

Thermal and storage stability of *Saccharomyces cerevisiae* (baker's yeast) allergens

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Summary

The effect of storage and high temperatures on the stability of *Saccharomyces cerevisiae* allergens was studied by immunoblotting. *Saccharomyces cerevisiae* allergic serum pool and ¹²⁵I- and galactosidase-labelled anti-IgE were used in the assays. Freeze-dried extracts were reconstituted with saline and with 50% glycerol and then stored at room (+20°C) and refrigerator temperature (+6°C) for different time periods.

The stability was better in 50% glycerol at +6°C than at room temperature without glycerol. However, after 1 month, two of the most important allergens of *S. cerevisiae*, the 48 and 32 kDa protein allergens, lost their IgE-binding capacity even in the extracts stored with 50% glycerol at +6°C. The 45 kDa allergen was, on the other hand, quite stable after storage for 9 months at +6°C. Although the beneficial effect of 50% glycerol was clear, storage at +6°C, even with 50% glycerol should not exceed 1 month for *S. cerevisiae* extracts.

Two commercially available *S. cerevisiae* extracts in solution with valid expiry dates were also analysed. They had only little allergenic potency, while a freeze-dried extract stored for 8 years showed good allergenic potency. Heating *S. cerevisiae* extracts resulted in precipitation, the precipitated fraction contained almost all the specific proteins as judged by electrophoresis and IgE detection. The supernatant fraction contained only a few allergens.

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Introduction

Diluted allergen extracts may lose their allergenic activity during storage and this is especially true for moulds which contain high enzymatic activity [1–4]. This circumstance raises the question of how stable the allergen extracts are for clinical purposes [1]. Of all preservatives and storage conditions that have been studied, 50% glycerol has turned out to be one of the best [2,5,6]. The potency of allergens is traditionally assayed with skin tests and RAST inhibition [7,8].

It has thus far been difficult to study the stability of *Saccharomyces cerevisiae* (baker's yeast) extracts, because the detailed allergen composition of the extract has not been known. IgE-immunoblotting studies have shown that a 48 kDa protein is the most abundant allergen followed by 32 and 10 kDa proteins and a

mannan polysaccharide [9]. The aim of this study was to monitor the stability of the identified allergens of *S. cerevisiae* in different storage conditions; immunoblotting was used for these studies.

Materials and methods

Preparation of baker's yeast extracts

The crude cytoplasmic antigen required for the extracts was prepared as previously reported [9]. The final extract solutions were produced by reconstituting the lyophilized antigen (10 mg/ml) with saline only and with saline +50% glycerol (Sigma, St. Louis, MO, USA). The glycerol-containing extracts were stored at +6°C for 9 months, 5 months, 4 weeks and 2 weeks, and extracts that lacked glycerol at +6°C and at room temperature (+20°C) for 6 months (Fig. 1). A *S. cerevisiae* extract prepared for studies 8 years earlier [10] and stored in a lyophilized form was also diluted for the studies.

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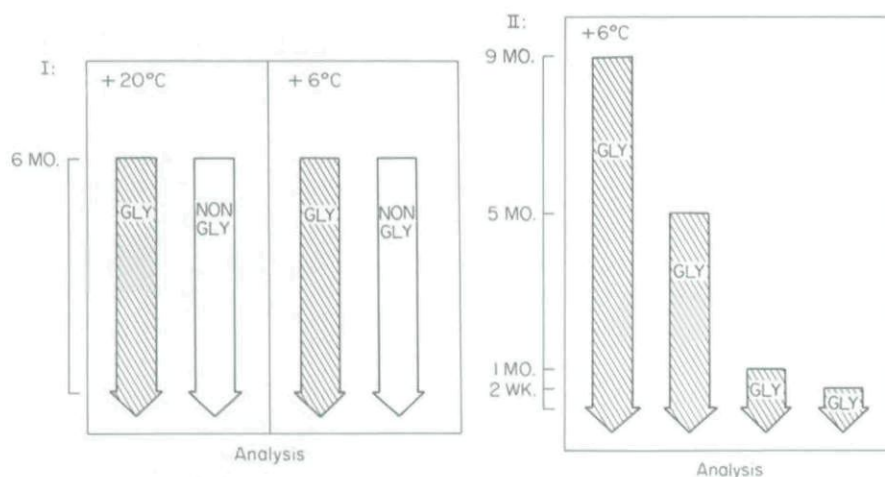


Fig. 1. Experimental design.

Thermal stability

The crude lyophilized cytoplasmic extract was diluted in water (10 mg of extract per ml of water). The samples were then placed in the water-bath into an oven heated to 200°C for 20 min or 250°C for 10 min.

Commercial extracts

Two commercial extracts, *S. cerevisiae* and *S. carlsbergensis*, were also studied by immunoblotting with the same serum pool. The manufacturer distributes these extracts stored in 50% glycerol and they were kept at +6°C according to the recommendations of the manufacturer. The expiry date of the *S. cerevisiae* extract was 8 months and of the *S. carlsbergensis* 1 month after the completion of these analyses.

Patients

A serum pool of seven *S. cerevisiae* IgE-positive patients with different specificities was used to detect a desired panel of IgE-binding. The pool contained sera from patients positive by *S. cerevisiae* RAST assays and by immunoblotting. The IgE-binding pattern of these sera showed that all important allergenic bands, including the 85, 75, 48, 45, 39, 32, 31, 25 and 10 kDa bands, and mannan were present. Thus, most prevalent allergens known to be present in *S. cerevisiae* extracts were recovered [9]. For a control, pooled sera from five non-atopic subjects was used.

SDS-PAGE and electrophoretic blotting

The extracts (antigen concentration 10 mg/ml) and molecular weight markers (Rainbow, Amersham, Buckinghamshire, UK) were separated by SDS-PAGE in

a 5.0–17.5% gradient gel as described [11]. Thirty microlitres of each extract was diluted with 15 µl of Tris buffer and incubated for 5 min at 100°C. The separated antigens were electroblotted onto nitrocellulose sheets (Schleicher & Schuell, Kassel, Germany) in a Transphor apparatus (LKB, Bromma, Sweden) as described previously [12].

IgE antibody detection

The separated allergens on the nitrocellulose sheet were identified by specific IgE binding as described previously [13]. The serum pools were not diluted. ¹²⁵I-labelled anti-IgE antibodies (Phadebas-RAST, Pharmacia Diagnostics, Uppsala, Sweden) were diluted to 20 000 cpm/ml in PBS-Tween before use. The ¹²⁵I-labelled bands were detected on an X-ray film in a cassette with intensifying screens after exposure at -70°C prior to development.

Results

The stored extracts were yellowish in colour but there was no precipitate.

Glycerol (50%) was superior compared with saline, as regards the preservation of the antigenic composition of *S. cerevisiae* extracts. Figure 2 shows the Coomassie brilliant blue-stained gels of the yeast extracts stored for 6 months, with and without glycerol, in comparison with the stain of a freshly diluted extract. The study extracts were stored at +20°C and at +6°C. Only a few bands could be identified in the preparations stored in saline alone. The extracts stored in glycerol were only slightly more stable at +6°C than at room temperature. In the glycerol-containing extract stored at +6°C for 6 months the 25 kDa protein was best preserved, followed by the clearly degraded 70, 55 and 15 kDa bands. The 45 kDa protein band was relatively intact. Degradation products were seen in the region below 14 kDa.

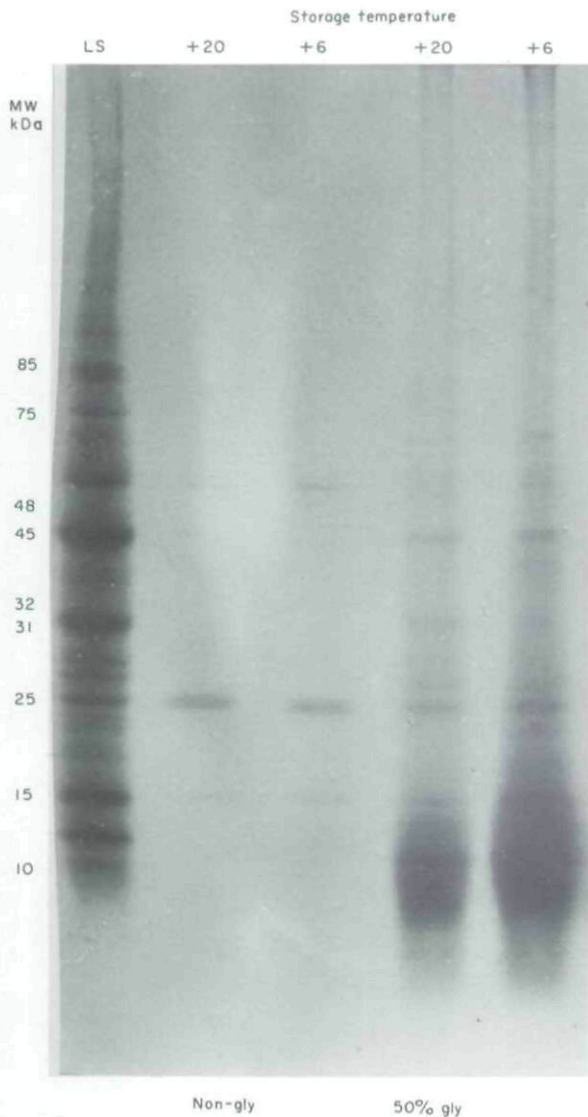


Fig. 2. Coomassie brilliant blue stained gel of yeast extracts stored at two temperatures in two preservatives. LS. Freshly diluted freeze-dried extract. Extracts stored for 6 months at room (+20°C) and refrigerator temperature (+6°C), in saline and 50% glycerol.

Signs of protein degradation were visible after 1 month of storage (Fig. 3). Protein staining of a freshly diluted preparation and the yeast extracts stored in 50% glycerol at +6°C for 9, 5 and 1 months and 2 weeks are shown. When extracts were stored for longer, only the low molecular weight protein bands and the 45 and 55 kDa bands were retained, although the intensity of these bands was also reduced.

IgE immunoblotting (Fig. 4) confirmed that allergen degradation had already taken place after 4 weeks of storage. An exception to this finding appeared to be the

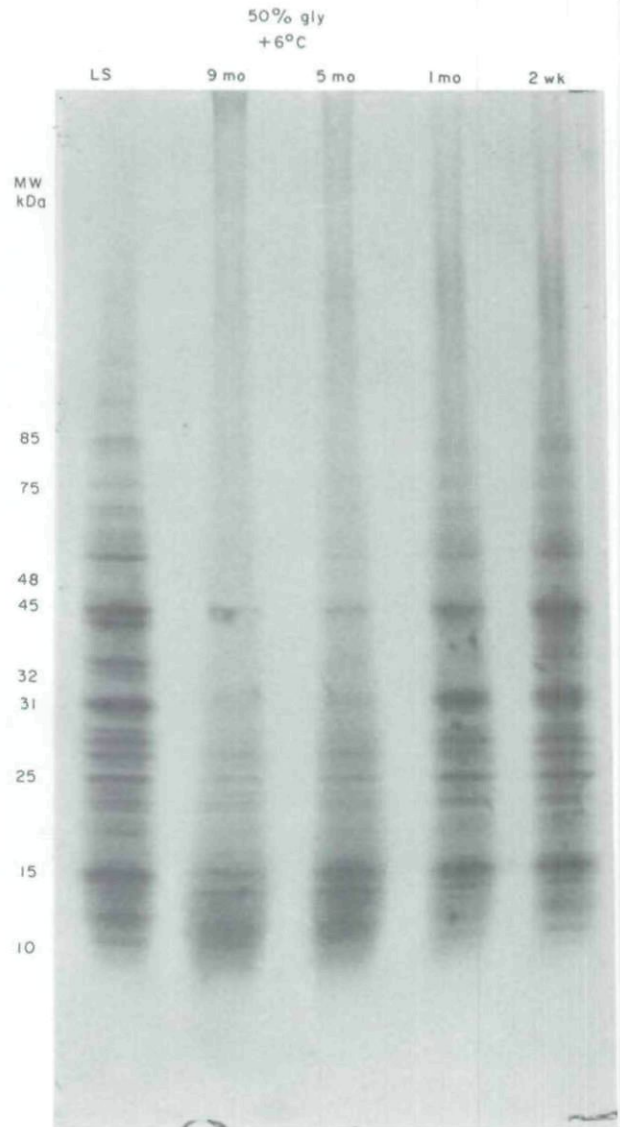


Fig. 3. Coomassie brilliant blue stained SDS-PAGE patterns of *Saccharomyces cerevisiae* extracts stored in 50% glycerol at +6°C for different time periods. LS. Freshly diluted extract. Extracts stored for 2 weeks, 1 month, 5 months and 9 months.

45 kDa allergen which was relatively stable in comparison with all the other allergens.

The lyophilized yeast extract which had been stored for 8 years had retained its protein composition well (Fig. 5). On the other hand, the two commercial extracts run in parallel did not stain or did so only very slightly.

The protein staining after heat treatment is shown in Fig. 6. Electrophoresis of the pellets after SDS treatment indicated that the proteins in the pellets were well preserved. Staining of the high molecular weight bands seemed to be more intense than that of the freshly diluted

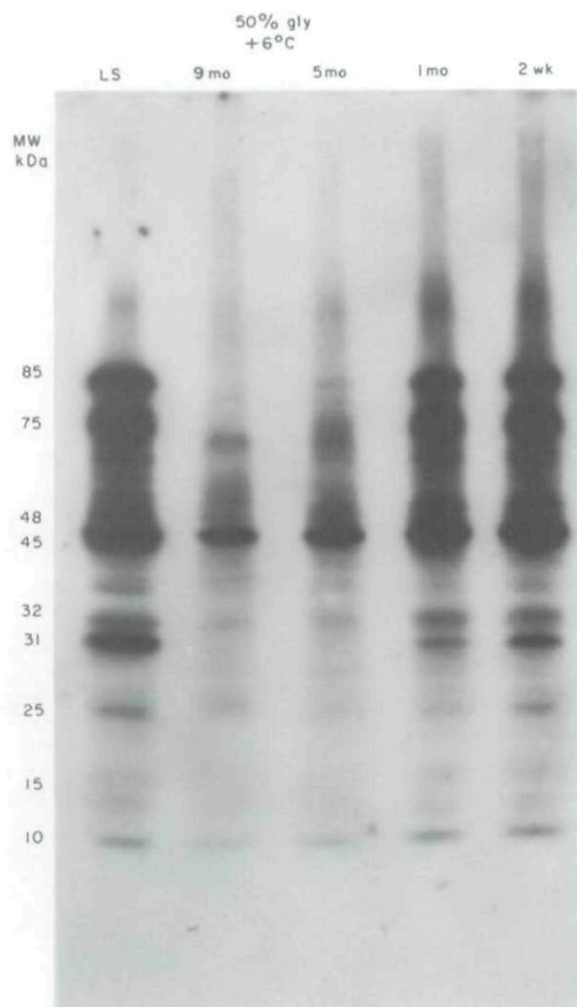


Fig. 4. IgE staining patterns with a patient serum pool of *Saccharomyces cerevisiae* extracts stored in 50% glycerol for periods from 2 weeks to 9 months. LS. Freshly diluted extract. Extracts stored for 2 weeks, 1 month, 5 months and 9 months.

extract. No protein bands were detected on electrophoresis of the supernatant. Consequently, most protein material probably precipitates when heated. IgE detection, however, revealed the important 10 kDa band and the typical high molecular weight diffuse mannan stain (50–150 kDa) [14] in the supernatant fluid of both heated extracts (Fig. 7). The 10 kDa band was equally intensive in both supernatants, but the mannan stain was markedly stronger in the extract heated for only 10 min. Heating of the extract for only 10 min also produced the 85 kDa band and for 20 min a weak 28 kDa band, not seen in the lyophilized control sample.

There was no IgE staining of control sera from non-atopic subjects.

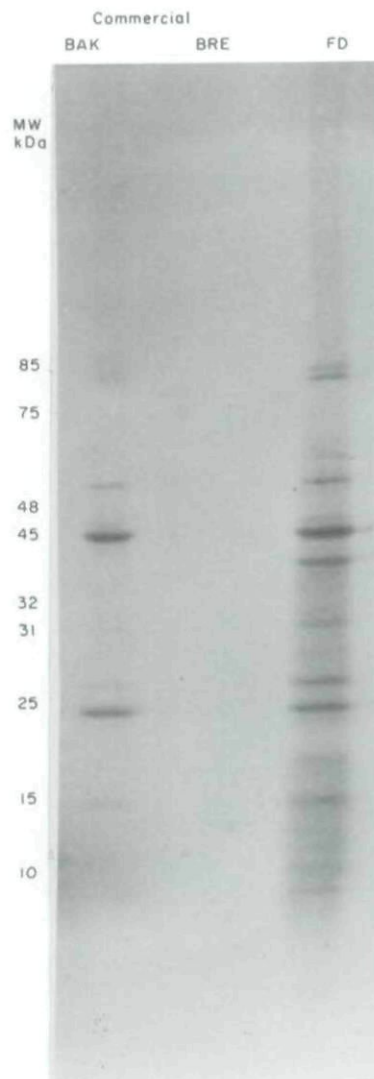


Fig. 5. Coomassie brilliant blue-stained SDS-PAGE analysis of two commercial extracts with expiry dates of November 1991 (BRE. Brewer's yeast) (*Saccharomyces carlsbergensis*) and June 1992 (BAK. Baker's yeast). The analysis was performed in October 1991. The freeze-dried *S. cerevisiae* extract (FD) was stored for 8 years and diluted prior to analysis.

Discussion

The finding that the high molecular weight *S. cerevisiae* protein bands stain more intensively after heat treatment is interesting. When heated, the *S. cerevisiae* extract clearly precipitates and it can be assumed that most of the protein components are precipitated and then solubilized after treatment with SDS. The proteins undoubtedly also precipitate during baking and the precipitated proteins are exposed to the harsh conditions of the gastrointestinal tract. However, the heat-stable IgE-binding components, the 10 kDa band and mannan present in the supernatant,

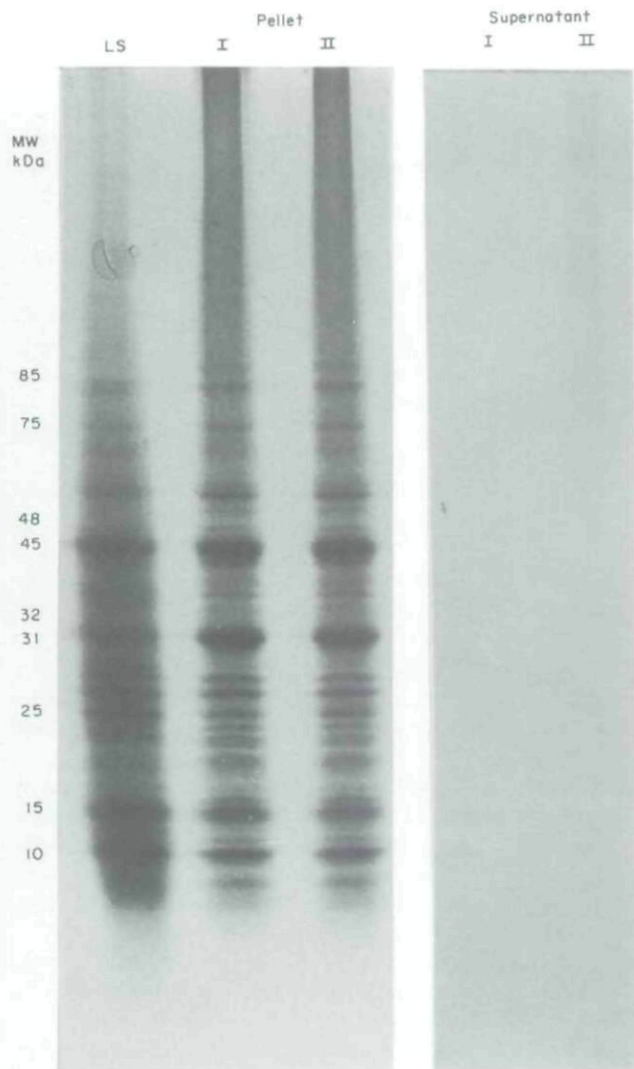


Fig. 6. SDS-PAGE analysis of baker's yeast extracts kept in a water-bath in an oven at 200°C for 20 min (I) and at 250°C for 10 min (II). A cloudy solution was obtained and centrifuged, the pellet and supernatant were analysed separately by dissolving them in SDS. LS. Lyophilized control sample.

were also preserved in the conditions simulating the gastrointestinal tract [15]. The 28 kDa band probably represents a degradation product of the 85 kDa band. These data suggest that the heating time is important for the degradation of allergens. Patients sensitized to mannan and the 10 and 85 kDa bands may become exposed to allergens of baker's yeast, if the duration of baking is too short.

The usual practice of allowing allergen extracts to remain at room temperature for several hours during the day while the skin tests are performed results in accelerated loss of allergenic potency [5]. The results of this study

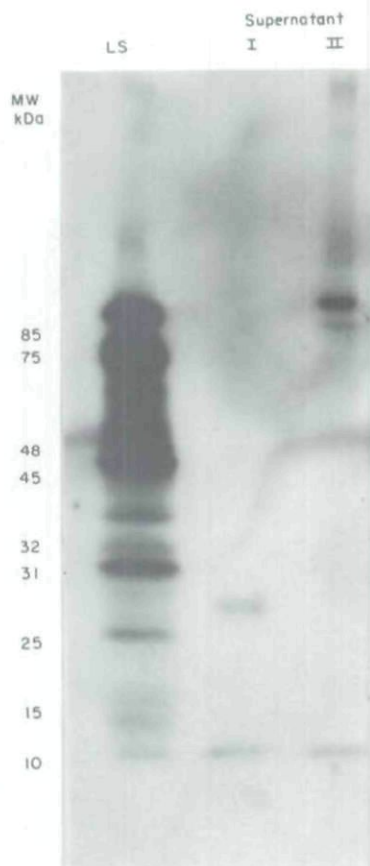


Fig. 7. IgE immunoblot of baker's yeast extracts kept in a water-bath in an oven at 200°C for 20 min (I) and at 250°C for 10 min (II). A cloudy solution was obtained and centrifuged and only the supernatant was studied by immunoblotting. LS. Lyophilized control sample.

indicate that the rapid decrease of *S. cerevisiae* allergens may cause false-negative results in skin-prick tests if extracts which have been stored at +6°C for only 1 month or more are used. The expiry date of the diluted extracts allows longer validity than is appropriate, as shown by the analysis of two commercial extracts.

Lyophilized extracts of ragweed pollen and moulds are much more stable over a range of temperatures and storage times than aqueous extracts [16,17]. The labile proteins in the *S. cerevisiae* extract, such as the 85, 48 and 31 kDa bands, were identifiable in the 8-year-old lyophilized extract, which also demonstrates the excellent stability of freeze-dried allergen of this yeast.

The finding that high molecular weight activity is enhanced in batches stored for a short time is of interest. This staining pattern resembles the pattern of a mannan polysaccharide [9]. Perhaps storage exposes new antigenic structures which become available for IgE binding. It is conceivable that the enhancement of the mannan band is

due to enzymatic cleavage of glycoproteins or carbohydrates.

It has recently been recommended that the assay of choice for immediate hypersensitivity to *Pityrosporum ovale*, another yeast, is skin-prick testing [18]. The stability of the *P. ovale* allergens in solution is not known. *Candida albicans* allergens are also poorly preserved during storage [19]. These observations question the reliability of skin-prick testing as a superior assay for type I hypersensitivity to yeasts when solubilized and longer stored commercial skin-test preparations are used.

This study allows the following recommendations regarding the storage and use of *S. cerevisiae* extracts as a reference material. *Saccharomyces cerevisiae* extracts should be stored as a freeze-dried material and should only be dissolved and diluted immediately before use. The maximum storage time of the diluted material is 1 month. Diluted *S. cerevisiae* should be stored at +6°C with glycerol (50% w/v).

Acknowledgements

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