.5 to 0.75 U of \( \alpha \)-galactosidase per ml could be achieved by growing the organism in shaker flasks on 2% nutrient 4S (E. A. Staley Co.), 0.5% yeast extract (Difco), 0.5% NaCl, 0.25% glycerol, and 2.0% lactose. Better yields could be obtained by simply culturing the organism on thin stationary layers of moist wheat bran. Extraction of 10 g of the bran culture with 100 ml of water showed yields of 3.0 to 3.7 U/ml. The work presented here is with enzyme produced on wheat bran. To 10 g of wheat bran in a 300-ml Erlenmeyer flask, 8 ml of distilled water was added. The flask was plugged with cotton, and the plug was covered with aluminum foil. Occasional shaking for 1 h evenly distributed the moisture. The flasks were then autoclaved at 120 °C for 20 min. Under these conditions, the moisture content was approximately 50%. The bran medium was inoculated with 0.2 ml of a spore suspension of NRRL 4869 and incubated 5 to 7 days at 35 °C as a stationary culture. The spore suspension was prepared by washing a week-old (room temperature) malt agar slant (Difco) of NRRL 4869 with 3 ml of sterile water. Although shaking flasks during incubation greatly increased sporulation, it significantly lowered enzyme yield and was avoided.

The \( \alpha \)-galactosidase was easily extracted from the bran culture by adding 100 ml of 2% NaCl to the contents of each flask. The flasks were held for 1 h with occasional agitation and then filtered. The residue was washed with 25 ml of 2% NaCl and the wash was added to the filtrate.

The activity of \( \alpha \)-galactosidase in the extracts was determined by the amount of glucose released from a 1% melibiose solution in 30 min at 40 °C and pH 5.0. Glucose was measured by the method of Hill and Kessler (2). One unit of \( \alpha \)-galactosidase is equivalent to the release of 1 \( \mu \)mol of glucose from melibiose per min under the conditions used. Although invertase is present in the crude extracts, it does not interfere with the assay of \( \alpha \)-galactosidase and is not considered detrimental to the objectives of our work.

\( A. \) awamori NRRL 4869 produces about 300 to 375 U of \( \alpha \)-galactosidase in 6 to 7 days on 10 g of wheat bran (Table 1). Enzyme yields by NRRL 4869, when tested with melibiose, compare favorably with those reported in the literature for Mortiella vinacea and Streptomyces olivaceus (6, 7). Other organisms (1, 3, 4) reportedly produce \( \alpha \)-galactosidase, but direct comparisons of activity with \( A. \) awamori are impossible because assays were done with p-nitrophenyl-\( \alpha \)-p-galactoside. In our hands, we found little relationship between activity on this phenylgalactosidase and activity on melibiose. However, NRRL 4869 \( \alpha \)-galactosidase degraded raffinose and stachyose in both aqueous solutions and soybean milk at rates comparable to melibiose hydrolysis. Paper chromatography of the digests showed that all the oligosaccharides were reduced to their constituent monosaccharides.

The crude \( \alpha \)-galactosidase concentrate described above was tested for its ability to hydrolyze soybean galactooligosaccharides in a modified Amicon DC-30 hollow-fiber dialyzer. The prefILTER was disconnected from the pump and inverted. In this position, the prefILTER served as a reservoir to hold the enzyme and still supplied a filter to prevent solid particles from entering the fibers (Fig. 1).

Stock solutions of \( \alpha \)-galactosidase from
NRRL 4869 were prepared by pooling NaCl extracts from 34 flasks (4,600 ml) and dialyzing overnight against cold tap water. The contents of the dialysis tubing were pooled and concentrated on a rotary evaporator to approximately 1 liter. This crude extract, which is stable for more than 6 months in the refrigerator, is transferred without further purification to the hollow-fiber reactor reservoir. The substrate, 18 liters of either 1% soybean milk or whey, was circulated around the outside of the fibers (185 ml/min) at 45 C. During operation the volume of enzyme solution decreased from 1 liter to 600 ml, which corresponds to the hold-up volume of the hollow-fiber cartridges.

It is possible to use glucose production as a measure of \(\alpha\)-galactosidase activity, because the crude enzyme also contains large quantities of invertase that hydrolyzes sucrose as rapidly as it is formed from raffinose and stachyose. About 4 h were required to convert raffinose and stachyose to their constituent monosaccharides (Table 2). Addition of soluble enzyme to treated soybean whey failed to increase the amount of glucose present, showing that the reaction went to completion in the hollow-fiber apparatus.

There are advantages to treating soybean whey or milk by the hollow-fiber technique. First, the enzyme is conserved and can be recovered. Secondly, proteolytic enzymes, present in the crude extracts, are prevented from contacting the soy milk protein that may lead to undesirable bitter flavors. Finally, no enzyme or other undialyzable components of the crude enzyme solution get into the product. Of course, dialyzable impurities must be removed from the \(\alpha\)-galactosidase solution before using it to treat soybean milk or whey.

Not only is \(\alpha\)-galactosidase produced in high yield on inexpensive wheat bran as the only medium ingredient, but also the enzyme is stable to both storage and operating conditions. It has been used discontinuously for periods of 5 h in a hollow-fiber reactor devised to convert soybean \(\alpha\)-D-galactooligosaccharides to their constituent hexoses.

### LITERATURE CITED

5. Rackis, J. J., D. H. Honig, D. J. Sessa, and F. R.