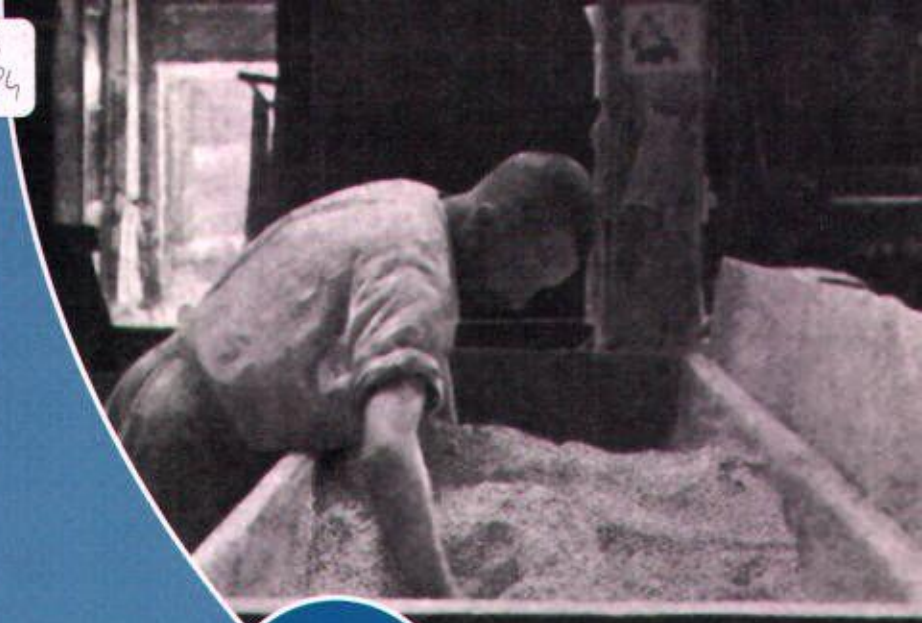
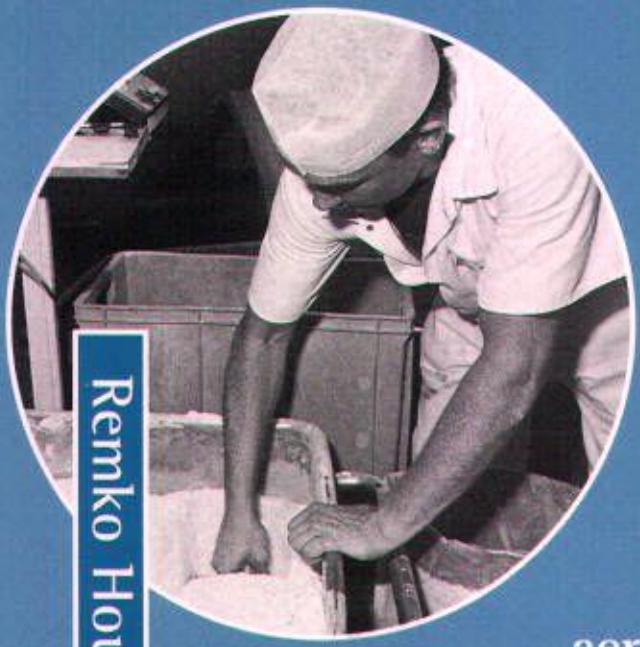


Occupational respiratory allergy in bakery workers



Remko Houba

Relationships with wheat and fungal α-amylase aeroallergen exposure

Stellingen

1. α-amylase, een enzym dat wordt toegevoegd als deegverbetermiddel bij de produktie van brood en beschuit, is evenals tarwemeel een belangrijk beroepsallergeen in de Nederlandse bakkerij-industrie.
   (dit proefschrift)

2. Er bestaat een zeer sterke relatie tussen blootstellingsniveaus aan tarwe- en α-amylase allergenen in de lucht in bakkerijen en de prevalentie van sensibilisatie tegen deze allergenen bij bakkerijmedewerkers.
   (dit proefschrift)

3. In de groep van gesensibiliseerde werknemers is de prevalentie van werkgerelateerde klachten eveneens afhankelijk van het niveau van allergeenblootstelling.
   (dit proefschrift)

4. Om een daling van het aantal bakkers met een beroepsgebonden luchtwegallergie te bewerkstelligen, hebben aanstellingskeuringen gericht op selectie en uitsluiting van werknemers met een allergische gevoeligheid minder waarde dan arbeidshygiënische maatregelen met als effect blootstellingsreductie op de werkplek.

5. Een toename van het gebruik van biotechnologisch vervaardigde enzymen in industriële processen zal, indien deze enzymen in aerosolvorm vrijkomen, leiden tot een toename van het aantal werknemers met een beroepsallergie.

6. Gerechtelijke procedures waarbij werkgevers financieel aansprakelijk zullen worden gesteld voor de beroepsgebonden gezondheidsschade bij hun werknemers, zullen door de afkalving van de sociale wetgeving in de toekomst wellicht geen uitzondering zijn.

7. De uitspraak van Heijermans, vaak beschouwd als de grondlegger van de Nederlandse arbeidshygiëne, dat meelstof bij inademing niet nadelig is voor de gezondheid van de bakker, is onjuist, mede gezien de stand van de toenmalige kennis over deze beroepsziekte.
   (In: Heijermans, 1926)

8. Een optimist gelooft dat we leven in de beste van alle mogelijke werelden. Een pessimist vreest dat dit waar is.
(Anonymus)

9. Een flexibele arbeidsmarkt wordt vaak gepropageerd door mensen die zelf al jaren niet meer van baan zijn veranderd.

10. Het Nederlandse drugsbeleid is verworden tot de kernproblematiek van Frankrijk.

11. Don't ask what the world needs.
Rather ask what makes you come alive.
Then go and do it!
Because what the world needs is people who have come alive.
(Howard Thurman)

12. Om in de wetenschap te promoveren moeten stellingen worden verdedigd. Om in het voetbal te kunnen promoveren zullen de verdedigende stellingen regelmatig moeten worden verlaten.

Stellingen behorend bij het proefschrift 'Occupational respiratory allergy in bakery workers: Relationships with wheat and fungal α-amylase aeroallergen exposure' van Remko Houba. Wageningen, 22 mei 1996.

Occupational respiratory allergy in bakery workers

Relationships with wheat and
fungal α-amylase aeroallergen exposure

Remko Houba

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Hoofddocent vakgroep Humane Epidemiologie en Gezondheidsleer

Remko Houba

Occupational respiratory allergy in bakery workers

Relationships with wheat and fungal α-amylase aeroallergen exposure

Proefschrift

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
dr C.M. Karssen
in het openbaar te verdedigen
op woensdag 22 mei 1996
des namiddags om vier uur in de Aula
van de Landbouwuniversiteit te Wageningen

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Voor mijn ouders

... of terwyl sy voor het noodig gebruik der Ademhaaling, de lugt inhalen, of sy vangen de vliegende Meel deeltjens op, die met het Quylzap gisten, en niet alleen de Keel maar ook de Maag en de Longen met een Deeg vullen; waar van dat sy zeer ligt aan den Hoest geraken, Kortademig en Hees, sijn en eind'lijk Aamborstig worden, de Longe Pyp en Longwegen overkorst en de vrije omgang met de Lugt belet en belemmert sijnde.

B. Ramazzini, De Morbis Artificium Diatriba, ± 1700
(Nederlandse vertaling, 1724)

Het Meelstof bestaat uit de bekende afgeronde amylumkorrels, welke geen nadeelige eigenschappen bezitten; slechts bijzonder groote hoeveelheden veroorzaken een hoestprikkel. Dat echter chronische bronchitis, longuitzetting, het zoogenaamde "bakker hoesten" te wijten zou zijn aan de inademing van meelstof, lijkt ons weinig waarschijnlijk; in zulke groote hoeveelheden wordt het meel gewoonlijk niet ingeademd, terwijl de afwezigheid van den prikkelenden invloed van het meel op de ademhalingswegen evenmin houvast geeft aan deze theorie.

L. Heijermans, Handleiding tot de kennis der Beroepsziekten, 1926

Abstract

In this thesis, results are presented of a cross-sectional epidemiological study among a few hundred bakery workers. Main focus was on the relationship between allergen exposure and the development of specific sensitization and respiratory allergy. Immunoassays were developed for measuring airborne allergens from wheat flour and fungal α-amylase, an enzymatic dough improver. A total of 571 personal inhalable dust samples were available from 230 workers. Wheat flour allergens were measured in 449 of these samples with an inhibition enzyme immuno assay, and an anti-wheat IgG_4 serum pool from bakery workers. Fungal α-amylase allergens were measured in 507 samples in a sandwich immunoassay, using affinity purified polyclonal rabbit IgG antibodies. The validity and specificity of both assays were extensively tested. All bakery workers were categorized into groups, based on their job histories and the wheat or α-amylase allergen exposure levels of their job titles. Optimal grouping strategies were tested. For both α-amylase and wheat allergens, a clear exposure-sensitization-relationship was found. Atopy appeared to be an important effect modifier of this relationship, and the steepest slope between allergen level and sensitization rate was found within the group of atopic workers. Twenty-three percent of the bakers reported work-related rhinitis or chest-tightness. Symptomatic bakers were divided into occupationally sensitized and non-sensitized workers. In both groups, a relation between allergen exposure and respiratory symptoms was found, but the slope of the relationship was steeper in sensitized bakers than in non-sensitized bakers.

Contents

1. Introduction

Background of the study

Adverse health effects from workplace exposures in the bakery industry have been described in a large number of studies. Especially bakery workers involved in dough production and dough forming have increased risks for occupational diseases. Several disorders have been related to the bakery occupation.

Baker's respiratory allergies

Respiratory disorders are by far the most prevalent occupational diseases among bakery workers. Epidemiologic surveys show prevalence rates of work-related eye/nose symptoms of 14-29%, while the prevalence rates of chest symptoms vary from 5-14%. This is also the main subject of this thesis and the available literature is reviewed in chapter 2. Respiratory allergies to cereal flours and powdered baking additives are probably responsible for a substantial part of these respiratory symptoms. Although a large number of studies have been performed on this subject, some important questions are still unanswered. Until now, little is known about the incidence of baker's allergy, due to the lack of well-designed cohort studies. Moreover, many investigators have tried to identify the causes of baker's allergy but most of them focused on host factors only, especially allergic disposition of the bakery workers. Only few have studied the environmental determinants of respiratory allergy among bakery workers, particularly the level of allergen exposure required for sensitization. The most important reason for the limited attention to environmental factors is that instruments for the measurement of personal exposure to airborne allergens were not available. Methods for the measurement of allergens in air require specialized sampling and assay techniques, which have been developed only for a limited number of occupational allergenic proteins (Reed *et al.* 1993; Gompertz 1994). Until recently, no assays had been developed for measuring allergens in the bakery industry.

Baker's eczema

Although respiratory allergy is the most important occupational disease among bakery workers, other disorders have also been described. Allergic contact-dermatitis among bakery workers has been reported from the beginning of this century, as reviewed by Grosfeld (1951), Bonnevie (1958) and Heyl (1970). Briefly,

in the years after World War I, chemical dough improvers were introduced and frequently used in the bakery industry. Soon, a rising frequency of baker's eczema was noted. Teleky (1927) noticed that before 1925 baker's eczema was a rare disease, but in that year 52 cases of eczema were diagnosed in a group of 640 bakers. It was not until 1930-1933 that ammonium persulphate and potassium persulphate were identified as the major offenders. In many countries, the use of persulphates was legally stopped during the 50s, and the incidence of baker's eczema drastically decreased (Forck 1968; Heyl 1970; Young 1974). Recent epidemiologic surveys, however, have shown that occupational skin disease may still be a serious problem in the bakery occupation, with prevalence rates varying from 5-8% (Järvinen *et al.* 1979; Hartmann 1986; Cullinan *et al.* 1994a). In Finland, 101 new cases of dermatoses among grain and flour dust workers were registered between 1980 and 1989, and the annual incidence for dermatoses in the bakery occupation was estimated to be 0.6 per 10.000 workers (Tossavainen & Jaakkola 1994). Many agents have been identified as potential sensitizers, among others cereal flours (Pigatto *et al.* 1987), cellulase and xylanase enzymes (Tarvainen *et al.* 1991a), fungal α-amylase (Schirmer *et al.* 1987; Morren *et al.* 1993), baker's yeast (Kortekangas-Savolainen *et al.* 1994), cinnamon oil en cinnamic aldehyde (Fisher 1975; Nethercott & Holness 1989), benzoyl peroxide (Baird 1945; Fisher 1989), chromium compounds in flour (Heine & Fox 1980), and certain emulsifiers (Foodmuls E 3137; Vincenzi *et al.* 1995) and antioxidants (propyl gallate; Bojs *et al.* 1987).

Baker's caries

Some epidemiologic surveys have indicated an increased incidence of dental caries in bakers and confectionery workers compared with the general population or with other industrial workers (Ackerman 1979; Masalin & Murtomaa 1992). This has been attributed to (flour and) sugar dust deposited on tooth surfaces during respiration (Ackerman 1979; Gardemin & Litta 1972). However, in only few studies exposure to sugar dust was measured to test this hypothesis. In a large Finnish confectionery, personal exposure levels of sugar dust were measured, suggesting that there are very few job titles with exposure to sugar dust in amounts needed to increase the risk of caries, at least theoretically (Masalin *et al.* 1988). Recently, an epidemiologic study has been performed among 294 employees in a Finnish confectionery (Masalin & Murtomaa 1992). In that study the dietary habits and

dental health behaviour (use of dental services, reasons for dental appointments, and the dental home-care habits) were studied. In addition, the caries status of each worker was recorded. A significant positive correlation was found between untreated caries and the number of sugary meals. The results suggested that the nature of between-meal snacks and the bakers' freedom to consume their products was the most important risk factor for dental caries.

Respiratory cancers

In some studies increased risks of respiratory cancers have been associated with the bakery occupation. Excess risks of nasal cancer among bakers and pastry cooks were reported in two case-control studies (Acheson *et al.* 1981; Luce *et al.* 1992). Using the Swedish Cancer-Environment Registry, which links cancer incidence with employment data, Malker *et al.* (1986) observed more nasal cancers for bakers and pastry makers than expected, on the basis of the age and sex-specific nasal cancer incidence rates of the Swedish population (6-fold increase). One have to keep in mind that sinonasal cancers are rare diseases. The annual incidence rate adjusted for age and sex, ranges from 0.3-1 case per 100,000 subjects in most countries (Leclerc & Luce 1994).

Significant excess mortality rates for cancer of the respiratory tract for bakery workers (SMR=166) were found in a Danish death registration cohort (Tüchsen & Nordholm 1986). The suspected carcinogens in that study were among others polycyclic aromatic hydrocarbons (PAH), reaction products of PAH, free radicals, and nitrodimethylamine, but none of these hypotheses has been confirmed. No increased risks of mortality from respiratory cancers (nasal cancer or lung cancer) were found for bakery workers in a retrospective cohort study among 327 male millers and bakers in England and Wales (Alderson 1987), and in a prospective cohort study of the US National Cancer Institute, among approximately 300,000 veterans of the US Army (Hrubec *et al.* 1995). In the last study, no information was available on the number of bakery workers in the study population.

Bakery industry in the Netherlands and population at risk

Table 1.1 gives an overview of the flour and bakery industry in The Netherlands. Four main categories can be distinguished: flour manufacturing, factories of bakery

requisites (e.g. bread improvers), bakeries for bread production, and bakeries making other products (e.g. confectionery). The table shows that approximately 50,000 people were employed in this industry in 1994. These numbers include administrative and commercial jobs. Assuming that in the large facilities about 50% of all employees are involved in production work, and 75% in the small facilities (< 20 employees), a rough estimate of the population potentially exposed to flour and flour related products, is approximately 32,000.

In the Netherlands about half of the bread production takes place in small bakeries (self employed bakers) (NBS 1994). The size of bakeries for bread production, however, may vary considerably between countries. In some countries, most bread is produced in large industrialized bakeries. In other countries, however, most of the production takes place in small traditional bakeries. This is illustrated in Table 1.2, where the number of bakeries per 100,000 inhabitants is shown (Bedrijfsschap voor het bakkersbedrijf 1988).

Table 1.1 Overview of flour and bakery industry in The Netherlands

Type of industry	Number of facilities/ plants	Number of workers in industry[6]
Flour manufacturing[1]	12	1,450
Factories of bakery requisites[2]	14	1,200
Cake, biscuit and rusk industry[3]	103	7,900
Bread industry		
facilities ≥ 20 employees[4]	189	12,050
facilities < 20 employees	2951	28,300
total[5]	3140	40,350
TOTAL	**3269**	**50,900**

[1] Only facilities with 20 or more employees. Source: CBS 1994a.
[2] Only facilities with 20 or more employees. Source: CBS 1993.
[3] Only facilities with 20 or more employees. Source: CBS 1994b.
[4] Source: CBS 1994b.
[5] Source: NBS 1994.
[6] Number of workers are round figures.

Table 1.2 Number of bakeries per 100,000 inhabitants by country

Belgium	74
France	63
Germany	44
The Netherlands	24
England	7

Aims of the study

This thesis is focused on occupational respiratory diseases among bakery workers. The aims of the study were:

1. To develop methods for measuring personal airborne allergen exposure in bakeries;
2. To explore relationships between allergen exposure levels and the prevalence of work-related allergies among bakery workers.

Structure of the thesis

In **chapter 2** the available literature on baker's respiratory allergy is extensively reviewed. The following issues are discussed: history of baker's asthma, potential allergens, exposure assessment in bakeries, the available epidemiologic surveys on baker's allergy, and the research on determinants of baker's allergy, especially the role of environmental factors.

In **chapter 3** and **chapter 4** methods are presented for measuring the two most important allergens in the bakery industry. Chapter 3 describes an assay for measuring wheat flour allergens. In chapter 4 an assay for measuring fungal α-amylase allergens is presented. The specificity, sensitivity and reproducibility of both assays are described. Furthermore, differences in dust and allergen exposure levels are discussed (by type of bakery and job titles of the bakers).

In **chapter 5** the characteristics of dust, wheat allergen and α-amylase allergen exposure are studied. The variability and the sources of variation for all three measures of exposure are discussed.

In **chapter 6** and **chapter 7** exposure-response-relationships are explored. Chapter 6 describes the relationship between fungal α-amylase allergen exposure and specific sensitization to α-amylase, measured by either skin prick testing or serum IgE. In chapter 7, exposure-sensitization-relationships for wheat allergens are described and work-related respiratory symptoms are related to both wheat flour and α-amylase allergen exposure.

Finally, **chapter 8** presents a discussion on the most important findings, potential biases and potential implications for the prevention of respiratory allergies in the flour and baking industry.

2. Occupational respiratory allergy in bakery workers - a review of the literature[1]

Remko Houba, Gert Doekes & Dick Heederik

Introduction

Occupational asthma is the most prevalent occupational respiratory disorder today (Chan-Yeung 1990; Meredith *et al.* 1991). It is a disease characterized by variable airflow limitation and/or airway hyperresponsiveness due to causes and conditions attributable to a particular occupational environment and not to stimuli encountered outside the workplace (Bernstein *et al.* 1993). In several countries, baker's asthma is reported to be one of the most frequent forms of occupational asthma (Thiel 1987; Hartmann 1989; Jeffrey 1992; Lagier *et al.* 1990; Nordman 1994), and information obtained from occupational disease registrations suggests that the number of cases with baker's asthma is steadily increasing (Nordman 1994; Thiel 1984; Baur 1993; Grieshaber & Rothe 1995).

Baker's asthma is, like other forms of occupational asthma, probably the most serious manifestation of occupational allergy among bakery workers. It is caused by immunologic sensitization and subsequent allergic reactions in the airways to occupational specific airborne allergens. Less severe types of baker's allergy are rhinitis (with frequent sneezing, nasal obstruction and rhinorrhoea) and conjunctivitis (with itching and inflamed, red eyes).

At present little is known about the incidence of baker's allergy. Although reliable data based on epidemiologic field studies are not available, some registry based incidence data have been published. In a surveillance scheme on work related and occupational respiratory diseases (SWORD) in clinical practices in the United Kingdom, 64 new cases of baker's asthma were diagnosed by chest and occupational physicians between 1989 and 1991 (Meredith & McDonald 1994), and 30 asthma cases were reported in 1993 (Sallie *et al.* 1994). In this project, the estimates of incidence of baker's asthma vary from 290-409 per million per year (Meredith 1993; Newman Taylor & Gordon 1993; Meredith & McDonald 1994). In

[1] *Submitted, supplemented with own study results*

the West Midlands Region of the United Kingdom, the incidence rate of bakers with diagnosed occupational asthma has been estimated to be 445 per million per year (95% confidence interval 121-821) (Gannon & Burge 1993). Reported cases of baker's asthma are probably only the tip of the iceberg. Because only bakers who sought specialized medical assistance were included, these incidence rates are likely to be an underestimation of the true incidence of baker's asthma.

The social and economic impact of allergic reactions in bakers caused by agents at work should not be underestimated. At the individual level, flour-induced rhinitis and asthma are frequently severe enough to cause considerable inconvenience, discomfort, and even abandonment of the trade. In a survey in the UK among patients with diagnosed asthma it has been shown that persons with occupational asthma had greater difficulty in finding new work, greater loss of income, and were less likely to be currently employed than those with asthma unrelated to work (Cannon *et al.* 1995). In Germany, more than 1200 bakers claim industrial injury compensation annually (Baur 1993; Grieshaber & Rothe 1995) and in that country the expenditure for disability compensation of bakers with work related asthma is substantial, e.g. 83 million D-mark in 1992 (Baur 1993). This illustrates that baker's asthma can have serious economic consequences on a national level as well.

Asthma mortality rates for bakery workers have been reported in a study performed in the US. In that study, 184 death certificates (of persons who died in Chicago) with asthma listed as the cause of death or a contributing cause of death, were examined for the given occupation. It appeared that bakers had markedly higher asthma mortality rates than expected, with 41 times the age- and race-adjusted national rate (DeMers & Orris 1994).

History

For a detailed description of the history of baker's asthma the reader is referred to two interesting historical reviews (Bonnevie 1958; Thiel & Kallweit 1984). Briefly, the first scientific description of baker's asthma was given by Ramazzini in 1700 in 'De Morbis Artificium Diatriba' (Ramazzini 1700). Until the beginning of this century, however, the actual causes of baker's asthma were in fact unknown. The aetiology was thought to be purely mechanical: flour mixed with worker's saliva formed a sticky substance, which would accumulate in the lung, block the

airways and hamper breathing. With the recognition of allergic sensitization and allergic diseases in the beginning of this century this theory was abandoned. Immediate skin reactivity to wheat extracts in a baker with asthma was first demonstrated in 1909 but it was not until 1929 that de Besche (1929) introduced the concept that baker's asthma is an allergic disease related to the bakery occupation. The first systematic investigation in the bakery trade was done by Baagöe (1933a&b). Since that time, many epidemiological surveys have been performed, estimating the prevalence of baker's allergy and trying to reveal the determinants of this occupational disease.

Allergens

Wheat flour and other cereal species

Reactivity to wheat flour (*Triticum aestivum*) and other cereal flours like rye flour (*Secale cereale*) and barley flour (*Hordeum vulgare*), has been reported as the main cause of baker's allergy. According to most authors, symptoms are induced through an (type I) IgE-dependent mechanism (Block *et al.* 1983; Prichard *et al.* 1985; Sutton *et al.* 1984). In sensitized subjects with symptoms, specific inhalation challenge tests with flour extracts induce immediate asthmatic reactions. Although these immediate type allergic reactions predominate, dual reactions are also reported, characterized by both an immediate and late reaction (Hendrick *et al.* 1976; Nakazawa *et al.* 1976).

Wheat flour is a complex mixture of peptide- and saccharide-containing substances, many of which must be considered as potential allergens that may induce specific IgE-sensitization after inhalation. The strongest in vitro reactivity have been observed with water-soluble proteins, particularly albumins (Baldo & Wrigley 1978; Prichard *et al.* 1985), but IgE antibodies against water-insoluble proteins have also been found (Walsh *et al.* 1985). Blands *et al.* (1976) detected 40 different antigens in wheat flour by crossed immunoelectrophoresis, of which 18 bound specific IgE from sera of sensitized bakers. In more recent studies, molecular weights of the IgE-binding proteins have been determined using electrophoresis and blotting techniques, which confirmed the allergenicity of many wheat flour proteins (Fränken *et al.* 1991; Pfeil *et al.* 1990; Sandiford *et al.* 1990; Sandiford *et al.* 1991; Sandiford *et al.* 1995). Most sera of sensitized bakers show IgE reactions with

many of these proteins, but the reaction profiles may differ markedly between individual sera (Sutton *et al.* 1984). However, reactions to some components (12-17, 30, and 46-47 kDa) are found with high frequencies, and these proteins are therefore considered to be so-called 'major allergens' (Sandiford *et al.* 1990; Gómez *et al.* 1990; Pfeil *et al.* 1990; Sandiford *et al.* 1991).

In other studies, attempts have been initiated to further characterize the allergens in wheat flour. These studies have shown that the main components of the 12-15 kDa bands belong to the α-amylase/trypsin inhibitor family (Boisen 1983; Fränken *et al.* 1994). The allergenicity of purified members of this enzyme inhibitor family has been shown both in vitro (Fränken *et al.* 1994; Sanchez-Monge *et al.* 1992) and in vivo (Armentia *et al.* 1993). Proteins of the same family can be found in other cereal species as well (Adachi *et al.* 1995; Garcia-Olmedo *et al.* 1987; Mena *et al.* 1992), and one barley protein (14.5 kDa) belonging to this family has been shown to be an important allergen associated with baker's asthma (Barber *et al.* 1989).

Another group of allergens are cereal amylases, which are present in cereal flour in its native form. Cereal amylases should be distinguished from fungal amylases added as dough improvers (see below). IgE antibodies reacting with α-amylases and/or ß-amylases from barley flour have been demonstrated, and molecular weights of these cereal amylases appeared to be 54, 59, and 64 kDa (Baur *et al.* 1994b; Sandiford *et al.* 1994b). Importantly, cereal and fungal amylases show only minimal immunologic cross-reactivity (Baur *et al.* 1994b; Sandiford *et al.* 1994b).

Many studies have demonstrated allergenic cross-antigenicity between different cereal flours, which parallels the closeness of the taxonomic relationship of the grain species (Baldo *et al.* 1980; Block *et al.* 1984; Sandiford *et al.* 1995). Similarly, cross-reactivity between cereal flours and pollens of several grasses has been described (Blands *et al.* 1976; Baldo *et al.* 1982). The cross-reactivity between different cereals can partly be explained by the presence of closely related enzymes or enzyme-inhibitors (Fränken *et al.* 1991; Sandiford *et al.* 1995), showing a high degree of homology (García-Casado *et al.* 1995).

Fungal amylase

Since the introduction of enzymatic dough improvers in the baking industry, and the increased use of these enzymes throughout the 1980s, several cases of bakers with occupational asthma due to sensitization to these enzymes have been reported. Of these, mostly *Aspergillus spp.* derived enzymes, α-amylase is the most

frequently reported cause of allergy. Fungal α-amylase (1,4-α-D-glucan glucanohydrolase), usually derived from *Aspergillus oryzae*, is a glycoprotein which catalyses the hydrolysis of internal α(1,4)-glycosidic linkages in various polysaccharides. It is routinely added to baking flour (in amounts of milligrams per kg flour) to hasten the baking process and improve bread quality. The first cases of α-amylase allergy have been described in the enzyme processing industry in the seventies (Flindt 1979). Since then, several individual case reports have been reported of baker's asthma caused by this starch cleaving enzyme, often in the absence of demonstrable reactivity to cereal allergens (Baur *et al.* 1986; Bermejo *et al.* 1991; Birnbaum *et al.* 1988; Blanco Carmona *et al.* 1991; Heyer 1983; Quirce *et al.* 1992; Tarvainen *et al.* 1991b; Valdivieso *et al.* 1994; Wüthrich & Baur 1990). In other studies, 24-55 % of bakers with respiratory symptoms attending medical services, were found to be sensitized to α-amylase (Baur *et al.* 1988b; Baur *et al.* 1989; Baur *et al.* 1994a; Wüthrich & Baur 1990).

With electrophoresis and immunoblotting, several IgE-binding proteins have been detected in crude amylase preparations. In all studies one dominating IgE-binding band in the immunoblots was found, with a molecular weight varying from 51-54 kDa (Baur *et al.* 1986; Baur *et al.* 1988b; Baur *et al.* 1994a; Quirce *et al.* 1992; Sandiford *et al.* 1994b). Baur and co-workers (Baur *et al.* 1994a) have demonstrated that this band represents the active α-amylase enzyme. In addition to this major allergen, commercially available extracts of fungal α-amylase appear to contain several other allergic components. The strongest reactions were found for proteins with molecular weights of 25-27 and 40 kDa (Baur *et al.* 1988b; Moneo *et al.* 1995; Sandiford *et al.* 1994b).

Recently it has been suggested that fungal α-amylase allergens in bread would be capable of inducing food associated allergy in bread consumers (Schata & Jorde 1992; Wüthrich 1994). Until now, three case studies have been presented, which showed that oral provocation with fungal α-amylase enzyme (Losada *et al.* 1992) and bread (Baur & Czuppon 1995; Kanny & Moneret-Vautrin 1995), may cause allergic respiratory symptoms in individuals sensitized through airborne exposure. Allergic reactions after consumption of α-amylase containing bread have not been reported yet in non-occupationally sensitized people. Some studies have shown a loss of allergenicity of heated α-amylase, suggesting that α-amylase in baked food products would indeed not be an important cause of allergic sensitization (Alday *et al.* 1995; Baur *et al.* 1994b). In contrast, however, Kanny & Moneret-Vautrin

(1995) reported that boiling the α-amylase extract did not completely abolish the allergenic activity of the enzyme, and Sander & Baur (1994) showed that enzyme-digested fragments of α-amylase were still capable of binding IgE antibodies.

Storage mites

Wheat flour in bakeries can be contaminated with storage mites (Armentia *et al.* 1992), and allergens from storage mites have been suggested as another cause of allergic symptoms in bakery workers (Revsbech & Dueholm 1990; Armentia *et al.* 1992). Epidemiologic studies showed high prevalence rates of sensitization to storage mites (*Acarus siro*, *Glycophagus domesticus*, *Lepidoglyphus destructor*, *Tyrophagus longior* & *Tyrophagus putrescentiae*) in bakery workers varying from 15 - 33 % (Cullinan *et al.* 1994a; De Zotti *et al.* 1994; Musk *et al.* 1989). However, no difference in sensitization to storage mites was found between bakery workers and controls (De Zotti *et al.* 1994; De Zotti *et al.* 1995), and others have also reported high prevalence rates of storage mite sensitization in non-occupationally exposed subjects (Korsgaard *et al.* 1985; Müsken & Bergmann 1992; Tee 1994). The latter studies suggest that storage mites are widespread in the environment and that positive skin responses to storage mites among bakers is more an indicator of atopy rather than a response to an occupational allergen specific to bakers. Moreover, part of the apparent anti-storage mite IgE reactions may be due to cross-reactivity with house dust mites (Müsken & Bergmann 1992; Johansson *et al.* 1994; Tee 1994), although according to some studies storage mites also possess own allergenic epitopes (Müsken & Bergmann 1992; Tee 1994).

Other allergens

Although cereal flours and fungal α-amylase are regarded as the most important allergens for the development of respiratory allergy in bakery workers, a large number of other potential allergens have also been reported in the literature. These are summarized in Table 2.1. Most noticeable are the different agents used as baking additives. In groups of asthmatic bakers, 5-10 % were sensitized to fungal glucoamylase and (hemi)cellulase (Baur *et al.* 1988b; Baur *et al.* 1989). Although the allergenic potency of these enzymes seemed to be less than the potency of α-amylase, their role must not be underestimated. Unfortunately, they have never been tested in epidemiological surveys. As can be seen from the table, sensitization to

Table 2.1 Overview of other potential occupational allergens in the bakery industry
All case studies refer to bakery workers with respiratory symptoms, except where noted.

Allergen	Reference	N	Pos. tests in case studies			Pos. tests in epid. surveys		Remarks
			skin tests	spec. IgE	spec. inhal. tests	skin tests	spec. IgE	
Baking additives								
Glucoamylase (*A. niger*)	Baur *et al.* 1988b	140		5%				
	Baur *et al.* 1989	261		5%				
	Wiewrodt *et al.* 1995	136	9%	6%				workers from enzyme manufacturing plant
(Hemi)cellulase (*A. niger*)	Losada *et al.* 1986	2	Yes	Yes	Yes			workers from pharmaceutical company
	Baur *et al.* 1988b	140		8%				
	Baur *et al.* 1989	261		10%				
	Tarvainen *et al.* 1991a	4	75%	100%				workers from enzyme manufacturing plant
	Quirce *et al.* 1992	5	80%	80%	80%			
	Wiewrodt *et al.* 1995	136	17%	7%				workers from enzyme manufacturing plant
Protease (*Bacillus subtilis*)	Baur *et al.* 1988b	100		1%				
	Baur *et al.* 1989	261		1%				
Papain (*Carica papaya*)	Wüthrich 1976	1	Yes					
	Baur *et al.* 1988b	100		1%				
	Baur *et al.* 1989	261		1%				
Cereal malt flour	Heyer 1983	8	88%		50%			
	Jorde *et al.* 1986	202	76%		20%			
	Wüthrich & Baur 1990	31		16%				
Soybean flour	Heyer 1983	8	75%		75%			
	Jorde *et al.* 1986	202	65%		18%			
	Bush *et al.* 1988	1	Yes	Yes	Yes			Worker in food processing company

Table 2.1 Continued

Allergen	Reference	N	Pos. tests in case studies			Pos. tests in epid. surveys		Remarks
			skin tests	spec. IgE	spec. inhal. tests	skin tests	spec. IgE	
Baking additives (continued)								
Soybean flour	Baur *et al.* 1988b	140		21%				
	Baur *et al.* 1989	261		32%				
	Wüthrich & Baur 1990	31		19%				
	Jeffrey 1992	205					3%	
Lecithin (from Soybean)	Heyer 1983	7	57%		29%			
	Wüthrich & Baur 1990	31	65%	39%				
	Lavaud *et al.* 1994	2	Yes	Yes	Yes			
Moulds & yeasts								
Aspergillus spp	Weiner 1960	1	Yes	?	Yes			
Various moulds	Bergmann *et al.* 1976	179	25-45%					Significantly different from controls
Alternaria & Aspergillus spp	Klaustermeyer *et al.* 1977	2	Yes		Yes			
Mucor, Clad. & Asp. spp	Wallenstein *et al.* 1980	354	30-40%					Significantly different from controls
Mould mix	Musk *et al.* 1989	259				2%		
Aspergillus fumigatus	Musk *et al.* 1989	259				<1%		
Bakers yeast	Dishoeck & Roux 1939	66	2%					Tested with scratch tests
	Baldo & Baker 1988	47	74%	68%				No bakery workers enolase identified as major allergen
	Musk *et al.* 1989	259				1%		
Egg material								
Yolk	Edwards *et al.* 1983	13	15%	31%				(only) 8 showed asthmatic like symptoms when exposed to egg spray

Table 2.1 Continued

Allergen	Reference	N	Pos. tests in case studies			Pos. tests in epid. surveys		Remarks
			skin tests	spec. IgE	spec. inhal. tests	skin tests	spec. IgE	
Egg material (continued)								
Egg white	Blanco Carmona *et al.* 1992	1	Yes	Yes	Yes			
Egg	Jeffrey 1992	205					3%	
Other allergens								
Buckwheat	Nakamura 1972	1	Yes	?	Yes			cook in buckwheat noodle shop
	Göhte *et al.* 1983	25	28%					plant products company
	Valdivieso *et al.* 1989	1	Yes	Yes	Yes			
alkaline hydrolysis wheat gluten derivative	Lachance *et al.* 1988	1	Yes	Yes	Yes			
Sesame seed	Keskinen *et al.* 1991	1	Yes	Yes	Yes			
Milk	Jeffrey 1992	205					2%	
Cacao	Zuskin *et al.* 1994b	71				31%		
Chocolate	Zuskin *et al.* 1994b	71				9%		
Hazelnut	Zuskin *et al.* 1994b	71				6%		
Almond	Zuskin *et al.* 1994b	71				6%		
Arthropodes	Popa *et al.* 1970	43	16-70%		16%			Also pos. inhalation test to cereals
Grain weevil	Rosenau *et al.* 1993	53		15%				
	Herling *et al.* 1994	66		40%				bakers not necessarily symptomatic
	Frangova Youroukova *et al.* 1995	31	53%					bakers not necessarily symptomatic
Flour beetle	Schulze-Werninghaus *et al.* 1991	125		7%				

N = Number of workers in the study; If the number of workers in the study < 4 than no percentages are presented

proteolytic enzymes (protease & papain) was rare (Baur *et al.* 1988b; Baur *et al.* 1989).

Although the addition of cereal malt flours decreased since the introduction of fungal amylases, the available studies show that this additive should also be regarded as a potent allergen (Heyer 1983; Jorde *et al.* 1986; Wüthrich & Baur 1990). The most important allergens in these malt flours are probably the cereal amylases mentioned before. Soybean flour has shown to be another potent allergen (Heyer 1983; Jorde *et al.* 1986; Baur *et al.* 1989; Wüthrich & Baur 1990). Soybean flour is often added to the dough because of its high content of lecithin, which act as emulsifier. In an epidemiologic study in Scotland, IgE to soybean flour has been tested in 205 bakery workers, of whom 3% appeared to be sensitized (Jeffrey 1992).

The role of moulds in baker's asthma is still controversial. Although it has been identified as the cause of asthma in some bakers (Weiner 1960; Klaustermeyer *et al.* 1977) and the prevalence of sensitization in symptomatic bakers significantly differed from groups of symptomatic controls (Bergmann *et al.* 1976; Wallenstein *et al.* 1980), the study of Musk *et al.* (1989) showed that sensitization was low in groups of unselected bakery workers. The prevalence of positive skin prick test to bakers yeast was low as well (Musk *et al.* 1989).

For most other allergens mentioned in Table 2.1, only sporadic case reports have been presented and their contribution to the high prevalence rates of baker's respiratory allergy is probably only minor.

Epidemiology

Sensitization

In many cross-sectional epidemiologic surveys, sensitization to wheat flour or fungal amylase has been tested, either by skin testing or by measurement of specific IgE-antibodies in sera. An overview of available cross-sectional studies among groups of unselected bakery workers, is presented in Table 2.2. The prevalence rates of sensitization varied from 5-28% for wheat flour and from 5-15% for α-amylase. For a good comparison of these prevalence rates, all tests should have been performed with standardized methods and extracts. However, the methods used varied from study to study. Moreover, no standardized extracts are available for bakery allergens, and as a result, the origin and concentration of the extracts used

for skin prick testing were quite different. These methodological differences hamper a valid comparison of the study results.

Table 2.2 Prevalence of sensitization to wheat flour and α-amylase in cross-sectional epidemiologic studies.

Reference	N	wheat flour		α-amylase		skin test used (conc. extract; cut-off point)
		skin tests	spec. IgE	skin tests	spec. IgE	
Herxheimer 1967	895	18%				? (?;?)
Thiel & Ulmer 1980	29	21%	28%			intracutaneous (0.1 % w/v;?)
Prichard *et al.* 1984	176	15%				prick test (1 mg/ml; 3 mm)
Hartmann *et al.* 1985	292	6%				intracutaneous (?;?) [only non-sympt. bakers!]
Baur *et al.* 1986	91		9%		2%	
Musk *et al.* 1989	259	5%				prick test (?; 2 mm)
Jeffrey 1992	205		24%		15%	
Bohadana *et al.* 1994	44	11%				prick test (10 mg/ml; >½ pos. control)
Cullinan *et al.* 1994a	344	5%		5%		prick test (10 mg/ml; 3 mm)
De Zotti *et al.* 1994	226	12%		8%		prick test (1 mg/ml; 3 mm)
Zuskin *et al.* 1994b	71	12%				prick test (0.05 % w/v; 3 mm)
Alday *et al.* 1995	228			7%		prick test (20% w/v; 3 mm)

N = Number of workers in the study

Respiratory symptoms

In many studies the prevalence of respiratory symptoms in bakery workers has been estimated. The vast majority of these epidemiologic surveys was cross-sectional in design. These studies, summarized in Table 2.3, clearly show that work-related respiratory symptoms suggestive of allergy are quite common in this occupation.

Many of the epidemiologic surveys, however, have important limitations. Especially in older studies, there is often a lack of information on e.g. participation rate and methods used to estimate symptom prevalence (diagnosis by physician, questionnaire by interview or self-administered questionnaire). Differences in prevalence rates may also have been caused by differences in prevalence of atopy or exposure levels. In only few studies, however, information on these potential

Table 2.3 Prevalence of respiratory symptoms reported in cross-sectional epidemiologic studies. All symptoms are reported to be work-related, except where noted in the last column.

Reference	Country	Type of bakery/bakeries	Number of workers in the study	Part. rate[1]	Exp. meas.[2]	Method used	Prevalence of symptoms		remarks
Baagöe 1933a	Denmark	22 small bakeries	66	?	no	?	rhinitis	12%	
Dishoeck & Roux 1939	The Netherlands	large & small bakeries and cake bakeries	152	100%[3]	no	?	flour illnesses of respiratory tract	26%	mill-workers excluded
Linko 1947	Finland	large & small bakeries and confectioneries	328	100%[3]	no	?	nasal symptoms asthma	18% 8%	
Pestalozzi & Schnyder 1955	Switzerland	5 large & medium sized bakeries	159	?	no	?	wheat rhinitis wheat asthma	26% 4%	
Gadborg 1956	Denmark	?	1316	78%	no	?	wheat rhinitis wheat asthma	15% 7%	reported prevalence rates are workers with respiratory symptoms *and* sensitization to flour
Beritic-Stahuljak 1976	Croatia	?	130	?	no	questionnaire	nasal catarrh bronchial asthma	30% 3%	only non-smokers in study; symptoms are not necessarily work-related
Järvinen *et al.* 1979	Finland	1 large bread bakery	234	?	no	questionnaire	rhinitis asthma	23% 9%	symptoms are not necessarily work-related
Thiel & Ulmer 1980	Germany	?	29	?	no	?	rhinitis asthma	21% 10%	
Charpin *et al.* 1984	France	?	154	91%	no	questionnaire	rhinitis asthma	22% 5%	symptoms are not necessarily work-related

Table 2.3 Continued

Reference	Country	Type of bakery/bakeries	Number of workers in the study	Part. rate[1]	Exp. meas.[2]	Method used	Prevalence of symptoms		remarks
Prichard *et al.* 1984	Australia	18 metropolitan bakeries	176	?	no	physician administered questionnaire	chest symptoms asthma	19% 5%	
Hartmann *et al.* 1985 & Hartmann 1986	Switzerland	1 large bakery	314	?	yes	questionnaire	respiratory allergy	7%	no further definition of respiratory symptoms
Thiel 1987	Germany	?	242	?	no	?	wheat rhinitis wheat asthma	14% 7%	
Musk *et al.* 1989	England	1 industrial bakery	279	88%	yes	self-administered questionnaire	nasal symptoms chest symptoms chest tightness	19% 13% 7%	
Rosenberg *et al.* 1991	France	bakeries and confectioneries	2088	?	no	questionnaire	rhinitis and/or asthma	6%	
Jeffrey 1992	Scotland	bakeries (< 50 workers)	224	?	yes	doctor administered questionnaire	lower respiratory symptoms nasal/eye symptoms	21% 27%	
Bohadana *et al.* 1994	France	1 industrial bakery	44	85%	yes	interviewer administered questionnaire	running nose dyspnoea wheat asthma	18% 18% 2%	symptoms are not necessarily work-related
De Zotti *et al.* 1994	Italy	105 small bakeries	226	82%	no	questionnaire	rhinoconjunctivitis asthma	14% 5%	
Cullinan *et al.* 1994a	England	3 large bakeries, 1 flour packing station & 3 flour mills	344	86%	yes	questionnaire	eye/nose symptoms chest symptoms	29% 14%	

Table 2.3 Continued

Reference	Country	Type of bakery/bakeries	Number of workers in the study	Part. rate[1]	Exp. meas.[2]	Method used	Prevalence of symptoms		remarks
Zuskin *et al.* 1994b	Croatia	1 large confectionery	71	?	no	questionnaire	nose secretions dyspnoea	24% 48%	exposure to various agents
Zuskin *et al.* 1994a	Croatia	1 large confectionery	78	85%	no	questionnaire	nose secretions dyspnoea occupational asthma	14% 41% 4%	symptoms only refer to flour exposed workers; diagnosis of occupational asthma was confirmed by medical records
Shamssain 1995	South Africa	?	63	?	no	questionnaire	nasal symptoms	54%	only non-smokers in study; symptoms are not necessarily work-related
Patussi *et al.* 1995	Italy	Bakeries, confectioneries & biscuit factories	320	?	?	questionnaire	rhinitis asthma	10% 7%	abstract from recent congress

[1] Participation rate
[2] Exposure assessment of bakery workers by personal dust sampling
[3] The authors claim that all workers from the bakeries were studied

risk factors was presented. These limitations hamper a good interpretation of study results. Moreover, a prerequisite for a comparison of study results is the use of validated and standardized questionnaires. However, the type of questionnaire and the definition of respiratory symptoms, varied considerably between studies. The large methodological differences and various definitions of symptoms used in each study makes a detailed comparison of prevalence rates impossible.

Another important drawback of these cross-sectional studies is that they are susceptible to survivor bias. Workers with severe work-related disease are likely to have left the bakery trade and therefore prevalence rates in cross-sectional studies may result in biased estimates of the actual risk. Cohort studies could reveal the magnitude of this selection bias, but are generally difficult to perform, time-consuming and expensive. The available cohort studies will be shortly discussed.

Cohort studies

Until now, only few cohort studies on baker's allergy have been conducted. The first, and still most comprehensive cohort study published until now, was performed by Gadborg (1956). The results have only been published in a thesis in Danish. A brief description can be found in Thiel & Kallweit (1984). Gadborg examined 1555 bakers in Copenhagen in a cross-sectional study. Five to six years later 500 randomly selected bakers were re-examined. From this population 487 bakers could be traced, and in this group 19 had developed wheat flour sensitization in the 5 year period, and 7 had developed a wheat flour induced respiratory allergy, defined as the presence of symptoms and sensitization. Although not calculated by the author, this study suggests incidence rates for wheat flour sensitization of 9.8 per 1000 workers per year, and for wheat allergy of 3.5 per 1000 per year. Unfortunately, no information is available on exposure levels or other potential determinants of baker's allergy. Because some assumptions had to be made in calculating these incidence rates[2], they have to be regarded as crude estimates of the true incidence rates. From the symptomatic bakers of the initial study, many stated that their symptoms had approved or even had disappeared, even though their exposure to flour had not been reduced. The same phenomenon was also found for sensitized bakers. Many bakers who were sensitized in the first study did not show positive

[2] For the calculation of incidence rates, sensitized bakers or bakers with symptoms were excluded from the population at risk, and a follow-up period of 5 years was assumed for every bakery worker.

test results in the second study.

A second cohort study has been performed in Germany. Herxheimer (1967 & 1973) followed the development of skin test reactivity to flour in bakers' apprentices over a five-year period in Berlin by performing annual skin test. At the first test, during the first month of work, already 8% of 880 apprentices had a positive skin test to flour. Although the flour sensitivity disappeared in half of the sensitized individuals within 12 months, the total number of positive tests steadily increased during the 5 year follow up (12% in second year, 19% in third, 27% in fourth and 30% in fifth year). At the beginning of the study, only 0.2% of the subjects had symptoms 'compatible of allergic rhinitis or asthma'. This figure increased to 7% after three years in the baking trade and fell back to 4.8% after five years. Importantly, Herxheimer was not able to complete the follow-up for the majority of bakery students from the initial study, and the number of subjects in the populations steadily decreased during the research period (baseline 880; 1st year 649; 2nd 421; 3rd 290; 4th 92 & 5th 37). Due to this immense loss to follow up (52% after 3 years and even 96% after 5 years), prevalence rates could be strongly biased. Again, no information on exposure levels was available in this study.

A more recent cohort study was performed in English bakeries, but until now only congress abstracts are available (Cullinan *et al.* 1995). In this study, a cohort of 103 newly exposed bakery or flour mill workers were surveyed at six-monthly intervals over three years, and incident rates were presented for specific sensitization and respiratory symptoms. The reported incidence rates of chest and eye/nose symptoms were 1.9 and 18.8 cases per 1000 person months respectively. Incident positive skin prick tests to α-amylase during follow-up were more common (incidence rate 5.8 per 1000 person months) than to the flour extract (0.6 per 1000 person months).

Determinants of specific sensitization and respiratory symptoms

Several investigators have tried to reveal the determinants of specific sensitization and work-related respiratory symptoms. The results are summarized in Table 2.4. In the majority of these studies only univariate analyses have been performed. Only few studies have presented stratified or multiple regression analyses, thus correcting for other potential determinants (Musk *et al.* 1989; Cullinan *et al.* 1994a; De Zotti *et al.* 1994; Bohadana *et al.* 1994). Moreover, many studies have focused on host factors only, especially on atopy. Only few studies have studied exposure levels as

a cause of sensitization and respiratory symptoms.

Table 2.4 Overview of identified determinants associated with specific sensitization or respiratory symptoms in bakery workers (+: positive association; 0: no association; -: negative association).

Reference	Potential determinants					
	Age	Atopy	Exposure level	Gender	Smoking	Years of exposure
	Specific sensitization to occupational allergens					
Prichard *et al.* 1984 & Prichard *et al.* 1985[1]	0	+			0	+
Hartmann *et al.* 1985 & Hartmann 1986[1]			+			
Musk *et al.* 1989	0[2]	+	+	0[2]	0[2]	+
Cullinan *et al.* 1994a	0[2]	+	0/+[3]	0[2]	0	
De Zotti *et al.* 1994		+		0	+	+
Popp *et al.* 1994[1]		+				
	Work-related respiratory symptoms					
Pestalozzi & Schneider 1955[1]		+				
Gadborg 1956[1]		+				
Järvinen *et al.* 1979[1]		+				
Prichard *et al.* 1984 & Prichard *et al.* 1985[1]	0	+			0	
Hartmann *et al.* 1985 & Hartmann 1986[1]		+	+			
Musk *et al.* 1989	0/-	0[2]	+	0	0[2]	0[2]
Rosenberg *et al.* 1991[1]		+			0	
Jeffrey 1992[1]			+[4]	+	0	
Bohadana *et al.* 1994	0[2]		0	0[2]	0	0[2]
Cullinan *et al.* 1994a	0[2]	0	+	0[2]	0/+[3]	
De Zotti *et al.* 1994	0	+		0	0	0

[1] Only univariate analyses
[2] According to method section this determinant was included in the multivariate analysis, but results were not mentioned in the text, most probably because no association was found
[3] A positive association was found in the univariate analysis, but not in the multivariate analysis
[4] high versus low exposed

Atopy is a very strong determinant for sensitization to occupational allergens. The reported odds ratios (OR) for atopy range from 5.1 to 16.3. In most studies atopic status was defined as a positive skin prick test to one or more common allergens (grass pollen, house dust mite etc). Some recent studies showed that exposure was also an important risk factor for sensitization, but this will be discussed in more detail in a separate section. Age and gender have not been reported to be determinants of sensitization. In an Italian study, sensitization was significantly associated with cigarette smoking (OR=2.7; De Zotti *et al.* 1994), but other studies did not find such an association.

For work-related respiratory symptoms, atopy and exposure appeared to be the most important risk factors. All studies showed an increased risk for atopics (OR: 3.5-11.7), with the exception of two English studies (Musk *et al.* 1989; Cullinan *et al.* 1994a). No associations were found between symptoms and age, gender or smoking habits, with a few exceptions. In one study, age was inversely associated with nasal symptoms (OR=0.7 per 10 years), but not with chest symptoms (Musk *et al.* 1989). In the study of Cullinan *et al.* (1994a), smokers had more work related symptoms than non-smokers, but no independent association was found in a multiple regression analysis. In another study, nasal/eye symptoms were more common in men than in women, although this was probably due to differences in duration and level of exposure (Jeffrey 1992).

In a recent study among 264 bakery workers, it has been suggested that work related respiratory symptoms predominantly had a non-allergic basis (Cullinan *et al.* 1994a). In that study, respiratory symptoms were not associated with atopic status, and no relation was found between symptoms and skin test results (wheat flour and α-amylase). According to the authors, one of the possible explanations was that 'symptoms were unlikely to be wholly specific for IgE associated allergic disease, but may also result from direct irritation'. Although some other studies did find a clear relationship between specific sensitization and work related symptoms (De Zotti *et al.* 1994; Prichard *et al.* 1985), there are also studies that support the theory that 'flour dust' can also act as an irritant in addition to its allergenicity. Bronchial hyperresponsiveness appeared to originate in bakers' apprentices soon after their exposure to flour, indicated by the higher number of positive responses to acetylcholine compared with a group of controls (Thiel & Ulmer 1980). Zuskin *et al.* (1994b) showed that the majority of respiratory symptoms in a group of confectionery workers did not correlate with immunologic findings, and the authors

concluded that exposure in this plant was associated with symptoms of an irritative nature. In a French study, bakery workers had an increased airway responsiveness compared with controls (Bohadana *et al.* 1994), and within the group bakery workers a positive relationship was found between responsiveness to metacholine and exposure to flour dust (with exposure modeled either as a categorical variable or expressed in terms of duration of exposure in years). In the study of Musk *et al.* (1989) also evidence of exposure related respiratory effects was obtained from measurements of non-specific bronchial reactivity. In a multivariate analysis, a positive metacholine challenge test was more prevalent among highly exposed workers compared with low exposed bakery workers (OR=2.3), corrected for all other potential determinants. Unfortunately, no information is available on the proportion of these workers sensitized to occupational or common allergens, and it is therefore impossible to distinguish between symptoms in presence or absence of sensitization to occupational or common allergens. Nevertheless, these studies suggest that apart from IgE-mediated allergies, work-related symptoms of bakery workers may also be caused by non-immunological responses.

Although a number of researchers have examined the relationship between the length of time spent in the baking trade and the appearance of specific sensitization and respiratory symptoms, results have often varied widely. Several studies among bakery students showed that sensitization can occur within a few months after start of exposure to flour and prevalence rates in these populations varied from 3 - 8 % for wheat flour sensitization and from 0 - 0.7 % for sensitization to α-amylase (De Zotti *et al.* 1995; Gautrin *et al.* 1995; Herxheimer 1967; Thiel & Ulmer 1980). In one study, the prevalence of positive wheat flour specific IgE tests in bakery students (4-20 weeks of exposure) was even 17% (Popp *et al.* 1994). In only few of these studies, however, the role of previous exposure was discussed. It is not unlikely that many students have worked in a bakery before the start of the study (for instance in the bakery of family members). In one of the studies it was shown that 29% of the bakery students had already worked in bakeries (De Zotti *et al.* 1995).

Other studies have estimated a latency period for the development of respiratory symptoms by asking asthmatic bakers for the time since first exposure and the onset of first symptoms. The mean latency period for each study population varied from 4-13 years (Järvinen *et al.* 1979; Popa *et al.* 1970; Hartmann *et al.* 1985; Diederichs & Lübbers 1955), but within each group this varied considerably between

individuals (range from a few months to >30 years). In an English study among 264 bakery workers, the median duration of employment before onset of chest symptoms was 1 year (range 1 month - 4.2 year), and 0.5 year (range 1 month - 3.3 year) for eye/nose symptoms (Cullinan *et al.* 1994a). However, bakers working in the trade longer than 4 years had been excluded from the study, and the median working years until onset of symptoms may have been larger for the total population of bakery workers. The spread in study results illustrates that there is still little understanding of the relationship between length and intensity of exposure to flour dust and the development of sensitization or respiratory symptoms. Only cohort studies can reveal this relation, but until now only few longitudinal studies have been published, and most of them have important methodological limitations. The available cross-sectional studies suggest that sensitization may occur soon after start of exposure, but it can take several years for the development of symptoms.

Exposure-response-relationships

Exposure measurements

The inhalation of cereal dusts and powdered baking additives may cause immunologic sensitization in bakery workers. However, little is known of the levels of exposure required for sensitization. Since wheat flour and fungal α-amylase are the most important allergens in the bakery occupation, exposure-response-relationships should be studied at least with measurement of wheat allergens or α-amylase allergens. Methods of measuring allergens in air, however, require specialized sampling and assay techniques, which have been developed only for a limited number of occupational allergenic proteins (Reed *et al.* 1993; Gompertz 1994).

Until now, in only one study a method for monitoring personal airborne allergens in the baking industry was presented. Sandiford *et al.* (1994a) have developed a method for measuring airborne flour allergens in flour mills and bakeries, using polyclonal rabbit IgG antibodies in a radioallergosorbent test (RAST) inhibition. A method for quantifying personal airborne fungal α-amylase allergen exposure has not been reported yet.

Because methods for measuring allergen exposure in bakeries were not available, many studies have measured dust exposure levels instead. However, monitoring dust

exposure has an important disadvantage. Dust levels may only partially correlate with the actual allergen concentration and it is questionable whether dust levels are a valid exposure parameter in occupations where IgE-mediated allergies predominate. In an English study, personal dust exposure levels were compared with wheat allergen exposure levels, measured with the assay developed by Sandiford *et al.* (1994a). Important differences in the relation between total dust and flour aeroallergen concentrations were found, depending on the use of products other than wheat flour and thus on the proportion of dust that consists of wheat flour (Nieuwenhuijsen *et al.* 1994). This shows that monitoring of dust exposure is not a valid approach for estimating flour allergen exposure levels of bakery workers, and that misclassification of exposure may occur in epidemiologic surveys when bakery workers are classified based only on dust exposure levels.

While dust sampling has long been the only available tool for exposure assessment in bakeries, even efforts to characterize personal dust exposure levels are of rather recent date. Some early reports have presented stationary dust measurements (Beritic-Stahuljak *et al.* 1976; Bergmann *et al.* 1976; Wallenstein *et al.* 1980), but usually these measurements have not been considered very reliable for quantitative exposure assessment in occupational environments. The first measurements of personal samples in bakeries have been described by Hartmann (1986), and since then several other studies have presented personal exposure levels, as summarized in Table 2.5. The sampling strategy and sampling equipment used varied from study to study, and for a good interpretation of the data, often essential information was missing, e.g. on sampled dust fraction, and duration of sampling. Only few studies have presented well-documented, full-shift personal exposure data (Musk *et al.* 1989; Jeffrey 1992; Nieuwenhuijsen *et al.* 1994; Lillienberg & Brisman 1994).

In Table 2.5 exposure levels for some occupational titles in bakeries are presented. Bakers working in the doughmaking area (doughmaking and bread forming) usually had the highest dust exposure with mean exposure levels varying from 2.3 - 8.6 mg/m^3. Mean dust exposure levels of oven workers varied from 1.1 - 3.2 mg/m^3. Bakery workers involved in slicing and packing of the bread or other products usually had exposure levels below 1 mg/m^3. In most studies, no important differences in dust exposure levels were found between bakeries with similar production process. In a British study, occupational titles of the bakery workers were considered as the most important determinant of dust exposure and the best

Table 2.5 Personal dust measurements in bakeries

Reference	sampling time (hours)	sampling head (dust fraction)	N	n	occupational title	GM or AM*	GSD or SD*	range
Hartmann 1986	3	?	31	31	all			0.2 - 19.8
Masalin *et al.* 1988	?	?	29	29	all			0.1 - 8.8
Musk *et al.* 1989	full-shift	Casella or Millipore (total dust)	79	10	doughmakers	2.7		0.6 - 14.1
				16	oven staff	1.7		0.0 - 37.6
Jeffrey 1992	full-shift	7-hole or Casella (total dust)	68	3	weighing/mixing	8.6	2.3	3.3 - 15.8
				16	dividing/moulding	4.7	2.0	1.6 - 19.1
Jauhiainen *et al.* 1993	4 - 7	3-piece cassettes (?)	20	13	making of dough	4.6*	3.6*	0.9 - 14.7
				7	making of bread	2.3*	0.9*	1.5 - 3.4
Kolopp-Sarda *et al.* 1994	?	?	10	10	all	4.9*	9.1*	
Bohadana *et al.* 1994	4	Millipore (total dust)	21	14	general baker	3.4*	3.7*	0.7 - 8.7
				6	oven handler	1.1*	0.9*	0.5 - 2.7
Lillienberg & Brisman 1994	full-shift	IOM (inhalable)	29	6	dough mixing	7.5*	5.4*	
				10	dough forming	2.5*	0.8*	
				3	oven control	3.2*	1.7*	
Burdorf *et al.* 1994	1-7	IOM (inhalable)	129	34	dough makers	5.5§	2.1§	1.2 - 16.9
				62	bread formers	2.7§	2.0§	0.6 - 14.2
				10	oven workers	1.2§	2.4§	0.2 - 4.0
Nieuwenhuijsen *et al.* 1994	full-shift	7-hole (total dust)	352	24	dispense/mixing	5.0	2.5	1.4 - 86.0
				32	roll production	2.4	2.5	0.4 - 21.1

N = Total number of personal samples
n = Number of samples taken in occupational title
AM = Arithmetic mean
GM = Geometric mean
SD = Standard deviation
GSD = Geometric standard deviation
* = Concentration and standard deviation in AM & SD (all others in GM & GSD)
§ = GM and GSD could be biased because of large variations in sampling time

way to categorize bakery workers into exposure groups (Nieuwenhuijsen *et al.* 1994; Nieuwenhuijsen *et al.* 1995a). However, these samples were taken in large and highly mechanized bakeries, and the determinants of dust exposure might be different for bakeries with different technologies, e.g. small traditional bakeries.

Some studies have performed task-based sampling or have measured peak exposures levels in bakeries. In a paper from Nieuwenhuijsen *et al.* (1995b), the highest dust exposure was found during cleaning operations, but bread- and roll-production were also identified as tasks with considerable dust exposure. Real-time dust monitoring was described in two studies using a Miniature Real Time Aerosol Monitor (Miniram) (Praml *et al.* 1986; Jongedijk *et al.* 1995). In the last study, peak exposures were measured in small bakeries by personal sampling with the Miniram for several hours. All peaks were assigned to corresponding activities using video monitoring during the measurements. Most peaks were caused by throwing wheat flour on the workbench during dough forming or by adding ingredients into the dough mixer. A high correlation was found between the number of peak exposures and the full shift time-weighted averages, suggesting that high peak exposures contribute strongly to the 8 hour exposure levels.

Occasionally, particle size determination of the flour dust have been performed, showing that particles larger than 10 μm predominate, and about 20% belong to the respirable fraction (Sandiford *et al.* 1994c; Burdorf *et al.* 1994).

Finally, some investigators have measured airborne fungi in bakeries. A large variety of fungal species have been found in bakeries (Charpin *et al.* 1971; Singh & Singh 1994; Klaustermeyer *et al.* 1977; Gemeinhardt & Bergmann 1977). The dominating mould species varied from study to study, suggesting that local circumstances in each bakery may be very important. Reliable quantitative exposure data of airborne fungi in bakeries are not available.

Exposure-response-relationships

As mentioned, in some studies the level of exposure to dust or specific allergens has been identified as a determinant of baker's allergy. This will be discussed in more detail.

Although not often cited, the first indications for an exposure-response-relationship can be found in a study among 314 bakery workers in Switzerland (Hartmann *et al.* 1985; Hartmann 1986). In this study 2-4 hour personal dust samples were taken in bakery workers, and based on these measurements all bakery

workers were classified into three exposure categories. In the group of workers without respiratory symptoms, a statistically significant positive association was found between dust exposure and the prevalence of sensitization to wheat flour. But also for symptoms a clear and positive relationship was found with the level of exposure. Although this was the first study on baker's allergy that examined the role of exposure, it has some important limitations. There is no information available on the sampled dust fraction (total dust, inhalable dust etc.), and additionally, the role of potential confounders or modifying factors like atopy was not studied.

The second study that related dust exposure to sensitization and respiratory symptoms was performed in the UK (Musk *et al.* 1989). In this study, all bakery workers were ranked based on their occupational title. This exposure ranking was based on 'perceived dustiness', not on actual exposure measurements. However, dust measurements were performed, which correlated well with rank. Using logistic regression analysis, it was shown that work-related symptoms were significantly associated with exposure rank. Skin sensitization was more common among highly exposed workers compared with low exposed workers (OR=3.0), also suggesting that exposure was an important determinant of baker's allergy.

In a more recent study, specific allergens have been measured in personal samples with immunochemical methods and these measures of exposure were related to specific sensitization and respiratory symptoms. Cullinan *et al.* (1994a) showed that the frequency of sensitization to wheat flour and α-amylase and the frequency of work-related symptoms tended to increase with intensity of wheat allergen exposure. In the multivariate analyses, however, no independent effect of aeroallergen exposure was found on the presence of positive skin prick tests or work-related chest symptoms. Only eye/nose symptoms were independently related to wheat allergen exposure (high versus low exposed workers: OR=3.1).

Discussion en conclusions

A large number of studies has been performed to study occupational respiratory allergies among bakery workers. These studies have shown that sensitization to occupational allergens and respiratory symptoms indicative of allergy are very common in the bakery industry. These effects are clearly apparent in bakery

workers exposed to flour dust levels between 2 to 5 mg/m^3, but may also occur at lower exposure levels. Although occupational allergy to a large variety of agents have been reported in bakery workers, the most important allergens are wheat flour (and other cereal flours) and fungal α-amylase, which is an important ingredient of baking additives. Most likely, the allergenic potency of α-amylase enzymes is much higher compared with the mix of wheat flour allergens. Although exposure to α-amylase allergens is about a factor 1000 lower than for wheat flour allergens (in terms of protein concentration), the prevalence of sensitization in bakery workers is comparable for both allergens.

Most epidemiologic surveys studying respiratory allergy among bakery workers, have been cross-sectional in design and several have important methodological shortcomings. Nevertheless, these studies showed that atopic status and exposure levels are the most important risk factors for both sensitization and work-related respiratory symptoms. The role of atopy has been described for a long time. In more recent studies, dust and wheat allergen exposure have been measured and identified as an important determinant of baker's allergy. Thus far, exposure-response relationships have been reported for flour dust and wheat allergens, but not for fungal α-amylase.

3. Wheat antigen exposure assessment for epidemiologic studies in bakeries using personal sampling and inhibition ELISA[1]

Remko Houba, Paula van Run, Dick Heederik & Gert Doekes

Abstract

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. In the present study we have investigated a method to measure wheat flour antigens with immunochemical methods. Wheat flour antigens were measured in 449 personal dust samples taken in bakeries, using ELISA inhibition and an anti-wheat IgG_4 serum pool. Western-blotting was performed to compare the wheat flour proteins detected by IgE and IgG_4. Electrophoresis and immunoblotting showed that many wheat flour proteins can bind IgG_4 and IgE, but also a reasonable similarity in major allergens detected by our IgG_4-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 relation 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 epidemiologic field studies. 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.

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[1]*Clinical and Experimental Allergy 1996;26:154-163.*

Introduction

Occupational respiratory diseases are common among bakery workers and others exposed to flour dust. According to most authors this affection appears to be an IgE-mediated type I allergic reaction to flour dust (Sutton *et al.* 1984; Prichard *et al.* 1985). 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 (Prichard *et al.* 1985; Baldo & Wrigley 1978; Baldo *et al.* 1982).

Epidemiologic 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 (Järvinen *et al.* 1979; Thiel & Ulmer 1980; Prichard *et al.* 1984; Musk *et al.* 1989). 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 (Meredith *et al.* 1991; Gannon & Burge 1993; Meredith & McDonald 1994; Nordman 1994). 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 to 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 (Musk *et al.* 1989; Brisman & Belin 1991; Jauhiainen *et al.* 1993; Burdorf *et al.* 1994), 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 RAST inhibition and an IgE serum pool from sensitized bakers (Tee *et al.* 1992) or polyclonal rabbit IgG antibodies (Sandiford *et al.* 1994a). 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 confectionery 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 IgG_4-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.

Materials and methods

Bakeries

Twenty one bakeries were studied during 1 to 20 days. These bakeries differed in a number of characteristics, such as number of employees, products, and technology level. Table 3.1 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 (Teflon) filters (Millipore; pore size 1.0 μm) and PAS-6 sampling heads at a flow rate of 2 l/min (ter Kuile 1984). Production workers were asked to participate for one to six days. After sampling, filters were weighed in a preconditioned room of 18-22 °C with 45-55 % humidity. Detection limit of this method is 0.17 mg/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 (Helsel 1990). Wheat antigens were recovered from the filters by extraction with 2.5 ml 0.15 M Phosphate Buffered Saline (PBS, pH 7.4) in a 10 ml centrifuge tube. Each tube was vortexed for 2 minutes, sonicated for 2 minutes, vortexed for 5 minutes and sonicated for 2 minutes, successively. The extract was centrifuged at 5,000 g for 15 minutes. The supernatant was collected and stored at -20°C for up to 6 months.

Table 3.1 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)

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 3.1). This wheat flour was extracted in the same way as the dust samples (but now 5 ml PBS per 500 mg wheat flour). After centrifugation, the supernatant was filtered through a 0.22 µm 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 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 IgG_4 against the standard wheat flour extract. Sera of 59 workers with intermediate and high IgG_4-titres (OD_{492} > 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.

SDS-Polyacrylamide gel electrophoresis & immunoblotting

Lyophilized wheat flour extract (1 mg protein per ml) was dissolved in sample buffer according to Laemmli (1970) and incubated for 10 minutes at 100°C. This extract was subjected in a 10*7.5*0.05 cm 13.5% polyacrylamidegel (30 µl extract per cm), using a Mini-Protean II slab cell unit (Biorad, Richmond, CA USA). Electrophoresis was performed at 100 Volt for 10 minutes and at 200 Volt for 50 minutes, 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 µm pore size), using a Mini-Trans blot transfer unit (Biorad). The transfer was performed at 3.33 mA/cm^2 for 1 hour at room temperature.

After blotting, the nitrocellulose sheet was cut into strips of 1.5 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 µl undiluted individual sera from the serum pool or from IgE-sensitized bakers.

For sera from the serum pool, a further 1.5-hour incubation at room temperature was performed with 1:1000 diluted mouse monoclonal anti-human IgG_4 conjugated with horseradish peroxidase (Central Laboratory for Blood Transfusion (CLB), Amsterdam, The Netherlands). Bound IgE from sensitized bakers was detected by subsequent 1 hour 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 minutes with TMB (Promega Corporation, Madison, WI USA).

ELISA inhibition procedure

Polystyrene high capacity microtiter plates (nr 655061, Greiner, Nuertingen, Germany) were coated overnight at 4°C with the standard wheat flour extract at a concentration of 5 µg/ml protein in 0.15 M PBS in volumes of 200 µl/well. Next morning the plate was washed with PBS-Tween and incubated for 30 minutes at 37°C with PBTG. 100 µl samples of 12 serial dilutions of the standard wheat flour antigen preparation (starting with 12.5 µg/ml protein in PBTG) and 100 µl of undiluted samples, were added to the wells and mixed with 100 µl subsequently added pooled serum (diluted 1:800 in PBTG). On each plate four blanks (200 µl

PBTG), four estimates of 0% inhibition (100 µl assay buffer), and four estimates of 100% inhibition (100 µl standard antigen of 12.5 µg/ml) were included. The plate was incubated for two hours at 37°C. After washing, bound IgG_4 was measured in each well by incubation (one hour at 37°C) with 200 µl conjugated anti-human IgG_4 (CLB) and finally an incubation (30 minutes in the dark at 20°C) with 200 µl o-phenylenediamine (OPD; 2 mg/ml) in 0.05 M citrate/phosphate buffer (pH 5.5), containing 0.015% H_2O_2. 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 OD_{492} 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 analyzed 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:

Flour aero-antigen (ng/m^3) = ([flour] (ng/ml) * volume of filter eluate (ml))/ (volume of sampled air (m^3)).

Statistical analyses

Differences in mean exposure levels between several groups were analyzed 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 & immunoblotting

The results of the blotting experiments with 24 randomly selected sera of the 59 sera in our IgG_4-serum pool are summarized in Table 3.2. 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.

Table 3.2 Molecular weights (kDa) of wheat flour proteins detected by 24 of the 59 sera in our IgG_4-serum pool.

Molecular weights (kDa)	Number of sera (%)
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)

Figure 3.1 shows an immunoblot of our IgG_4 serum-pool and sera from three allergic bakers (in duplicate), with high anti-wheat IgE-titers (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 IgG_4-serum pool.

Detection limits, reproducibility and specificity of the inhibition ELISA

Figure 3.2 shows the inhibition curve of our wheat flour 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 m^3, this results into a detection limit for personal antigen measurements of 50 ng/m^3. As for the dust measurements, values below the detection limit (n=70) were considered as being two-thirds of this limit. The mean OD_{492} 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.

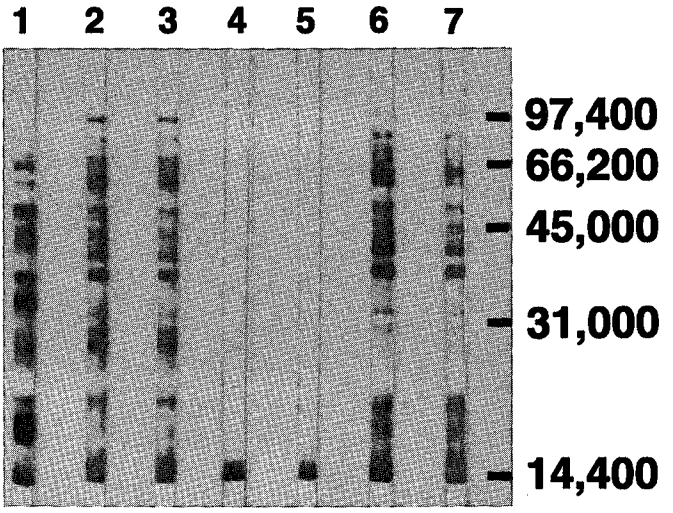

Figure 3.1 Wheat flour immunoblots with human IgG_4 serum pool (track 1) and human IgE (from sensitized bakers; track 2-7).

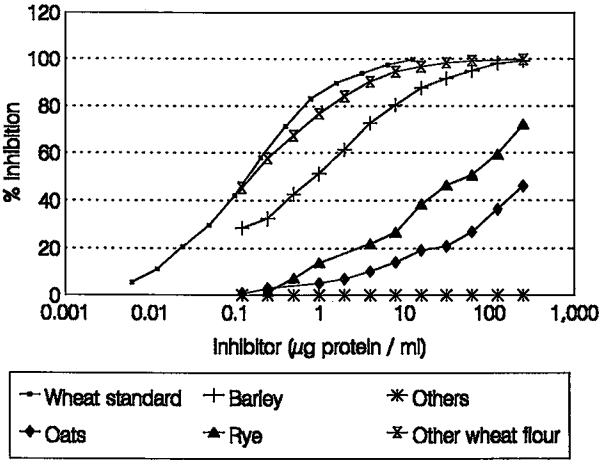

Figure 3.2 Inhibition of wheat flour standard with other grain species (barley, rye and oats), other ingredients frequently used in bakeries (baker's yeast, fungal α-amylase and egg), and several mould extracts (see text).

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 Figure 3.2, extracts from closely related grain species showed some inhibition, suggesting cross reactivity 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 extract, 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.

Airborne dust and wheat flour 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 3.3. 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 0.4-37.7 mg/m^3). Most doughmakers spent 80 - 90 % of their time in a relatively small section 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 mg/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

Table 3.3 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)
	N	GM	range	N	GM	range	
Total	546	1.0	0.1 - 37.7	449	684	33 - 252,407	1,450
Large bakeries							
Doughmakers	105	3.0[§]	0.4 - 37.7	76	5,323[§]	33 - 252,407	2,850[§]
All-round staff	66	0.9[*]	0.1 - 26.8	54	992[#]	33 - 68,159	1,550[#]
Oven staff	81	0.6	0.1 - 5.1	71	322	33 - 28,079	1,150
Slicers, packers & transport workers	132	0.4[§]	0.1 - 2.8	109	77[§]	33 - 7,736	350[§]
Production managers	20	0.6	0.1 - 4.9	17	505	33 - 74,614	2,100[†]
Maintenance & cleaning workers	27	0.7	0.3 - 5.5	20	242	33 - 2,539	600
Small bakeries							
Bread baker	36	3.3[§]	1.2 - 8.8	31	5,989[§]	1,289 - 53,275	1,950[¶]
Mixed baker	57	2.0[§]	0.3 - 14.2	55	2,729[§]	261 - 44,200	1,600
Confectioner	22	0.7[§]	0.1 - 3.7	16	614[§]	33 - 3,812	1,550

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 & cleaning workers ($p<0.05$)
[†] significantly different from maintenance & cleaning workers ($p<0.05$)
[¶] significantly different from confectioner's ($p<0.05$)

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 3.3). More importantly, significant differences were found in antigen exposure levels between groups with 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 3.3. 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 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 3.1). 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 Figure 3.3 and 3.4 clearly show that there was a distinct difference in this relationship between the samples from bread factories and confectioneries and from bread bakers in factories and small bakeries.

Within each occupational title (from Table 3.3) 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 3.4.

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 5 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 confectioneries. No significant difference in dust exposure levels could be found between these groups. For oven staff, differences between dust- and antigen

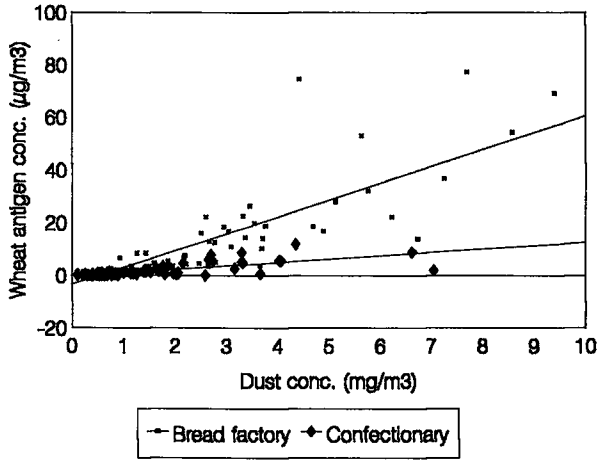

Figure 3.3 Relation between gravimetric dust measurements and wheat flour antigen concentration in large industrial bread factories (N=158) and confectioneries (N=76).

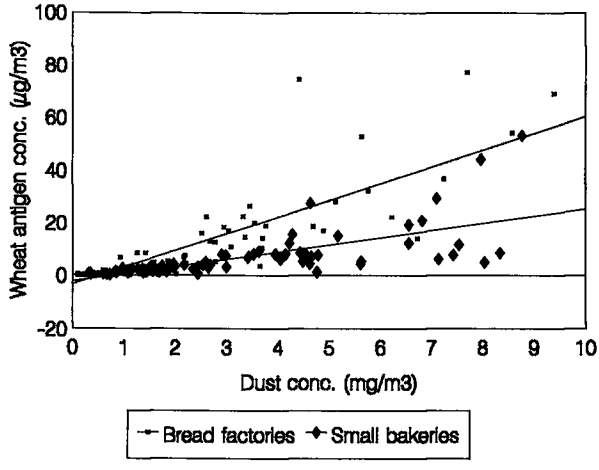

Figure 3.4 Relation between gravimetric dust measurements and wheat flour antigen concentration for bread bakers in small (N=102) and large bakeries (N=158).

Table 3.4 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)
	N	GM	range	N	GM	range	
All doughmakers	105	3.0	0.4 - 37.7	76	5,323	33 - 252,407	2,850
Wheat-bread production sites	37	4.5[§]	0.9 - 37.7	32	16,511[§]	1,597 - 252,407	4,750[§]
Confectioner's	32	2.4	0.4 - 7.4	25	1,969[§]	33 - 12,024	1,300
Crispbakes production site	29	3.1	0.9 - 8.9	15	5,104[§]	1,425 - 11,468	1,900[§]
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	1,150
Wheat-bread production sites	34	0.7	0.1 - 5.1	30	771[§]	33 - 28,079	2,050[§]
Confectioner's	21	0.6	0.1 - 3.2	21	302	33 - 2,600	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 ($p<0.05$)

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 measure airborne wheat flour antigens, using an anti-wheat IgG_4 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 (Sutton *et al.* 1984; Blands *et al.* 1976; Baldo & Wrigley 1984; Gómez *et al.* 1990). 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 (Theobald *et al.* 1986). 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 (Gómez *et al.* 1990; Pfeil *et al.* 1990; Sandiford *et al.* 1990). These are also the major protein bands reacting with our serum pool of IgG_4-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 titer, is limited. It is therefore difficult to get a serum pool which will be sufficient for many assays. The availability of IgG_4-positive sera is much larger and antibody titers 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 analyzed over a considerable period of time. A recent review of the role of IgG_4 in allergic diseases, reported that there is a great similarity between IgE and IgG_4 with respect to the type of antigens recognized (Aalberse *et al.* 1993).

Moreover, our own immunoblotting results showed this also applies to the wheat allergens, serum IgE and IgG_4-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 cross-reactivity between wheat flour and other cereal species, particularly barley, but no cross-reactivity 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 (Gómez *et al.* 1990; Barber *et al.* 1989; Armentia *et al.* 1993). It is known that proteins from other cereal species belong to the same protein family (Garcia-Olmedo *et al.* 1987) and one barley protein (14.5 kDa) of this family was shown to be a major allergen associated with baker's asthma (Barber *et al.* 1989). Because of the similarity in proteins in several cereal species, some degree of cross-reactivity 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/m^3 for slicers and packers to 4.5 mg/m^3 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 epidemiologic studies to baker's asthma is that it doesn't measure the true etiological 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/m^3, differences in dust exposure levels are very small, whereas approximately 80 % of the work force 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/m^3. Antigen exposure levels in these occupational titles, however, varied from 77 to 992 ng/m^3, the first being 13 times higher than the

latter. Expedition workers have a dust exposure of 0.4 mg/m^3, 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 3.4 and Figures 3.3 and 3.4 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.* (1992) 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/m^3 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/m^3, where only little variation in flour aero-allergen concentration was found. Sandiford *et al.* (1994a) 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.* (1994) 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 confectioneries. Exposure data in small bakeries (<10 workers) have never been published in the international literature, whereas almost 2/3 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 epidemiologic 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 spend 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 epidemiologic 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.

4. Airborne levels of α-amylase allergens in bakeries[1]

Remko Houba, Paula van Run, Gert Doekes, Dick Heederik & Jack Spithoven

Abstract

In the baking industry, the use of enzymes has increased throughout the 1980s. Several studies have reported sensitization and respiratory disorders among bakery workers caused by enzymes in dough improvers. Fungal α-amylase is the most frequently reported cause of allergy. α-Amylase allergen exposure levels in the bakery industry, however, have not been reported yet. Main objective of this study is to quantify personal α-amylase exposure levels of bakery workers. Alpha-amylase allergens were measured in 507 personal samples of airborne dust taken in bakeries, using a newly developed sandwich enzyme immuno assay (EIA) with affinity-purified polyclonal rabbit IgG antibodies. A cascade impactor was used to estimate the size of dust particles carrying α-amylase allergens. IgE from sensitized bakers and the rabbit IgG antibodies used in the assay had similar reaction profiles to a commercially available α-amylase extract. The EIA appeared to be highly specific for fungal amylase. Allergen exposure levels varied considerably among bakery workers, depending on the type of the bakery and the job category of the workers (range 0-40 ng/m^3). In confectioneries, no α-amylase allergens were detected. In other bakeries, α-amylase exposure was only found for workers directly involved in doughmaking. Measurements of the particle size distribution in these bakeries showed that α-amylase allergens are most likely to deposit in the nose and ciliated airways. This study shows that personal monitoring of fungal amylase allergen exposure in bakeries is possible. This enables the identification of high risk tasks and allergen sources, as well as to study exposure-response relationships.

[1]*Submitted in revised form to the Journal of Allergy and Clinical Immunology*

Introduction

The production of enzymes with biotechnical methods, and the commercial use of these enzymes, has been associated with the occurrence of allergic sensitization and occupational asthma in exposed individuals in a wide variety of industries (Bernstein & Malo 1993; Chan-Yeung & Malo 1994). In the baking industry, the use of enzymes has increased throughout the 1980s and since, several studies have reported sensitization and respiratory disorders among bakery workers caused by enzymes in dough improvers.

The following enzymes have been reported to cause sensitization in bakery workers: α-amylase, amyloglucosidase, cellulase, glucoamylase, hemicellulase, lipoxygenase, papain, pectinase, and xylanase (Baur *et al.* 1988a; Quirce *et al.* 1992; Tarvainen *et al.* 1991a; Wiewrodt *et al.* 1995). Of these, mostly *Aspergillus spp.* derived, enzymes, α-amylase is the most frequently reported cause of allergy. Several individual case reports have been reported of baker's asthma caused by this starch cleaving enzyme (Bermejo *et al.* 1991; Birnbaum *et al.* 1988; Blanco Carmona *et al.* 1991; Latil *et al.* 1987). Other reports show that 24 - 34% of bakers with respiratory symptoms are sensitized to α-amylase (Baur *et al.* 1986; Baur *et al.* 1988a). Recently, epidemiologic studies have been performed in which α-amylase sensitization was assessed in open populations of bakery workers. In these studies it appeared that 5 - 15% of all bakers were sensitized to α-amylase, as shown by either skin prick testing or specific IgE analysis (Cullinan *et al.* 1994a; De Zotti *et al.* 1994; Griffin *et al.* 1994; Houba *et al.* 1996c). These studies convincingly showed that occupational exposure to fungal α-amylase increase the risk of respiratory allergy in bakery workers.

This increased risk results into an obvious need to quantify personal α-amylase exposure levels in this type of industry. Personal monitoring of enzyme allergens in other industries has been developed before for the proteolytic enzymes Esperase® and papain (Agarwal *et al.* 1986; Swanson *et al.* 1992). To our knowledge, however, levels of personal exposure to airborne α-amylase allergens have not been reported yet. We have therefore developed a sensitive sandwich enzyme immuno assay (EIA) using affinity-purified polyclonal rabbit IgG antibodies to measure α-amylase allergens. The EIA was used to assess personal α-amylase allergen exposure levels of bakery workers in various bakeries.

Materials and methods

Personal dust sampling and filter extraction

Personal inhalable dust samples were collected in the workers' breathing zone during full-shift periods of 6-8 hours, using polytetrafluoroethylene (Teflon) filters (Millipore; pore size 1.0 μm) and PAS-6 sampling heads at a flow rate of 2 l/min (ter Kuile 1984). Production workers in 21 bakeries were asked to participate for one to six days. These bakeries differed in a number of characteristics, such as number of employees, type of products, and technology level. All bakery workers were classified into nine job categories. More information on bakery characteristics, job categories, and dust- and wheat flour allergen exposure levels in each job category can be found elsewhere (Houba *et al.* 1996a). Filters were stored at -20°C.

α-Amylase allergens were recovered from the filters by extraction at room temperature with 2.5 ml 0.15 M Phosphate Buffered Saline (PBS, pH 7.4) in a 10 ml centrifuge tube. Each tube was vortexed for 2 minutes, sonicated for 2 minutes, vortexed for 5 minutes and sonicated for 2 minutes, successively. The extract was centrifuged at 5,000 g for 15 minutes. The supernatant was stored at -20°C.

Particle size determination

Particle size distribution was measured in small bakeries and doughmaking areas of large bakeries by stationary air sampling with an Andersen 1 CFM Ambient Fractionating Sampler (Andersen, Atlanta, GA USA) at a flow rate of 28.3 l/min, using glass fiber filters (Nenimij BV, Zoetermeer, The Netherlands). This cascade impactor separates the airborne particles into 9 fractions from ≥ 10 μm down to 0.4 μm diameter. The sampler was located at about 1 meter from the working areas of the bakers. Allergens were recovered from the filters in 15 ml PBS in the same way as described for the personal samples. The extract was centrifuged at 5,000 g for 5 minutes, the supernatant centrifuged again at 5,000 g for 15 minutes, and the supernatant was collected and stored at -20°C.

α-Amylase allergen standard

A standard α-amylase allergen preparation was made, using lyophilized powder of Fungamyl 1600S® (NOVO Nordisk, Denmark; kindly provided by the Department of Occupational Medicine, Sahlgren Hospital, Göteborg). This fungal α-amylase (derived from *Aspergillus oryzae*) was dissolved in PBS and filtered

through a 0.22 μm filter (Millipore). Protein content of the α-amylase preparation, as measured with the BCA-protein assay (Pierce; Rockford, IL USA), was 44% of total dry weight.

Anti-α-amylase antibodies

Anti-α-amylase antibodies were raised in a New Zealand white male rabbit (Broekman Institute, Someren, The Netherlands), by subcutaneous immunization with the complete Fungamyl 1600S® preparation. For primary immunization, 1 ml containing 0.5 mg protein was given, mixed 1:1 with Freund's complete adjuvant. Booster injections were given at 4 week intervals with the same amount of antigen in incomplete Freund's adjuvant. Serum was collected at 2 week intervals after booster injections and stored at -20°C.

Immunoglobulins were isolated from mixed batches of serum (60 ml; 3 bleedings) by ammonium sulphate precipitation (0.2 g/ml serum). The precipitate was resuspended in 24 ml PBS, dialysed against PBS for 48 hr, filtered through a 0.22 μm filter (Millipore), followed by affinity chromatography on Sepharose-coupled amylase. The immune adsorbent was prepared by coupling 20 ml of an α-amylase solution (10 mg freeze-dried Fungamyl 1600S®/ml) to 40 ml CNBr-activated Sepharose-4B (Pharmacia LKB; Uppsala, Sweden), following the instructions of the manufacturer. For isolation of immunospecific antibodies 6 ml of the immunoglobulin solution was applied at a flow rate of 0.25 ml/min on the packed affinity column (25 ml). After extensive washing, bound antibodies were eluted with 0.1 M Glycine adjusted to pH 2.8 with HCl. Fractions of 2 ml were collected at a flow rate of 0.25 ml/min and immediately neutralized with Tris-buffer (1 M; pH 7.2). Fractions with OD_{280}>0.1 were pooled and dialysed against 7 changes of ammoniumbicarbonate for 48 hr, freeze-dried and resuspended in 5 ml PBS. The affinity-purified antibodies were stored at -20°C at a concentration of 2.35 mg protein/ml.

Part of the antibodies was biotinylated to be used as detector antibody. Biotinylation was performed using 1 ml (1 mg/ml) antibody solution, which was incubated with 10 μl 1 M carbonate buffer (pH 8.6) and 180 μl biotin solution for 4 hours at 20°C. One mg biotin-N-hydroxysuccinimide ester (Boehringer Mannheim GmbH, Mannheim, Germany; art.nr. 1008960) per ml dimethylsulfoxide (Sigma, St. Louis, MO USA; art.nr. D-8779) was used. To remove the non-bound biotin, the biotinylated antibodies were dialysed at 4°C against 6 changes of PBS for 48 hours

and stored at 4°C.

SDS-Polyacrylamide gel electrophoresis & immunoblotting

The validity of the assay was tested by immunoblotting. The standard α-amylase preparation (1 mg protein/ml) was made in sample buffer according to Laemmli (1970), incubated for 10 minutes at 100°C, and subjected to SDS-electrophoresis in a 10*7.5*0.05 cm 12% polyacrylamydegel (29.3 µl extract per cm), using a Mini-Protean II slab cell unit (BioRad, Richmond, CA USA). Electrophoresis was performed at 100 Volt for 10 minutes and at 200 Volt for 50 minutes, 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 µm pore size), using a Mini-Trans blot transfer unit (BioRad), at 3.33 mA/cm^2 for 1 hour at room temperature.

After blotting, the nitrocellulose sheet was cut into strips of 1.5 mm, washed three times (10 min each, in PBS containing 0.1% Tween 20), incubated for 30 minutes with PBS-Tween containing 0.2% (w/v) gelatin (PBTG), and washed again. Strips were incubated overnight at room temperature with 140 µl undiluted sera from 5 bakers with high IgE-titer for α-amylase and with 1:50 and 1:100 diluted biotinylated affinity-purified rabbit anti-α-amylase antibodies. Bound IgE from sensitized bakers was detected by subsequent 1 hour incubations with 1:500 diluted mouse-anti-human IgE (Central Laboratory for Blood Transfusion (CLB), Amsterdam, The Netherlands), 1:500 diluted biotinylated rabbit anti-mouse immunoglobulins, and 1:200 diluted peroxidase-conjugated avidin (both Dakopatts (DAKO), Copenhagen, Denmark). Biotinylated rabbit IgG antibodies were detected by incubation with 1:200 diluted peroxidase-conjugated avidin. Bound peroxidase was detected by incubation for 30 minutes with tetramethylbenzidine (TMB; Promega Corporation, Madison, WI USA). Several blanks were included to test for non-specific binding of the reagents.

Sandwich-EIA procedure

Polystyrene high capacity microtitre plates (no 655061, Greiner) were coated overnight at 4°C with affinity-purified rabbit anti-α-amylase antibodies (2.35 µg/ml) in volumes of 200 µl per well. Next morning the plate was washed with PBS-Tween and incubated for 30 minutes at 37°C with PBTG. 200 µl of undiluted test

samples and 12 serial dilutions of the standard α-amylase antigen preparation (range 47 - 2,000 pg/ml) were added to the wells. On each plate several positive and negative controls (blanks) were tested. The plate was incubated for 1 hour at 37°C. After washing, 200 µl 1:500 diluted biotinylated affinity-purified rabbit anti-α-amylase antibodies were added to each well, followed by an incubation with 200 µl 1:2,000 diluted peroxidase-conjugated avidin (DAKO) (1 hr at 37°C) and an incubation (30 minutes in the dark at 20°C) with 200 µl o-phenylenediamine (OPD; 2 mg/ml) in 0.05 M citrate/phosphate buffer (pH 5.5), containing 0.015% H_2O_2. The reaction was stopped by adding 50 µl 2 M HCl and the absorbance of each well was measured at 492 nm with an EIA-reader (Thermomax microplate reader, Molecular Devices Corporation (MDC); Menlo Park, CA USA).

The dose-response curve for the standard preparation was obtained by 4-parameter curve fitting, using the SOFTmax software package (MDC). Allergen concentrations of samples were determined by interpolation on this curve.

Materials for specificity tests

The specificity of the assay was tested by measuring the following allergen extracts (all from ALK Benelux, Houten, The Netherlands): *Asp. flavus* (22.11), *Asp. niger* (22.02), *Asp. oryzae* (22.12), Baker's yeast (*Saccharomyces cerevisiae*; 52.01), Barley flour (52.02), House dust mite (mixture of *D. farinae* and *D. pteronyssinus*; SQ510), *Pen. expansum* (25.02), Penicillium mix (*Pen. brevicompactum*, *Pen. expansum*, *Pen. notatum*, and *Pen. commune*; 25.00), Rye flour (52.05), and Storage mite mix (*L. destructor*, *T. putrescentiae*, and *A. siro*; 35.00). A wheat-extract was obtained from a batch of wheat flour from one of the bakeries (Houba *et al.* 1996a).

Statistical analyses

Differences in mean α-amylase exposure levels between groups of workers were tested using a T-test (SAS software; PROC ANOVA). Differences with $p<0.05$ (two-sided) were considered significant. Because of the log-normal distribution of exposure data within groups, these tests were applied to log-transformed allergen concentrations.

Results

Assay characteristics

The validity of the assay was assessed by immunoblotting experiments, in which the reaction profile of the polyclonal rabbit IgG antibodies was compared with that of IgE antibodies in the sera of α-amylase sensitized bakery workers (Figure 4.1). Electrophoresis showed that the fungal α-amylase extract contains several proteins. Several of these components appeared to be immunogenic and/or allergenic. Both human IgE and rabbit IgG antibodies showed a strong reaction with a protein of approximately 52 kDa. Two other important bands of 40 and 27 kDa also reacted with both rabbit IgG and human IgE antibodies. In addition to these three prominent bands, some other proteins in the α-amylase extract were detected (at 29, 33, 35, 42, and 44 kDa), often by both human IgE and rabbit IgG, but the intensity of these bands was much lower. In general, and especially for the three main bands, the reaction profile of human IgE and rabbit IgG antibodies was similar, as can be seen in Figure 4.1. The reaction profile of the non-biotinylated rabbit antibodies, which were used for coating in the sandwich-EIA and for the preparation of the biotin-conjugate, was identical to the profile shown in Figure 4.1 (data not shown). No reaction was found for any of the controls (see legend of Figure 4.1).

As shown in Figure 4.2, a steep dose-response curve was obtained with the standard α-amylase preparation, at low protein concentrations. Maximum OD_{492} values were reached at approximately 2,000 pg/ml. Only samples with an OD_{492} higher than the OD of the reagent blank (no α-amylase) + 5 SD were considered positive, which implied a detection limit of the sandwich assay of 100 pg/ml.

The reproducibility of the assay was estimated by computing coefficients of variation (CV) for the duplicate analysis of α-amylase allergens in personal samples. Of the 93 samples with a detectable concentration of α-amylase, 75 % were analyzed in duplicate on two different days. The inter-day CV in the analysis was 22%, which was independent of the allergen level.

The results of the specificity tests are presented in Figure 4.2. For reasons of clarity, not all extracts tested are shown in this figure. A substantial reactivity was noted for the extract of the mould species *Aspergillus oryzae*, from which the α-amylase is derived. Nevertheless, reactivity was about 100 times less compared with the α-amylase standard. Cross-reactivity was also found for some other *Aspergillus* species, especially *Aspergillus flavus*. Some reactivity in the EIA was found for

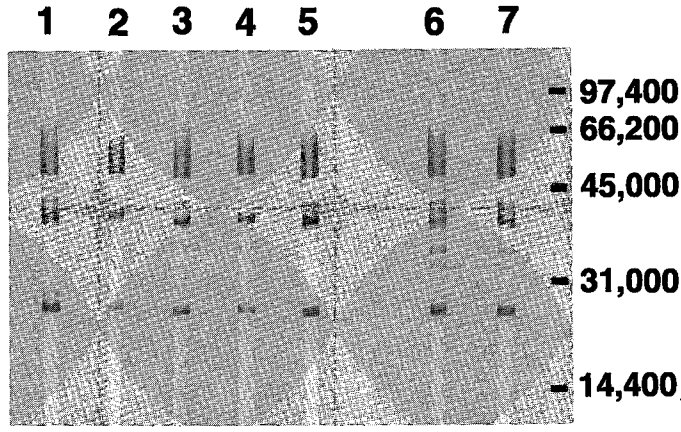

Figure 4.1 Immunoblotting of a commercially available α-amylase preparation. Reaction with human IgE from sensitized bakers (track 1-5), or with affinity-purified rabbit polyclonal antibodies (track 6 & 7).

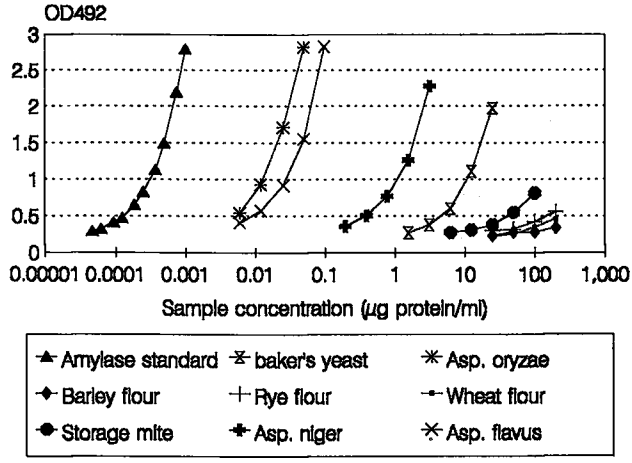

Figure 4.2 Reactivity of several extracts in the sandwich EIA for fungal α-amylase.

extracts of baker's yeast and storage mites, but at much higher protein concentrations. Very low reactivity was found for extracts of wheat flour, barley flour, and rye flour. Finally, no reactivity in our EIA was found for extracts of *Penicillium* species and house dust mite (data not shown).

Dust samples from bakeries usually contain large amounts of wheat allergen (Houba *et al.* 1996a). We therefore particularly investigated whether the simultaneous presence of wheat extract could affect the measurement of α-amylase allergens in dust samples. Titration curves were produced for the α-amylase preparation diluted in either PBTG or PBTG containing various concentrations of wheat flour. Wheat flour protein concentrations up to 10-25 µg/ml had no influence on the α-amylase standard curve. Measured OD_{492} values were slightly higher (0.1-0.4 OD-units) when the amylase standard was dissolved in solutions containing 100 µg/ml of wheat flour proteins.

Personal airborne α-amylase allergen measurements

A total of 550 personal measurements were available from 224 workers in all bakeries. Results of dust exposure levels are described in detail elsewhere (Houba *et al.* 1996a). Briefly, based on dust exposure levels, production workers could be classified into nine job categories (see Table 4.1). The highest airborne dust levels were found for doughmakers of large industrialized bakeries (geometric mean (GM) = 3.0 mg/m^3) and bread bakers in small bakeries (GM=3.3 mg/^{3}), and the lowest levels were found for slicers, packers and transport workers (GM=0.4 mg/m^3).

α-Amylase allergens were measured in 507 personal samples from 21 Dutch bakeries. Because all filters from personal samples were extracted in 2.5 ml and the sampled volume for eight hour sampling is around 1 m^3, this resulted into a detection limit for personal allergen measurements of 250 pg/m^3. In the statistical analysis, a value of two-thirds of this limit was assigned to all samples with concentrations below this detection limit (N=414), as proposed in the literature (Helsel 1990). Samples with high α-amylase concentrations (> 1.5 ng/ml; N=43) were retested at higher dilutions. Several samples were tested at more than one dilution and for each dilution the allergen concentration was determined. These tests showed parallelism between the standard curve and sample curves.

Ninety three samples (18%) had detectable concentrations of α-amylase. α-Amylase allergens could only be measured in samples of bakers working in, or close to the doughmaking areas, where bread improvers are added to the dough.

Table 4.1 Personal α-amylase exposure levels of bakery workers classified by job category and, if relevant, by type of bakery (N=507)

Job category		α-amylase (ng/m³)				
		N	AM	GM	GSD	range
LARGE INDUSTRIALIZED BAKERIES (> 50 WORKERS)						
1.	Doughmakers					
	Wheat-bread*	32	3.2	0.8	5.14	< det. - 33.1
	Pastry	32	all < detection limit			
	Crispbakes*	27	39.4	18.1	4.56	0.2 - 221.8
	Rye-bread	7	0.2	0.2	1.48	< det. - 0.5
2.	All-round staff					
	Wheat-bread	35	0.6	0.2	2.35	< det. - 14.3
	Pastry	4	all < detection limit			
	Crispbakes*	26	14.5	1.3	11.02	< det. - 150.2
3.	Oven staff	77	0.2	0.2	1.45	< det. - 2.3
4.	Slicers, packers & transport workers	119	0.2	0.2	1.44	< det. - 8.8
5.	Production managers	20	all < detection limit			
6.	Maintenance & cleaning workers	27	0.2	0.2	1.33	< det. - 0.7
SMALL TRADITIONAL BAKERIES (1-6 WORKERS)						
7.	Bread bakers	31	0.8	0.3	3.09	< det. - 11.2
8.	Mixed bakers[1]	54	0.3	0.2	1.81	< det. - 2.2
9.	Pastry cooks	16	all < detection limit			

N = number of samples
AM = arithmetic mean
GM = geometric mean
GSD = geometric standard deviation
* = exposure significantly different from all other groups within job category ($p < 0.05$)
[1] = bakers involved in both bread and pastry production

Characteristics of personal α-amylase allergen exposure levels are presented in Table 4.1. Doughmakers in large industrialized bakeries had the highest α-amylase exposure. However, not all doughmakers had equal exposure to α-amylase allergens. Fungal amylase is not used in the production of pastry. As a result, no α-amylase allergens were detected in personal samples in those bakeries, although dust exposure levels were comparable to the other doughmakers (Houba *et al.* 1996a). In other bakeries, α-amylase exposure levels varied strongly. Highest exposure levels were found for doughmakers producing crispbakes (a kind of toast), followed by doughmakers in wheat-bread production sites, and bread- and mixed bakers in small traditional bakeries.

α-Amylase allergens were also detected in personal samples from all-round staff. These workers have no specific job, but sometimes work as doughmaker, packer etc. In all-round workers, full shift personal α-amylase allergen exposure levels varied strongly, depending on the type of work performed on the day of sampling. For some job categories (production managers in large bakeries and pastry cooks in small bakeries) all measurements were below the detection limit, and in oven staff and slicers & packers, 96% and 98% of all samples were below detection limit respectively.

Figure 4.3 shows a histogram of all 93 samples with α-amylase levels above the detection limit. This distribution suggests a distinction of three groups of exposure levels: ≤ 5 ng/m^3, 6-15 ng/m^3, and ≥ 15 ng/m^3. In the last category, all samples but one were from the facility producing crispbakes (the other is from a doughmaker of a large bakery). Most of the samples from bread bakers or mixed bakers of small traditional bakeries show levels below 5 ng/m^3; only one sample had a higher α-amylase concentration. Samples from large (wheat bread producing) bakeries, were distributed among the first (≤ 5 ng/m^3) and second (6-15 ng/m^3) group.

Particle size distribution

In Table 4.2 results of the measurements with the Andersen sampler are presented. In each stage of the cascade impactor, α-amylase allergens were determined. As can be seen in the table, α-amylase allergens are predominantly present in particles larger than 5 μm. In general, more than half of the α-amylase allergens were found in the dust fraction with a particle size > 9 μm.

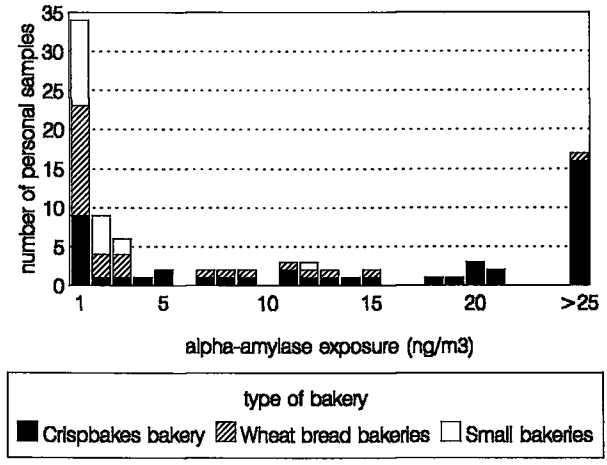

Figure 4.3 Histogram of personal α-amylase allergen exposure levels measured in three types of bakeries. Only samples above the detection limit are presented (n=93). The last column (>25) represents concentrations of more than 25 ng/m^3 α-amylase.

Table 4.2 Distribution of α-amylase allergens in different stages of the cascade impactor. All samples are taken in doughmaking areas from several types of bakeries (N=7). Sampling time varied from 7 - 28 hours.

Type of bakery	Total dust concentration (mg/m^3)	α-amylase concentration (ng/m^3)	Particle size fraction of the dust (μm)[1]				
			> 9.0	5.8 - 9.0	4.7 - 5.8	3.3 - 4.7	<0.4 - 3.3
wheat bread	0.24	1.2	14%	11%	75%	-	-
crispbakes	1.45	229.3	62%	24%	11%	1%	1%
	0.50	3.4	73%	18%	7%	3%	-
	0.76	17.2	90%	9%	-	-	-
small bakery	1.13	4.5	70%	13%	9%	8%	-
	1.03	0.3	51%	49%	-	-	-
	2.39	59.1	64%	33%	-	-	4%

[1] In some samples the sum of percentages of all stages is not exactly 100%, because individual percentages were rounded.

Discussion

In our study personal α-amylase allergen exposure levels in bakeries were measured with a sandwich EIA, using affinity-purified polyclonal rabbit IgG antibodies. Several tests were performed to study the validity and specificity of this method. Immunoblotting revealed a very similar reaction profile for IgE from sensitized bakery workers and the rabbit antibodies. Three major bands were found. A very strong band around 52 kDa, and two other important bands around 40 and 27 kDa bound both human IgE and rabbit IgG antibodies. In addition, some reactivity was found for proteins of 29, 33, 35, 42, and 44 kDa. Several other studies also found a dominant IgE-binding band in immunoblots with molecular weights varying from 51-54 kDa (Quirce *et al.* 1992; Sandiford *et al.* 1994b; Baur *et al.* 1994a; Moneo *et al.* 1995). Baur and co-workers (1994a) have recently demonstrated that this band represents the active α-amylase. In addition to this major allergen, commercially available extracts of fungal α-amylase appear to contain several other allergic components and as a result also other (often weaker) bands were detected by IgE from sensitized bakery workers. Sandiford *et al.* (1994b) found two IgE-binding proteins at 40 and 27 kDa in three out of eleven sensitized bakery workers. In another study, immunoblots were made using serum from two α-amylase allergic bakers. One bakery worker had IgE-antibodies against a 25 kDa protein, but no reactivity was found against the major protein around 52 kDa (Moneo *et al.* 1995). Baur *et al.* (1994a) also observed IgE reactions with several other components of a commercially available extract of fungal amylase, but no molecular weights of these bands were reported.

Alpha-amylase preparations as used in the baking industry contain several allergenic proteins. In principle, measurement of only one specific major allergen, in this case the active enzyme itself, can be preferred, e.g. in a monoclonal antibody based assay. However, although the exact nature of the other components detected by the rabbit antibodies is not known, they appear to be allergenic, which justify their inclusion in this allergen exposure assay.

Our polyclonal antibodies appeared to be sufficiently specific for use in a bakery environment. An extract of *Aspergillus oryzae*, the mould species from which the amylase is derived, showed the highest reactivity in the assay. Cross-reactivity was also detected against extracts of some other *Aspergillus spp.*, but for all tested preparations the level of reactivity was less than 1 % compared to the amylase

standard. Extracts of *Penicillium spp.* had no influence on our test results. Some reactivity in the EIA was found for extracts of baker's yeast and storage mites, but only at high protein concentrations. It is very unlikely that airborne exposure to baker's yeast and storage mites reach such levels in bakeries, and in practice no false positive results are therefore expected in the α-amylase assay. Hardly any reactivity was also found for barley flour, rye flour, and wheat flour. Wheat flour proteins are present in personal samples in considerable amounts, and should therefore be considered as a potential disturbing factor. It is, however, not very likely that the presence of wheat flour have affected the measured amylase exposure levels. Most of the personal dust samples in bakeries (98%) have flour protein levels below 60 μg/m^3, which is equivalent to protein levels in extracted samples of approximately 25 μg/ml (Houba *et al.* 1996a). At this concentration, the wheat flour extract gave in our amylase assay OD_{492} values that hardly differed from the background (Fig 2), and did not interfere with the measured α-amylase levels. At 100 μg wheat flour protein/ml the apparent amylase concentrations were somewhat increased. As mentioned before, these concentrations are very unusual for personal samples. Moreover, in all personal samples from pastry cooks and confectionery workers, no reactivity was found in our sandwich-EIA, although wheat flour protein concentrations in these samples are high (up to 25 μg/ml) (Houba *et al.* 1996a).

In some previous studies the enzyme activity of α-amylase was measured in airborne dust samples, to evaluate α-amylase exposure levels in a protein processing factory (Brisman & Belin 1991) and in bakeries (Jauhiainen *et al.* 1993; Burdorf *et al.* 1994). For the exposure assessment of fungal amylase allergens, however, these methods have two important limitations. First, inactive or denaturated enzymes are not measured, but can possibly still act as allergen. Sander & Baur (1994) showed that digested fragments of α-amylase were still able to bind IgE antibodies. In this case, the amylase allergen exposure may be underestimated if enzyme activity is measured. Second, these methods measure the amylase activity of both fungal and cereal origin. Amylase (ß more than α) is already present in flour, but there appeared to be only minimal immunological cross-reactivity between cereal and fungal amylases (Sandiford *et al.* 1994b). Therefore, measurements of enzyme activity may have limited validity in quantifying exposure to allergens of fungal amylase, and methods based on immunochemical techniques may be preferred.

For bakery workers large differences in α-amylase allergen exposure were found, with mean exposure levels of occupational titles varying from 0 to 40 ng/m^3. The

type of bakery in which the bakers are working is very important for the level of α-amylase exposure. In confectioneries, α-amylase is not used in doughmaking and therefore no fungal amylase exposure exist. In the rye-bread producing facility, amylase allergen exposure appeared to be very low. Higher exposure levels were found in the large wheat bread and crispbakes producing bakeries and for bakers working in small facilities. Within those bakeries, α-amylase exposure was only found for workers directly involved in doughmaking, or for workers present in the doughmaking area. Between these doughmakers, however, differences in α-amylase exposure were also large. Doughmakers working in large industrialized wheat bread producing factories had a mean α-amylase exposure which was four times higher than the amylase exposure of bread bakers in small bakeries, but which was 10 times lower than that of doughmakers from the crispbakes factory. In all these groups there is a high day to day variability in α-amylase allergen exposure.

Measurements with the cascade impactor showed that α-amylase allergens are present in particles ≥ 5 μm. In most bakeries, more than half of the α-amylase is in the dust fraction with particle sizes larger than 9 μm. This means that α-amylase allergens are most likely to deposit in the nose and the ciliated airways (bronchi and bronchioles) (Lippmann *et al.* 1980). This is in agreement with effects of α-amylase allergen exposure (rhinitis and asthma) described in the literature.

This study shows that personal monitoring of fungal amylase allergens in bakeries is possible. The exposure assessment of airborne α-amylase allergens enables the identification of allergen sources, as well as to study exposure-response relationships. Analyses with data from our epidemiologic study showed that the range of α-amylase exposure levels measured in this study strongly associated with enhanced risk of α-amylase sensitization of the bakery workers (Houba *et al.* 1996c). The availability of an assay and the (first) indications for an exposure-response relationship opens a way to standard setting as for proteolytic enzymes of *Bacillus subtilis* (ACGIH 1986). With the possibility of identifying allergen sources, the assay can also assist in recognizing high risk activities to be targeted for modification and prevention strategies.

5. Grouping strategies for exposure to inhalable dust, wheat allergens and α-amylase allergens in bakeries[1]

Remko Houba, Dick Heederik & Hans Kromhout

Abstract

This paper describes repeated measurements of inhalable flour dust, wheat allergens and α-amylase allergens in the bakery industry. A total of 571 full-shift personal dust samples were collected. Wheat allergens and α-amylase allergens were measured in 449 and 507 samples, respectively, by the use of recently developed immunoassays. For all three measures of exposure, the main components of exposure variability were determined. Different grouping strategies for studying exposure-response-relationships were compared. The specific job of a bakery worker was identified as the most important source of variability in inhalable flour dust concentrations. For exposure to wheat allergens, the job performed also was the most important source of variation, but type of bakery explained some variability as well. For α-amylase allergen exposure, information on type of bakery was more important then job information. For exposure to inhalable dust and wheat allergens, a classification by job title would lead to sufficient contrast in average exposure levels. In contrast, a grouping strategy based on a combination of job and type of bakery appeared to be essential to obtain a meaningful classification of exposure to α-amylase allergens.

Introduction

The inhalation of cereal dusts and powdered baking additives may cause immunologic sensitization and respiratory symptoms in exposed bakers. It is clear that both host factors (atopy) and environmental factors (exposure) are important determinants of respiratory symptoms. Many epidemiologic studies in the bakery

[1] *Submitted*

industry have focused on host factors only (Järvinen *et al.* 1979; Prichard *et al.* 1984; Rosenberg *et al.* 1991; De Zotti *et al.* 1994). Recently conducted studies have attempted to relate dust or allergen exposure levels to specific sensitization and respiratory symptoms (Hartmann 1986; Musk *et al.* 1989; Cullinan *et al.* 1994a). However, very little is known about the levels of flour dust or specific allergen exposure that can cause allergic sensitization or lead to the development of respiratory symptoms.

The limited understanding of dose-response-relationships in baker's allergy has two reasons. First, information on levels of exposure to inhalable dust or specific allergens is still limited. In only few studies, exposure data of inhalable dust were presented (Musk *et al.* 1989; Lillienberg & Brisman 1994; Nieuwenhuijsen *et al.* 1994). Methods for quantifying personal allergen exposure levels in the baking industry have only recently been developed for wheat flour (Sandiford *et al.* 1994a; Houba *et al.* 1996a) and fungal α-amylase (Houba *et al.* 1996b). Second, in only few studies the exposure assessment strategy was designed with the aim of studying exposure-response-relationships. For studying such relationships, bakery workers have to be categorized into exposure groups in such a way that, within each group, workers are more or less 'uniformly' or 'homogeneously' exposed (Hawkins *et al.* 1991; Rappaport 1991). Moreover, contrast in exposure level between exposure groups is a prerequisite for detection of an exposure-response-relationship (Prais & Aitchinson 1954; Kromhout & Heederik 1995). Large between-worker variation within exposure groups may bias the exposure-response-relationship (Heederik & Miller 1988; Armstrong *et al.* 1992). For designing an optimal grouping strategy, components of exposure variability have to be determined. The relevant sources of variability of exposure can be studied when repeated measurements on individuals are available (Boleij *et al.* 1995).

Thus far, only two studies have performed repeated measurements of flour dust of bakery workers (Burdorf *et al.* 1994; Nieuwenhuijsen *et al.* 1995a). In both studies, the authors have classified workers into exposure groups based on estimated components of exposure variability in dust exposure. However, studying exposure-response-relationships in the bakery industry based on dust exposure levels, has an important disadvantage. Dust levels may have a moderate correlation with actual allergen concentrations (Houba *et al.* 1996a; Houba *et al.* 1996b), and it is questionable whether dust levels are a valid exposure parameter in occupations where IgE-mediated allergies are being studied. Sources of variation of exposure to

specific allergens may be different from variance components of dust exposure and as a result, optimal grouping strategies for allergen exposure may be different. Until now, however, repeated measurements of allergen exposure in the bakery industry have not been published and consequently, grouping strategies for allergen exposure (in particular wheat flour and α-amylase) have not been studied.

This paper describes results of analyses of variance of exposure to inhalable flour dust, wheat allergens, and fungal α-amylase allergens in the bakery industry. For each measure of exposure, some alternative grouping strategies will be compared. Differences in optimal grouping strategies for inhalable dust and both types of allergens are discussed.

Materials and methods

Sampling methods and strategy

Exposure levels were measured in 5 large industrialized bakeries and 16 small bakeries. Most of the large bakeries had several independent sections with autonomous production lines. In the analyses, each department was considered to be a separate bakery. Four types of large bakeries were distinguished with regard to the type of product: wheat bread production (n=4), production of confectionery (n=4), production of crispbakes or Dutch toast (n=1), and rye bread production (n=1). Workers of the large industrialized bakeries could be divided into 6 occupational groups according to their job description, namely (1) doughmakers, (2) all-round staff, (3) oven staff, (4) slicers, packers and transport workers, (5) production managers, and (6) maintenance and cleaning workers. Bakers in the 16 small bakeries were divided into 3 occupational groups according to whether they produced bread, pastry, or a mixture of the two. Within each occupational title, randomly selected bakers were asked to carry a personal air sampler. Participation was on a voluntary base, but most of the bakery workers cooperated. In total, two-hundred and thirty workers in the bakeries were measured on 571 occasions during full-shifts. Most of the workers were measured on more than one occasion (with a maximum of 6 measurements per workers). However, for some workers only one measurement was available.

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

filters (Millipore; pore size 1.0 μm) and inhalable dust (PAS-6) sampling heads at a flow rate of 2 l/min (ter Kuile 1984). The PAS-6 sampler was recently assessed as accurate for exposure to inhalable dust in indoor situations with low wind speed (Kenny *et al.* 1996). After sampling, filters were weighed in a preconditioned room of 18-22°C with 45-55% humidity. Detection limit of this method was 0.17 mg/m^3, computed by dividing the mean plus three times the standard deviation of 24 blanks by the mean sampled volume. Forty four dust samples (8%) were below the detection limit. Wheat flour and α-amylase allergens were recovered from the filters by extraction with 2.5 ml Phosphate Buffered Saline, and the allergen concentrations were measured by inhibition enzyme immunoassay (EIA) for wheat flour (Houba *et al.* 1996a), or sandwich EIA for fungal α-amylase (Houba *et al.* 1996b). The assay for the measurement of wheat allergens had a reproducibility of 19.5%, and the detection limit for personal wheat allergen measurement was 50 ng/m^3. Seventy samples (16%) were below the detection limit. The detection limit for personal α-amylase allergen measurement was much lower (0.25 ng/m^3), but 82% of all samples were below this limit. The reproducibility of the α-amylase assay was 22%, based on the duplicate analysis of 70 samples with detectable concentrations of α-amylase. In the analyses, values below the detection limit were considered as being two-thirds of this limit, as described by Helsel (Helsel 1990).

Statistical analyses

All statistical analyses were performed using SAS software (version 6.09). For descriptive analyses PROC MEANS and PROC UNIVARIATE were used. Statistical analyses to estimate variance components were performed using PROC NESTED. Groups, workers within those groups, and their subsequent measurement periods were assumed to be selected at random. Therefore, random effect models were used. The structure of the exposure data was regarded as hierarchical (nested) (Samuals *et al.* 1985). A one-way nested random effects ANOVA model was used to estimate the within-worker variance component (σ^2_{ww}) and the between-worker variance component (σ^2_{bw}), both for the total population and for each group separately. The estimates of variance components σ^2_{ww} and σ^2_{bw} will be designed as S^2_{ww} and S^2_{bw}, respectively, and were used to calculate the GSD_{ww} ($\exp(S_{ww})$) and the GSD_{bw} ($\exp(S_{bw})$). The variance components were also used for the calculation of the estimated within-worker to between-worker variance ratio: $\hat{\lambda} = S^2_{ww}/S^2_{bw}$ (Heederik *et al.* 1991a), and the ratios of the 97.5th and 2.5th percentiles of the log-

normally distributed exposures of each group of workers: ${}_{bw}R_{.95} = \exp(3.92*S_{bw})$ (Rappaport 1991). Both statistics ($\hat{\lambda}$ and ${}_{bw}R_{.95}$) were used to evaluate the homogeneity of exposure within occupational titles. A two-way nested random-effects ANOVA model was also applied to estimate a between-group variance component (σ^2_{bg}), a within-group variance component (σ^2_{wg}), and a within-worker variance component (σ^2_{ww}) for different grouping schemes (Kromhout & Heederik 1995). The estimates of the variance components σ^2_{bg}, σ^2_{wg} and σ^2_{ww} will be designated as S^2_{bg}, S^2_{wg} and S^2_{ww}, respectively. Using these variance components, the elasticity was calculated as a measure of contrast in exposure level between groups: elasticity = $S^2_{bg}/(S^2_{bg}+S^2_{wg})$ (Kromhout & Heederik 1995). The data used in the analysis of variance were the log-transformed estimates of inhalable dust and allergen exposure.

Results

A total of 571 personal dust samples were collected. Wheat allergens and α-amylase allergens were measured in 449 and 507 samples respectively. Descriptive statistics and results of the one-way analyses of variance are presented in Table 5.1, 5.2, and 5.3, for exposure to inhalable dust, wheat allergens and α-amylase allergens, respectively. In each table, exposure data are presented for the total population and for each of the 22 occupational titles separately. The definition of occupational title is based on job of the bakery worker and type of bakery where he or she is employed. For inhalable dust and wheat allergen exposure, the exposure data followed a log-normal distribution for nearly all occupational titles. For α-amylase allergen, however, a log-normal distribution of exposure data was only found in a few occupational titles. In most groups the exposure data were not log-normally distributed due to the high number of samples without detectable levels of α-amylase allergens.

For inhalable flour dust, the highest dust concentrations were found for doughmakers in large industrialized bakeries and for bakers involved in bread production in the small bakeries, and to a lesser extend for the all-round staff of the large bakeries. Overall, the between-worker exposure variability was greater than the within-worker variability (λ=0.58). After grouping, the within-worker variation appeared to be larger than the between-worker variation for 14 of the 22

Table 5.1 Characteristics of inhalable flour dust exposure by job and type of bakery.

Occupational title	N	k	AM	GM	GSD_{bw}	GSD_{ww}	λ	$_{bw}R_{.95}$
Overall	571	230	2.0	1.0	2.5	2.0	0.6	34.2
Large industrialized bakeries								
Doughmakers								
Bread bakeries	37	15	6.8	4.5	2.1	1.7	0.5	18.2
Confectioneries	40	18	2.8	2.2	1.5	1.8	2.5	4.5
Crispbakes factory	40	16	3.2	2.8	1.3	1.5	2.2	3.1
Rye bread bakery*	7	3	1.0	0.9				
All-round staff								
Bread bakeries	37	16	1.4	0.9	2.0	2.3	1.5	13.8
Confectioneries*	4	2	7.5	1.2				
Crispbakes factory	29	12	1.3	1.1	1.2	1.8	16.8	1.8
Oven staff								
Bread bakeries	34	17	1.0	0.7	2.1	2.0	0.9	17.4
Confectioneries	21	10	0.9	0.6	1.0	2.4	∞	1.0
Crispbakes factory	24	11	0.8	0.7	1.4	1.4	0.7	4.2
Rye bread bakery*	5	3	0.4	0.4				
Slicers, packers, transport								
Bread bakeries	66	28	0.5	0.3	1.6	2.3	3.0	6.3
Confectioneries	26	11	0.5	0.4	1.6	2.1	2.6	5.8
Crispbakes factory	22	11	0.8	0.8	1.0	1.3	∞	1.0
Rye bread bakery	17	9	0.5	0.5	1.2	1.6	4.4	2.3
Production managers								
Bread bakeries	13	6	1.3	0.7	1.0	3.7	∞	1.0
Confectioneries*	3	1	0.5	0.5				
Crispbakes factory*	4	2	0.7	0.6				
Maintenance & cleaning workers	27	9	0.9	0.7	1.0	2.1	∞	1.0
Small traditional bakeries								
Bread bakers	36	18	3.8	3.3	1.6	1.3	0.5	5.7
Mixed bakers	57	27	2.7	2.0	1.9	1.8	0.8	11.3
Pastry cooks	22	9	1.0	0.7	1.0	2.3	∞	1.0

N = Number of measurements in a group
k = Number of workers in a group
AM = Arithmetic mean (mg/m^3)
GM = Geometric mean (mg/m^3)
GSD_{bw} = Between-worker geometric standard deviation
GSD_{ww} = Within-worker geometric standard deviation
λ = Variance ratio of within- and between-worker variance ($= \sigma^2_{ww}/\sigma^2_{bw}$)
$_{bw}R_{.95}$ = Ratio of the 97.5th and 2.5th percentiles of the between-worker distribution
* = The number of samples were to small to estimate variance components

Table 5.2 Characteristics of wheat allergen exposure by job and type of bakery.

Occupational title	N	k	AM	GM	GSD_{bw}	GSD_{ww}	λ	$_{bw}R_{95}$
Overall	449	206	5,360	684	6.5	3.1	0.4	1578
Large industrialized bakeries								
Doughmakers								
Bread bakeries	32	14	35,068	16,511	2.7	2.3	0.7	52.2
Confectioneries	25	13	3,571	1,969	2.8	2.4	0.7	59.6
Crispbakes factory	15	9	5,853	5,104	1.0	2.1	∞	1.0
Rye bread bakery*	4	2	503	364				
All-round staff								
Bread bakeries	35	16	4,377	997	3.3	4.1	1.4	102
Confectioneries*	4	2	18,505	2,487				
Crispbakes factory	16	9	2,305	876	1.0	5.4	∞	1.0
Oven staff								
Bread bakeries	30	17	3,653	771	6.4	2.6	0.3	1400
Confectioneries	21	10	720	302	1.6	4.6	10.4	6.5
Crispbakes factory	15	8	152	128	1.5	1.6	1.1	5.0
Rye bread bakery*	5	3	37	36				
Slicers, packers, transport								
Bread bakeries	56	26	173	74	2.2	2.3	1.1	21.2
Confectioneries	22	9	102	62	1.7	2.1	1.9	7.6
Crispbakes factory	17	10	599	150	1.0	4.4	∞	1.0
Rye bread bakery	13	9	40	38	1.0	1.4	∞	1.0
Production managers								
Bread bakeries	13	6	7,745	859	2.4	7.2	5.0	31.5
Confectioneries*	3	1	79	60				
Crispbakes factory*	1	1	304	304				
Maintenance & cleaning workers	20	8	528	242	1.3	3.8	28.5	2.7
Small traditional bakeries								
Bread bakers	31	17	8,687	5,989	2.0	1.7	0.6	14.6
Mixed bakers	55	26	5,298	2,729	2.1	2.3	1.3	17.4
Pastry cooks	16	7	1,183	614	2.4	3.4	1.9	32.9

N = Number of measurements in a group
k = Number of workers in a group
AM = Arithmetic mean (ng/m^3)
GM = Geometric mean (ng/m^3)
GSD_{bw} = Between-worker geometric standard deviation
GSD_{ww} = Within-worker geometric standard deviation
λ = Variance ratio of within- and between-worker variance ($= \sigma^2_{ww}/\sigma^2_{bw}$)
$_{bw}R_{95}$ = Ratio of the 97.5th and 2.5th percentiles of the between-worker distribution
* = The number of samples were to small to estimate variance components

Table 5.3 Characteristics of fungal α-amylase allergen exposure by job and type of bakery.

Occupational title	N	k	AM	GM	GSD_{bw}	GSD_{ww}	λ	$_{bw}R_{.95}$
Overall	507	214	3.3	0.3	3.1	2.4	0.6	85.7
Large industrialized bakeries								
Doughmakers								
Bread bakeries	32	14	3.2	0.8	4.6	2.0	0.2	403
Confectioneries	32	16			all < detection limit			
Crispbakes factory	27	11	39.4	18.1	2.6	3.3	1.5	44.9
Rye bread bakery*	7	3	0.2	0.2				
All-round staff								
Bread bakeries	35	16	0.6	0.2	2.1	1.5	0.3	19.7
Confectioneries	4	2			all < detection limit			
Crispbakes factory	26	11	14.5	1.3	3.1	8.5	3.7	80.8
All other jobs in large bakeries	243	111	0.2	0.2	1.0	1.4	∞	1.0
Small traditional bakeries								
Bread bakers	31	17	0.8	0.3	2.4	2.1	0.8	29.3
Mixed bakers	54	26	0.3	0.2	1.0	1.9	-	1.0
Pastry cooks	16	7			all < detection limit			

N = Number of measurements in a group
k = Number of workers in a group
AM = Arithmetic mean (ng/m^3)
GM = Geometric mean (ng/m^3)
GSD_{bw} = Between-worker geometric standard deviation
GSD_{ww} = Within-worker geometric standard deviation
λ = Variance ratio of within- and between-worker variance ($= \sigma^2_{ww}/\sigma^2_{bw}$)
$_{bw}R_{.95}$ = Ratio of the 97.5th and 2.5th percentiles of the between-worker distribution
* = The number of samples were to small to estimate variance components

occupational titles. For each group, the between-worker exposure variability wasconsiderably lower compared with the whole population. For some groups, the number of samples is very small, and for these groups, no variance components were estimated. With the exception of doughmakers, only small differences in inhalable dust levels were found between several types of bakeries.

For wheat allergen exposure a similar pattern was found. For the total population the between-worker variability was large and was substantially lower for each occupational title separately. An important difference with inhalable dust exposure levels is that differences between bakery types within each job were larger. In each type of bakery, the proportion of the dust consisting of wheat flour was different, due to the use of ingredients other than wheat flour (Houba *et al.* 1996a). Compared

with dust exposure, the ${}_{bw}R_{.95}$ of the total group of bakery workers was much higher (1578 versus 34).

For α-amylase allergen exposure the picture was remarkably different. For several occupational titles, all samples were below the detection limit. In fact, α-amylase exposure was limited to bakers involved in dough production in some types of bakeries (bread production and production of crispbakes). Between those groups, however, large differences in α-amylase allergen exposure were found. Allergen exposure in the crispbakes factory was much higher compared with bread producing bakeries. The α-amylase exposure in large bread-producing bakeries was considerably higher compared with bread bakers in the small traditional bakeries. For most occupational titles, the between-worker variation was smaller compared with the whole population, which implies that the occupational title mean exposures were an acceptable exposure estimate for each member of that group. An exception is the group of doughmakers from the bread factories, where a high between-worker variation was found.

In Table 5.4 three alternative grouping strategies were compared for each measure of exposure. For each grouping strategy, the total variability of exposure was partitioned into a between-group component, a between-worker component, and a within-worker component. For inhalable dust and wheat allergens all available samples were used. For α-amylase, the analysis of variance was restricted to those occupational titles with detectable levels of α-amylase allergens. For inhalable dust exposure, the job of a bakery worker explained approximately 50% of the exposure variability. Grouping workers based on type of bakery was not meaningful for exposure to inhalable dust. For wheat allergen exposure, job was also the main explanatory variable, although bakery type also explained variability in wheat allergen exposure. However, the increase in contrast is limited when workers were grouped by the combination of job and type of bakery. In contrast, for exposure to α-amylase allergens type of bakery was the most important source of exposure variability.

Table 5.4 Between- and within-group exposure variability for several grouping schemes.

Grouping variables	g	k	S^2_{bg}	S^2_{wg}	S^2_{ww}	elasticity
Flour dust exposure (n=571)						
job	9	253	0.69	0.27	0.43	0.72
type of bakery	4	230	0.02	0.79	0.48	0.03
combination	22	253	0.67	0.22	0.43	0.75
Wheat allergen exposure (n=449)						
job	9	222	2.98	1.10	1.14	0.73
type of bakery	4	206	0.88	2.99	1.26	0.23
combination	22	222	3.11	0.73	1.14	0.81
Fungal amylase exposure (n=205)						
job	4	95	1.50	1.76	1.12	0.46
type of bakery	2	85	3.92	1.41	1.06	0.73
combination	6	95	2.53	0.77	1.12	0.77

g = number of groups
k = number of workers
S^2_{bg} = estimated variance component due to groups
S^2_{wg} = estimated variance component due to workers (between workers within groups)
S^2_{ww} = estimated variance component due to days (between days within workers)
elasticity = $S^2_{bg}/(S^2_{bg}+S^2_{wg})$

Discussion

When studying the relationship between exposure and response, an accurate quantitative estimate of exposure is needed for each study subject. Basically, two quantitative exposure assessment strategies can be distinguished. First, each worker of the population under study can be measured repeatedly, and an average exposure estimate is calculated for each worker. In the second approach, subgroups of workers are defined based on work characteristics, and a random sample of workers in each group is monitored. Exposure assessment strategies in occupational epidemiology are almost always based on grouping strategies, rather than on individual-based strategies. Desirable goals in grouping strategies would be division of workers into groups that are more or less uniformly exposed, and exposure groups with clearly distinguishable levels of exposure. Insight into exposure

variability is crucial when designing an appropriate exposure assessment strategy. In this study, the variability of exposure to inhalable flour dust, to wheat allergens, and to α-amylase allergens was studied using nested random effects ANOVA models. Exposure variability of inhalable flour dust was described before (Burdorf *et al.* 1994; Nieuwenhuijsen *et al.* 1995a). However, variability of allergen exposure levels has not been described before.

An underlying assumption of the grouping strategy is that the average exposure of an individual worker is supposed to be indistinguishable from the average exposure of the total group (Boleij *et al.* 1995). For epidemiologic research, no strict criteria can be given for a required level of homogeneity. The inhomogeneity which is acceptable is determined by the magnitude of the variation in exposure in the whole population and the variation within the exposure groups (Heederik *et al.* 1991a). For compliance strategy purposes, Rappaport (1991) has suggested that the difference between the highest and lowest exposed worker in a group should be below a factor 2, as reflected by a ${}_{bw}R_{.95}<2$. According to this strict definition, only few occupational titles in our study could be considered as a uniform exposed groups. However, several studies in other industries have shown that only few exposure groups can meet this rather strict criterion (Spear *et al.* 1987; Heederik *et al.* 1991a; Rappaport *et al.* 1993; Kromhout *et al.* 1993). For inhalable dust and both types of allergens, the ${}_{bw}R_{.95}$ for most occupational titles was considerably smaller than for the total group, which implies that the mean exposure of the occupational title was an acceptable exposure estimate for each member of that group. Besides, there was no information available for further subdivision of workers into smaller groups.

The formation of uniform exposed groups is helpful in making an optimal estimate of the exposure for each individual. As a result, the estimates of the relationship between exposure and response will be more precise, due to the reduction of random exposure measurement error. However, a strict definition of a uniformly exposed group is not a prerequisite for identifying a relationship between an exposure and a health outcome. Large within-group variation is acceptable as long as there is limited overlap (Heederik *et al.* 1991b). Therefore, the variability in exposure between groups have to be substantially larger than the exposure variability within exposure groups. In our study, the between-group variance was the most important source of variability of exposure for all three measures of exposure. A grouping strategy in which bakery workers were grouped based on a

combination of job and type of bakery resulted in a high contrast in exposure with elasticity of 0.75, 0.81 and 0.77 for inhalable flour dust, wheat allergen and α-amylase allergen, respectively. However, for exposure to inhalable dust and wheat allergens, a classification by job title alone would lead to a comparable contrast in average exposure levels. For α-amylase allergen exposure, information on type of bakery was the main explanatory variable found in our analysis of variance. However, the samples of many occupational titles had to be excluded from the analyses, because all samples were below detection limit of the α-amylase assay. As a result, the estimated components of exposure variability are probably only applicable for a small number of jobs in the bakery industry, and job should also be considered as an important explanatory variable of α-amylase allergen exposure. Therefore, a grouping strategy based on a combination of job and type of bakery is probably essential for a meaningful classification of exposure to α-amylase allergens. The elasticity for the different grouping strategies described in Table 5.4 is high compared with the elasticity found in other industries (Heederik *et al.* 1991a; Kromhout & Heederik 1995; Kromhout *et al.* 1996).

In total, twenty one bakeries were included in the study, and most of them were small traditional bakeries. Only a limited number of large industrialized bakeries were studied, and although some of them included several types of separate bakeries, only 1 plant was available for some types of bakeries (crispbakes factory and rye bread factory). For these types of bakeries we are in fact unable to give any information about possible differences between bakeries. For other types of bakeries, however, several were included in the study and only small differences in exposure levels between bakeries were found.

As mentioned, two other studies have performed repeated exposure measurements in bakery workers (Burdorf *et al.* 1994; Nieuwenhuijsen *et al.* 1995a). In both studies, the specific job of the bakery workers was also identified as the most important source of variance of inhalable dust exposure, and a similar elasticity for this grouping strategy was found (approximately 0.75 in both studies). However, no variability of allergen exposure was described in these studies. Our study results showed that for exposure to wheat allergens, job of a bakery worker was by far the most important source of variation, and contrast in exposure levels could only be slightly increased when information on type of bakery was incorporated in the grouping strategy. For exposure to α-amylase allergens, however, information of the type of bakery was essential, and using information on jobs in the grouping

strategies as well, led to similar estimates of contrast.

6. Exposure-sensitization relationship for α-amylase allergens in the baking industry[1]

Remko Houba, Dick Heederik, Gert Doekes & Paula van Run

Abstract

Fungal α-amylase is an important occupational allergen in the bakery industry. Epidemiologic studies focusing on the relationship between α-amylase allergen exposure and work related respiratory allergy, however, have not been reported yet. In this cross-sectional study, sensitization to occupational allergens and work related symptoms were studied in 178 bakery workers and related to allergen exposure. Alpha-amylase allergen concentrations were measured in personal dust samples, using a sandwich enzyme immunoassay. All workers were categorized into groups on the basis of their job histories and the α-amylase exposure levels of their job titles. Of all workers 25% had one or more work related symptoms. As much as 9% of the bakery workers showed a positive skin prick test reaction to fungal amylase, and in 8% amylase-specific IgE was demonstrated. Alpha-amylase exposure and atopy appeared to be the most important determinants of skin sensitization, with prevalence rate ratios for atopy of 20.8 (95% CI 2.74-158) and for medium and high α-amylase exposure groups of 8.6 (95% CI 1.01-74) and 15.9 (95% CI 1.95-129) respectively. Furthermore, a positive association was found between positive skin prick tests to α-amylase and work-related respiratory symptoms. In conclusion, this study shows that there is a strong and positive relationship between α-amylase allergen exposure levels in bakeries and specific sensitization in bakery workers.

Introduction

Work related respiratory allergy is highly prevalent among bakery workers, occurring in approximately 10-20% of all bakers (Järvinen *et al.* 1979; Thiel & Ulmer 1980; Prichard *et al.* 1984; Musk *et al.* 1989). In several countries, baker's

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asthma is one of the most frequent occupational lung diseases (Baur *et al.* 1988a; Lagier *et al.* 1990; Wüthrich & Baur 1990; Nordman 1994). The majority of patients with baker's asthma is sensitized to wheat and rye flour, but the last 10-15 years have shown an increased interest in the role of baking additives, especially α-amylase. Fungal α-amylase (1,4-α-D-glucan glucanohydrolase), usually derived from *Aspergillus oryzae*, is a glycoprotein that catalyses the hydrolysis of internal α(1,4)-glycosidic linkages in various polysaccharides. It is routinely added to baking flour (in milligrams per kg flour) to hasten the baking process and improve bread quality.

The first cases of α-amylase allergy have been described in the enzyme processing industry in the seventies (Flindt 1979). Since then, several other studies have reported respiratory allergies in bakery workers caused by α-amylase, often in the absence of demonstrable reactivity to wheat allergens (Wüthrich & Baur 1990; Birnbaum *et al.* 1988; Bermejo *et al.* 1991; Blanco Carmona *et al.* 1991; Losada *et al.* 1992; Quirce *et al.* 1992; Valdivieso *et al.* 1994) Baur and co-workers showed in a study of 140 bakers who visited a clinic with work related symptoms, that 24% were sensitized to α-amylase (Baur *et al.* 1988a).

Although the number of studies on α-amylase allergy in patients with baker's asthma is growing, epidemiologic studies in bakery workers focusing on relationship between α-amylase exposure and type I sensitization or work related respiratory symptoms have not been reported yet. Some recent cross-sectional studies showed that in a population of bakery workers 5 - 7.5 % were sensitized to α-amylase, as shown by skin prick testing (Cullinan *et al.* 1994a; De Zotti *et al.* 1994). In neither of these studies, however, was information available on airborne α-amylase exposure levels.

Exposure to α-amylase in airborne dust has been measured by assessing enzyme activity of the dust in a protein processing factory (Brisman & Belin 1991), and bakeries (Jauhiainen *et al.* 1993; Burdorf *et al.* 1994). For two reasons, however, measurements of enzyme activity may have a limited validity in quantifying allergen exposure. First, inactive or denaturated enzymes are not measured, but they possibly still act as allergens. Second, these methods measure the amylase activity of both fungal and cereal origin, and are therefore not specific. For these reasons, measurement methods of fungal α-amylase based on immunochemical techniques are to be preferred. Although allergens of proteolytic enzymes have been measured before in personal air samples (Agarwal *et al.* 1986; Swanson *et al.* 1992),

immunochemical measurements of airborne α-amylase in bakeries have only recently been presented (Belin *et al.* 1994).

To our knowledge no study has been published before in which α-amylase allergen exposure data were applied in an epidemiologic survey. In this report results are presented of the relationship of α-amylase allergen exposure in bakeries with sensitization to fungal amylase and respiratory symptoms. The results of this cross-sectional study 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 relationship between allergen exposure and work-related asthma. In a previous report, an enzyme immuno assay (EIA) for measuring wheat antigens for the same population was presented (Houba *et al.* 1996a).

Materials and methods

Population

The health survey was carried out between October 1992 and July 1993 and comprised 203 production workers in 14 Dutch bakeries. Maintenance workers were excluded from the analysis because of potential other exposures (eg. welding fumes), leaving 178 bakery workers in the study. All workers completed a short self-administered Dutch version of an internationally accepted respiratory questionnaire (MRC 1966), supplemented with questions on work related symptoms. Symptoms were considered to be work related if they were reported by the subject as being provoked by contact with flour or process related products (eg. baking additives) during work ('Do you have any of the following allergic symptoms during work, after contact with certain agents at work?'). Work related rhinitis was defined as the presence of sneezing or running nose (production of nasal secretions) during work. Work related conjunctivitis was defined as the presence of itchy or teary eyes. Symptoms reported to be caused by irritants (smoke, potash, etc.) were not included. Additionally, smoking habits and job histories were obtained. Forced expiratory lung function measurements were conducted as described previously (Smid *et al.* 1992). Population characteristics are given in Table 6.1. Venous blood samples were taken from 169 workers for analysis of IgE antibodies and 169 workers underwent skin prick tests (SPT), as described below. For 167 workers both serum samples and SPT results were available. Informed written consent was

obtained from all subjects, before the start of the study, according to Dutch legal requirements.

Skin prick tests (SPT)

Conventional SPTs were applied using a lancet with a 1 mm point, which was pressed at a 90-degree angle to the skin surface through a drop of allergen solution. All SPTs were performed by two trained technicians. The following occupational allergens were tested: wheat flour (ALK Benelux, Houten, The Netherlands (ALK), 52.06), rye flour (ALK, 52.05), fungal α-amylase (from *Aspergillus oryzae*; ALK, 52.52), bakers yeast (*Saccharomyces cerevisiae*; ALK, 52.01), and storage mites (ALK, 35.00; 1000 NE/ml). All extracts with occupational allergens had a concentration of 5 mg/ml, except where noted. Subjects were classified as atopic if they had at least one positive response to one of five tested common allergens: house dust mite (ALK, SQ 510), grass pollen (ALK, SQ 293), tree pollen (ALK, SQ 197), cat fur (ALK, SQ 555), and dog fur (ALK, SQ 553). Positive and negative controls consisted of phosphate-buffered saline (PBS) with or without histamine (10 mg/ml), respectively. All tests were read after 15 minutes and were considered positive if the mean wheal diameter was at least 3 mm greater than the negative control.

Specific IgE determination

Sera were stored at -20°C until IgE analysis. Specific IgE determination to wheat flour was performed by a commercial immunoassay (AlaSTAT; DPC, Apeldoorn, The Netherlands) (El Shami & Alaba 1989). Sera of class 2 or higher (> 0.7 kU/l) were considered positive. Specific IgE to α-amylase was assessed with a modification of a previously described EIA (Wessels *et al.* 1991). Microwells were coated overnight at 4°C with a semi-purified preparation of α-amylase from *Aspergillus oryzae* (Fungamyl 1600S®, NOVO Nordisk, Denmark; kindly provided by the Department of Occupational Medicine, Sahlgren Hospital, Göteborg) at a concentration of 12.5 μg/ml of protein. Serum samples were incubated at a 1/5 dilution in PBS-Tween containing 0.2% gelatin (PBTG), and bound IgE was measured by subsequent incubations of 1 hr at 37°C with monoclonal mouse anti-human IgE (1/16,000; Central Laboratory of the Blood Transfusion Service (CLB), Amsterdam, The Netherlands), biotinylated affinity-purified rabbit anti-mouse Ig (1/5,000; Dakopatts (DAKO), Copenhagen, Denmark) and avidin-peroxidase (1/2,000; DAKO), and finally an incubation for 30 min. at room temperature with

o-phenylenediamine (OPD). An OD_{492} exceeding the OD + 0.05 of the reagent blank (no serum control) was interpreted as a positive reaction.

Alpha-amylase allergen exposure measurements

In all bakeries, personal dust samples were collected in the workers breathing zone during full-shift periods of 6-8 hours, using polytetrafluoroethylene (Teflon) filters (Millipore; pore size 1.0 μm) and inhalable dust (PAS-6) sampling heads at a flow rate of 2 l/min (ter Kuile 1984). α-Amylase allergens were recovered from the filters by extraction with 2.5 ml PBS in a 10 ml centrifuge tube. Each tube was vortexed for 2 minutes, sonicated for 2 minutes, vortexed for 5 minutes and sonicated for 2 minutes, successively. The extract was centrifuged at 5,000 g for 15 minutes, and the supernatant was collected and stored at -20°C. The α-amylase concentration in each sample was measured with a sandwich-EIA. Microtiter plates were coated with affinity-purified polyclonal rabbit anti-α-amylase antibodies. After washing, undiluted samples and 12 dilutions of a standard α-amylase antigen preparation (Fungamyl 1600S®) were added to the wells and the plate was incubated for 1 hour at 37°C. After another wash, wells were incubated with biotinylated affinity-purified rabbit anti-α-amylase antibodies, and subsequently with peroxidase-conjugated avidin and OPD. The reaction was stopped by adding HCl and the absorbency at 492 nm of each well was measured with an EIA-reader. The dose-response curve for the standard preparation was obtained by 4-parameter curve fitting, and allergen concentrations of samples were determined by interpolation in this curve. Only samples with an optical density higher than the optical density of the reagent blank (no α-amylase) + 5 SD were considered, resulting in a detection limit of the sandwich assay of 100 pg/ml. This resulted into a detection limit for personal allergen measurements of 250 pg/m^3. Samples with high concentrations (> 1.5 ng/ml) were re-tested at higher dilutions. The validity and specificity of the assay have been investigated extensively. The amylase assay appeared to be highly specific for fungal amylase and immunoblotting revealed a very similar reaction profile for IgE from sensitized bakery workers and the rabbit antibodies (Houba *et al.* 1996b).

Statistical analyses

All statistical analyses were performed using SAS software (version 6.09). Differences in mean exposure levels between groups were tested using a T-test

(PROC ANOVA). Exposure-response relationships were studied with univariate, stratified and multivariate analysis. As proposed by several investigators (Lee 1994; Axelson 1994) prevalence rate ratios (PRR) were calculated, by using Cox's proportional hazards model (Cox 1972), as modified by Breslow (1974) with SAS software (PROC PHREG). Differences at $p < 0.05$ (two sided) were considered significant.

Results

Symptoms, SPTs and IgE measurements

An overview of the prevalence of respiratory symptoms, work-related symptoms and positive IgE and SPT reactions is presented in Table 6.1. Forty-four workers (25 %) had one or more work-related symptoms, with the highest prevalence for rhinitis and the lowest for chest tightness. Of the 28 workers (16 %) who reported work-related rhinitis or chest tightness, 19 (68 %) had rhinitis only, 2 (7 %) had asthmatic symptoms only and 7 (25 %) reported these symptoms simultaneously.

Sixty five (38 %) of the workers had a positive skin test to at least one common allergen, and they were defined as atopic. Twenty five (15 %) had a positive skin test to one or more occupational allergens (wheat flour, rye flour, α-amylase or baker's yeast). There was a reasonable agreement between α-amylase sensitization measured by skin prick testing and IgE-analysis. Of the 13 IgE positive workers 77% also had a positive SPT to α-amylase; 63% of the SPT positive workers were also IgE positive. At our department, another occupational asthma cohort study was performed in laboratory animal workers. In that study skin prick tests were also performed with the fungal amylase extract as a control for the bakery study. Seven out of 416 laboratory animal workers (1.7 %) had a positive reaction to α-amylase. This was significantly different from the prevalence rate in bakery workers.

α-Amylase exposure

A total of 546 personal dust samples were available. On the basis of dust exposure levels, production workers were classified in eight job titles (Houba *et al.* 1996a). Because exposure data were lognormal distributed, the geometric means (GM) and geometric standard deviations (GSD) were computed next to the arithmetic mean (AM). The geometric means of full-shift airborne dust levels varied

Table 6.1 Characteristics of the group of bakery workers (N=178)

	Mean	SD	Range
Age (yr)	34.0	10.0	20 - 61
Years in bakery industry	10.2	8.7	0.2 - 43
Years smoked	11.2	10.6	0 - 42
FVC (l)	5.31	0.97	3.09 - 7.36
FEV_1 (l/s)	4.25	0.86	2.17 - 6.37
Tiffenau-index (%)	80.1	7.8	46.8 - 95.4
PEF (l/s)	10.5	2.5	4.8 - 18.0
	n	**%**	
Smokers	97	54 %	
Ex-smokers	35	20 %	
Nonsmokers	46	26 %	
% Male	157	88 %	
Respiratory symptoms (N=178)			
Chronic cough	18	10 %	
Chronic phlegm	13	7 %	
Shortness of breath	7	4 %	
Ever wheezing	38	21 %	
Frequent wheezing	7	4 %	
Chest tightness	18	10 %	
Work related symptoms (N=178)			
Rhinitis	26	15 %	
Conjunctivitis	10	6 %	
Chest tightness	9	5 %	
Skin symptoms	19	11 %	
Positive SPT reactions (N=169)			
House dust mite	41	24 %	
Grass pollen	35	21 %	
Tree pollen	15	9 %	
Cat fur	28	17 %	
Dog fur	28	17 %	
Wheat flour	14	8 %	
Rye flour	8	5 %	
Fungal α-amylase	16	9 %	
Bakers yeast	2	1 %	
Storage mites	19	11 %	
Positive IgE-response (N=169)			
Wheat flour	9	5 %	
Fungal α-amylase	13	8 %	

from 3.0 mg/m^3 for doughmakers of large industrialized bakeries to 0.4 mg/m^3 for slicers, packers and transport workers.

In 480 personal samples from production workers, α-amylase exposure levels were determined. The other samples were taken in maintenance workers, which were excluded from this study (n=27), were used during the development of the α-amylase assay, other assays or for endotoxin analysis (n=23), or were lost before filter elution (n=16). Alpha-amylase exposure levels varied considerably, depending on job title and type of bakery. Bakers involved in dough production had a much higher α-amylase exposure than other bakery workers (oven staff, packers). In confectioneries and rye bread production sites, however, no amylase was used in the production process, and α-amylase exposure levels in these bakeries were all close to the detection limit. Four exposure categories were defined based on frequency of measurements above detection limit and level of α-amylase exposure in each job title and each type of bakery (see Table 6.2). Category I consisted of all doughmakers working in the factory producing crispbakes (a kind of toast), where α-amylase exposure was very high. Category II consisted of all other production workers handling α-amylase frequently (doughmakers and all-round staff from wheat bread producing bakeries and bread- and mixed bakers from small bakeries), and category III of all workers handling α-amylase only occasionally. Although the range in α-amylase allergen exposure levels in each category was large, mean exposure levels were significantly different. Workers in these categories can be seen as more or less homogeneously exposed groups. For workers in one job category, however, α-amylase exposure was difficult to determine. In all-round staff in the crispbakes-producing bakery, full shift personal α-amylase exposure levels varied strongly (GSD=8.2), depending on the type of work performed on the day of sampling. In fact, this job title was a heterogeneous group of workers, of which some could be classified as high exposed and some as low exposed, depending on the number of days worked as doughmaker, packer, etc. Because detailed information on these determinants were not available, workers in this exposure category could not be divided into high or low exposed. Therefore, this group was treated as a separate exposure category (category IV in Table 6.2). All workers were classified in one of these categories, on the basis of their total job history (highest exposed category ever worked in).

Table 6.2 Exposure categories for α-amylase allergens

		α-amylase exposure (ng/m³)							Number of workers in category N=169
		N	below detection limit	above detection limit					
			n (%)	n	AM	GM	GSD	range	
I	high*	27	0 (0)	27	39.4	18.1	4.6	0.2 - 221.8	23
II	medium*	152	109 (72)	43	3.4	1.3	3.8	0.2 - 33.1	39
III	low*	275	268 (98)	7	1.9	0.7	4.0	0.2 - 8.8	71
IV	indistinct / strong varying*	26	11 (42)	15	25.1	6.1	8.2	0.2 - 150.2	36

N = number of personal samples
n = number of personal samples below or above detection limit
AM = arithmetic mean of samples above detection limit
GM = geometric mean of samples above detection limit
GSD = geometric standard deviation of samples above detection limit
* = exposure significantly different from all other categories ($p < 0.05$)

Exposure-response-relationship

Figure 1 shows the relationship between α-amylase allergen exposure and prevalence of a positive SPT for this enzyme. In the whole population, sensitization rate increased from 1.4% in the low exposed workers, and 12.8% in the medium exposed workers, to 30.4% in the high exposed workers. Positive SPT reactions were more common among atopic workers, and in this group especially a strong exposure-response relation was found, with more than 50% sensitization in the high exposed atopic workers. No clear relationship was found in non-atopic workers. In a multivariate analysis, α-amylase exposure and atopy appeared to be the most important determinants of skin sensitization (Table 6.3), with significant prevalence rate ratios (PRR) for atopy (PRR=20.8) and for high and medium α-amylase exposure groups (PRR=15.9 and 8.6, respectively). Several other potential determinants were tested but none of them was significantly associated with a positive SPT for α-amylase: current smoker (PRR 0.62; 95% confidence interval (CI) 0.23-1.68), ever smoker (PRR 0.76; CI 0.26-2.18), age (PRR 1.01; CI 0.97-1.06) and years in bakery industry (PRR 1.00; 0.95-1.06). Addition of these variables to the multivariate model hardly changed the prevalence rate ratios for atopy and α-amylase exposure.

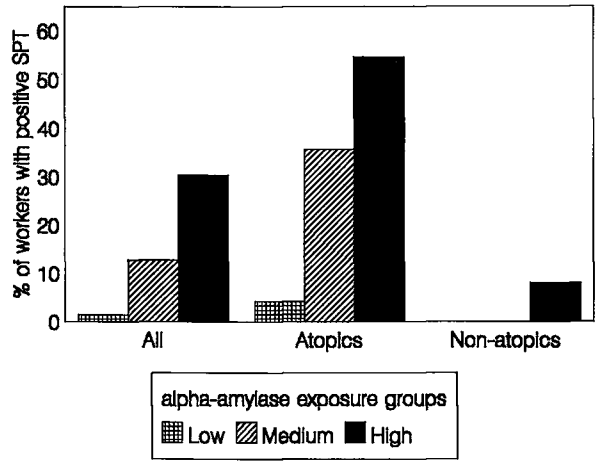

Figure 1 Prevalence of positive skin prick test to α-amylase by exposure group and atopic status. The number of workers in each group are:
High exposed: 23 bakery workers (12 non-atopics & 11 atopics)
Medium exposed: 39 bakery workers (25 non-atopics & 14 atopics)
Low exposed: 71 bakery workers (47 non-atopics & 24 atopics)

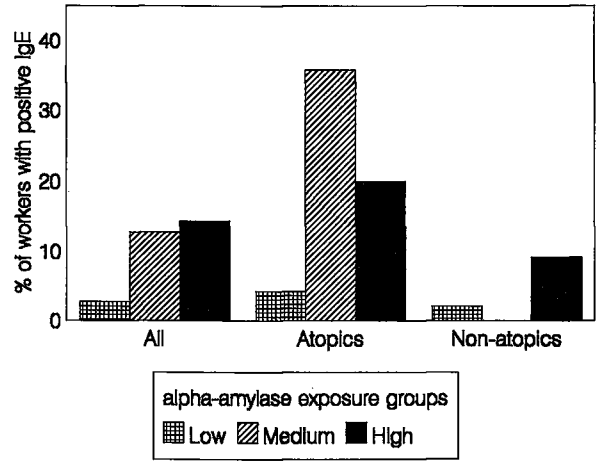

Figure 2 Prevalence of positive IgE to α-amylase by exposure group and atopic status. For numbers of workers per group, see legend of figure 1.

Table 6.3 Multivariate analysis of sensitization to α-amylase in relation to atopy and α-amylase exposure category in a group of bakery workers

	PRR†	95 % Confidence Interval	p
Model 1: skin prick tests (n=169)			
atopy	20.8	2.74 - 158	< 0.01
high α-amylase exposure*	15.9	1.95 - 129	< 0.01
medium α-amylase exposure*	8.6	1.01 - 74	< 0.05
low α-amylase exposure	1.0		
indistinct α-amylase exposure*	4.6	0.48 - 45	0.18
Model 2: IgE (n=167)			
Atopy	8.3	1.84 - 38	< 0.01
high α-amylase exposure*	3.9	0.65 - 24	0.13
medium α-amylase exposure*	4.6	0.85 - 22	0.08
low α-amylase exposure	1.0		
indistinct α-amylase exposure*	2.4	0.40 - 14	0.33

* low exposed group is reference category
† Prevalence Rate Ratio

Similar analyses were performed with IgE-sensitization, as illustrated in Figure 2 and Table 6.3. In the univariate analysis, the prevalence of positive IgE tests tended to increase with intensity of exposure. After stratification for atopy, however, there was no clear exposure-response-relation. The multivariate analysis shows that the only statistically significant determinant of positive IgE was atopic status (Table 6.3). Prevalence rate ratios of positive IgE to α-amylase by exposure group were elevated, but statistically not significant. No association could be found between positive IgE and some other potential determinants: current smoker (PRR 0.93; CI 0.31-2.76), ever smoker (PRR 1.09; CI 0.30-3.94), age (PRR 0.99; CI 0.93-1.05) and years in bakery industry (PRR 0.98; 0.91-1.05). Addition of any of these to a multivariate model with atopy and exposure did not change the results of Table 6.3.

Table 6.4 shows the relationship between SPT sensitization to α-amylase and the prevalence of work-related respiratory symptoms. For the total population a strong and positive association was found. Prevalence of work-related rhinitis was almost 5

times higher in α-amylase-SPT positive workers, and the prevalence of work-related chest symptoms was almost 12 times higher. The most important potential confounder in this relationship was atopic status, and because all but one of the positive SPT reactions to α-amylase were found in the atopic workers, the analysis was also performed for this group of workers only. Although prevalence ratios were smaller, there was still a statistically significant association between positive α-amylase SPT and work-related respiratory symptoms. Similar results were obtained for the relation between positive α-amylase IgE and work-related symptoms (also statistically significant).

Table 6.4 Prevalence ratios of work-related respiratory symptoms in relation to skin prick test sensitization to α-amylase in a group of bakery workers

Symptom	Total n (%)	Positive SPT α-amylase n (%)	Negative SPT α-amylase n (%)	Prevalence Rate Ratio	95% confidence interval
All workers	N=169	N=16	N=153		
rhinitis	24 (14)	8 (50)	16 (10)	4.78	2.05 - 11.2
chest symptoms	9 (5)	5 (31)	4 (3)	11.95	3.21 - 44.5
Atopic workers only	N=65	N=15	N=50		
rhinitis	14 (22)	7 (47)	7 (14)	3.33	1.17 - 9.50
chest symptoms	7 (11)	4 (27)	3 (6)	4.44	1.00 - 19.9

Only part of the workers with positive SPTs to α-amylase had work-related respiratory symptoms (8 out of 16). Most of the workers without symptoms (five of eight) worked in the high exposed group. We therefore looked at the degree of anti α-amylase reactivity, by comparing the mean wheal diameters of SPT positive workers within each exposure category, as given in Table 6.5. Although positive SPT reactions were most prevalent in the high exposed group of workers, the largest wheal diameters were found in the medium exposed category and the smallest in the highest exposed group. The difference was statistically significant. Anti-α-amylase IgE reactivity of the IgE positive workers in the high exposed group was also lower compared with the IgE reactivity in the medium exposed group. This difference, however, was not statistically significant.

Table 6.5 Mean wheal diameter of SPT to α-amylase for skin prick test positive bakery workers

Exposure category		number of positive skin prick tests	mean wheal diameter	range
I	high	7	3.9	3.2 - 5.1
II	medium	5	9.2	6.6 - 14.9
III	low	1	4.1	-
IV	indistinct/ strong varying	3	7.2	5.5 - 10.0

Discussion

Fungal amylase appeared to be an important occupational allergen in the bakery industry. As much as 9 % of the bakery workers in this study showed a positive SPT to this enzyme, and 8% had a positive IgE test. In our study considerable effort has been put into measuring and modelling α-amylase allergen exposure of the bakery workers. The number of bakery workers in our study was relatively small. Nevertheless, a strong and positive association was found between α-amylase-allergen exposure and SPT reactivity to this enzyme. For IgE sensitization, there was also a positive trend with α-amylase exposure, but this association did not reach statistical significance in the multivariate analysis. Atopic status was another important determinant of α-amylase sensitization. For skin prick test sensitization, the clearest exposure-response relationship was found within the group of atopic workers. No clear association was found in non-atopic bakers. However, prevalence of positive SPT reactions in this group was low (only one bakery worker), and an exposure-response relationship might have been found if a larger population had been studied. It might also be that non-atopic workers become sensitized at higher allergen exposure levels than do atopic workers. No association was found between α-amylase sensitization and smoking habits, age or years in industry.

Alpha-amylase exposure occurred mainly in doughmaking activities. Bakers working in these occupational titles were also exposed to other allergens, especially wheat flour. In a previous study, an EIA for measuring wheat flour antigens was presented, and wheat flour exposure of all bakery workers was measured (Houba *et al.* 1996a). Although there was a low correlation between wheat flour allergens and

α-amylase allergens if individual measurements were considered (r=0.19), some undesirable correlation existed when grouping workers in exposure categories (r=0.30). Therefore, wheat flour allergen exposure should be considered as a potential confounder, when estimating exposure response relationships for α-amylase in bakery workers. The relationships described in this study, however, are most likely to be caused by α-amylase exposure and not by wheat flour exposure. First, in this study we looked at specific endpoints for α-amylase (SPT and IgE). It is not very likely that exposure to wheat flour has caused sensitization to fungal amylase. Second, if we restricted the analyses to workers with high exposure to wheat flour (all doughmakers and all workers in small bakeries; n=96), the high and medium exposed α-amylase categories were not changed, but the low exposed group was restricted to doughmakers from confectioneries and the rye bread producing bakeries (oven staff, packers etc. excluded). In this sub-group, the exposure response-relation presented in Figure 1 remained unchanged. Third, in another analysis we calculated the mean α-amylase and wheat flour allergen exposure for each individual worker for whom we had exposure measurements. We then performed a multivariate analysis with skin prick test sensitization as the dependent variable and either the mean personal wheat flour exposure or the mean personal α-amylase exposure as the continuous independent variable. A significant association could be found for α-amylase exposure. Wheat flour exposure also showed an elevated prevalence ratio, although not significant. When both α-amylase and wheat flour were entered into the model, the relation for wheat flour exposure disappeared, whereas the prevalence ratio for α-amylase exposure remained unchanged. This shows that α-amylase exposure is the best explanatory variable in our exposure-response analyses. In all models, atopic status was included as covariate.

Another potential confounder is exposure to fungi in the bakeries. In a study among wheat millers, Moneo and coworkers (Moneo *et al.* 1994) suggested that sensitization to fungal α-amylase could have been caused indirectly by *A. oryzae* growing on milled wheat because of cross-reactivity between this fungus and α-amylase (Quirce *et al.* 1992). It is our opinion, however, that this phenomenon has not biased our study results. Measurements of airborne fungi in the bakeries revealed only low concentrations of fungi and only a small proportion appeared to be *Aspergillus spp.* (1-2%; data not shown). It is unlikely that these exposure levels caused sensitization to α-amylase, but if they did, α-amylase sensitization would

also be expected in doughmakers with similar exposure to fungi, but without exposure to fungal α-amylase (doughmakers from confectioneries and rye-bread production sites). However, no α-amylase sensitization was observed in this group.

In this study α-amylase exposure was related to specific sensitization, and subsequently α-amylase sensitization was related to work-related respiratory symptoms. Fungal amylase allergen exposure was not directly related to respiratory symptoms. Work-related respiratory symptoms are not specific health endpoints for α-amylase exposure. The role of α-amylase allergens in the development of symptoms can not be studied without taking into account the role of exposures to other allergens in the workplace such as cereal allergens. Wheat flour especially contains a large number of proteins that appear to be allergenic, and this cereal flour is an important factor in baker's asthma. At this moment, analyses are underway in which work-related symptoms are related to both α-amylase allergen and wheat flour allergen exposure.

Exposure-response relationships found in our study may have been influenced by selection bias. Bakers with respiratory allergies may have switched to other job titles with low α-amylase exposure, or they may have exchanged the bakery work for another job without allergen exposure. The removal to lower exposed jobs have been taken into account to some extent, since we modelled α-amylase exposure for each baker as the highest exposure category ever worked in. Control of selection bias caused by workers who have left the bakery industry was not possible, but it may have played a role in our study. In the highest exposed group of workers, the highest prevalence of positive SPT and IgE reactions to α-amylase were found, but the prevalence of work related symptoms and the degree of in vitro and in vivo reactivity to fungal amylase was low (measured as wheal diameter of the SPT and the optical density in the IgE analysis, respectively). It is possible that, especially in this category, workers who develop respiratory symptoms, are likely to leave, because of the high allergen exposure levels. Personal communications with employers in the bakeries confirmed this impression. Because this selection is dependent on α-amylase exposure, it is likely that underestimation of the observed exposure-response relationship has occurred. This selection bias, however, can be studied in detail only in a longitudinal study. At this moment most of the bakery workers in this study are part of a longitudinal study.

Two other epidemiologic studies focused on α-amylase sensitization. Cullinan and coworkers (Cullinan *et al.* 1994a) found positive SPT reactions in 5 % of

English mill and bakery workers. In this study, a positive SPT response was significantly and independently associated with atopy, but no independent effect could be found for smoking, dust exposure, or wheat allergen exposure. Amylase exposure was not measured in this study. In an Italian study 7.5 % of the bakery workers were sensitized to α-amylase, derived by skin prick testing (De Zotti *et al.* 1994). Atopy appeared to be the most important risk factor for sensitization to occupational allergens (α-amylase and wheat flour), and additionally, positive associations were found for smoking habits and years worked in the industry. Again, measurements of α-amylase allergens were not performed. Prevalence of positive SPT in these two studies are difficult to compare with our results, because the concentration of the fungal α-amylase extract was different in each study. Furthermore, differences in α-amylase exposure may be another important explanation for differences in SPT response. As mentioned, no information was available on α-amylase allergen exposure levels in both studies, so the role of exposure could not be established.

Exposure-response relationships among workers in bakeries have been reported before, for flour dust exposure and wheat aeroallergen exposure (Musk *et al.* 1989; Cullinan *et al.* 1994a; Hartmann *et al.* 1985). No exposure-response relationships have been reported for α-amylase exposure yet. Our study results might have implications for the management and prevention of occupational asthma in the bakery industry. This study suggests that reduction of α-amylase allergen exposure levels may lead to a reduction of the number of sensitized bakery workers. If this finding is confirmed by other (longitudinal or preferably by intervention) studies, this type of information can be used to establish exposure limits for α-amylase allergen exposure below which sensitization and development of occupational asthma are less likely to occur. Before that, however, much work has to be done in the development of a standardized assay for measuring α-amylase exposure. In our study we used polyclonal serum, and although this has no influence on the validity of our study results, the development of an assay based on monoclonal serum and one common standard α-amylase allergen extract might be necessary, not only for a good comparison of α-amylase allergen exposure levels in several epidemiologic studies, but also for standard setting.

In conclusion, this study has shown that α-amylase allergen exposure, together with atopy, is a major determinant of allergen specific type I sensitization. Sensitization to α-amylase is strongly associated with work-related respiratory

symptoms indicative of allergy. In addition to cereal flours, fungal α-amylase allergens play an important role in the development of occupational allergy among bakery workers.

7. Occupational allergy among bakery workers in relation to wheat flour and α-amylase allergen exposure[1]

Remko Houba, Dick Heederik & Gert Doekes

Abstract

In this study, the determinants of specific sensitization and work-related respiratory symptoms were studied in a cross-sectional epidemiologic survey among 393 bakery workers, with a special focus on the role of allergen exposure levels. Wheat allergens and fungal α-amylase allergens were measured in 449 and 507 personal inhalable dust samples of bakery workers, respectively. A strong and positive association was found between wheat allergen exposure level and the prevalence of wheat sensitization. In our study population, 23% of the bakers reported work-related respiratory symptoms. Only a proportion of the symptomatic bakery workers (30%; 7% of all bakery workers) was sensitized to wheat flour or α-amylase. Therefore, two groups of symptomatic bakers were distinguished, based on the presence or absence of sensitization for these occupational allergens. In both groups, a relation between allergen exposure (to wheat and α-amylase) and respiratory symptoms was found, but the slope of the relationship was steeper in sensitized bakers than in non-sensitized bakers. It is not obvious what may have caused the work-related symptoms in the group of bakers without sensitization to wheat flour or α-amylase. Sensitization to other occupational allergens and a non-specific hyper-responsive reaction to the dusty environment in bakeries are the most plausible explanations. This is supported by the observation that the majority of these workers had IgE antibodies to non-occupational allergens, had a history of allergic symptoms to common allergens, or reported chronic respiratory symptoms not related to the work environment. In conclusion, our study results suggest that only a proportion of all bakery workers with respiratory symptoms have IgE-mediated allergic reactions to wheat flour and fungal amylase. For those subjects, however, clear exposure-response-relationships were found for both allergens.

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[1] *Submitted*

Introduction

The inhalation of cereal flours and powdered baking additives may cause immunologic sensitization in bakery workers with subsequent allergic respiratory symptoms in sensitized bakers. Allergic reactions in the eyes and nose predominate as shown by recent cross-sectional epidemiologic studies among bakery workers in which prevalence rates for work-related eye and nose symptoms vary from 14 to 29% (Musk *et al.* 1989; Rosenberg *et al.* 1991; De Zotti *et al.* 1994; Cullinan *et al.* 1994a). Asthma is probably the most serious manifestation of baker's allergy, and may be found in 3-7% of all bakers (Prichard *et al.* 1984; Thiel 1987; Musk *et al.* 1989; De Zotti *et al.* 1994). According to the present knowledge, the most important allergens involved in baker's allergy are cereal flours and fungal α-amylase. The prevalence rate of sensitization varies from 5 to 15% for wheat flour and from 5 to 9% for α-amylase (Prichard *et al.* 1984; Cullinan *et al.* 1994a; De Zotti *et al.* 1994; Houba *et al.* 1996c).

Several investigators have tried to reveal the determinants of specific sensitization and work-related respiratory symptoms. In most studies, atopic status of the bakery workers has been shown to be an important risk factor for both sensitization and the development of respiratory symptoms (Järvinen *et al.* 1979; Prichard *et al.* 1984; Musk *et al.* 1989; De Zotti *et al.* 1994; Houba *et al.* 1996c). Age, gender, and smoking habits have usually not been reported as significant risk factors, although one study showed a positive association between symptoms and smoking (Cullinan *et al.* 1994a). In addition to atopy, the level of exposure to flour dust or specific allergens in bakeries have also been identified as important determinants of baker's allergy. The first indications for an exposure-response-relationship were reported by Hartmann (1986) and Musk *et al.* (1989). In these studies, a positive association was found between levels of flour dust exposure and the prevalence rate of respiratory symptoms and sensitization to wheat flour. More recent studies have measured specific allergens in personal samples with immunochemical methods and related these measures of exposure to health endpoints. In an epidemiologic survey in the UK, wheat flour allergens were measured with an inhibition immunoassay (Sandiford *et al.* 1994a), and work-related eye/nose symptoms were independently and positively related to wheat allergen exposure levels (Cullinan *et al.* 1994a).

An exposure-response-relationship for α-amylase allergens was described recently (Houba *et al.* 1996c). In a cross-sectional study among 178 bakery workers, a

strong and positive relationship between airborne α-amylase allergen exposure levels and α-amylase specific sensitization was observed. In addition, specific sensitization was strongly associated with work-related respiratory symptoms. However, the relationship between α-amylase exposure levels and respiratory symptoms of the bakery workers was not analyzed.

Until now, no studies have been published in which respiratory symptoms were related to both wheat allergen and α-amylase allergen exposure. In this paper, the determinants of work-related respiratory symptoms are studied in a cross-sectional epidemiologic survey among bakery workers, with a special focus on the role of exposure levels to both wheat flour and fungal amylase allergens. The slopes of the exposure-response relationships will be compared, and the proportion of work-related symptoms attributable to both allergens estimated.

Materials and methods

Population

The health survey was carried out between april 1991 and july 1993 and comprised 427 production workers in 21 Dutch bakeries (participation rate 75%). Most of the non-response was caused by (short-term and long-term) absence during the research period (holidays, illness), and in some cases by the high pressure of work. Only few workers refused to take part in the study (6% of all eligible workers). Non-responders were equally distributed among all bakeries and all types of jobs.

Five bakeries were large industrialized bakeries (40-200 employees), the others were small traditional bakeries (1-6 employees). These bakeries differed in a number of other characteristics, such as type of product, and technology level, as reported elsewhere (Houba *et al.* 1996a). Maintenance workers were excluded from the analyses because of potential exposures to other respiratory hazards (eg. welding fumes), leaving 393 bakery workers in the study. From 347 workers, venous blood samples were taken and analyzed for specific IgE antibodies, as described below. The other 46 workers were equally spread over all job titles and the frequency of work-related symptoms in this group was slightly higher compared with the bakers with blood samples, but not significantly different (28% versus 22%; p=0.38).

Questionnaire

All workers completed a short self-administered Dutch version of an internationally accepted respiratory questionnaire (MRC 1966), supplemented with questions on work related symptoms. Symptoms were considered to be work related if they were reported by the subject as being provoked by contact with flour or process related products (eg. baking additives) during work ('Do you have any of the following allergic symptoms during work, after contact with certain agents at work?'). Symptoms reported to be attributable to smoke production when starting up the ovens, were not included in this definition. Work related rhinitis was defined as the presence of sneezing or running nose (production of nasal secretions) during work. Work related conjunctivitis was defined as the presence of itchy or teary eyes. In addition, questions were asked about personal history of allergic symptoms to common allergens ('Are you allergic or have you been allergic to one or more agents?'). If answered positively, further questions were asked on type of symptoms and putative causal agents (house dust, pollen and/or domestic animals). Finally, complete records of smoking habits and job histories were obtained.

IgE-antibodies

Sera were stored at -20°C until IgE analysis. Specific IgE antibodies to wheat flour were measured with a commercial immunoassay (AlaSTAT; DPC, Apeldoorn, The Netherlands) (El Shami & Alaba 1989). Sera of class 1 or higher (> 0.35 kU/l) were considered positive.

Specific IgE antibodies to house dust mite (*Dermatophagoides pteronyssinus*), grass pollen (1:1 mixture of *Lolium perenne* and *Phleum pratense*), birch pollen (*Betula verrucosa*), cat allergen, and fungal α-amylase were assessed with a previously described EIA (Doekes *et al.* 1996). Microwells were coated overnight at 4°C with commercially available lyophilized extracts (ALK Benelux, Houten, The Netherlands), or a semi-purified preparation of α-amylase from *Aspergillus oryzae* (Fungamyl 1600S®, NOVO Nordisk, Denmark). Coating concentrations were 25 μg/ml of protein for the common allergens and 12.5 μg/ml for α-amylase. Diluted sera (1/10 for common allergens and 1/5 for α-amylase; in PBS-Tween containing 0.2% gelatin (PBTG)) were incubated for 2 hours at 37°C, and bound IgE was measured by subsequent incubations with monoclonal mouse anti-human IgE, biotinylated affinity-purified rabbit anti-mouse Ig, avidin-peroxidase, and o-phenylenediamine (OPD). An OD_{492} exceeding the OD + 0.05 of the reagent blank

(no serum control) was interpreted as a positive reaction. Results of this assay correlated very well with commercially available test kits and the assay had a similar sensitivity and specificity with regard to skin prick tests (Doekes *et al.* 1996).

Total IgE was measured by a sandwich EIA (Doekes *et al.* 1996). Briefly, mouse monoclonal anti-IgE was coated in microwells. Sera were added in four dilutions and incubated for 2 hours at 37°C. Bound IgE was measured by incubation with peroxidase-labelled mouse monoclonal anti-IgE and OPD as the peroxidase substrate. Each microtiter plate included a serially diluted reference sample (Kabi-Pharmacia, 10-9123-01).

The presence of atopy was assessed on the basis of IgE serology data. Two parameters were used separately as indicators of atopy: a total serum IgE $\geq$ 100 kU/l, and the presence of specific IgE to at least one of the common allergens of the test panel.

Allergen exposure assessment

In all bakeries, personal dust samples were collected in the workers breathing zone during full-shift periods of 6-8 hours, using polytetrafluoroethylene (Teflon) filters (Millipore; pore size 1.0 μm) and inhalable dust (PAS-6) sampling heads at a flow rate of 2 l/min (ter Kuile 1984). Wheat flour and α-amylase allergens were recovered from the filters by extraction with 2.5 ml PBS, and the allergen concentrations were measured by inhibition EIA for wheat flour (Houba *et al.* 1996a), or sandwich EIA for fungal α-amylase (Houba *et al.* 1996b).

Wheat allergen and α-amylase allergen exposure varied considerably among bakery workers, depending on the job of the bakery worker (e.g. doughmaker or packer) and the type of the bakery (Houba *et al.* 1996a; Houba *et al.* 1996b). Based on these two characteristics, 22 occupational titles could be distinguished and each bakery worker was classified into one of these occupational titles. The 22 job titles were used for further classification into (larger) exposure categories as described below.

Statistical analyses

All statistical analyses were performed using SAS software (version 6.09). Differences in mean exposure levels were tested using Student's t-tests (PROC ANOVA). In some occasions exposure levels were not (log)normally distributed and

the nonparametric Wilcoxon rank-sum test was used (PROC NPAR1WAY). The determinants of work-related respiratory symptoms were analyzed by multiple regression techniques. Prevalence rate ratios (PRR) were calculated by using a proportional hazards model (Cox' regression using PROC PHREG) (Lee & Chia 1993; Axelson *et al.* 1995). For the analysis of the relation between allergen exposure and sensitization, all bakery workers were classified into an exposure category based on their total job history (highest exposed category ever worked in). For the analyses of acute respiratory symptoms, however, the classification into exposure groups was based on the job title in the last year of employment. In all analyses, differences with $p < 0.05$ (two sided) were considered significant.

Results

Allergen exposure categories

Five exposure categories of fungal α-amylase exposure could be distinguished as shown in Table 7.1. Only 35% of all bakery workers had been exposed to fungal α-amylase. Occupational titles without detectable α-amylase allergen exposure served as the reference category in the epidemiologic analyses (group I). Group II consisted of mixed and bread bakers from small facilities plus all-round workers from large industrial bread-producing bakeries, who were exposed to α-amylase on a regular basis. Doughmakers from industrialized bakeries, frequently handling dough improvers, formed exposure group III. In one bakery (crispbakes factory), doughmakers had clearly higher α-amylase exposure levels compared with other doughmakers, and were classified into exposure group IV. For all-round staff from this crispbakes-factory, α-amylase allergen exposure varied strongly. For this group (group V), no reliable mean α-amylase allergen exposure level could be estimated, and workers in this group could not be classified as high or low exposed (Houba *et al.* 1996c). This exposure group is included in all epidemiologic analyses, but is not presented in the tables for reasons of clarity. Except for category V, the α-amylase allergen exposure in each group was significantly different from all other groups.

In contrast to fungal α-amylase exposure, no (extreme) highly exposed job titles could be identified for wheat allergen exposure. Ranking all 22 occupational titles, the mean wheat allergen exposure gradually increased, and there were no obvious cut-off points for the formation of exposure groups. Therefore, three exposure

Table 7.1 Definition of exposure categories for wheat flour and α-amylase allergens, and the number of workers in each category based on the job title in the last year of employment.

Exposure category		N	samples < detection limit	AM	GM	GSD	range	Number of workers in category (N=393)
		α-amylase allergen exposure (ng/m^3)						
I*	low	275	98 %	0.2	0.2	1.4	0.2 - 8.8	255
II*		120	83 %	0.5	0.2	2.3	0.2 - 14.3	90
III*		32	31 %	3.2	0.8	5.1	0.2 - 33.1	9
IV*	high	27	0 %	39.4	18.1	4.6	0.2 - 221.8	13
V	indistinctive / strong varying	26	42 %	14.6	1.3	11.0	0.2 - 150.2	26
		wheat allergen exposure (μg/m^3)						
I*	low	151	38 %	0.2	0.1	3.1	0.03 - 7.7	143
II*	medium	120	9 %	3.5	0.7	6.4	0.03 - 74.6	118
III*	high	178	1 %	11.0	3.8	4.4	0.03 - 252.4	132

N = number of personal samples
AM = arithmetic mean
GM = geometric mean
GSD = geometric standard deviation
* = exposure significantly different from all other categories ($p < 0.05$)

groups were formed with approximately equal number of bakery workers (tertile analyses; see Table 7.1). Some alternative ways of classification have been tested (with different cut-off points), but these produced similar results in the epidemiologic analyses. The group with the highest wheat exposure levels (group III), consisted mostly of doughmakers from the industrialized bakeries including workers from small traditional bakeries. Group II consisted of all-round staff, oven staff and production managers. All other workers were classified into group I. The wheat allergen exposure was significantly different between all groups. To give an indication of inhalable dust exposure levels in the bakeries, the geometric mean dust exposure for the three wheat exposure groups were 0.46, 0.78, and 2.37 mg/m^3, respectively.

Population characteristics

Population characteristics are given in Table 7.2, including an overview of the

Table 7.2 Characteristics of the group of bakery workers (N=393)

	Mean	SD	Range
Age (yr)	33.9	9.9	17 - 61
Years in bakery industry	9.0	8.1	0.1 - 43
Years smoked	10.0	10.1	0 - 43
Pack-years	6.5	8.2	0 - 43
	n	**%**	
Smokers	189	48 %	
Ex-smokers	85	22 %	
Nonsmokers	119	30 %	
% Male	348	89 %	
Respiratory symptoms (N=393)			
Chronic cough	46	12 %	
Chronic phlegm	26	7 %	
Shortness of breath	26	7 %	
Ever wheezing	91	23 %	
Frequent wheezing	30	8 %	
Chest tightness	41	10 %	
Work related symptoms (N=393)			
Rhinitis	83	21 %	
Conjunctivitis	58	15 %	
Chest tightness	29	7 %	
IgE serology (N=347)			
Total IgE ≥ 100 kU/l	87	25 %	
House dust mite	69	20 %	
Grass pollen	53	15 %	
Birch pollen	16	5 %	
Cat allergens	15	4 %	
Wheat flour	36	10 %	
Fungal α-amylase	26	7 %	
Positive IgE-response *and* work-related symptoms (rhinitis and/or chest tightness (N=347)			
Wheat flour	15	4 %	
Fungal α-amylase	12	3 %	

prevalence of chronic respiratory symptoms, work-related symptoms and results of the IgE-analyses.

Work-related symptoms were highly prevalent among the bakery workers, ranging from 29 bakers (7%) with chest tightness to 83 (21%) with rhinitis. Most workers with chest tightness also reported rhinitis (21 out of 29). Because of this highly associated occurrence, subsequent analyses with work-related symptoms were not performed for each symptom separately, but for bakers who reported rhinitis *and/or* chest tightness.

As mentioned, two parameters were used as indicator of atopy: elevated total IgE levels or the presence of specific IgE to at least one of the common allergens. Eighty seven bakery workers (25%) had total-IgE levels greater than 100 kU/l. A positive IgE-test to any of the four common allergens was found in 94 bakers (27%). Both parameters of atopy were closely associated (χ^2=43; p<0.001). Wheat flour specific IgE was detected in 36 bakery workers (10%) and 26 (7%) had IgE to fungal amylase. Only 6 workers were sensitized to both wheat flour and α-amylase.

Symptoms versus sensitization

Based on work-related symptoms and sensitization to occupational allergens, four groups of workers could be distinguished, as shown in Table 7.3. A relatively small group of 23 workers had symptoms and were sensitized to wheat or α-amylase (group A), whereas most of the bakery workers with work-related symptoms (70%) had no detectable IgE against wheat flour or fungal amylase (group B). A high proportion of the workers in group B had enhanced total serum IgE (40%) or specific IgE to one of the common allergens (38%). The prevalence of respiratory symptoms in general (i.e. not work-related) was also high in this group, with 27% reporting a personal history of allergic symptoms to common allergens, and 55% reporting at least one of the chronic respiratory symptoms. Seventy eight percent (78%) of the bakers in group B were positive for one of the IgE tests, or reported at least one of the symptoms mentioned above.

Determinants of work-related symptoms (other than allergen exposure)

Table 7.4 shows the association between work-related symptoms (rhinitis and/or chest tightness) and potential determinants. The only variables significantly associated with symptoms were atopy, defined either as elevated total IgE or the presence of specific IgE to common allergens, and a personal history of allergic

Table 7.3 Characteristics of workers grouped by work-related symptoms and sensitization to occupational allergens (wheat flour or α-amylase) (N=347)

GROUP	**A**	**B**	**C**	**D**
work-related symptoms	+	+	-	-
sensitization to wheat flour or α-amylase	+	-	+	-
N	23	55	33	236
Positive IgE responses:				
Total IgE ≥ 100 kU/l	70%*	40%*	24%	17%
House dust mite	56%*	27%*	15%	15%
Grass pollen	52%*	24%*	21%	9%
Birch pollen	22%*	7%	3%	3%
Cat allergens	30%*	7%*	6%*	1%
Any of the IgE-tests (total or specific IgE)	83%*	58%*	39%	30%
Allergic symptoms to common allergens	39%*	27%*	13%	6%
Any of the chronic respiratory symptoms	74%*	55%*	36%	23%

* = Significantly different from non-symptomatic/non-sensitized group ($p<0.05$)

Table 7.4 Work-related respiratory symptoms (rhinitis and/or chest tightness) in relation to potential determinants of symptoms, other than allergen exposure (all univariate analyses).

	PRR	95 % Confidence Interval	p
Potential determinants of symptoms (other than allergen exposure)			
Total IgE ≥ 100 kU/l	2.84	1.82 - 4.43	< 0.001
Positive IgE to any of the common allergens	2.56	1.64 - 3.99	< 0.001
Personal history of allergy to common allergens	3.35	2.17 - 5.19	< 0.001
Current smoker	0.89	0.59 - 1.34	0.56
Ever smoker	1.09	0.69 - 1.71	0.72
Years smoked	1.01	0.99 - 1.03	0.48
Pack-years smoked	1.01	0.99 - 1.04	0.22
Age	1.00	0.98 - 1.02	0.87
Years of employment in bakery	1.00	0.98 - 1.03	0.76
Gender (male=0; female=1)	0.45	0.18 - 1.11	0.08

PRR = Prevalence rate ratio

symptoms to common allergens. Age, years of employment, and all indicators of smoking habits were not related to work-related respiratory symptoms. When work-related rhinitis and chest tightness were analyzed separately, current smokers showed a lower prevalence of chest tightness than never-smokers, but this difference was not statistically significant (PRR 0.47; 95% confidence interval (CI) 0.20-1.12). No difference between smokers and never-smokers was found for rhinitis (PRR 0.91; CI 0.54-1.51). Although smoking habits were not related to work-related symptoms, they were positively associated with most of the chronic respiratory symptoms from the standardized respiratory symptom questionnaire.

Although only borderline significant, work-related symptoms were more common among male workers compared with female workers. However, male workers were more likely to be exposed to higher allergen levels, since the proportion of males was higher in the higher exposure categories.

Exposure-response-relationships

Figure 7.1 shows the relationship between wheat allergen exposure and wheat specific IgE-sensitization. A clear exposure-response-relationship was found, both for atopic and non-atopic workers. The relationship was strongest within the group of atopic bakers. In a multivariate analysis with atopy and wheat allergen exposure groups, the prevalence rate ratio for atopy was 1.89 (CI 0.97-3.70), and 1.73 (CI 0.47-6.45) and 3.15 (CI 1.10-9.01) for medium and high wheat allergen exposure respectively. In this analyses, the group of workers with low wheat allergen exposure was used as reference group. Several other potential determinants were tested (smoking habits, gender, age, and years in bakery industry), but none of them was significantly associated with sensitization, and addition of these variables to the multiple regression model hardly changed prevalence rate ratios for atopy and wheat allergen exposure. Similar exposure-sensitization-relationships were found in analyses with atopy defined as a positive IgE-test to common allergens, or a combination of total and specific IgE.

The relationship between α-amylase allergen exposure and α-amylase specific sensitization was described in a previous publication (Houba *et al.* 1996c). However, also in this population the frequency of α-amylase sensitization clearly increased with intensity of α-amylase allergen exposure. In the whole population, the sensitization rate for α-amylase increased from 5% in the low exposed workers (group I in table 7.1), to 9% in group II, 10% in group III, and 14% in group IV.

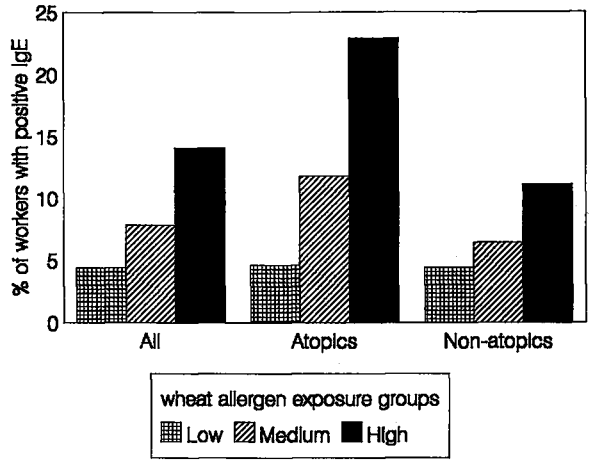

Figure 7.1 Prevalence of wheat flour sensitization by wheat allergen exposure category and atopy defined as a total serum IgE ≥ 100 kU/l (N=347). Because bakery workers were classified in one of these categories based on their total job histories, rather than on the actual allergen exposure, the number of workers in each category differs from the numbers presented in the last column of Table 7.1.

The relationship between allergen exposure and work-related symptoms was analyzed with multiple regression analyses. Results are summarized in Table 7.5. These relationships were described for two groups of workers which were distinguished on the basis of the presence or absence of sensitization to these occupational allergens. For α-amylase allergens, work-related symptoms tended to increase with increasing exposure, except for the highest α-amylase exposure group, which showed a lower prevalence rate ratio. The strongest exposure-response-relationship was found when sensitization to α-amylase was present, with prevalence rate ratios of 2.6 and 7.5 for α-amylase groups II and III respectively. In the group with highest α-amylase exposure, however, no bakery worker reported work-related symptoms in combination with α-amylase specific sensitization. For the group of bakers without sensitization to α-amylase, the prevalence rate ratios also increased with increasing allergen exposure levels. However, this relationship was less pronounced. For wheat allergen exposure, similar results were found. The prevalence of work-related symptoms increased in groups with higher exposure levels. Again, this association was most obvious for workers with sensitization to wheat flour. Also in non-sensitized workers a relation between wheat allergen

Table 7.5 Multivariate analyses of work-related symptoms (rhinitis and/or chest tightness) in relation to atopy, allergen exposure, and allergen specific sensitization (N=347).

	allergen specific sensitization	PRR	95% Confidence Interval
α-amylase exposure			
atopy		2.5	1.6 - 4.1
I (low)*	yes and no	1.0	
II	no	1.8	1.1 - 2.9
III	no	2.3	0.6 - 9.5
IV (high)	no	0.5	0.1 - 3.9
II	yes§	2.6	1.1 - 6.1
III	yes§	7.5	1.0 - 55
IV (high)	yes§	-#	-
wheat allergen exposure			
atopy		2.7	1.7 - 4.2
I (low)*	yes and no	1.0	
II (medium)	no	1.4	0.7 - 2.6
III (high)	no	1.6	0.9 - 2.9
II (medium)	yes†	2.4	0.8 - 7.2
III (high)	yes†	2.7	1.2 - 5.8

PRR = prevalence rate ratio
atopy = total IgE levels ≥ 100 kU/l
* = low exposed group is reference category
\# = no bakers in this category have work-related symptoms in combination with sensitization
§ = IgE-sensitization to α-amylase allergens
† = IgE-sensitization to wheat allergens

exposure and symptoms was found, but the individual prevalence rate ratios did not reach statistical significance. When all bakers sensitized to α-amylase and/or wheat flour were excluded from the analyses, a weak exposure-related trend could still be found for both allergens.

In Table 7.5, the definition of atopy was based on a positive test for total IgE. Alternatively, atopy was defined as a positive IgE-test to one of the four common

allergens. Using this definition in the analyses, similar prevalence rate ratios for atopy were found (PRR=2.3), and the exposure-response-relationships were essentially identical. When a personal history of allergic symptoms to common allergens was used as an indirect indicator of atopy (instead of IgE serology), similar exposure-response-relationships were found. Atopy and allergen exposure were the only significant determinants of work-related symptoms. Addition of any of the potential confounders and modifying factors to the models (age, gender and smoking habits) did not change the results.

Discussion

In this study a high prevalence of work-related respiratory symptoms in bakers was found, i.e. 7% for chest tightness, and 21% for rhinitis. Atopy and the level of allergen exposure appeared to be the most important determinants of these symptoms, whereas age, gender, and smoking habits were not associated with the prevalence of work-related symptoms. This is the first study in which work-related symptoms were shown to be independently associated with exposure levels of both wheat flour and α-amylase allergens.

Atopy has been identified as an important risk factor for work-related respiratory symptoms in many other studies (Järvinen *et al.* 1979; Prichard *et al.* 1985; Hartmann 1986; Rosenberg *et al.* 1991; De Zotti *et al.* 1994). As mentioned, exposure-response relations have been less systematically investigated, but were reported for estimates of dust exposure (Hartmann 1986; Musk *et al.* 1989) and for wheat allergen exposure (Cullinan *et al.* 1994a). Fungal α-amylase is another important allergen in the baking industry, but until now, no studies have been published in which the prevalence of respiratory symptoms was related to airborne α-amylase allergen exposure levels.

Considerable effort has been put into measuring and modelling fungal α-amylase allergen exposure and wheat allergen exposure. For both allergens clear exposure-response-relationships were found. However, two types of exposure-response-relationships should be distinguished. First, the relationship between allergen exposure and allergen specific sensitization. Second, the relationship between allergen exposure and the occurrence of work-related respiratory symptoms in sensitized individuals. In this study, a clear exposure-sensitization-relationship was

found for wheat allergens. Atopy appeared to be an important effect modifier of this relationship, and the steepest slope between allergen level and sensitization rate was found within the group of atopic workers. However, sensitization to wheat flour was also found in non-atopic bakery workers and for this group of workers an exposure-response-relation was found as well. In a previous study, strong exposure-sensitization relationships were described for α-amylase allergens using skin prick test as response variable (Houba *et al.* 1996c), and confirmed in this stud, using specific IgE as response variable.

Once sensitized, bakers may develop allergic symptoms upon renewed exposure to the specific allergen. It is often suggested that sensitized individuals may develop attacks of asthma at very low exposure levels. This conclusion, however, is probably mainly based on the experience of patients visiting medical centres for their usually severe asthmatic symptoms. In fact, exposure-response-relationships for sensitized individuals have not been studied yet in open populations of bakery workers. Our study results revealed that there is also an exposure-related response within the group of sensitized bakers, both for α-amylase and wheat allergens. However, the number of sensitized individuals in each exposure group was small, and the results should therefore be interpreted with some caution. Nevertheless, the existence of an positive relationship between exposure and symptoms implicates the possibility of exposure intervention also for sensitized individuals or there may be possibilities for individual medical guidance of allergic bakers. The relationship between exposure and symptoms should therefore be an important scope for further research.

The exposure-response-relationships may have been biased by the healthy worker effect. This is illustrated for the group with very high α-amylase allergen exposure (mean α-amylase level 39 ng/m^3), in which the prevalence of work related symptoms was very low. This is possibly due to selection processes. Sensitization to α-amylase is most prevalent in workers who are working or had worked in this category. However, the prevalence of work related symptoms and the degree of in vitro and in vivo reactivity to fungal amylase (measured as the titres in the IgE-analysis) at the time of the cross-sectional survey was low (Houba *et al.* 1996c). No baker in this exposure group had work-related symptoms in combination with α-amylase sensitization. It is possible that, especially in this category, workers who developed respiratory symptoms due to α-amylase exposure, have left their job location, because of the high allergen exposure levels. Personal communications

with employers in the bakeries confirmed this impression. A better estimate of this selection bias, however, can only be studied in a longitudinal survey.

Although work-related symptoms were associated with wheat allergen and α-amylase allergen exposure, not all symptoms could be explained by an IgE response to one of these allergens. In this study 23% of the bakery workers reported work-related rhinitis and/or chest-tightness, but in only 30% of this group (7% of all bakery workers) IgE-sensitization to wheat or α-amylase could be demonstrated. This suggests that the group of bakers with work-related respiratory symptoms is probably heterogeneous with regard to the actual cause of these symptoms.

It is not very obvious what may have caused the work-related symptoms in the group of workers without sensitization to occupational allergens. Our results showed that these symptoms were also positively correlated with allergen exposure levels, although the slope of this association was steep. This suggests that the potential causes of work-related symptoms of bakers without simultaneous sensitization to wheat flour or α-amylase, may be correlated with the allergen exposure measured in our study. There are some possible explanations for this finding. First, our IgE-assays may have failed to detect sensitization in some individuals, although the sensitivity and specificity were comparable to other IgE assays. Second, many other allergens have been identified in case series of asthmatic bakers, for instance other cereal flours (Sutton *et al.* 1984; Thiel & Ulmer 1980) and several baking additives like soybean flour (Jorde *et al.* 1986; Baur *et al.* 1988b) and other fungal enzymes such as glucoamylase and (hemi)cellulase (Baur *et al.* 1988b; Quirce *et al.* 1992). These allergens may have caused allergic reactions in some bakery workers. However, several studies among asthmatic bakery workers showed that the number of asthmatic bakers sensitized to these allergens is much lower compared with the number of symptomatic bakers sensitized to wheat flour and α-amylase. For this reason, it is not very likely that specific sensitization to other occupational allergens is the explanation for all symptoms that can not be attributed to wheat flour or α-amylase (70% of all work-related symptoms). A non-specific reaction to the dusty environment in the bakeries is probably an additional explanation. The majority (78%) of the workers with these work-related symptoms had IgE antibodies to non-occupational allergens (58%), had a history of allergic symptoms to common allergens (27%), or reported chronic respiratory symptoms outside the job environment (55%).

A non-specific nature of work-related symptoms among bakery workers was also

suggested in another epidemiologic survey (Cullinan *et al.* 1994a). In that study, no relation was found between symptoms and skin test results for wheat flour and α-amylase. According to the authors, one of the possible explanations is that symptoms may also result from direct irritation. Some other studies have found a positive relation between non-specific bronchial reactivity and exposure to flour dust (Musk *et al.* 1989; Bohadana *et al.* 1994). Unfortunately, no information was available on the proportion of these workers sensitized to occupational or common allergens, and it is therefore impossible to distinguish between symptoms in the presence or absence of sensitization to occupational allergens as we have done in our study. Nevertheless, these studies suggest that apart from IgE-mediated allergies, work-related symptoms of bakery workers may also have been caused by non-immunologic responses. These type of symptoms should be distinguished from work-related symptoms associated with IgE-mediated allergic reactions to occupational allergens, when specific exposure-response-relationships for these occupational allergens are studied. Our study results suggested that the slope of the relationship between allergen exposure (to wheat and α-amylase) and respiratory symptoms was steeper in sensitized bakers than in non-sensitized bakers.

Although wheat flour and α-amylase allergens are probably responsible for only a proportion of the work-related symptoms reported by the bakery workers, they may have been responsible for the more severe symptoms. In our study, a higher percentage of workers with chest tightness was found in the group of symptomatic bakers with sensitization to wheat or α-amylase, compared with the symptomatic bakers without sensitization (44% versus 26%). In the analyses we have not discriminated on the basis of severity of the work-related symptoms. Unfortunately, it was not possible to perform separate analysis for eye/nose symptoms and chest tightness, due to the small numbers of asthmatic workers.

Although an exposure-related association with symptoms was found for both wheat allergens and α-amylase allergens, some differences can be observed. For α-amylase allergens, the association was stronger compared with wheat allergens, especially when the exposure-sensitization-relationships for wheat flour described in this study is being compared with the equivalent exposure-sensitization-relationship found for α-amylase allergens described in our previous paper (Houba *et al.* 1996c). Increased risks were found at α-amylase exposure levels just above the detection limit for this allergen in personal samples (> 0.25 ng/m^3). This suggests that fungal α-amylase is an extremely potent allergen. However, the number of bakery workers

exposed to α-amylase allergens is much lower. In our study, 6.6% of all bakery workers have work-related symptoms in combination with sensitization to occupational allergens: 3.4% for α-amylase and 4.3% for wheat flour. This shows that in our study population, the proportion of work-related symptoms attributable to wheat flour allergens is comparable with α-amylase allergens, despite the lower potency of the mixture of allergens in wheat flour. Besides, the range in wheat allergen exposure in our study was relatively small, and wheat flour may form a greater health hazard in groups where exposure levels are higher (eg. flour mills).

The described exposure-response-relationships may have important consequences for the occupational health care in bakeries. The findings of exposure-response gradients provide important evidence of causation and suggest that occupational asthma is to some extend preventable by reducing exposure levels. This is an important step when standard setting is considered for allergen exposure in bakeries. We fully realize that this is the first study that describes such exposure-response-relationships, and that these findings have to be confirmed by other studies (preferably cohort-studies) before standard setting can be considered. Moreover, considerable work has to be done to standardize assays for measuring wheat allergen and fungal α-amylase allergen exposure.

In conclusion, 23% of the bakery workers in this study reported work-related rhinitis and/or chest tightness. Our study results suggest that only a third of these workers have IgE-mediated allergic reactions to wheat flour and fungal α-amylase. For those subjects, however, clear exposure-response-relationships were found for wheat allergen exposure and fungal α-amylase allergen exposure.

8. General discussion

Respiratory disorders in bakery workers caused by exposure to agents at work is a serious occupational health problem. Epidemiologic surveys show prevalence rates of work-related eye/nose symptoms of 14-29%, while the prevalence rates of chest symptoms vary from 5-14% (Prichard *et al.* 1984; Musk *et al.* 1989; Cullinan *et al.* 1994a; De Zotti *et al.* 1994). Many of these symptoms are attributed to immunologic sensitization and subsequent allergic reactions to specific airborne allergens. Baker's asthma is one of the most serious manifestations of occupational allergy among bakery workers and is often severe enough to cause permanent working disability. There are no reliable data available on the incidence of baker's asthma, but occupational disease registrations in several countries show that baker's asthma is one of the most frequent forms of occupational asthma (Thiel 1987; Hartmann 1989; Jeffrey 1992; Lagier *et al.* 1990; Nordman 1994). In Germany, more than 1200 bakers with occupational asthma claim industrial injury compensation annually (Baur 1993; Grieshaber & Rothe 1995). At the start of this project, no information was available on the prevalence or incidence of baker's asthma in The Netherlands.

Main findings

Allergen exposure assessment

Only a few investigators have studied the environmental determinants of baker's asthma. Exposure characterization is a topic in occupational asthma research that, until recently, has received remarkably little attention, not only in the bakery industry, but also in general. One of the reasons was the absence of reliable methods for measuring aero-allergen exposure. In this study, immunoassays have been developed for quantifying airborne exposure levels to allergens from wheat flour (Chapter 3) and from fungal α-amylase (Chapter 4), which are considered as the most important allergens in the bakery industry. The validity and specificity of both methods have been tested extensively. These assays have been used for measuring allergen concentrations in a few hundred personal inhalable dust samples of bakery workers. To our knowledge this is the most comprehensive exposure assessment that has been performed in the bakery industry until now.

Levels of inhalable dust exposure were mainly determined by the job of the

bakery workers. Relatively small differences were found in flour dust exposure levels between bakeries. The job title of the bakery worker was also the most important determinant of wheat allergen exposure. However, within each job title clear differences in wheat allergen exposure levels were found between bakeries, depending on the use of other products than wheat flour. Fungal α-amylase exposure was only apparent in bakery workers involved in dough production in bakeries where bread or crispbakes were produced. There was some correlation between levels of inhalable dust and wheat allergens (r=0.77; n=449), but a very low correlation was found between dust exposure and α-amylase exposure (r=0.19; n=507). These results showed that quantification of dust exposure in the bakery industry could not be used as a surrogate for describing allergen exposure levels, especially for α-amylase allergens.

Levels of inhalable dust exposure in large industrialized bakeries were comparable to dust exposure levels measured in large bakeries in the UK, Finland and Sweden (Musk *et al.* 1989; Jauhiainen *et al.* 1993; Lillienberg & Brisman 1994; Nieuwenhuijsen *et al.* 1994), and, as in our study, the specific job of the bakery workers was also the major determinant of dust exposure levels in British bakeries (Nieuwenhuijsen *et al.* 1995a). In many countries, a substantial proportion of all bread production takes place in small traditional bakeries (<10 workers per bakery). This is the first study describing dust and allergen exposure levels in small bakeries. In these bakeries, the inhalable dust exposure levels were comparable with dust levels of doughmakers in large industrialized bakeries. However, this may be different for other countries. In the framework of a European collaborative project, personal inhalable dust levels were measured in two small bakeries in Germany, using the same sampling equipment and protocol. Dust exposure levels in these bakeries (n=30; GM=6.9 mg/m^3; range=0.9-118 mg/m^3) were twice as high compared with Dutch bakeries (n=36; GM=3.3 mg/m^3; range=1.2-8.8 mg/m^3) (unpublished results). It is not clear what might have caused the higher dust exposure, but differences in dough production may be a possible explanation. Observations during the measurements suggested that the amount of flour used to spread over the dough during dough forming was higher compared with Dutch bakeries.

Work-related respiratory symptoms

This study showed that there was a high prevalence of work-related respiratory

symptoms in bakers, i.e. 7% for chest tightness, and 21% for rhinitis, measured by self-administered questionnaires. In theory, two main groups of symptomatic bakers should be distinguished. First, bakers with an IgE-mediated allergic reaction to occupational allergens. Second, (atopic) workers with a non-specific hyper-responsive reaction to agents at work.

The first group of symptomatic workers are bakers with an IgE-mediated allergic reaction to occupational allergens. Immunologic investigations among selected bakers with work-related asthma have shown that wheat flour and α-amylase are the allergens to which most asthmatic bakers had produced IgE-antibodies (Baur *et al.* 1989; Wüthrich & Baur 1990; Jorde *et al.* 1986). In our study population, however, only 30% of the symptomatic bakery workers had detectable IgE antibodies against wheat or α-amylase. This suggested that only a proportion of the work-related symptoms could be attributed to IgE mediated allergies to wheat and α-amylase. However, these allergens may have been responsible for the more severe work-related symptoms. In our study, a higher percentage of asthmatics was found in the group of symptomatic workers with sensitization to wheat and α-amylase, compared with the symptomatic bakers without sensitization.

In the majority of symptomatic bakers in our study, no IgE antibodies to occupational allergens were found. The actual cause of respiratory symptoms in this group was unknown. However, it is possible that some of these symptoms can still be attributed to an IgE-mediated allergy to occupational allergens. First, our IgE-assays may have failed to detect sensitization to wheat or α-amylase in some individuals, although the sensitivity and specificity were comparable to other (commercial) IgE assays. In the subpopulation in which also skin prick tests were performed (chapter 6), a positive skin-prick test was occasionaly found for wheat flour or α-amylase in bakers with a negative IgE-test. Second, although wheat and α-amylase are generally considered as the most important occupational allergens in the baking industry, many other airborne agents in the bakery industry are capable of inducing IgE-mediated allergic reactions in the respiratory tract (see chapter 2). Most noticeable are probably rye flour, enzymatic baking additives such as glucoamylase and cellulase (both from *Aspergillus niger*), cereal malt flour, soybean flour, or lecithin from soybean flour. In our study, only rye flour was included in the panel of occupational allergen extracts in the skin prick tests. Of the 169 bakery workers tested, eight (5%) were sensitized to rye flour. However, most of the bakers sensitized to rye flour were also sensitized to wheat flour (63%), probably due to

cross-reactivity of cereal allergens (Baldo *et al.* 1980; Block *et al.* 1984; Sandiford *et al.* 1995). The other potential allergens have not been tested in our study, nor in other epidemiologic surveys, with the exception of soybean flour (Jeffrey 1992). In that study, 3% of the 205 bakery workers in the study had detectable IgE antibodies to soybean flour. Further research to the sensitizing capacities of other potential allergens in population studies is needed.

The second group of symptomatic workers that could be distinguished were (atopic) bakers with a nonspecific airway hyperresponsiveness and/or bakers with a pre-existing allergy or asthma exacerbated at work. This is probably a large proportion of the symptomatic bakers in our study population. In the group of symptomatic bakers without sensitization to wheat or α-amylase, the majority (78%) had IgE-antibodies to non-occupational allergens (58%), had a history of allergic symptoms to common allergens (27%), or reported chronic respiratory symptoms outside the job environment (55%). It is possible that these individuals have symptoms not only during work, but also outside the working-environment. We don't have much information about the severity of these work-related symptoms. As mentioned, the proportion of bakers with chest-tightness in this group of responders was lower compared with the bakers with sensitization to occupational allergens. Repeated peak flow measurements in these individuals could provide more information about the severity and the work-relatedness of these symptoms.

In our study population, a third group of symptomatic workers were bakers who reported work-related symptoms but were not sensitized to occupational or common allergens, nor reported any other respiratory symptom outside the bakery environment. It is not clear what may have caused these symptoms, but they may have been due to a non-specific reaction to the dusty environment in the bakery industry. Incidentally, high levels of personal dust exposure levels were found, e.g. eight percent of all personal samples in our study were above 5 mg/m^3.

In several other epidemiologic surveys among bakery workers high prevalence rates of work-related respiratory symptoms have been found as well, varying from 14 to 29% (Prichard *et al.* 1984; Musk *et al.* 1989; Cullinan *et al.* 1994a; De Zotti *et al.* 1994). In most of these studies, the causes of respiratory symptoms reported by the bakery workers have not been discussed. Based on the information from case studies among selected asthmatic bakery workers, one would have expected that the majority of the symptoms were caused by cereal flours and α-amylase. However, our study results suggest that allergic reactions to these allergens account for only a

small proportion of the symptoms reported in cross-sectional studies. This was also suggested by Cullinan and co-workers (1994a), who found only a weak association between symptoms and specific sensitization (to wheat flour and α-amylase) in a cross-sectional study among 264 bakery workers in the UK. They concluded that respiratory symptoms of bakery workers predominantly had a non-allergic basis. A possibility may be inflammatory reactions induced by certain agents at the workplace. One possibly important component may be glucans that are present in cereal flours (Stone & Clarke 1992). Recently, it has been suggested that ß-1,3-glucans from moulds can play a role in bio-aerosol induced inflammatory responses and result in respiratory symptoms and complaints (Rylander *et al.* 1992; Fogelmark *et al.* 1994). It is possible that glucans in plants are also capable of inducing inflammatory reactions. Further identification of the causes and mechanisms of respiratory symptoms among bakery workers is needed.

Exposure-response-relationships

This study showed that the level of exposure to allergens was an important determinant of baker's allergy. Two types of exposure-response relationships should be distinguished. First, the relationship between allergen exposure and allergen specific sensitization. Second, the relationship between allergen exposure and the occurrence of work-related respiratory symptoms in sensitized individuals.

The relation between allergen exposure and sensitization was studied in detail for α-amylase (chapter 6). A very strong and positive relation was found, especially among atopic workers. An exposure-sensitization-relationship for wheat flour was described in chapter 7. Also for this occupational allergen a statistically significant association was found between allergen exposure level and the prevalence rate of sensitization. Again, atopy appeared to be an important effect modifier of this relationship, and the strongest relationship was found within the group of atopic workers. The interpretation of the exposure-sensitization relationships is relatively simple, as both exposure and health-endpoint are specific for the allergen that was studied. The strong associations between exposure and response suggest that the incidence of sensitization and IgE-mediated allergic reactions can be effectively reduced by a reduction of allergen exposure levels.

Once sensitized, workers may develop allergic symptoms upon renewed exposure to the specific allergen. It is often stated that strongly sensitized individuals may develop attacks of asthma at very low exposure levels. This conclusion, however, is

probably mainly based on the experience of patients visiting medical centres for their usually severe asthmatic symptoms. In fact, exposure-response-relationships for sensitized individuals have not been studied yet in open populations of bakery workers. Our study results revealed that there is also an exposure-related response within the group of sensitized bakers, both for α-amylase and wheat allergens. These results should be interpreted with some caution, because the number of sensitized individuals in our study was small. Moreover, the degree of anti-allergen reactivity (measured as the wheal diameter of the skin prick tests or the titer the IgE analysis) was different for each individual and the exposure-response-relationship may vary from individual to individual. Nevertheless, the demonstration of an exposure-response-relationship within the range of prevailing exposure levels, implicates the possibility of intervention, also for sensitized individuals. The confirmation and characterization of this relationship between exposure and symptoms should be an important scope for further research. Repeated peak flow measurements could be used to reveal the severity of airway responsiveness in relation to allergen exposure levels.

Some indications for exposure-response-relationships for baker's asthma have been described earlier for inhalable flour dust (Hartmann 1986; Musk *et al.* 1989) and wheat allergens (Cullinan *et al.* 1994a). No exposure-response-relationships have been reported for α-amylase exposure yet. Associations between various indicators of exposure and the occurrence of occupational asthma have also been found in other occupational environments, e.g. in laboratory animal facilities (Kibby *et al.* 1989; Cullinan *et al.* 1994b; Hollander *et al.* 1996), in red cedar sawmills (Vedal *et al.* 1986; Brooks *et al.* 1981), in the electronic industry (colophony fumes from soldering; Burge *et al.* 1979; Burge *et al.* 1981), and among workers exposed to epoxy resins (acid anhydride exposure; Venables 1989). However, most studies used surrogates of exposure, such as perceived dustiness, the number of hours per day worked with animals, etc. Only few investigators have studied exposure-response relationships based on quantitative exposure assessment as we have done in our study. The finding of exposure-response gradients for occupational asthma provides important evidence of causation and suggests that occupational asthma is to some extent preventable by reducing exposure levels. In the enzyme detergent industry, reduction of exposure levels of potentially asthma-provoking substances has dramatically reduced the proportion of sensitized workers (Gilson *et al.* 1976; Juniper *et al.* 1977). However, it is not clear if the reduction in disease can only be

attributed to the reduction of exposure levels. Simultaneous to measures for exposure reduction, atopic job applicants were refused to start working at the enzyme factory.

Studies of occupational asthma may also provide a useful model for studying asthma in general, caused by non-occupational exposures at home or elsewhere. In those situations it is often difficult to detect exposure-response-relationships due to disease driven exposure reduction. As a result, exposure levels have sometimes even been found to be inversely related with symptom prevalence (Verhoeff *et al.* 1994).

In some reviews on occupational asthma, it has been suggested that short periods of high exposure are more important for developing asthma than an equivalent dose accumulated at a lower exposure level over a longer period of time (Malo & Chan-Yeung 1993; Venables 1994). However, this hypothesis has not been tested for baker's asthma. It would need detailed information on peak exposure levels in bakeries, but until now, only few data are available. As part of our project, peak exposures were measured in small bakeries by personal sampling with a Miniature Real Time Aerosol Monitor (Miniram) for several hours (Jongedijk *et al.* 1995). In addition, time-weighted average dust exposure levels with personal sampling equipment were determined during the same shift. A high correlation was found between the number of peak exposures and the full shift time-weighted averages, suggesting that high peak exposures contributed strongly to the 8 hour exposure levels, and that high peak exposures and high daily averages were difficult to disentangle. It implicates that the importance of peak exposures as a major cause of occupational asthma will be difficult to prove.

Bias and confounding

A major problem in cross-sectional epidemiologic studies on respiratory disorders is selection bias caused by the healthy worker effect. One of the factors that contribute to the healthy worker effect is pre-employment selection. Persons with respiratory symptoms may tend not to choose jobs that burden their respiratory system if they already suffer from airway diseases, or may be denied by occupational physicians during pre-employment screening. Unfortunately, we have no information on these potential selection processes and it could therefore not be evaluated whether this selection introduced bias in the exposure-response-

relationships. The second kind of selection takes place during employment. Bakers with respiratory allergies may have left to other job titles with low allergen exposure, or may have exchanged the bakery work for another job without allergen exposure. Exposure-response-relationships found in our study may have been influenced by this selection bias. This is illustrated in chapter 6 and chapter 7 for the group with very high α-amylase allergen exposure. Sensitization to α-amylase is most prevalent in workers who are working or had worked in this category, but the prevalence of work-related symptoms and the degree of in vitro and in vivo reactivity in this group was low. It is possible that workers who developed respiratory symptoms have left their job location, because of the high allergen exposure levels. However, because this selection is dependent on exposure level, it is likely that underestimation of the observed exposure-response relationships has occurred. The magnitude of the selection bias can only be studied in a longitudinal study. Unfortunately, cohort studies in which respiratory allergies among bakery workers were investigated are not very common due to lack of funding and the long time span between the start of the study and the actual results.

Information bias occurs when the procedures of conducting exposure or effect measurements result in associations that are different from the true association between exposure and effect. Observer bias may have occurred if the determination of the endpoints would depend on knowledge of the exposure status. Such bias may have occurred for the respiratory symptoms reported by the bakery workers. It may be possible that some workers in our study had been incorrectly classified as having work-related symptoms. However, most of the exposure-response-relationships described in our study were studied with more objective health endpoints as sensitization or respiratory symptoms in combination with specific sensitization. It is therefore not very likely that observer bias have influenced our study results. Information bias may also have occurred due to random errors in the measurement of the exposure variables. However, since this nondifferential misclassification generally reduces the power to detect existing associations, it does not explain the observed exposure-response-relationships.

Confounding bias occurs when the observed association between exposure and effect differs from the true association because of a third variable that is correlated with the exposure variable and the health endpoint. This third variable has to be an independent risk factor of the health endpoint. For the health endpoints in our study (specific sensitization and respiratory symptoms), age and smoking habits are

usually considered as potential confounding factors. In our analyses, we have adjusted for these confounders, and therefore, confounding will probably not have affected our study results. However, it can not be excluded that due to error in the measurement of confounders, some bias may have occurred (Rothman 1986).

Measuring airborne allergens

In this study immunoassays have been developed for measuring airborne allergens of wheat flour and fungal α-amylase. The main goal of our epidemiologic study was to explore relationships between the level of allergen exposure and the prevalence of specific sensitization or respiratory symptoms caused by these allergens. Therefore, immunoassays were needed that had to meet certain requirements on validity, specificity, sensitivity and reproducibility. The results in chapter 3 and 4 showed that both assays met all requirements of validity and specificity and that the assays were sensitive enough to discriminate between several levels of aero-allergen exposure in personal samples. Moreover, the same assay materials were used during the entire project and therefore the results of the assays were consistent during the study period.

Although the exposure assessment in our study was internally consistent, problems may arise when allergen exposure levels in our study are to be compared with exposure levels reported in other studies. Assay characteristics may differ from study to study, and especially the use of different reference preparations and antibody sources may hamper the comparison of study results. Assuming that there is only a systematic difference between the assays, the exchange of samples and reagents could reveal a conversion factor. Such experiments have been done for our α-amylase assay. At the department of Occupational Medicine of the Sahlgren Hospital in Göteborg (Dr L Belin & Dr A Ståhl), an alternative assay was developed for measuring fungal α-amylase allergens in environmental samples. In a series of sample extracts, α-amylase allergen concentrations were measured with both assays, as shown in Figure 8.1 (unpublished results). There was a very high correlation between the results of both assays. However, the absolute concentrations were quite different, with the Dutch assay resulting in six times higher α-amylase concentrations. The extracts that were tested in the assays were coming from exactly the same personal samples. Differences could therefore only be attributed to

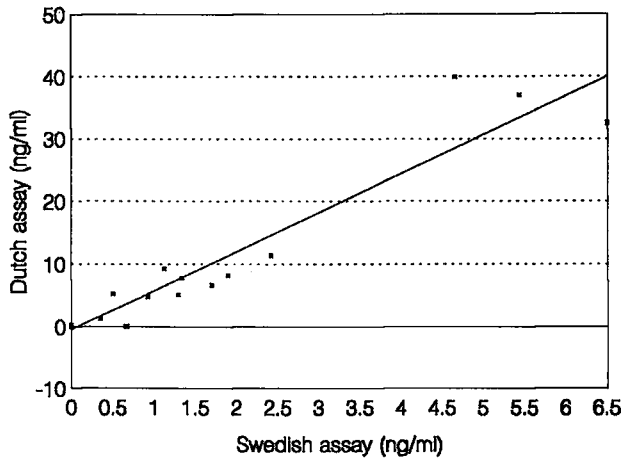

Figure 8.1 Comparison of two sandwich-assays for measuring fungal α-amylase allergens. Results are expressed as protein-concentration.
For all data (N=29): Y = -0.49 + 6.23*X (corr. coeff. = 0.96)
For data>detection limit (N=12): Y = -0.80 + 6.35*X (corr. coeff. = 0.94)

differences in assay and not to differences in sampling procedures or processing of samples. This clearly shows that absolute allergen exposure levels reported in different studies can not be compared when different assays have been used, but that a conversion factor needs to be determined.

One possibility for establishing inter-laboratory comparison, is the development of more standardized assays. This is necessary not only for a good comparison of allergen exposure levels reported in several epidemiologic studies, but especially when occupational exposure limits for these allergens are considered. A desirable goal would be to assess allergen concentrations with the use of a purified protein standard - preferentially a major allergen - for each specific allergen and monoclonal antibodies to these allergens. If the allergen is a single protein, this would be a realistic approach. This may be possible for α-amylase allergens. Although commercially available extracts of fungal α-amylase contain several allergens (Sandiford *et al.* 1994b; Baur *et al.* 1994a; Moneo *et al.* 1995; Houba *et al.* 1996b), one major protein was detected in all studies and all tested sera from allergic bakers had IgE antibodies to this protein. Baur and co-workers (1994a) have demonstrated that this protein is the active α-amylase, and have shown the allergenicity of a highly purified enzyme preparation. If a monoclonal antibody

against this purified allergen can be produced, it is possible to develop a standard assay which could become commercially available. Although this is probably a realistic approach for the development of a standardized assay for fungal α-amylase allergens, it may not be a suitable approach for allergens from wheat flour. Wheat flour is a complex mixture of many allergenic molecules (Blands *et al.* 1991; Fränken *et al.* 1991; Pfeil *et al.* 1990; Sandiford *et al.* 1990; Sandiford *et al.* 1995). Sera from sensitized bakers show IgE reactions with many of these proteins, but importantly, the reaction profile may differ markedly between individual sera. As noticed by Sandiford (Sandiford *et al.* 1994a), a standard assay for measuring airborne allergens from wheat flour would entail the use of an immense number of monoclonal antibodies in order to detect the large number of proteins in flour that are potentially allergenic. Assessment of a 'marker' protein would be an alternative. However, some of the major allergens detected in wheat flour belong to certain enzyme inhibitor families, which can be found in other cereal species or grass species as well (Adachi *et al.* 1995; Garcia-Olmedo *et al.* 1987; Mena *et al.* 1992). Immunoassays based on these monoclonal antibodies and standards will not be specific for one cereal flour.

Implications for occupational health and the bakery industry

Prevention of baker's asthma

Because baker's asthma is a common disease among bakery workers that can cause permanent working disability, surveillance and prevention programs should be developed to reduce the incidence of this occupational disease. In order to achieve an effective control, the causal agents and other determinants of the disease must be identified, the relationship between exposure and response must be quantified, and the effectiveness of control measures in reducing the incidence of asthma must be measured (Venables 1994). This study and several other studies have determined atopy as an important risk factor for baker's asthma (Järvinen *et al.* 1979; Hartmann 1986; Rosenberg *et al.* 1991; De Zotti *et al.* 1994). Moreover, recent studies have identified the level of exposure as an important determinant (Hartmann 1986; Musk *et al.* 1989; Cullinan *et al.* 1994a), and this study has provided important additional information on exposure-response-relationships. No risk factors other than atopy and exposure have been described for baker's asthma. Therefore, prevention strategies

should be aimed at these two determinants.

One way to prevent baker's asthma is reduction of environmental exposures to the causative agents. The exposure-response-relationships described in chapter 6 and 7 suggested that reduction of allergen exposure levels may reduce the number of sensitized bakery workers. With the information on exposure-response-relationships, it is possible that levels of exposure could be determined that would preclude sensitization and the development of occupational asthma. However, two levels should be distinguished: the level of allergen exposure which will elicit symptoms in sensitized individuals and the levels of exposure sufficient to induce the sensitized state in persons who are not yet sensitized (Venables 1994). The best approach would probably be to aim for reduction of exposure levels below that which is known to induce sensitization. The development of immunoassays for wheat flour and fungal α-amylase has made it possible to identify thresholds for the risk of sensitization and symptoms for these agents. Moreover, allergen sources can now be more precisely determined, and the effects of a reduction in allergen exposure better evaluated.

Identification and exclusion of atopic workers may be another option for preventing occupational asthma. Atopy is shown to be a predisposing factor for baker's asthma. However, the prevalence of atopy in the general population is high, and the association between atopy and occupational allergy is not absolute. For instance, in our study population described in chapter 6, 38% of all workers could be classified as atopic, but only a minority of them (37%) was sensitized to one of the occupational allergens, and only half of the sensitized workers reported work-related respiratory symptoms. Depending on how atopy is defined, this approach may mean denying employment to approximately one third of all job applicants, of whom only a proportion would develop occupational asthma (Venables 1994). Moreover, a substantial proportion of subjects who will develop respiratory symptoms is not atopic. In practical terms, the risk of developing symptoms is not sufficiently high to justify the exclusion of atopics from work in the bakery industry. Atopy is of considerable academic interest for studying the development of occupational asthma, but using it as pre-employment screening factor is questionable (Hendrick 1994). This means that there are insufficient data available to provide criteria for the exclusion of individuals from certain jobs on the basis of pre-employment screening, except for a known history of sensitization to a specific agent present in the workplace (Balmes 1991). More valuable could be a

surveillance program of workers perceived to be at risk (based on atopic status or exposure level), so that the development of baker's asthma is recognized early in it's course, thereby preventing progression to moderate or severe disease with its associated morbidity and disability (Hendrick 1994).

Standard setting

At the moment, no specific standards are available for occupational exposures in the bakery or flour industry. Most hygienist have considered flour dust as nuisance dust, for which the standard in The Netherlands is set at 10 mg/m^3 for an eight-hour time weighted average. However, flour dust contains several individual active components which can cause sensitization and allergic symptoms in exposed workers. In all published studies, health-effects have been demonstrated in bakery workers exposed to flour dust levels between 2-5 mg/m^3, but allergic and other inflammatory reactions probably occur at much lower levels. Recently, the National Health Council in the Netherlands has proposed an occupational exposure limit for grain dust of 2 mg/m^3 as an eight hour average. However, in that document, the definition of grain dust has explicitly excluded flour dust from milled grain, because the constituents of grain dust and flour dust are often quite different, and the type of respiratory disorders caused by grain dust (predominantly chronic bronchitis) is different from the respiratory disorders caused by flour dust (predominantly allergic rhinitis and asthma). For flour dust, a separate standard should be considered.

First of all, it has to be decided for which agent a standard should be established: a rather non-specific standard for inhalable dust, or separate standards for each specific allergen. The primary aim should be the prevention of sensitization to occupational allergens. Therefore, separate standards for wheat and α-amylase exposure should be determined. However, since there is a reasonable correlation between flour dust and wheat allergens, a standard for inhalable flour dust could probably be used as an practical alternative for wheat allergens. In most situations, a reduction of inhalable dust levels would also lead to a reduction of wheat allergen levels, and a standard for inhalable dust may be the most practical approach. Our study results showed a statistically increased risk for wheat flour sensitization at dust exposure levels of 2.4 mg/m^3. However, increased prevalence rates of wheat flour sensitization were also found in bakery workers exposed to flour dust levels of approximately 1 mg/m^3, although not significantly different from the lowest exposed group of bakery workers. This may mean that an occupational exposure limit for

flour dust must be lower than 1 mg/m^3, to prevent sensitization to wheat allergens.

For fungal α-amylase enzymes a separate exposure limit should be established. For this allergen we have established a very strong exposure-response-relationship, and the correlation with inhalable dust or flour allergen levels is low. Dose dependent sensitization was found at very low allergen concentrations (0.25 ng/m^3, measured as time weighted average over 8 hour). As mentioned before, the introduction and successful application of standards for allergen exposure will highly depend on the existence of methods of exposure assessment that are reproducible in different labs. Much work has still to be done in the development of standardized assays, before quantitative exposure limits can be established for occupational allergens.

Summarizing conclusions

The results of this study lead to the following summarizing conclusions:

1. Wheat flour and the enzymatic dough improver fungal α-amylase, appeared to be important occupational allergens in our population of bakery workers, with prevalence rates of sensitization of 10% and 7% respectively.

2. It is possible to measure allergens from wheat flour and fungal α-amylase in personal samples of bakery workers.

3. Large differences in α-amylase allergen exposure levels were found between bakeries. Small but significant differences in wheat allergen exposure levels were found between bakeries.

4. Personal inhalable dust levels can not be used as a surrogate for describing α-amylase allergen exposure levels.

5. There is a high prevalence of work-related respiratory symptoms in bakery workers, i.e. 7% for chest tightness, and 21% for rhinitis.

6. Work-related rhinitis or asthma may have been caused by IgE-mediated reactions to occupational allergens, but also by non-allergic or inflammatory

responses to other components of the flour dust. The IgE-mediated allergies are probably responsible for the more severe work-related symptoms, as more asthmatics were found in the group of workers with specific sensitization to occupational allergens.

7. A strong and positive relation was found between full-shift personal allergen exposure levels and specific sensitization in bakery workers, both for α-amylase allergens and for wheat flour allergens.

8. Atopy is an important modifier of these exposure-response-relationships, with the strongest relationships found within the group of atopic workers.

9. Fungal α-amylase is a very potent allergen, for which increased risks of sensitization were found at very low allergen exposure levels.

10. Sensitization to occupational allergens is strongly associated with work-related symptoms.

11. Our study results also suggest an exposure-related response for symptoms within the group of sensitized workers, both for α-amylase and wheat allergens.

12. Reduction of allergen exposure may lead to a substantial reduction in the incidence and prevalence of occupational sensitization and symptomatic allergy among bakery workers.

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Summary

Chapter 1 summarises the background of the study. In the Netherlands, approximately 50,000 people are employed in the flour and bakery industry. It is estimated that the population potentially exposed to flour and flour related products, is approximately 32,000. Adverse health effects from workplace exposures in the bakery industry have been described in a large number of studies. Especially bakery workers involved in dough production and dough forming have increased risks for occupational diseases. Several disorders have been related to the bakery occupation and are shortly discussed in chapter 1: baker's respiratory allergies, baker's eczema, baker's caries, and respiratory cancers. Respiratory allergies are by far the most prevalent occupational disease among bakery workers and this is also the main subject of this thesis.

In **Chapter 2**, a review of the literature on occupational respiratory allergy in bakery workers is presented. Respiratory disorders in bakery workers caused by exposure to agents at work is a serious occupational health problem. Baker's asthma is probably the most serious manifestation of occupational allergy among bakery workers and is often severe enough to cause permanent working disability. It is caused by immunologic sensitization and subsequent allergic reactions in the airways to occupational specific airborne allergens. Less severe types of baker's allergy are rhinitis and conjunctivitis. There are no reliable data available on the incidence of baker's allergy, but occupational disease registrations in several countries show that baker's asthma is one of the most frequent forms of occupational asthma. In Germany, more than 1200 bakers with occupational asthma claim industrial compensation annually. No information is available on the prevalence or incidence of baker's allergy in The Netherlands. Immunologic investigations among selected bakers with work-related asthma have shown that cereal flours and fungal α-amylase are the allergens to which most asthmatic bakers produce IgE-antibodies. Fungal α-amylase is an enzyme that is routinely added to baking flour (in milligrams per kg flour) to hasten the baking process and improve bread quality. However, several other allergens have been identified in case series of asthmatic bakers, e.g. glucoamylase, (hemi)cellulase, cereal malt flours, soybean flour, lecithin from soybean flour, moulds, and egg material. Many epidemiologic surveys have been performed, estimating the prevalence of baker's allergy. Most epidemiologic surveys have been cross-sectional in design and several have

important methodological shortcomings. Available studies show prevalence rates of work-related eye/nose symptoms of 14-29%, while prevalence rates of chest symptoms vary from 5-14%. The prevalence rate of sensitization varies from 5 to 15% for wheat flour and from 5 to 9% for α-amylase. Many investigators have tried to identify the causes of baker's allergy but most of them focused on host factors only, especially allergic disposition of the bakery worker. Only few have studied the environmental determinants of respiratory allergy among bakery workers, particularly the level of allergen exposure required for sensitization or the occurrence of symptoms. The most important reason for the limited attention to environmental factors is that instruments for the measurement of personal exposure to airborne allergens were not available. Nevertheless, available studies have shown that atopy and exposure are the most important risk factors for both sensitization and work-related symptoms. Age, gender, and smoking habits have usually not been reported as significant risk factors. The role of atopy has been described for a long time. In more recent studies, dust and wheat allergen exposure was measured and identified as an important determinant of baker's allergy. Thus far, exposure-response-relationships have been reported for flour dust and wheat allergens, but not for fungal α-amylase.

Chapter 3 describes an assay for measuring airborne wheat flour allergens in bakeries. In twenty one bakeries, personal inhalable dust samples were collected in the workers breathing zone during full-shift periods of 6-8 hours. A total of 546 personal measurements were available from 221 workers. In 449 of these samples wheat flour allergens were determined, using an inhibition enzyme immuno assay (EIA), and an anti-wheat IgG_4 serum pool from bakery workers. Western-blotting was performed to compare the wheat flour proteins detected by IgE from allergic bakers and IgG_4 from our serum pool. This showed that many wheat flour proteins can bind IgG_4 and IgE, but also a reasonable similarity in major allergens detected by both types of antibodies. 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. Bakers working in the doughmaking area usually had the highest dust exposure with (geometric) mean exposure levels varying from 0.9-4.5 mg/m^3. Mean dust exposure levels for other workers in large bakeries was below 1 mg/m^3. Dust exposure levels in small traditional bakeries was comparable with dust exposure levels for

doughmakers in the large industrialized bakeries. For personal wheat allergen exposure a similar trend was found, with doughmakers, again, in the highest exposure category. However, for several groups clear differences in wheat allergen exposure existed, where no differences in dust exposure levels could be found. The relation between dust and wheat allergen exposure varied considerably, depending on the specific bakery occupation, the size of the bakery, and the type of product produced by the bakery. Therefore, dust exposure levels will often be only a crude approximation of wheat allergen levels. Measurement of airborne wheat allergens in bakeries will probably be essential for epidemiologic field studies focusing on exposure-response-relationships.

In **Chapter 4** an assay for measuring airborne allergens from fungal α-amylase is described. In several studies sensitization and respiratory disorders among bakery workers were reported, caused by enzymes in dough improvers. Fungal α-amylase is the most important cause of allergy. α-Amylase allergen exposure levels in the bakery industry, however, have not been reported yet. We have therefore developed a sensitive sandwich enzyme immunoassay, using affinity purified polyclonal rabbit IgG antibodies, to measure α-amylase allergens in 507 personal samples of airborne dust taken in bakeries. The validity of the assay was assessed by immunoblotting experiments, in which the reaction profile of the polyclonal rabbit IgG antibodies was compared with that of IgE antibodies in the sera of α-amylase sensitized bakery workers. IgE from sensitized bakers and the rabbit IgG antibodies had similar reaction profiles to a commercially available α-amylase extract. The assay appeared to be specific for fungal α-amylase, although some reactivity was noted for extracts of the mould species *Aspergillus oryzae* and some other *Aspergillus spp.*. The detection limit of the sandwich assay was 100 pg/ml. Ninety three samples (18%) had detectable concentrations of α-amylase. Allergens from α-amylase could only be measured in samples of bakers working in, or close to the doughmaking areas, where bread improvers are added to the dough. Allergen exposure levels varied considerably among bakery workers, with mean exposure levels for occupational titles varying from 0 to 40 ng/m^3. The type of bakery in which the bakers were working was very important for the level of α-amylase exposure. In confectioneries, α-amylase is not used in doughmaking, and therefore no fungal amylase exposure exist. A cascade impactor was used to estimate the size of dust particles carrying the α-amylase allergens, showing that α-amylase allergens

are present in particles ≥ 5 μm. In most bakeries, more than half of the α-amylase is in the dust fraction with particle sizes lager than 9 μm. This means that α-amylase allergens are most likely to deposit in the nose and the ciliated airways.

In **Chapter 5** the characteristics of exposure to dust, wheat allergens and α-amylase allergens are studied. For each measure of exposure, repeated measurements on individuals were available, and the main components of exposure variability were determined. Different grouping strategies for studying exposure-response relationships were compared. Bakers of the large industrialized bakeries were divided into 6 occupational groups according to their job description (e.g. doughmaker, packer, etc.). Bakers from small traditional bakeries were divided into 3 occupational groups according to whether they produced bread, pastry, or a mixture of the two. Furthermore, four types of bakeries were distinguished with regard to the type of product: wheat bread production, production of confectionery, production of crispbakes, and rye bread production. The specific job of a bakery worker was identified as the most important source of variability in inhalable flour dust concentrations. For exposure to wheat allergens, the job performed also was the most important source of variation, but type of bakery explained some variability as well. For α-amylase allergen exposure, information on type of bakery was more important then job information. For exposure to inhalable dust and wheat allergens, a classification by job title would lead to sufficient contrast in average exposure levels. In contrast, a grouping strategy based on a combination of job and type of bakery appeared to be essential to obtain a meaningful classification of exposure to α-amylase allergens.

Chapter 6 describes exposure-sensitization-relationships for α-amylase allergens. In a cross-sectional study, sensitization to occupational allergens and work-related symptoms were studied in 178 bakery workers and related to allergen exposure. All workers were categorized into groups, based on their total job histories and the α-amylase exposure levels of their job titles. As much as 9% of the bakery workers showed a positive skin prick test reaction to fungal α-amylase and in 8% amylase-specific IgE was demonstrated. In the whole population, the sensitization rate increased from 1.4% in the low exposed group, and 12.8% in the medium exposed group, to 30.4% in the high exposed group. Atopy appeared to be an important effect modifier of this relationship, and the steepest slope between allergen level

and sensitization rate was found within the group of atopic workers. No clear exposure-response-relationship was found in non-atopic workers. In a multivariate analysis, α-amylase exposure and atopy appeared to be the most important determinants of skin sensitization with prevalence rate ratios for atopy of 20.8 (95% Confidence Interval (CI) 2.74-158) and for medium and high α-amylase exposure groups of 8.6 (95% CI 1.01-74) and 15.9 (95% CI 1.95-129), respectively. No association was found between α-amylase sensitization and smoking habits, age or years in industry. Furthermore, a positive association was found between positive skin prick tests to α-amylase and work-related respiratory symptoms. This is the first study describing exposure-response-relationships for α-amylase allergens.

Chapter 7 presents the results of a cross-sectional epidemiologic survey among 393 bakery workers. In this chapter, the determinants of specific sensitization and work-related respiratory symptoms were studied, with a special focus on the role of allergen exposure levels. A strong and positive association was found between wheat allergen exposure level and the prevalence of wheat sensitization. Again, atopy appeared to be an important effect modifier of this relationship, and the steepest slope between allergen level and sensitization rate was found within the group of atopic workers. However, sensitization to wheat flour was also found in non-atopic bakers and for this group of workers an exposure-response-relationship was found as well. In our study population, 23% of the bakers reported work-related rhinitis or chest-tightness. Only a proportion of the symptomatic bakery workers (30%; 7% of all bakery workers) was sensitized to wheat flour or α-amylase. Therefore, two groups of symptomatic workers were distinguished, based on the presence or absence of sensitization for these allergens. In both groups, a relation between allergen exposure (to wheat and α-amylase) and respiratory symptoms was found, but the slope of the relationship was steeper in sensitized bakers than in non-sensitized bakers. It is not obvious what may have caused the work-related symptoms in the group of bakers without sensitization to wheat flour or α-amylase. Our results showed that these symptoms were also positively correlated with allergen exposure levels. Sensitization to other occupational allergens and a non-specific hyper-responsive reaction to the dusty environment in bakeries are the most plausible explanations. Although wheat flour and α-amylase allergens are probably responsible for only a third of the work-related symptoms reported by the bakery workers, they may have been responsible for the more

severe symptoms. In our study, a higher percentage of workers with chest-symptoms was found in the group of symptomatic bakers without sensitization.

Chapter 8 presents a discussion on the most important findings, potential biases and potential implications for the prevention of respiratory allergies in the flour industry. The results of this study lead to the following summarizing conclusions:

- Wheat flour and the enzymatic dough improver fungal α-amylase, appeared to be important occupational allergens in our population of bakery workers, with prevalence rates of sensitization of 10% and 7% respectively.
- It is possible to measure allergens from wheat flour and fungal α-amylase in personal samples of bakery workers.
- Large differences in α-amylase allergen exposure levels were found between bakeries. Small but significant differences in wheat allergen exposure levels were found between bakeries.
- Personal inhalable dust levels can not be used as a surrogate for describing α-amylase allergen exposure levels.
- There is a high prevalence of work-related respiratory symptoms in bakery workers, i.e. 7% for chest tightness, and 21% for rhinitis.
- Work-related rhinitis or asthma may have been caused by IgE-mediated reactions to occupational allergens, but also by non-allergic or inflammatory responses to other components of the flour dust. The IgE-mediated allergies are probably responsible for the more severe work-related symptoms, as more asthmatics were found in the group of workers with specific sensitization to occupational allergens.
- A strong and positive relation was found between full-shift personal allergen exposure levels and specific sensitization in bakery workers, both for α-amylase allergens and for wheat flour allergens.
- Atopy is an important modifier of these exposure-response-relationships, with the strongest relationships found within the group of atopic workers.
- Fungal α-amylase is a very potent allergen, for which increased risks of sensitization were found at very low allergen exposure levels.
- Sensitization to occupational allergens is strongly associated with work-related symptoms.
- Our study results also suggest an exposure-related response for symptoms within the group of sensitized workers, both for α-amylase and wheat allergens.

- Reduction of allergen exposure may lead to a substantial reduction in the incidence and prevalence of occupational sensitization and symptomatic allergy among bakery workers.

Samenvatting

Hoofdstuk 1 beschrijft de achtergrond van de studie. In Nederland werken ongeveer 50.000 mensen in meelfabrieken, bakkerijen en fabrieken voor bakkerijgrondstoffen. Het aantal mensen dat blootgesteld is aan meelstof of aan meelstof gerelateerde produkten, wordt geschat op ongeveer 32.000. Verschillende beroepsziekten zijn tot nu toe in verband gebracht met de bakkerij industrie en worden achtereenvolgens kort besproken: luchtwegallergieën (bakkersallergie), bakkerseczeem, bakkers cariës en neus- en longkanker. Met name bakkers betrokken bij de deegbereiding hebben over het algemeen een verhoogd risiko voor deze beroepsgebonden aandoeningen. Van de genoemde aandoeningen komen luchtwegallergieën verreweg het meest voor en dit is ook het belangrijkste onderwerp van deze studie.

In **hoofdstuk 2** wordt een overzicht gegeven van de beschikbare literatuur op het gebied van beroepsgebonden luchtwegallergieën bij bakkers. Bakkers astma is de meest ernstige verschijningsvorm van de beroepsallergie, die vaak leidt tot ziekteverzuim of zelfs uitval naar de WAO. Minder ernstige verschijningsvormen van de ziekte zijn regelmatig terugkerende niesaanvallen en/of geïrriteerde ogen. Klachten ontstaan over het algemeen na een immunologische sensibilisatie voor één of meer beroepsallergenen. Er zijn geen betrouwbare gegevens beschikbaar over de incidentie van bakkersallergie. In sommige landen wordt echter een nauwkeurige registratie van beroepsziekten bijgehouden en hieruit blijkt dat bakkersastma één van de meest voorkomende vormen van beroepsastma is. In Duitsland komt een bakker met beroepsastma in aanmerking voor een uitkering of voor omscholing, en in dat land worden jaarlijks 1200 nieuwe gevallen van bakkersastma geconstateerd. Er is geen informatie beschikbaar over het voorkomen van bakkersallergie in Nederland, wegens het ontbreken van betrouwbare registraties. Immunologisch onderzoek bij astmatische bakkers heeft aangetoond dat meelstof van verschillende granen en enzymatische broodverbetermiddelen de belangrijkste oorzaken zijn van bakkersallergie. Van de enzymen is met name α-amylase in verband gebracht met luchtwegallergieën bij bakkers. Alpha-amylase is een enzym dat zorgt voor de aanmaak van suikers uit zetmeel; het versnelt het produktieproces en verbetert de kwaliteit van het brood. Er zijn echter ook andere allergenen beschreven die kunnen leiden tot beroepsallergie, bijvoorbeeld andere enzymen in broodverbetermiddelen (gluco-amylase, cellulase), mout van granen, sojabloem, lecitine uit sojabloem,

bepaalde schimmels en bepaalde ei-bestanddelen. Er zijn veel epidemiologische studies gedaan, die de prevalentie van bakkersallergieën hebben beschreven in een open populatie bakkerijmedewerkers. Dit zijn voor het overgrote deel dwarsdoorsnede-onderzoeken geweest, waarvan er veel belangrijke methodologische tekortkomingen hebben. De beschikbare studies tonen echter aan dat werkgerelateerde neus- en oogklachten gerapporteerd worden door 14 tot 29% van de bakkers, terwijl benauwdheidsklachten voorkomen bij 5 tot 14%. Sensibilisatie tegen de twee belangrijkste beroepsallergenen, tarwe en α-amylase, komen voor bij respectievelijk 5-15% en 5-9% van alle bakkers. Veel onderzoekers hebben geprobeerd de oorzaken van bakkersallergie in kaart te brengen. De meesten hebben zich uitsluitend gericht op persoonsgebonden faktoren, waarvan atopie de belangrijkste is. Slechts enkelen hebben de omgevingsfaktoren in kaart gebracht, met name de blootstellingsniveaus die nodig zijn voor sensibilisatie of het ontwikkelen van klachten. Eén van de belangrijkste reden voor deze beperkte belangstelling voor blootstelling, is het ontbreken van instrumenten om de allergeenblootstelling in kaart te brengen. De beschikbare gegevens laten echter zien dat niveaus van blootstelling aan stof en een atopische status van bakkerijmedewerkers de belangrijkste risikofaktoren zijn voor het ontwikkelen van een bakkersallergie. Leeftijd, geslacht en rookgewoonten blijken niet van belang te zijn. De rol van atopie was al in veel onderzoeken beschreven. Recentelijk zijn er echter ook een paar studies gepubliceerd waarin blootstellingsmetingen zijn verricht en waaruit blijkt dat de hoogte van blootstelling aan stof of allergenen ook van belang is. Tot nu toe zijn er aanwijzingen voor een blootstellings-respons-relaties voor stof en tarwe-allergenen. Voor α-amylase zijn nog geen blootstellings-respons-relaties beschreven.

Hoofdstuk 3 beschrijft een immunoassay voor het meten van allergenen van tarwe in de omgevingslucht in bakkerijen. In 21 bakkerijen zijn persoonsgebonden stofmetingen uitgevoerd gedurende een volledige werkdag. In totaal zijn er 546 metingen beschikbaar, afkomstig van 221 bakkerijmedewerkers. In 449 van deze monsters zijn tarwe-allergenen bepaald, met een nieuw ontwikkelde inhibitie-immunoassay, waarbij gebruik wordt gemaakt van een pooled serum van bakkers met verhoogde IgG_4-titers tegen tarwe. Met behulp van electroforese en immunoblotting is het reaktiepatroon vergeleken van de serum-pool die gebruikt is in onze immunoassay, met het reaktiepatroon van IgE-antilichamen van allergische bakkers. Deze experimenten laten zien dat een groot aantal eiwitten in tarwe in staat

is om IgG_4 en IgE te binden. Belangrijker is echter dat het reaktiepatroon voor beide antilichaambronnen vergelijkbaar is, wat aangeeft dat onze meetmethode biologisch relevante stoffen meet. Inhibitie-testen met andere bakkerijgrondstoffen lieten zien dat er enige kruisreaktiviteit was met meel van andere graansoorten (rogge, gerst), maar er werd geen reaktie gemeten tegen andere potentiële verstorende stoffen. Binnen bakkerijen worden grote verschillen gevonden in blootstelling aan stof, die voornamelijk afhangt van de functie van de bakkerijmedewerker. De gemiddelde blootstelling van deegmakers is over het algemeen het hoogst en varieert van 0,9 tot 4,5 mg/m^3 in de verschillende bakkerijen. Voor andere werknemers in grote industriële bakkerijen is de gemiddelde blootstelling vaak onder de 1 mg/m^3. De blootstellingsniveaus in kleine ambachtelijke bakkerijen zijn vergelijkbaar met de niveaus gemeten bij de deegmakers van de grote bakkerijen. Voor tarwe-blootstelling wordt een vergelijkbare trend gevonden, maar er blijken ook belangrijke verschillen te bestaan. Vaak blijkt de blootstelling aan tarwe tussen groepen werknemers duidelijk van elkaar te verschillen, terwijl de blootstelling aan stof vergelijkbaar is. De verhouding stof/tarwe-allergenen blijkt sterk te variëren en is met name afhankelijk van het soort werkzaamheden van de bakker, de grootte van de bakkerij en het soort produkt dat door de bakkerij wordt gemaakt. Hieruit blijkt dat stofmetingen vaak alleen een ruwe schatting kunnen geven van de blootstelling aan tarwe-allergenen. Het meten van allergenen met de door ons ontwikkelde assay is een meer valide en een specifieker instrument om de blootstelling aan tarwe-allergenen in kaart te brengen en is wellicht van essentieel belang voor epidemiologische veldstudies die de relatie onderzoeken tussen blootstelling en effect.

In **hoofdstuk 4** wordt een assay beschreven voor het meten van allergenen van α-amylase. In verschillende studies zijn beroepsallergieën beschreven bij bakkers, veroorzaakt door enzymen uit broodverbetermiddelen. Alpha-amylase is hiervan de belangrijkste. Tot nu toe zijn er echter nog geen gegevens voorhanden over de blootstellingsniveaus van dit enzym in bakkerijen. In dit hoofdstuk wordt een sandwich immuno assay beschreven, waarbij gebruik wordt gemaakt van affiniteits gezuiverde polyclonale konijne antilichamen tegen α-amylase. Met deze methode is de concentratie α-amylase allergenen gemeten in 507 persoonlijke stofmonsters. De validiteit en specificiteit van de methode zijn uitgebreid getest. De polyclonale antilichamen van het konijn en IgE van gesensibiliseerde bakkers zijn gericht tegen

dezelfde eiwitten in het α-amylase extract. De assay blijkt specifiek te zijn voor α-amylase uit schimmels. Wel wordt enige kruisreaktiviteit gevonden met extracten van *Aspergillus oryzae*, waaruit het amylase wordt gewonnen, en enkele andere *Aspergillus* schimmelsoorten. De detectielimiet van de assay was 100 pg/ml. Desondanks werden in slechts 93 extracten detecteerbare hoeveelheden α-amylase allergenen gevonden. Het allergeen kon alleen worden gemeten in monsters afkomstig van bakkers die in of in de buurt van de deegmakerijen werkzaam waren. Er bleken enorme verschillen te bestaan in blootstellingsniveaus voor α-amylase tussen bakkers met verschillende functies. Ook bleken er grote verschillen te bestaan in allergeenblootstelling tussen verschillende bedrijfstypen. In banketbakkerijen wordt over het algemeen geen amylase toegevoegd aan het deeg en in deze bakkerijen konden dus ook geen allergenen van amylase worden gemeten. In een aantal bakkerijen is de deeltjesgrootte gemeten van het stof in bakkerijen. In elke fractie zijn vervolgens ook allergenen van amylase gemeten. De meeste allergenen van amylase bleken zich te bevinden in de grotere stoffracties (met deeltjesgrootte ≥ 5 μm).

In **hoofdstuk 5** wordt de variabiliteit in blootstelling aan stof, α-amylase allergenen en tarwe allergenen beschreven. Voor alle drie blootstellingsmaten zijn herhaalde metingen per individu beschikbaar. Voor onderzoek naar blootstellings-respons-relaties moeten werknemers worden ingedeeld in groepen met verschillende blootstelling. Dit moet zo worden gedaan dat het contrast in blootstelling tussen de groepen zo groot mogelijk is en dat de spreiding in blootstelling binnen de groepen zo klein mogelijk is. Indelingen zijn gemaakt op grond van de functie van een bakker en het type bakkerij waarin deze persoon werkzaam was. In de grote industriële bedrijven worden een 6-tal functies onderscheiden. Bakkers in kleinere ambachtelijke bakkerijen zijn ingedeeld in drie groepen: broodbakkers, banketbakkers en bakkers die beide werkzaamheden verrichten. Verder worden een viertal type bakkerijen onderscheiden op grond van het soort produkt: brood, banket, beschuit of roggebrood. Voor stofblootstelling blijkt functie de meeste variabiliteit in blootstelling te verklaren. Voor tarwe allergenen was functie ook de belangrijkste variantiebron, maar ook het type bakkerij bleek enige variantie in blootstelling te verklaren. Voor zowel stof als tarwe allergenen bleek een indeling op grond van functie voldoende contrast in blootstelling op te leveren. Voor allergenen van α-amylase was informatie over het type bakkerij echter van

essentieel belang om werknemers goed in te kunnen delen in groepen.

Hoofdstuk 6 beschrijft de relatie tussen blootstelling aan α-amylase allergenen en α-amylase specifieke sensibilisatie bij bakkers. In een dwarsdoorsnede-onderzoek zijn sensibilisatie en het voorkomen van werkgerelateerde klachten beschreven bij 178 bakkers. Werknemers werden ingedeeld in een aantal groepen met verschillende blootstelling aan α-amylase allergenen. In de totale populatie bleek 9% een positieve huidtest te hebben tegen amylase, terwijl in 8% van de sera IgE antilichamen tegen amylase konden worden aangetoond. De prevalentie van positieve huidpriktesten varieerde van 1,4% in de laag blootgestelde groep, tot 12,8% in de groep met middelmatige blootstelling, tot 30,4% in de groep bakkers met een hoge blootstelling aan α-amylase. Atopie bleek een belangrijke effect modifier te zijn en de sterkste blootstellings-respons-relatie werd gevonden binnen de groep atopische bakkers. Bij niet-atopici kon geen blootstellings-respons-relatie worden aangetoond. In een multivariate analyse bleken atopie en blootstelling de belangrijkste determinanten te zijn van sensibilisatie, met prevalentie ratio's voor atopie van 20,8 (95% betrouwbaarheidsinterval (btbhi) 2,74-158), en voor middelhoge en hoge blootstelling aan amylase respectievelijk 8,6 (95%btbhi 1.01-74) en 15,9 (95%btbhi 1.95-129). Leeftijd, rookgewoonten en aantal jaren werkzaam in de bakkerij bleken geen belangrijke determinanten te zijn van sensibilisatie. Wel was sensibilisatie voor α-amylase sterk geassocieerd met werkgerelateerde klachten bij bakkers. Dit is de eerste studie die blootstellings-respons-relaties beschrijft voor allergenen van α-amylase.

In **hoofdstuk 7** worden de resultaten gepresenteerd van een dwarsdoorsnede onderzoek bij 393 bakkers. De determinanten van sensibilisatie tegen beroepsallergenen en werkgerelateerde klachten zijn bestudeerd. Er bleek een sterk positieve relatie te bestaan tussen niveaus van blootstelling aan tarwe allergenen en het voorkomen van tarwe sensibilisatie. Ook hier bleek atopie een belangrijke effect modifier te zijn en de sterkste relatie werd weer gevonden binnen de groep atopische bakkers. Ook bij niet atopici bleek echter een duidelijke blootstellings-respons-relatie te bestaan. Een groot aantal bakkers in deze studie (23%) rapporteerde werkgerelateerde luchtwegklachten. Slechts een deel hiervan bleek echter gesensibiliseerd te zijn voor tarwe of α-amylase (30% of 7% van de totale populatie). Voor de beschrijving van de relaties tussen blootstelling en klachten

werden daarom twee groepen werknemers onderscheiden: werknemers met en zonder sensibilisatie tegen het betreffende allergeen. Voor beide groepen werd een positieve relatie gevonden tussen blootstelling en werkgerelateerde klachten, maar deze relatie was veel steiler voor de groep gesensibiliseerde bakkers. Een groot aantal klachten konden dus waarschijnlijk niet worden toegeschreven aan een IgE-gemedieerde allergische reaktie tegen tarwe of α-amylase. Het is niet helemaal duidelijk wat de oorzaak is van de overige klachten, maar een allergische reaktie tegen andere beroepsallergenen en aspecifieke reakties tegen stof in bakkerijen zijn de meest plausibele verklaringen. Hoewel allergenen van tarwe en amylase waarschijnlijk maar een deel van de klachten bij bakkers konden verklaren, zijn ze wellicht wel de oorzaak van de meer ernstige klachten. In de groep gesensibiliseerde bakkers bleek een hoger percentage mensen benauwdheidsklachten te hebben vergeleken met de niet gesensibiliseerde werknemers.

In **hoofdstuk 8** worden de belangrijkste bevindingen uit dit proefschrift bediscussieerd en de potentiële vormen van bias besproken. Verder worden de mogelijke implicaties voor de meelverwerkende industrie en de bedrijfsgezondheidszorg bediscussieerd. De samenvattende conclusies van het onderzoek zijn de volgende:

- Tarwe en α-amylase bleken binnen onze onderzoekspopulatie belangrijke beroepsallergenen te zijn. De prevalentie van sensibilisatie was respectievelijk 10% en 7%;
- Het blijkt mogelijk te zijn allergenen van tarwe en α-amylase te meten in persoonlijke stofmonsters van bakkerijmedewerkers;
- Er worden grote verschillen gevonden in α-amylase blootstelling tussen verschillende typen bakkerijen. Voor blootstelling aan tarwe worden kleinere verschillen gevonden tussen soorten bakkerijen, maar deze verschillen zijn wel significant;
- Resultaten van stofmetingen kunnen niet worden gebruikt om de blootstelling aan α-amylase allergenen te beschrijven;
- De prevalentie van beroepsgebonden luchtwegklachten bij bakkers is hoog, variërend van 7% voor benauwdheidsklachten tot 21% voor neus- en oogklachten;
- Een deel van de klachten wordt veroorzaakt door een IgE-gemedieerde allergische reaktie tegen tarwe en α-amylase, maar aspecifieke of

ontstekingsreakties spelen waarschijnlijk ook een belangrijke rol. De IgE-gemedieerde reakties zijn waarschijnlijk wel verantwoordelijk voor de meer ernstige klachten;

- Voor zowel tarwe als α-amylase worden sterk positieve relaties gevonden tussen het niveau van allergeenblootstelling en de prevalentie van allergeen-specifieke sensibilisatie;
- Atopie blijkt een sterke effect modifier te zijn. De sterkste blootstellings-respons-relaties worden gevonden bij atopische bakkers;
- α-Amylase uit schimmels blijkt een zeer potent beroepsallergeen te zijn. Sensibilisatie bij bakkers treed op bij lage blootstellingsniveaus;
- Sensibilisatie tegen beroepsallergenen blijkt sterk geassocieerd te zijn met het voorkomen van werkgerelateerde luchtwegklachten;
- Voor zowel tarwe- als amylase allergenen, lijkt ook binnen de groep gesensibiliseerde werknemers een relatie te bestaan tussen het niveau van blootstelling en het voorkomen van klachten;
- Reduktie van blootstelling aan allergenen in bakkerijen kan leiden tot een verlaging van de incidentie en prevalentie van beroepsgebonden allergieën bij bakkers.

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Curriculum Vitae

Remko Houba werd geboren op 6 november 1963 in Wageningen. In 1982 behaalde hij het VWO diploma aan het Marnix College te Ede. Hij studeerde Milieuhygiëne aan de Landbouwuniversiteit in Wageningen van 1982 tot 1988, met als specialisatie Arbeidshygiëne. Hij deed doctoraalonderzoeken bij de Vakgroep Gezondheidsleer en de Vakgroep Luchthygiëne en -verontreiniging. Na het behalen van het doctoraalexamen is hij anderhalf jaar voor beide vakgroepen werkzaam geweest als projektmedewerker. In deze tijd heeft hij meegewerkt aan een onderzoek naar respiratoire effecten als gevolg van blootstelling aan stof en endotoxine in de Nederlandse graan- en mengvoederindustrie. In het daaropvolgende jaar heeft hij zijn militaire dienstplicht vervult. In juli 1990 startte hij zijn promotieonderzoek bij de Vakgroep Humane Epidemiologie en Gezondheidsleer waarvan de resultaten in dit proefschrift zijn beschreven. Sinds april 1996 werkt hij in de Verenigde Staten bij het National Institute of Occupational Safety and Health te Morgantown, in het kader van een Research Associateship beurs van the National Research Council.