

Wheat antigen exposure assessment for epidemiological studies in bakeries using personal dust sampling and inhibition ELISA

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Summary

Background Asthma in bakery workers caused by exposure to wheat flour proteins is an important occupational health problem. Until recently, gravimetric dust measurements were the only available technique for quantitative exposure assessment in bakeries. However, it is questionable whether dust levels are a good exposure parameter or only give a crude approximation of the actual flour allergen concentration.

Objective In the present study we have investigated a method to measure wheat flour antigens with immunochemical methods.

Methods Wheat flour antigens were measured in 449 personal dust samples taken in bakeries, using enzyme-linked immunosorbent assay (ELISA) inhibition and an anti-wheat IgG4 serum pool. Western-blotting was performed to compare the wheat flour proteins detected by IgE and IgG4.

Results Electrophoresis and immunoblotting showed that many wheat flour proteins can bind IgG4 and IgE, but also a reasonable similarity in major allergens detected by our IgG4-serum pool and IgE-positive sera. Inhibition tests showed some cross-reactivity with some cereal species, but not with other ingredients used in bakeries. In bakeries, large differences in personal airborne flour levels were found between occupational titles. For several groups clear differences in wheat antigen exposure levels existed, where no differences in dust exposure levels could be found. The relationship between dust and wheat antigen exposure varied considerably, depending on the specific bakery occupation, the size of the bakery, and the type of product produced by the bakery. This study also shows that personal sampling of wheat antigens is possible on a large scale and can be used for epidemiological field studies.

Conclusion Measurement of airborne wheat antigens in bakeries is a more specific and sensitive measurement tool than measuring dust samples, and will probably be essential for epidemiologic field studies focusing on exposure-response relationships.

Keywords: occupational allergy, occupational asthma, bakery workers, wheat flour, airborne allergens, exposure, inhibition ELISA

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Introduction

Occupational respiratory diseases are common among bakery workers and others exposed to flour dust.

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According to most authors this affliction appears to be an IgE-mediated type I allergic reaction to flour dust [1,2]. IgE antibodies to a number of wheat flour components have been demonstrated in allergic bakers' sera, and the strongest reactivity was observed with water-soluble proteins, particularly albumins [2-4].

Epidemiological studies among bakery workers show prevalence rates of allergic rhinitis of 15-20%, while baker's asthma is prevalent in 5-7% of the bakers [5-8]. At present little is known about the incidence of baker's allergy, and although reliable data based on field studies are not available, some registry based incidence data have been published [9-12]. These data showed that this profession can be placed in the group with high risk for occupational lung diseases.

Until now little is known about the relation between exposure levels in bakeries and the prevalence or incidence of work-related allergies. Although a large number of studies of baker's asthma have been published, information about exposure levels in bakeries is very limited. In a few studies exposure to flour dust was assessed using gravimetric methods [8,13-15], but available data are not sufficient to be used to explore exposure-response relationships. Moreover, it is questionable whether dust levels are a good exposure parameter or only give a crude approximation of the actual flour allergen concentration. Direct measurements of the wheat protein allergens with immunochemical methods would probably be a more valid approach.

Two recent papers have described an application of these methods in bakeries and flour mills, using radio-allergosorbent test (RAST) inhibition and an IgE serum pool from sensitized bakers [16] or polyclonal rabbit IgG antibodies [17]. In these studies wheat flour allergens were measured in personal dust samples taken in large industrial English bakeries and mills. In most other

European countries a lot of bread and confectionary production takes place in small bakeries, which differ in several aspects from the large industrial bakeries (e.g. type of products and sort of equipment). Wheat flour allergen levels in these bakeries have never been published. In all situations, little is known about the relation between levels of dust and wheat flour allergens.

In the present study we have investigated an alternative method to measure wheat flour antigens, using ELISA inhibition and bakers' sera with high anti-wheat IgG4 titres. With this method, the concentration of wheat flour antigen was measured in 449 personal dust samples taken in small and large bakeries. Special attention was paid to the relation between dust and aero-antigen exposure in different types of bakeries.

The presented results are part of a large prospective study among a few hundred bakery workers in The Netherlands. Primary aim of the whole project is to study exposure-incidence relationships between allergen exposure and asthma.

Materials and methods

Bakeries

Twenty-one bakeries were studied over 1-20 days. These bakeries differed in a number of characteristics, such as number of employees, products, and technology level. Table 1 shows some of the characteristics of the bakeries.

Dust sampling and filter extraction

In all bakeries, personal inhalable dust samples were collected in the workers breathing zone during full-shift periods of 6-8 hours, using polytetrafluoroethylene

Table 1. Characteristics of the bakeries in the study

Bakery	Number of employees	Main products	Days measured	Number of employees measured	Number of personal measurements	Automation ¹
1	200	Wheat bread & pastry	12	73	188	++
2	40	Wheat bread	2	21	39	++
3	170	Crispbakes, pastry & Rye bread	20	72	182	++
4	10 ²	Pastry	3	3	9	+
5	25 ²	Biscuits	3	5	13	++
6-21	1-6	Bread & pastry	1-6	47	115	0/+

¹ Automation of production: 0 = low, + = fair, ++ = high.

² Only production area (slicers, packers & transport workers not measured).

(Teflon) filters (Millipore; pore size $1.0\ \mu\text{m}$) and PAS-6 sampling heads at a flow rate of $2\ \text{L}/\text{min}$ [18]. Production workers were asked to participate for 1–6 days. After sampling, filters were weighed in a preconditioned room of $18\text{--}22^\circ\text{C}$ with 45–55% humidity. Detection limit of this method is $0.17\ \text{mg}/\text{m}^3$, computed by dividing the mean plus three times the standard deviation of 24 blanks by the mean sample volume. In the statistical analysis, values below the detection limit ($n = 44$) were considered as being two-thirds of this limit, as proposed by Helsel [19]. Wheat antigens were recovered from the filters by extraction with $2.5\ \text{mL}$ $0.15\ \text{M}$ Phosphate Buffered Saline (PBS, pH 7.4) in a $10\ \text{mL}$ centrifuge tube. Each tube was vortexed for 2 min, sonicated for 2 min, vortexed for 5 min and sonicated for 2 min, successively. The extract was centrifuged at $5000g$ for 15 min. The supernatant was collected and stored at -20°C for up to 6 months.

Extraction of wheat antigen standard

A standard wheat antigen preparation for use in the ELISA inhibition was obtained from a batch of wheat flour from bakery 1 (see Table 1). This wheat flour was extracted in the same way as the dust samples (but now $5\ \text{mL}$ PBS per $500\ \text{mg}$ wheat flour). After centrifugation, the supernatant was filtered through a $0.22\ \mu\text{m}$ filter (Millipore), dialysed against distilled water, freeze-dried and stored at 4°C . Before use in the ELISA inhibition, freeze-dried extract was diluted in PBS and protein concentration of the wheat flour extract was measured (approximately $0.45\ \text{mg}$ protein per mg freeze-dried material) using the BCA-protein assay (Pierce; Rockford, IL, USA).

Sera

Sera from 140 workers of bakery number 1 were collected and screened by ELISA for the presence of specific IgG4 against the standard wheat flour extract. Sera of 59 workers with intermediate and high IgG4-titres (OD492 >0.5 at serum dilution 1:400; maximum OD of ELISA-reader was 3.0) were pooled, and subsequently used as the antibody source in the inhibition ELISA.

Sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE) & immunoblotting

Lyophilized wheat flour extract ($1\ \text{mg}$ protein per mL) was dissolved in sample buffer according to Laemmli [20] and incubated for 10 min at 100°C . This extract was subjected in a $10 \times 7.5 \times 0.05\ \text{cm}$ 13.5% polyacrylamide gel ($30\ \mu\text{L}$ extract per cm), using a Mini-Protein II slab cell

unit (Biorad, Richmond, CA, USA). Electrophoresis was performed at $100\ \text{V}$ for 10 min and at $200\ \text{V}$ for 50 min, successively. Low molecular weight markers (Biorad) were included in separate lanes in each gel.

After SDS-PAGE, proteins were blotted onto a nitrocellulose sheet (Sleicher & Schuell; $0.22\ \mu\text{m}$ pore size), using a Mini-Trans blot transfer unit (Biorad). The transfer was performed at $3.33\ \text{mA}/\text{cm}^2$ for 1 h at room temperature.

After blotting, the nitrocellulose sheet was cut into strips of $1.5\ \text{mm}$, washed three times (10 min each in PBS (pH 7.4), containing 0.1% Tween 20), incubated with PBS-Tween containing 0.2% (w/v) gelatin (PBTG), and washed again. Strips were incubated overnight at room temperature with $140\ \mu\text{L}$ undiluted individual sera from the serum pool or from IgE-sensitized bakers.

For sera from the serum pool, a further 1.5-h incubation at room temperature was performed with 1:1000 diluted mouse monoclonal anti-human IgG4 conjugated with horseradish peroxidase (Central Laboratory for Blood Transfusion (CLB), Amsterdam, The Netherlands). Bound IgE from sensitized bakers was detected by subsequent 1 h incubations with 1:500 diluted mouse-anti-human IgE (CLB), 1:500 diluted biotinylated rabbit anti-mouse immunoglobulins, and 1:200 diluted peroxidase conjugated avidin (both Dakopatts, Copenhagen, Denmark). Bound peroxidase was detected by incubation for 30 min with TMB (Promega Corporation, Madison, WI, USA).

ELISA inhibition procedure

Polystyrene high capacity microtitre plates (nr 655061, Greiner, Nuertingen, Germany) were coated overnight at 4°C with the standard wheat flour extract at a concentration of $5\ \mu\text{g}/\text{mL}$ protein in $0.15\ \text{M}$ PBS in volumes of $200\ \mu\text{L}/\text{well}$. Next morning the plate was washed with PBS-Tween and incubated for 30 min at 37°C with PBTG; $100\ \mu\text{L}$ samples of 12 serial dilutions of the standard wheat flour antigen preparation (starting with $12.5\ \mu\text{g}/\text{mL}$ protein in PBTG) and $100\ \mu\text{L}$ of undiluted samples, were added to the wells and mixed with $100\ \mu\text{L}$ subsequently added pooled serum (diluted 1:800 in PBTG). On each plate four blanks ($200\ \mu\text{L}$ PBTG), four estimates of 0% inhibition ($100\ \mu\text{L}$ assay buffer), and four estimates of 100% inhibition ($100\ \mu\text{L}$ standard antigen of $12.5\ \mu\text{g}/\text{mL}$) were included. The plate was incubated for 2 h at 37°C . After washing, bound IgG4 was measured in each well by incubation (1 h at 37°C) with $200\ \mu\text{L}$ conjugated anti-human IgG4 (CLB) and finally an incubation (30 min in the dark at 20°C) with $200\ \mu\text{L}$ *o*-phenylenediamine (OPD; $2\ \text{mg}/\text{mL}$) in $0.05\ \text{M}$ citrate/phosphate buffer (pH 5.5), containing 0.015%

H₂O₂. The reaction was stopped by adding 50 µL 2 M HCl and the optical density was read at 492 nm with an ELISA-reader (SLT, EAR 340 AT).

A standard curve of OD492 against the log concentration of standard antigen was calculated with 4-parameter curve fitting, using the SOFTmax software package (Molecular Devices Corporation; Menlo Park, CA, USA). Antigen concentrations of samples were calculated by the same computer program. For high concentration samples, additional sample dilutions were tested if necessary. All samples were analysed in triplicate on three different days, and the mean value was used for further statistical analysis. The flour aero-antigen concentration was calculated as follows:

$$\text{Flour aero-antigen (ng/m}^3\text{)} = ([\text{flour}] \text{ (ng/mL)} * \text{volume of filter eluate (mL)}) / (\text{volume of sampled air (m}^3\text{)}).$$

Statistical analyses

Differences in mean exposure levels between several groups were analysed using SAS software (PROC ANOVA). Differences with $P < 0.05$ (two-sided) were considered significant. Because of the log-normal distribution of exposure data, these tests were done with log-transformed dust and wheat antigen concentration.

Results

SDS-PAGE and immunoblotting

The results of the blotting experiments with 24 randomly selected sera of the 59 sera in our IgG4-serum pool are summarized in Table 2. Although different patterns were obtained with regard to the number, molecular weight and intensity of the bands recognized by each individual serum, some prominent bands could be found. In the serum pool, which is used in the inhibition ELISA, 67% of the sera reacted to the 14 kDa band and 50% to the 30–32 kDa band. Figure 1 shows an immunoblot of our IgG4 serum-pool and sera from three allergic bakers (in duplicate), with high anti-wheat IgE-titres (class 3 or higher). Again, there is heterogeneity in the number and molecular weights of bands recognized by the IgE-positive sera. Most bands detected by the IgE-positive sera are also detected by our IgG4-serum pool.

Detection limits, reproducibility and specificity of the inhibition ELISA

Figure 2 shows the inhibition curve of our wheat flour

Table 2. Molecular weights (kDa) of wheat flour proteins detected by 24 of the 59 sera in our IgG4-serum pool

Molecular weights (kDa)	Sera n (%)
14	16 (67)
19	9 (38)
22	3 (13)
25	3 (13)
32	12 (50)
38	5 (21)
45	2 (8)
50	7 (30)
57	6 (25)
86	6 (25)

extract. Wheat flour concentration of samples were only determined between 20% and 85% inhibition. Samples with inhibition greater than 85% were diluted and tested again. This means that the detection limit of the assay is between 20 ng/mL and 1.6 µg/mL. Because all personal samples are diluted in 2.5 mL and the sampled volume is around 1 m³, this results into a detection limit for personal antigen measurements of 50 ng/m³. As for the dust measurements, values below the detection limit ($n = 70$) were considered as being two-thirds of this limit. The mean OD492 of the 0% inhibition controls was 2.1 (reactivity of 800-diluted serum).

The reproducibility of the assay was estimated by computing coefficients of variation (CV) for the triplicate analysis of the wheat antigens in all personal samples. The inter-day coefficient of variation in the analysis was 19.5%, which was independent of the antigen level.

The specificity of this ELISA was determined by inhibition tests with other ingredients which are frequently used in the bakeries, and some commercial mould extracts (ALK Benelux, the Netherlands). As shown by Fig. 2, extracts from closely related grain species showed some inhibition, suggesting crossreactivity with wheat antigens. As judged from the protein concentrations required for 50% inhibition, the inhibitory capacities of barley, rye and oats extracts were approximately 10, 500 and 2000 times less than that of the wheat flour extracts, respectively. Extracts of baker's yeast, egg, fungal α-amylase (which is added as a dough improver) and all tested mould extracts (*A. candidus*, *A. flavus*, *A. niger*, *A. oryzae*, *A. versicolor*, *C. herbarium*, *P. aurantiogr.*, *P. brevicompactum*, and *P. expansum*) gave no inhibition.

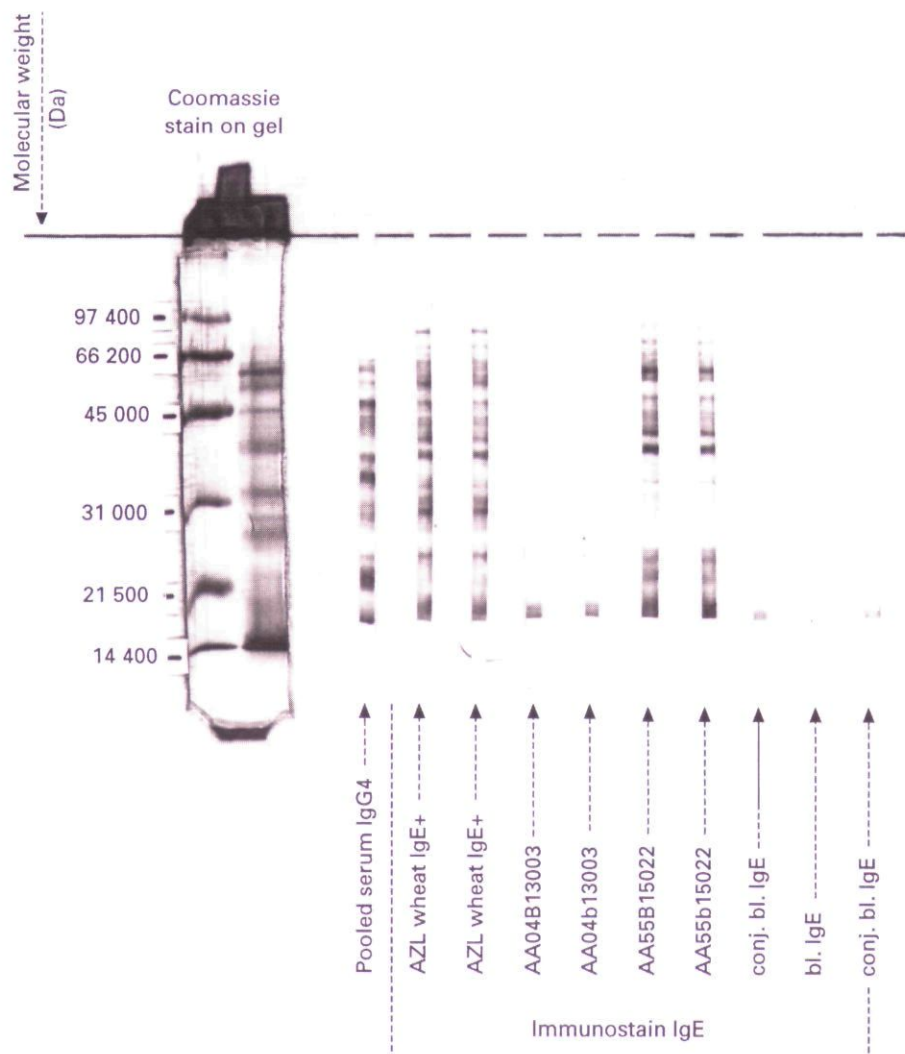


Fig. 1. Wheat flour immunoblots with human IgG4 serum pool (track 1) and human IgE (from sensitized bakers; tracks 2–7).

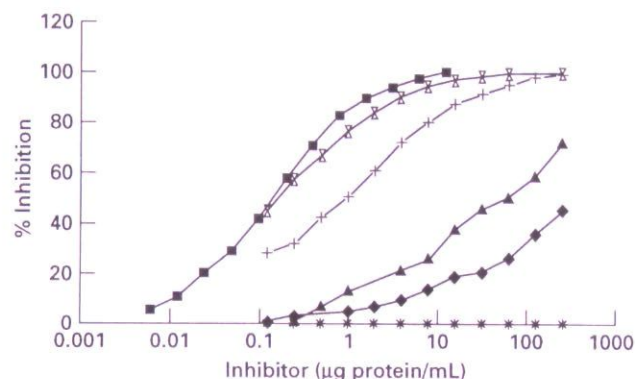


Fig. 2. Inhibition of wheat flour standard with other grain species (barley, rye and oats), other ingredients frequently used in bakeries (baker's yeast, fungal α -amylase and egg), and several mould extracts (see text). ■, Wheat standard. +, Barley. ♦, Oats. ▲, Rye. *, Others. —x—, Other wheat flour.

Airborne dust and wheat antigen measurements

A total of 546 personal measurements were available from 221 workers in all bakeries. In 449 of these samples wheat flour antigen levels were determined (the other samples were used to set up the wheat-flour inhibition assay ($n = 24$), were used for the development of other assays or endotoxin analysis ($n = 57$), or were lost before filter elution ($n = 16$)). Workers were grouped into nine exposure categories by job title. The exposure categories consisted of one or more jobs with similar tasks. Results of the dust and antigen measurements are summarized in Table 3. Because of the lognormal distribution of the exposure measurements, geometric means are presented.

In large bakeries the highest personal inhalable dust concentrations were found for doughmakers (GM = 3.0 mg/m^3 ; range $0.4\text{--}37.7 \text{ mg/m}^3$). Most doughmakers spent 80–90% of their time in a relatively small section

Table 3. Characteristics of all personal dust measurements ($n = 546$) and its wheat antigen content ($n = 449$) by job category

Job category	Personal dust exposure (mg/m^3)			Personal wheat antigen exposure (ng/m^3)			Mean ratio antigens/dust (ng/mg)
	<i>n</i>	GM	Range	<i>n</i>	GM	Range	
Total	546	1.0	0.1–37.7	449	684	33–252 407	1450
<i>Large bakeries</i>							
Doughmakers	105	3.0§	0.4–37.7	76	5323§	33–252 407	2850§
All-round staff	66	0.9*	0.1–26.8	54	992#	33–68 159	1550#
Oven staff	81	0.6	0.1–5.1	71	322	33–28 079	1150
Slicers, packers and transport workers	132	0.4§	0.1–2.8	109	77§	33–7736	350§
Production managers	20	0.6	0.1–4.9	17	505	33–74 614	2100†
Maintenance and cleaning workers	27	0.7	0.3–5.5	20	242	33–2539	600
<i>Small bakeries</i>							
Bread baker	36	3.3§	1.2–8.8	31	5989§	1289–53 275	1950¶
Mixed baker	57	2.0§	0.3–14.2	55	2729§	261–44 200	1600
Confectioner	22	0.7§	0.1–3.7	16	614§	33–3812	1550

n, number of measurements. GM, geometric mean. § Significantly different from all other job categories in large or small bakeries ($P < 0.05$). * Significantly different from oven staff ($P < 0.05$). # Significantly different from oven staff and maintenance and cleaning workers ($P < 0.05$). † Significantly different from maintenance and cleaning workers ($P < 0.05$). ¶ Significantly different from confectioner's ($P < 0.05$).

of the bakery, where dough is prepared for the production of bread, crispbakes (a kind of toast), biscuits etc. Other job titles in large bakeries spent no (e.g. packers) or only part of their time (e.g. all-round staff) in the dough-production section. Outside this section the contact with dust is considerably less and geometric means of exposure levels drop below $1 \text{ mg}/\text{m}^3$. Except for the doughmakers, there appeared to be only minor differences in dust exposure levels between the various exposure groups.

For personal wheat antigen exposure a similar trend was found, with doughmakers, again, in the highest exposure category. The wheat antigen exposure of the other occupational titles was considerably lower. Although there was a similar trend in dust and antigen exposure, some important differences were noticed. Differences between the various occupational titles were much larger for antigen exposure compared with dust exposure. For instance, doughmakers had a 7.5 times higher dust exposure than slicers and packers, but wheat antigen levels were on average 69 times higher (see Table 3). More importantly, significant differences were found in antigen exposure levels between groups and low dust exposure levels. Differences between exposure groups are also illustrated by the ratio of dust and antigen levels given in the last column of Table 3. This ratio represents the average amount of wheat antigens per mg dust, and

was highest for doughmakers, and clearly lower for workers who spend only part of their working time in the dough production area (all-round staff, production managers and oven staff). The lowest ratio was found for slicers, packers and transport workers, who usually will

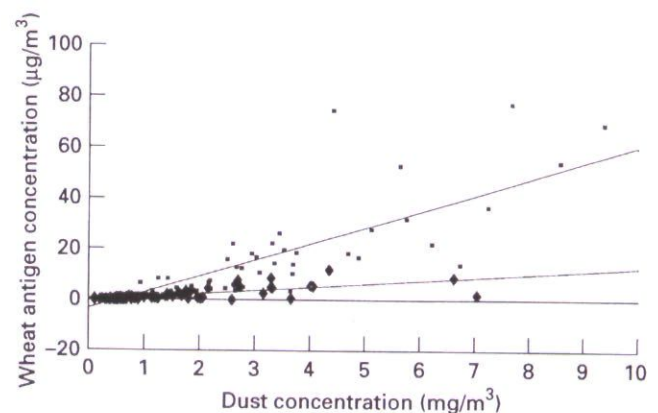


Fig. 3. Relation between gravimetric dust measurements and wheat flour antigen concentration in large industrial bread factories ($n = 158$) and confectionaries ($n = 76$). —■—. Bread factory. —◆—. Confectionary.

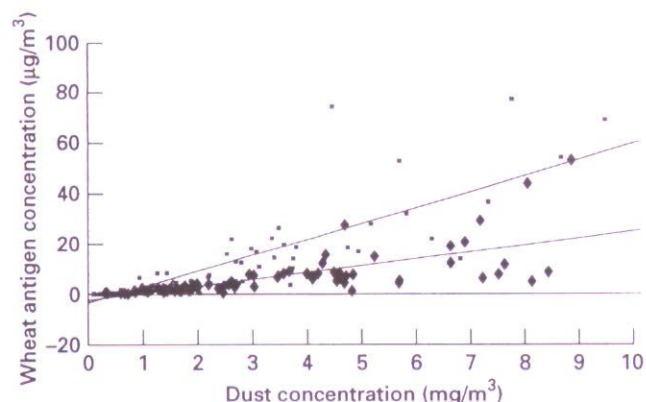


Fig. 4. Relationship between gravimetric dust measurements and wheat flour antigen concentration for bread bakers in small ($n = 102$) and large bakeries ($n = 158$). —■—, Bread factories. —◆—, Small bakeries.

not work in the dough production area. Hardly any differences in the antigen/dust ratio were found between occupational titles in small bakeries, presumably because in the small bakeries all bakers work in the same room.

Bakeries involved in this study varied with respect to the types of products that were made (see Table 1). In some of the bakeries, only breads made from wheat flour are produced, and therefore wheat flour is by far the most frequently handled product. In other bakeries, wheat flour is only one of the many ingredients (confectionery, small bakeries) or is only used in small amounts (rye-bread production sites). In each of these

groups we investigated the relationship between dust- and wheat antigen levels. In general, a good relationship existed, but Figures 3 and 4 clearly show that there was a distinct difference in this relationship between the samples from bread factories and confectionaries and from bread bakers in factories and small bakeries.

Within each occupational title (from Table 3) we therefore divided workers into four categories: wheat-bread production, pastry production, crispbakes production and rye-bread production. For two occupational titles exposure characteristics of these four groups are summarized in Table 4.

Also in this analysis, differences in exposure between the groups are larger for wheat antigens than for inhalable dust, i.e. doughmakers of wheat-bread production sites have a five times higher dust exposure, whereas the mean wheat antigen exposure is about 45 times higher. The mean wheat antigen exposure of doughmakers from crispbakes production sites is significantly higher than the mean antigen exposure from the confectionaries. No significant difference in dust exposure levels could be found between these groups. For oven staff, differences between dust- and antigen levels are even more pronounced. No differences could be found in dust exposure levels, but mean wheat antigen levels were significantly different for nearly all groups.

Discussion

We have developed an ELISA inhibition method to

Table 4. More detailed characteristics of personal dust measurements and its wheat antigen content for two job categories

Job category	Personal dust exposure (mg/m^3)			Personal wheat antigen exposure (ng/m^3)			Mean ratio antigens/dust (ng/mg)
	<i>n</i>	GM	Range	<i>n</i>	GM	Range	
All doughmakers	105	3.0	0.4–37.7	76	5323	33–252 407	2850
Wheat-bread production sites	37	4.5§	0.9–37.7	32	16511§	1597–252 407	4750§
Confectioner's	32	2.4	0.4–7.4	25	1969§	33–12 024	1300
Crispbakes production site	29	3.1	0.9–8.9	15	5104§	1425–11 468	1900§
Rye-bread production site	7	0.9§	0.4–1.9	4	364§	98–972	550
All oven staff	81	0.6	0.1–5.1	71	322	33–28 079	1150
Wheat-bread production sites	34	0.7	0.1–5.1	30	771§	33–28 079	2050§
Confectioner's	21	0.6	0.1–3.2	21	302	33–2600	750§
Crispbakes production site	21	0.7	0.4–1.3	15	128	55–413	250
Rye-bread production site	5	0.4	0.3–0.6	5	36§	33–51	100

n, Number of measurements. GM, Geometric mean. § Significantly different from all other job categories in doughmakers or oven staff ($P < 0.05$).

measure airborne wheat flour antigens, using an anti-wheat IgG4 serum pool. SDS-PAGE and immunoblotting showed that our serum pool reacted with a large spectrum of antigens in wheat flour. Several previous studies have demonstrated that many proteins from wheat flour can be IgE-binding allergens and that bakers with occupational asthma differ markedly with respect to the allergens recognized by their serum IgE antibodies [1,21–23]. Our immunoblots of IgE-positive sera, are consistent with these findings. A similar diversity has been found in IgG-reactions from sera of allergic bakers [24]. Although wheat flour is a mixture of many allergenic molecules, some major IgE-binding allergens have been identified with electrophoresis and blotting techniques. The proteins with molecular mass of 14–15 kDa and 30 kDa are the most frequently reported bands [23,25,26]. These are also the major protein bands reacting with our serum pool of IgG4-positive bakers, suggesting that the serum pool measures the most important IgE-binding allergens. Use of a IgE serum pool of allergic bakers would undoubtedly be the most direct way to measure wheat allergens in air samples, because it has the advantage of measuring the substance causing the disease. However, it also has some important limitations. The availability of IgE-positive sera, especially with high antibody titre, is limited. It is therefore difficult to get a serum pool which will be sufficient for many assays. The availability of IgG4-positive sera is much larger and antibody titres are usually very high. An important consequence is that the serum pool can be diluted many times (800-fold in our study), so the collected serum will suffice for many assays. This is of great benefit for the application in epidemiologic studies, where usually large amounts of samples have to be analysed over a considerable period of time. A recent review of the role of IgG4 in allergic diseases, reported that there is a great similarity between IgE and IgG4 with respect to the type of antigens recognized [27]. Moreover, our own immunoblotting results showed this also applies to the wheat allergens, serum IgE and IgG4-pool used in our study. Thus, the inhibition ELISA most probably measures antigens with biological relevance. At this moment analyses are being performed in which these measurements are studied in relationship to clinical responses.

Inhibition results showed some crossreactivity between wheat flour and other cereal species, particularly barley, but no crossreactivity was found for other ingredients (baker's yeast, egg and fungal α -amylase) and several mould extracts. Allergen identification studies showed that the main components of the 15 kDa band in wheat flour belong to the α -amylase inhibitor family [23,28,29]. It is known that proteins from other cereal species belong

to the same protein family [30] and one barley protein (14.5 kDa) of this family was shown to be a major allergen associated with baker's asthma [28]. Because of the similarity in proteins in several cereal species, some degree of crossreactivity can not be avoided. Besides, barley flour is not commonly used in the baking process, and influence on test results will therefore be limited. Rye flour is used more frequently, but exposure levels in the working environment have to be about 500 times higher than wheat flour levels to give a similar inhibition.

The presented method is useful for a wide exposure range. Mean flour dust exposure varied from 0.4 mg/m³ for slicers and packers to 4.5 mg/m³ for doughmakers in bread producing bakeries. In most samples wheat antigen levels could be measured, even in samples where dust exposure levels were below detection limit. Until now, quantitative exposure assessment in bakeries using gravimetric dust is a general practice and in most cases the only available technique for measuring airborne exposure. However, a great disadvantage of the use of dust exposure levels in epidemiological studies to baker's asthma is that it doesn't measure the true aetiological agent, but only is a surrogate for the actual flour allergen concentration. Another problem is that only small differences exist between different occupational titles. Especially between occupational titles with exposure levels below 1 mg/m³, differences in dust exposure levels are very small, whereas approximately 80% of the workforce in large bakeries are working in these groups. Average personal dust exposure levels in these occupational titles varied from 0.4 to 0.9 mg/m³. Antigen exposure levels in these occupational titles, however, varied from 77 to 992 ng/m³, the first being 13 times higher than the latter. Expedition workers have a dust exposure of 0.4 mg/m³, but this hardly contains any wheat flour. For other occupational titles, who perform part of their tasks in the dough production area, the wheat flour contents of the dust was higher. Therefore, if wheat antigen levels are used, clear differences between these groups existed. These differences will be of great importance for epidemiologic studies among bakery workers.

Table 4 and Figs 3 and 4 showed that there are important differences in wheat antigen exposure levels between bakeries depending on the type of product that was made. Bread producing bakeries, where wheat flour is by far the most handled product, have the highest antigen exposure levels. Other bakeries, where wheat flour is only one of the ingredients, had a considerably lower wheat antigen exposure, where only minor differences in dust exposure levels could be found. For the different types of bakeries, clear differences existed in the relationship between dust exposure and wheat antigen exposure.

As mentioned before, only two papers describe the measurement of wheat flour allergens in bakeries. Tee *et al.* [16] measured 55 stationary and personal dust samples of one industrial bakery. The assay used anti-wheat IgE antibodies from a serum pool of sensitized bakery workers. Above 5 mg/m³ dust exposure, a good correlation was found between dust and flour allergen levels in the air. The method appeared less suitable in samples with dust concentrations below 5 mg/m³, where only little variation in flour aero-allergen concentration was found. Sandiford *et al.* [17] developed a method using polyclonal rabbit IgG antibodies. Detection limit of this method was 1 µg/mL and a wide range of airborne flour concentrations were found in flour mills, but a smaller range in bakeries. Unfortunately, detection limit and flour exposure levels cannot directly be compared with our results because of differences in the assays (in particular difference in standards). Nieuwenhuijsen *et al.* [31] studied the relationship of these wheat flour levels with dust exposure levels. In contrast to our study, differences in mean flour aeroallergen exposure between occupational titles were much smaller than for mean total dust. They also found some differences in the relationship between total dust and flour aeroallergen in large industrial bread bakeries and confectionaries. Exposure data in small bakeries (<10 workers) have never been published in the international literature, whereas almost two out of three of the Dutch bakers work in these small bakeries. Our study shows strong differences in relation dust/wheat antigens for this type of bakery, compared with large industrial bakeries.

This study shows that personal sampling of wheat antigens using ELISA inhibition, is possible on a scale large enough for epidemiological studies. There appeared to be considerable differences in the relation between dust- and wheat antigen exposure for several groups, depending on the variety of products that were handled and the time spent in the dough production area. For some groups, clear differences in antigen exposure levels existed, where no differences in dust exposure levels could be found. Therefore, dust exposure levels will often be only a crude approximation of wheat antigen levels. This will have considerable negative consequences for epidemiological field studies among bakery workers focusing on exposure-response relationships. If occupational wheat allergies are studied, exposure estimates based on flour dust levels alone can lead to serious exposure misclassification of bakery workers, and exposure response relations can be missed or biased. Measurement of wheat antigens can also be important for taking effective control measures for reducing exposure, and interventions based on dust alone may not be adequate.

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