

Stability of cereal allergens

E. VARJONEN, F. BJÖRKSTÉN* and J. SAVOLAINEN†

Departments of Dermatology and *Allergology, Helsinki University Central Hospital and †Department of Clinical Allergology, University of Turku, Finland

Summary

Background Ingested cereals and inhaled cereal dusts can cause IgE-mediated allergy. Testing for these allergies yields variable, often undependable results, which could be due to the use of unsuitable or degraded testing materials.

Objective We studied the effect of storage media, time and temperature on the stability of protein allergens extracted from wheat, rye, barley and oats flour. We also examined the effect of heat processing similar to that used when preparing foods on the stability of allergenic proteins present in wheat flour.

Methods After storage experiments proteins in allergen extracts were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then visualized using immunoblotting or staining with Coomassie brilliant blue. After heat processing wheat flour proteins were extracted and their IgE-binding capacity was estimated.

Results Extracted proteins from wheat, rye and barley flour appeared stable for at least 21 months when stored at 4°C in an aqueous solution containing 50% glycerol and 0.45% sodium chloride. In a medium with 0.4% phenol and 0.9% sodium chloride degradation of many proteins became apparent already after 1 month of storage. Oats extracts lost their IgE-binding capacity more rapidly and the maximal storage time appeared to be 10 months at 4°C in a solution containing 50% glycerol and 0.45% sodium chloride. The IgE-binding capacity of the allergenic proteins decreased as heat-processing temperature and heating time increased. No processing conditions entirely abolished IgE-binding.

Conclusion Cereal allergen preparations can be stored either in lyophilized form or at 4°C in 50% glycerol. Unheated materials should be used also when testing for food allergy, as heating reduces allergenicity and thus sensitivity at testing.

Keywords: allergen, barley, glycerol, heat, IgE, oats, phenol, rye, wheat

Clinical and Experimental Allergy, Vol. 26, pp. 436–443. Submitted 30 December 1994; revised 26 July 1995; accepted 16 October 1995.

Introduction

Allergen preparations, for instance extracts, tend to lose allergenicity, also termed potency, as they are stored. Storage stability varies from one allergen to another, generally it decreases with increasing storage temperature and improves with lyophilization [1–3]. Poor stability has been a major problem with many allergen preparations, especially when extracts are diluted for testing or therapy [4–6]. Relatively good stability has been observed for

allergens stored in 50% glycerol, which is frequently used as a solvent for skin-test preparations [3,6,7].

Ingested cereals cause symptoms of allergy particularly in children [8–10] and inhaled cereal dust in bakers [11–13]. Cereals in foods are usually heated before they are eaten and it would be logical to use similarly heated cereal allergens for testing patients with suspected food allergy, but this is not generally done.

The purpose of the present study was to examine the stability of cereal antigens and allergens during storage for different time periods, in various media and at various temperatures. For wheat flour also heat processing conditions were included to mimic food preparation.

Correspondence: Dr E. Varjonen, Department of Dermatology, Helsinki University Central Hospital, Meilahdentie 2, FIN-00250 Helsinki, Finland.

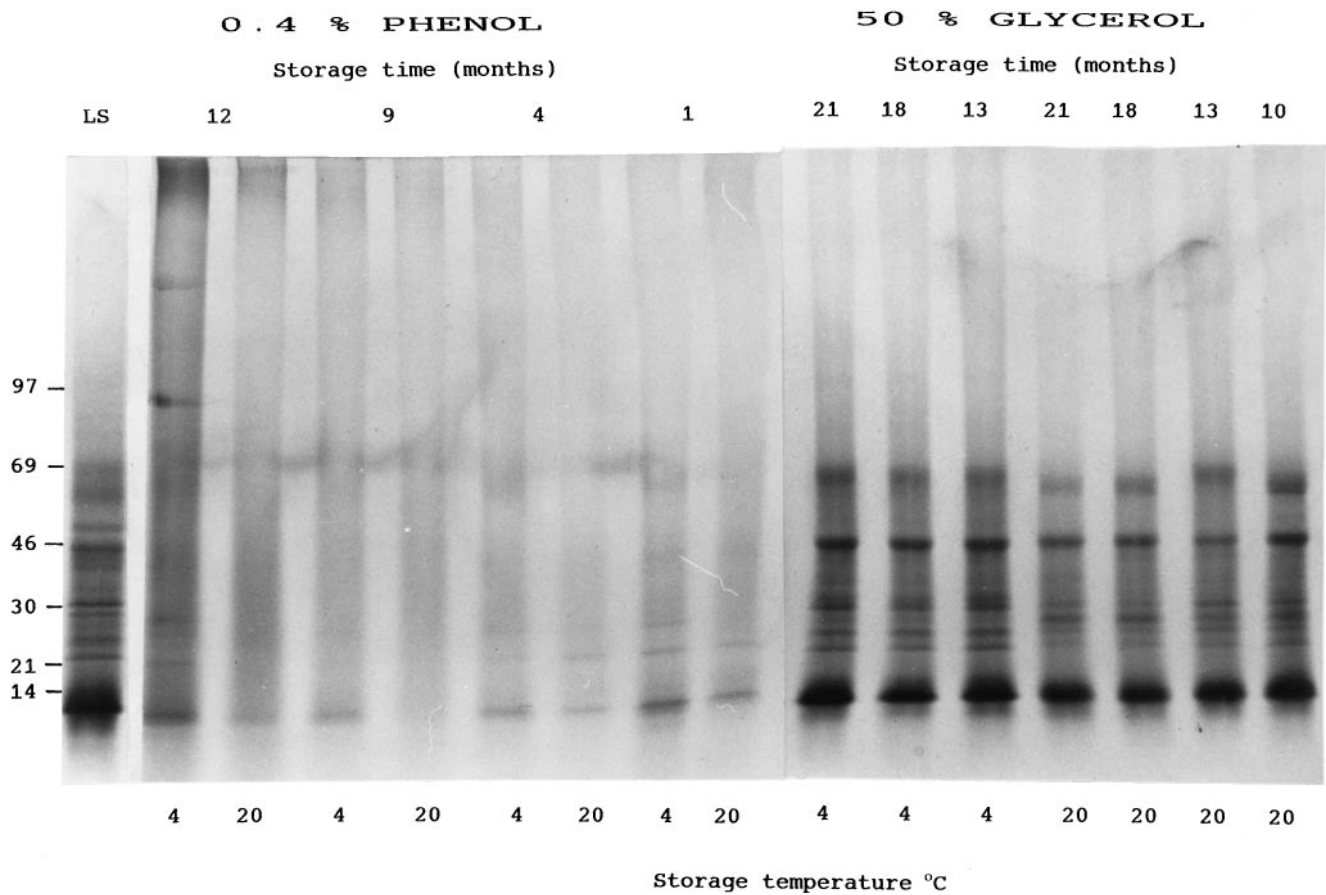


Fig. 1. Wheat-flour extracts in 0.9% sodium chloride solution were stored for various periods at 4°C or 20°C with either 0.4% phenol or 50% glycerol added as preservative. The figure shows protein patterns after SDS-PAGE separation and Coomassie blue staining. LS. Freshly-prepared and run lyophilized reference extract.

Materials and methods

Storage stability experiments

Neutral and acidic extracts of wheat, rye, barley and oats were prepared as previously described [12,14]. In brief, to obtain neutral extracts, flour samples were extracted with potassium phosphate buffer, pH 7.0, containing 400 mmol/L sodium chloride, and to obtain acidic extracts, with 50 mmol/L sodium acetate buffer, pH 3.8. Protein contents were determined according to Lowry *et al.* [15]. Extracts were lyophilized and stored at -20°. The final solutions for storage experiments were prepared by combining equal quantities of proteins obtained through neutral and acidic extraction and dissolving them in 0.9% sodium chloride solution with either 0.4% phenol or 50% glycerol added as preservative. Batches of phenol and glycerol containing solutions were then stored at 4° and at 20° for 1, 4, 9, 12, 13, 18 and 21 months.

Experiments to determine stability during heat processing

Wheat flour was heat processed in sealed cans of the kind used for foods. Flour was placed into the cans as such (residual water 11%) and as wet dough containing 50% added water. Various cans were then heated in an autoclave at 80°, 100° and 120° for 10, 20 and 60 min. After heating, neutral and acidic extracts were prepared as described above and allergens were coupled from the extracts onto radioallergosorbent test (RAST) discs as previously described [12].

Sera

To prepare a serum pool, sera of 12 adult patients with atopic dermatitis (AD) (aged 17–48 years, mean 29 years) who had positive reactions to cereals in skin-prick tests (SPT) and/or radioallergosorbent assays (RASTs) were combined. In a previous study these sera had been found to have IgE

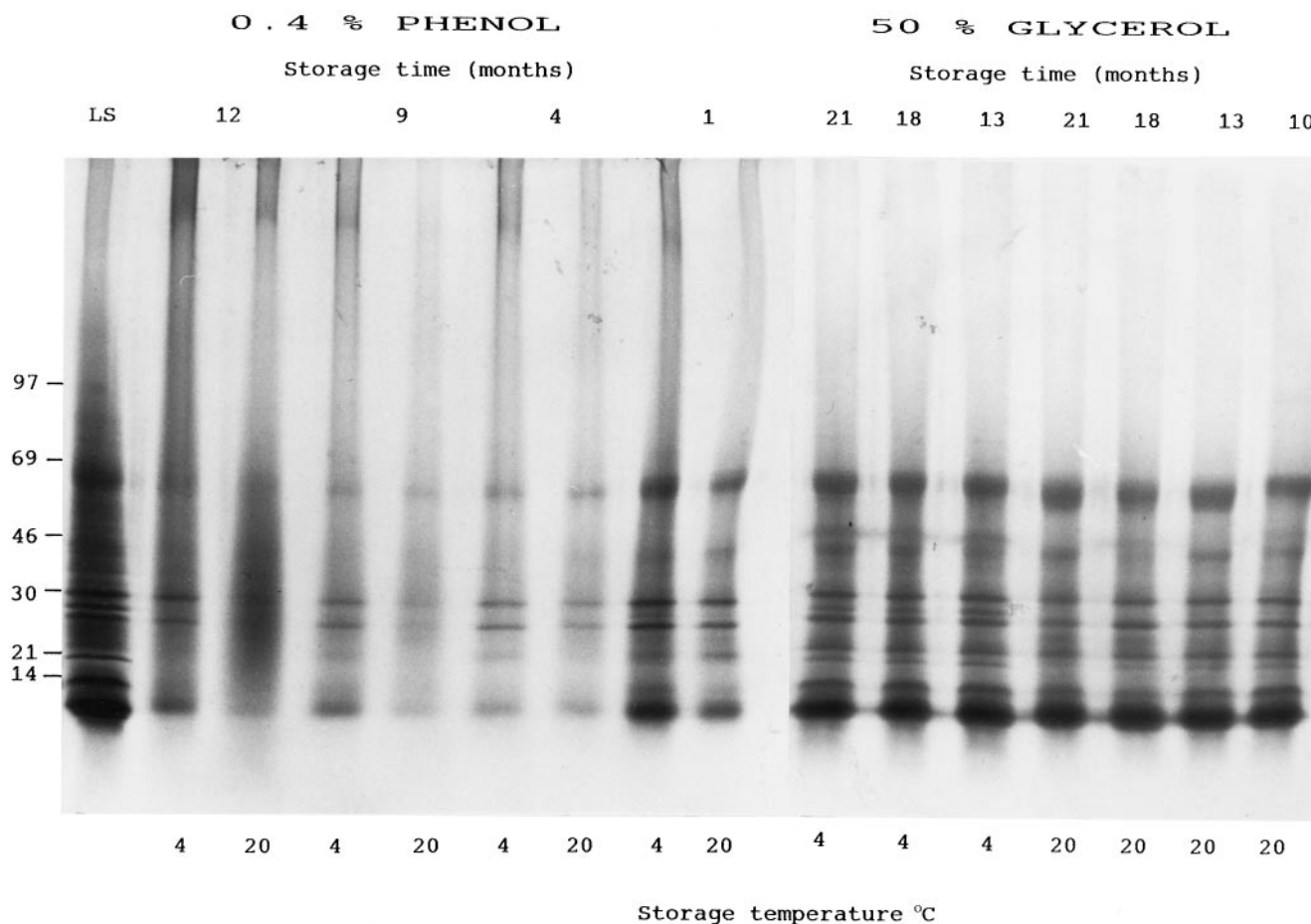


Fig. 2. Rye-flour extracts stored and analysed as in Fig. 1.

antibodies which bound strongly to various cereal proteins [14]. The serum pool was used for immunoblotting in the storage stability part of the study.

A second pool contained equal volumes of sera from 13 paediatric patients with AD (aged 2–12 years, mean 4.3 years) with positive cereal RASTs. The pool and individual sera from 16 children similar to those just described were used in the heat processing stability part of this study.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

After various storage periods and conditions the proteins in cereal extracts and molecular weight markers (Rainbow, Amersham, UK) were separated by SDS-PAGE according to Laemmli [16]. Equal amounts of cereal proteins were loaded into the slots (100 µg/slot) and were separated in a large 5–17% gradient gel under non-reducing conditions. Separated antigens were then electroblotted to nitrocellulose in a Transphor apparatus (LKB, Bromma, Sweden)

according to Towbin *et al.* [17]. The protein bands on the gel were stained with Coomassie brilliant blue.

Thereafter, the nitrocellulose sheets were blocked with PSB-Tween 0.5% for 1 h at 37°C. To detect IgE-binding proteins, nitrocellulose sheets were incubated at room temperature overnight in the undiluted serum pool. Sheets were then washed three times for 10 min with a solution containing 0.9% sodium chloride and 0.5% Tween and thereafter incubated for 4 h at room temperature with ¹²⁵I-labelled anti-human IgE (Pharmacia, Uppsala, Sweden) diluted 1 : 10 with phosphate-buffered saline (PBS) containing 0.5% Tween. The ¹²⁵I-labelled bands were detected through autoradiography using x-omatic film (Kodak, USA) an intensifying screen and storage at –70°.

The radioallergosorbent test (RAST)

Equal volumes of neutral and acidic extracts prepared from heat processed wheat flour were combined and used for coupling proteins onto cyanogen bromide activated paper

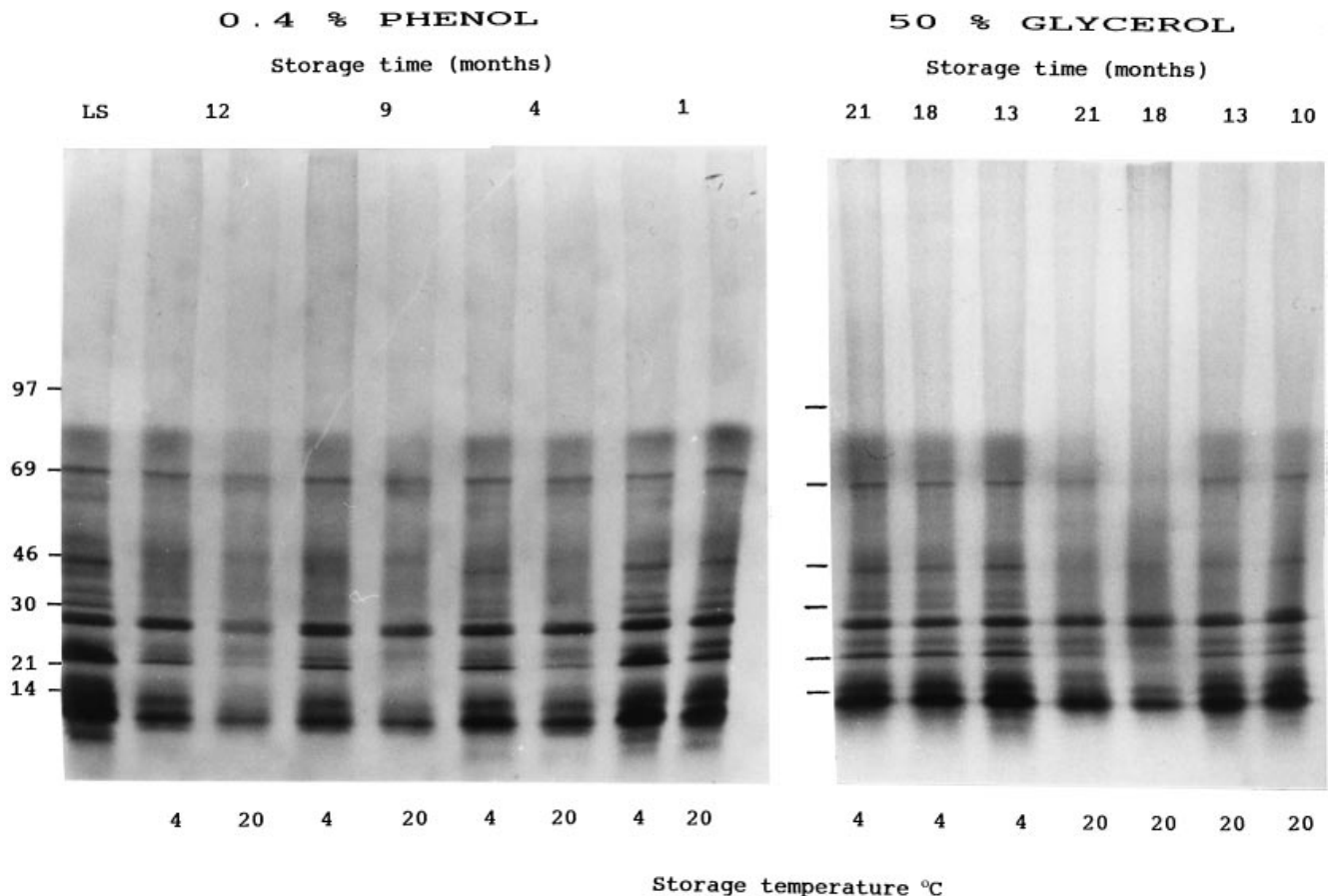


Fig. 3. Barley-flour extracts stored and analysed as in Fig. 1.

discs [18]. Other RAST reagents, including standards, were obtained from Pharmacia (Uppsala, Sweden).

Results

Storage stability in 50% glycerol

Proteins appeared to remain largely undegraded after storage in 50% glycerol at 4° for at least 21 months and at 20° for 10 months as judged by the permanence of Coomassie brilliant blue stained protein bands after SDS-PAGE separation of proteins from preparations stored under various conditions. This was true for both wheat (Fig. 1), rye (Fig. 2) and barley (Fig. 3), while the stability of oats proteins appeared somewhat poorer as the 66 kDa major IgE-binding protein in oats was seen to degrade after 13 months at 4° (Fig. 4).

The stability of proteins in 50% glycerol was further evaluated by immunoblotting of SDS-PAGE separated proteins. It was evident that the binding pattern of glycerinated extracts stored at 4° for 21 months and at 20° for 10

months were generally comparable to the immunoblotting pattern obtained with freshly prepared lyophilized extract. Again oats preparations had the poorest stability with the 66 kDa main IgE-binding protein stable for a maximum of 10 months (Fig. 5).

Storage stability in the presence of 0.4% phenol

When stability in the presence of 0.4% phenol was similarly evaluated it was found to be much poorer. Most wheat and rye protein bands had deteriorated already after 1 month of storage (Figs 1, 2, 5A and 5B). IgE-binding proteins of barley were more stable; the main protein bands were detected in the immunoblotting patterns even when stored for 10 months at 4° (Fig. 5C). The 66 kDa main IgE-binding protein in oats was similarly stable for 10 months at 4° in 0.4% phenol (Fig. 5D).

Stability during heat processing

Proteins extracted from heated flours were coupled to RAST discs and the capacity of the discs to bind IgE from pooled

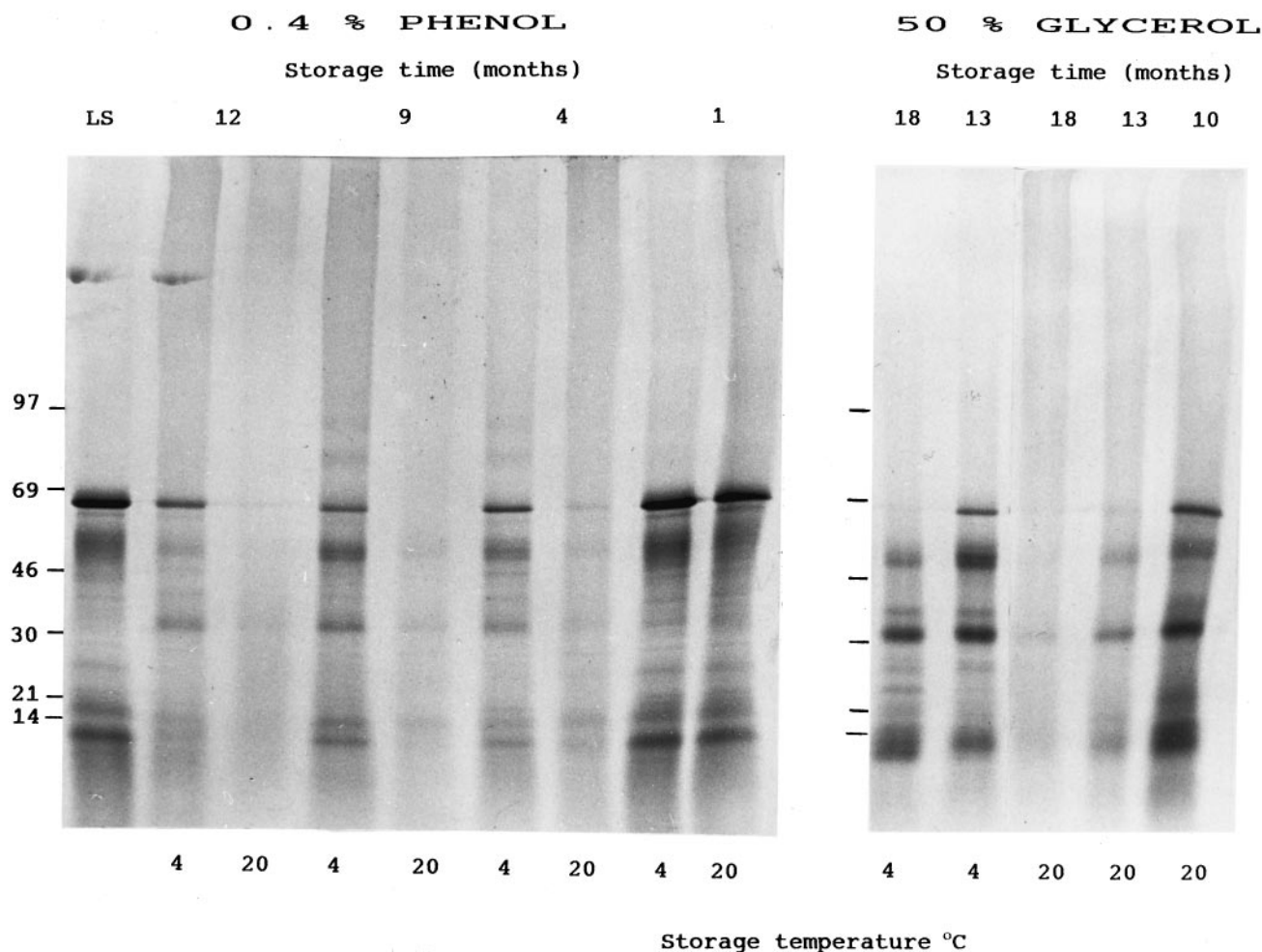


Fig. 4. Oats-flour extracts stored and analysed as in Fig. 1.

children's sera was examined. The binding capacity fell with the heating time, heating temperature and water content during heating (Fig. 6). Similar observations were made with individual sera from 16 children with wheat-specific IgE. Discs to which unheated wheat antigen had been coupled always bound the largest quantity of IgE (Fig. 7).

Discussion

We found that IgE-binding proteins in cereal extracts made from wheat, rye and barley were reasonably stable when stored in 50% glycerol for up to 21 months at 4°C or for 10 months at 20°C. Oats preparations were found to deteriorate somewhat more rapidly, the 66 kDa main IgE-binding protein was stable for 10 months at 20°C in 50% glycerol as judged by SDS-PAGE followed by immunoblotting. When 0.4% phenol was used as

preservative most proteins from all cereal preparations were lost already after a 1-month storage (Figs 1–5).

The stability of cereal flour extracts has previously been difficult to study, as the antigenic and allergenic composition has been poorly known. Our previous immunoblotting results with adults and cereal allergic children with atopic dermatitis showed most frequent IgE-binding to wheat proteins at 26, 28 and 79 kDa in adults [14] and to 26 and 69 kDa in children [19]. All these bands could be visualized in wheat extract stored for 21 months in glycerol at 4°C. The important 66 kDa allergen in oats both with sera from adults and children was visible in oats extract stored for 10 months.

Our storage stability results are similar to those reported in several preceding studies with other allergen extracts. Center *et al.* [1] found that glycerinated and lyophilized ragweed pollen extracts retained their allergenic content for over 15 months, while phenol was destructive to those allergens. Anderson *et al.* [20] reported that there was a

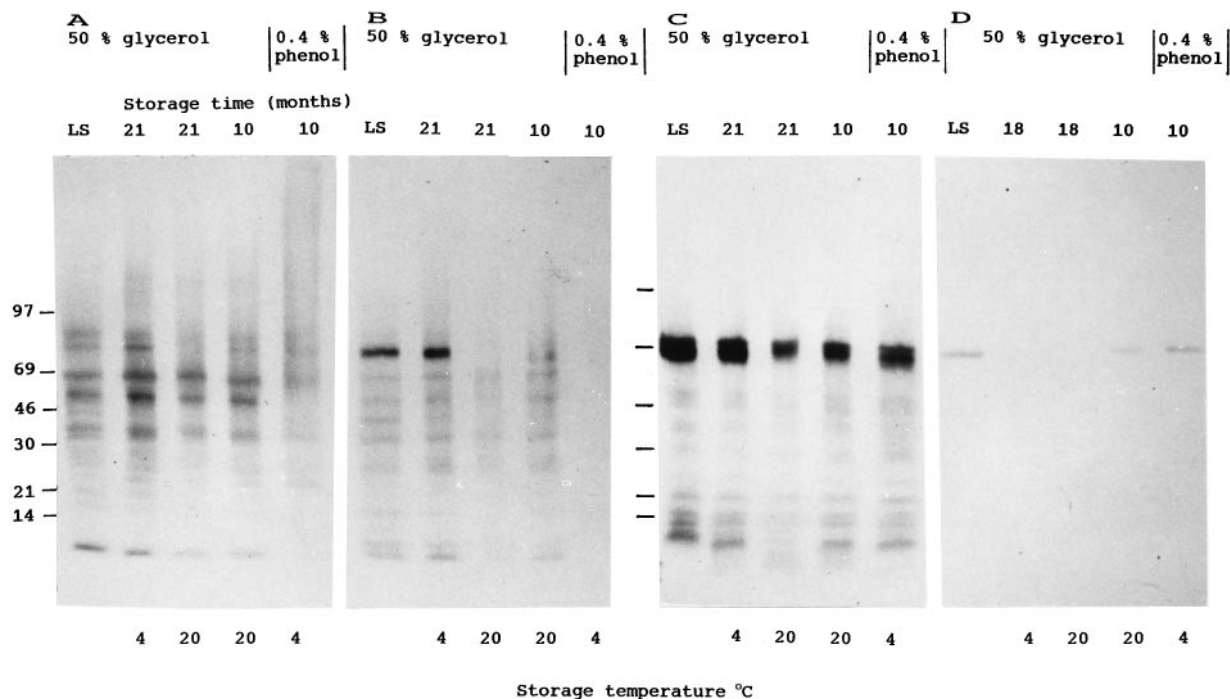


Fig. 5. Wheat, rye, barley and oats-flour extracts stored as in Fig. 1. The figure shows IgE-binding protein patterns after SDS-PAGE separation. The cereal-specific IgE came from a serum pool obtained from adult AD patients. Bound IgE was detected with ^{125} I-labelled anti-human IgE. LS. Freshly prepared and run lyophilized reference extract. A. Wheat. B. Rye. C. Barley. D. Oats.

rapid drop of allergenic activity when timothy grass pollen extracts were stored at 35°C, slower changes occurred at 22°C and no changes at 4°C. Stability is a property which varies from one allergen to another. Thus, the cereal allergens studied now were more stable than similarly stored mould [21] and yeast [3,7] allergens, the preparations containing high enzymatic activity. When cereal proteins are extracted according to protein solubility [22] the salt-solutions are known to extract also metabolically active minor protein groups, i.e. enzymes, along with albumins and globulins [23]. In oats this salt-soluble protein fraction accounts for over half of the total protein content whereas in wheat, rye and barley the other protein fractions, prolamins and glutelins without enzyme activity, predominate [24,25]. It might be that oats extract contains higher enzymatic activity, which may account for the more rapid breakdown of oats proteins compared with that of the other cereals together with the previous finding that oats revealed only half the amount of IgE stained bands compared to wheat [14,19].

It can be concluded that 50% glycerol is a good storage medium [3,7,20] which stabilizes and presumably inhibits the adsorption to surfaces of many proteins. Phenol will effectively inhibit microbial growth, but it is a reactive chemical — also reactive with proteins — and it is poorly suited for the preservation of allergen preparations.

We found that the allergenicity of wheat flour proteins was reduced but not eliminated, by heating flour under conditions similar to those used in food processing. Allergenic proteins coupled to RAST discs from heated flour preparations bound less wheat-specific IgE from sera of children with AD and positive wheat-specific RASTs than unheated allergens. When wet dough containing 50% water was heated even less IgE-binding capacity usually remained, but this could have been due to poor extraction of proteins from the dough (Fig. 6).

Previous heating studies have given similar results. Thus, Sutton *et al.* [10] observed that gluten (wheat protein) was rendered less allergenic by heat treatment, as vital gluten gave higher RAST uptakes than devitalized (heated) gluten. Shibasaki *et al.* [26] found that when rice glutelin and globulin fractions were heated RAST activities were reduced to 40–70% of that with native preparations. In both studies sera came mostly from children with AD, but similar results were also obtained with a few sera from persons allergic to flour dust.

We conclude that allergen preparations to be used for patient testing should either be stored lyophilized or at 4°C in 50% glycerol. Although it would seem logical to test for food allergy using heated cereal allergens, we suggest the use of unheated materials, as sensitivity will otherwise be reduced.

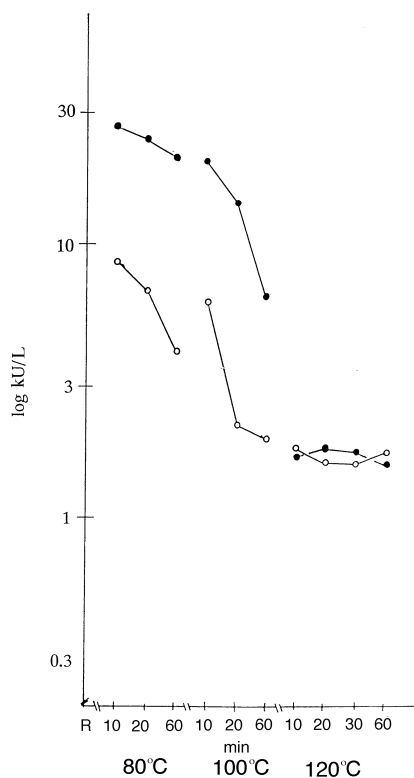


Fig. 6. Heat-moisture effects on the IgE-binding capacity of wheat flour proteins measured with the RAST method using a serum pool from children with AD. ●—, Dry heated. —○—, Wet heated.

Acknowledgments

This study was financially supported by a grant from the Finnish Allergy Research Foundation. Mrs Leena Kavén-Honka gave skillful technical assistance.

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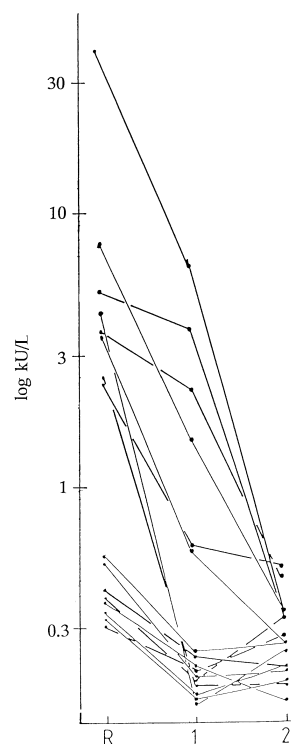


Fig. 7. Effect of wet heating on the IgE-binding capacity of wheat flour proteins. Individual sera from 16 children with AD and with wheat-specific IgE were used in measurements with the RAST. R. Unheated wheat flour. 1. Wet wheat flour heated at +80°C for 10 min. 2. Wet wheat flour heated at +120°C for 20 min.

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