

Physical and Biochemical Properties of Airborne Flour Particles Involved in Occupational Asthma

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Aerosol particles which deeply penetrate the human airways and which trigger baker's asthma manifestations are known to represent only a part of flour and of airborne particles found in bakeries. They were a major focus of this study. To this end, aerosols were produced from different wheat and rye flours, using an automatic generator designed for bronchial challenge. Particles were characterized for their size distribution, their ability to be deposited in the airways, their protein content, their histological composition and their reactivity with immunoglobulin E (IgE) present in sera from asthmatic bakers. Like dust particles collected in the bakery, the aerosols produced showed increased protein content but decreased IgE reactive protein content when compared to the corresponding bulk flours. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of these particles showed a predominance of endosperm gluten proteins. Under scanning electron microscopy, flour particles displayed various tissue fragments with entrapped large A-starch and small B- or C-starch granules, whereas aerosol particles appeared primarily as a mixture of the endosperm intracellular interstitial protein matrix and small B- or C-starch granules free or still associated. These observations showed that aerosols supposed to penetrate deeply the airways, mainly correspond to intracellular fragments of endosperm cells enriched in gluten proteins but with lower amount of allergens belonging to albumins or globulins.

Keywords: aerosols; cereal flours; flour dust; inhaled particles; occupational asthma; rhinitis; *Secale cereale*; *Triticum aestivum*

INTRODUCTION

Flour for bread making is the most common source of allergens responsible for occupational asthma in France and other European countries. It induces baker's asthma and rhinitis with high incidence from inhaled airborne particles directly issued from wheat or rye flour or from flour additives such as fungal amylase (Houba *et al.*, 1998; Brisman, 2002; Ameille *et al.*, 2003; Bensefa *et al.*, 2004). Most of the allergens of cereal grains involved in asthma and rhinitis

belong to the albumins and globulins protein fraction of the grain. They are mainly enzymes, stress or defense proteins (Donovan and Baldo, 1993; Weiss *et al.*, 1997; Amano *et al.*, 1998; Baur and Posch, 1998). Their localization in the different tissue is not known with precision. Albumins and globulins are the only proteins present in the grain germ, which encompasses the embryo and the scutellum, and in the aleurone cell layer, which surrounds the starchy endosperm. In this last tissue, other albumins and globulins coexist with large amounts (~70 to 80%) of storage protein, mainly gliadins and glutenins which form the gluten and which are deposited exclusively in this tissue (Gianibelli *et al.*, 2001). At the cellular level, gliadins and glutenins look as an amorphous protein matrix deposited between starch

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granules in the endosperm cells. Starch granules are generally divided into large lenticular A type (10–38 μm) and small, round or polygonal B type (<10 μm). A third C type (<5 μm) has also been described (Bechtel and Wilson, 2003). Whole meal flours correspond to the grinding of the whole grain, encompassing the starchy endosperm tissue and the bran formed by the germ and by the aleurone layer attached to the outer pericarp. Normal white flour corresponds to enrichment in the starchy endosperm tissue (Evers and Bechtel, 1988).

For asthmatic patients, the inhaled dose of flour particles and their dose rate (amount of particles inhaled per time units) are the main factors in the onset of bronchial obstruction (Choudat *et al.*, 1999, 2002; Heederik and Houba, 2001). Little is known about these inhaled particles. They have a mean aerodynamic diameter <10 μm (Sandiford *et al.*, 1994a; Bogdanovic *et al.*, 2006). They correspond to a minor part of dust particles present in the atmosphere of bakeries (Sandiford *et al.*, 1994a). Most of the studies on the biochemical and allergenic composition of airborne particles have been done on these dust particles (Sandiford *et al.*, 1994b; Houba *et al.*, 1996). They showed not only the similarities of composition with flour but also the variability of composition of the collected samples, which depended on the location within the bakery.

There are different ways to artificially produce aerosol particles similar to those inhaled by bakers. Among them, the more convenient method is the use of an automatic aerosol generator (Système automatisé de génération d'aérosols solides—SAGAS) (Fabriés *et al.*, 2000). This device was originally designed to test patients with occupational asthma triggered by flour cereals. It allows the reproducible delivery of particles with designed constant properties, compatible with deep penetration in the human bronchial airways.

In this work, the SAGAS device was used to collect particles which trigger strong reactions to asthmatic bakers. Their nature and their physical and biochemical properties, with special attention to their content in immunoglobulin E (IgE) reactive proteins, were studied. The particles were compared to the flours from which they derived and to aerosols collected in a bakery.

MATERIAL AND METHODS

Flours

Normal bread wheat flours (type 55 and whole meal) and rye flours (type 85 and 130) manufactured by the Grands Moulins de Paris were used in this study. They were devoid of technological additives. They were kept at -20°C in sealed bags. Great care was taken to avoid humidification of the flour before

use. Wheat whole meal was passed through a 1-mm sieve.

Particle size analysis of the flour used to generate airborne particles

A Mastersizer X laser diffractometer (Malvern Instruments Ltd, Malvern, UK) was used to measure particle size of the flours. The flour particles were dispersed in the air by using a system combining a vibrating hopper and an air driven Venturi ejector, before to be drawn in the measurement cell of the diffractometer. The particle diameter measurements were in the range 0.5–600 μm . In this method, the diffraction pattern of light scattered by particles present in an optical sensing volume is measured at different angles. The intensity of light measured at a given angle is a function of the diameter of the particle. The obtained intensity distribution was further converted by calculation into volume distribution of particles as function of an equivalent diffraction diameter.

Generation of aerosols from wheat and rye flours

An automatic solid aerosol delivery apparatus called here the SAGAS, designed for bronchial challenge of patients, was used to generate airborne particles from wheat and rye flours. A detailed description of the system is given in Fabriés *et al.* (2000). Briefly, the equipment encompasses a controlled dry air circuit with a flour particle injector and a cyclone separator coupled to an inhalation chamber from which airborne particles are delivered to the patient through a mouthpiece after particle counting. The flour remaining is collected separately. The system is fully computerized to measure the amount of aerosol inhaled by the patient and to calculate the dose intake (amount of flour inhaled per time units). In automatic mode, the apparatus stabilizes the delivery of aerosols mimicking natural airborne particles of flour, at a concentration around a predetermined value, usually 3 mg m^{-3} .

Aerosol particle sampling

Aerosols generated by the SAGAS system were collected from a line diverted from the usual delivering outlet used for the patient challenge. This line was connected to a Bravo sampling pump (Tecora, Milan, Italy) set to a flow rate of 10 l min^{-1} . For optical microscopy imaging, 25-mm cellulose ester membranes type AA, with 0.8- μm pore diameter (Millipore Corp., Bedford, MA, USA), were used. For scanning electron microscopy, 37-mm nuclepore membranes with 0.8- μm pore diameter (Whatman Int. Ltd, Maldstone, UK) were used with a flow rate of 20 l min^{-1} for sampling >5–10 min. For the dosage and electrophoretic separations of aerosol proteins, particles were collected on 37-mm GF/A glass filters (Whatman Int. Ltd), as for imaging.

Sampling was >15 min with five replicates. Experiments were reproduced three times.

Natural airborne particles were collected similarly from the atmosphere of an industrial bakery producing white bread. Samples (S) were collected on individual workers (S1–S3) and at different fixed places of the bakery (S4–S9), >1 h 30 min to 3 h, with a flow rate of 2 l min⁻¹. Average temperature was 22°C and humidity 55%. All samples were collected at the level of the airways of the workers. They were analysed only for their protein content.

Particle size analyses using optical microscopy imaging

The membranes with the collected particles were exposed to acetone vapors to make them transparent. They were then treated using glycerol triacetate and covered with a cover glass. Imaging was performed on a Leitz Laborlux S optical microscope (Wild Leitz Ltd, Heerbrugg, Switzerland) fitted with a JVC Y-F50 digital color video camera (Japan Victor Company Ltd, Yokohama, Japan). Images were processed to measure the surface distribution of the particles as a function of their equivalent surface diameters. From this distribution, a volume distribution was then calculated using Granix software version 5.2.0 (Microvision Inst., Evry, France).

Particle size analyses using an optical particle counter

The aerosol particles generated by the SAGAS system were also counted and their size distribution measured using a GRIMM 1105 optical particle counter (Grimm Labortechnik GmbH, Ainring, Germany), which was set online with the aerosol delivering outlet. In this method, particles entering in an optical sensing volume are counted and the light scattered is measured at different angles of incidence. The intensity of the scattered light at a given angle depends not only on the size but also on the refraction index of the particles. The distribution obtained is the number distribution of particles as a function of the equivalent diameter determined by light scattered by the particles. The distribution of the number of particles counted was transformed by calculation into volume distribution. Over the total measurement range of the counter was 0.75–25 µm; eight classes of size were compared. Counting was integrated over a period of 60 s. In the absence of patient, the air was pumped at a flow rate of 1.3 l min⁻¹ to aspirate the aerosols.

Scanning electron microscopy observation

Aerosols generated by the SAGAS system and collected on nuclepore membranes as described above were processed for scanning electron microscopy. Fragments of the membranes were cut and glued onto 25-mm brass stubs with quick drying silver paint and further gold coated using a sputter coater SCD040

(Balzers Union, Liechtenstein). For bulk powder samples, the flour was sprinkled on top of a conductive double-sided adhesive carbon disc G3348 (Agar Scientific, Stansted, UK), glued onto the brass stub and gold coated as above. Observations were made on a cold cathode field emission scanning electron microscope 7400-F (Jeol, Tokyo, Japan).

Calculation of the aerosol deposit in the airways

The Lung Dose Evaluation Program (LUDEP) software, version 2.07 (ACJ & Associates, Inc., Richland, WA), developed by International Commission on Radiological Protection (ICRP) was used (ICRP, 1994; Jarvis and Birchall, 1994). From the distribution of the aerosol particle sizes observed, the software evaluates the deposition of these particles in the different parts of the airways of a normal man. The following parameters were used for calculations. (i) Respiratory activity parameters: the respiration is oral, the reference aerosol is the aerosol inhaled via the mouth. Tidal volume: 833 cm³; respiratory rate: 12 min⁻¹ and volumetric flow rate of inspired air: 20 l min⁻¹. (ii) Physiological parameters corresponding to a seated adult Caucasian man, 1.76 m high. Residual functional capacity: 3301 cm³; extrathoracic dead volume: 50 cm³; dead space of the trachea and bronchi: 49 cm³; dead space of the bronchioles: 47 cm³; diameter of the trachea: 1.65 cm and diameter of the first bronchioles: 0.165 cm. (iii) Physical characteristics of the aerosol: specific mass ρ of the particles was set to 1 g cm⁻³; the dynamic shape factor χ of the particles to 1 and the particle aerodynamic diameter D_{ae} was deduced from the following formula: $D_{ae} = D_v \frac{\sqrt{\rho}}{\sqrt{\chi}}$, where D_v is the volume equivalent diameter.

Protein extraction

Total proteins from samples (100 mg) of the crude wheat and rye flours, the residual flours after use in the SAGAS apparatus and the total aerosol particles trapped on each glass filters were extracted under gentle mixing with 1 ml of Laemmli's sample buffer (Laemmli, 1970), 63 mM Tris-HCl pH 6.8, 10% (w/v) glycerol, 2% (w/v) sodium dodecyl sulfate (SDS) containing 5% (v/v) 2-mercaptoethanol, for 1 h, then boiled for 5 min. After centrifugation (5 min at 11 000 g), the supernatant was recovered.

Protein content determination

Total proteins solubilized in the previous extracts were determined using the turbidic method of Vera (1988), with 40% trichloroacetic acid as precipitant.

Electrophoretic analysis of flour and aerosol proteins

Proteins solubilized in the previous extracts were separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) performed on Novex NuPAGE®

4–12% gels, using 2-(N-Morpholino)ethanesulfonic acid buffer, according to the manufacturer specifications (Invitrogen, Carlsbad, CA). Separated proteins were stained using Coomassie Blue according to Neuhoﬀ *et al.* (1988). Densitometric analyses were performed on digitalized image of the gel using the Bio 1D software (Vilber Lourmat, Torcy, France).

Immunoblotting analyses of IgE reactions from patients

The same amounts of proteins from wheat and rye flours and from the corresponding aerosols collected on the SAGAS apparatus were separated using SDS–PAGE as above. They were transferred on polyvinylidene difluoride (PVDF) membrane according to Laurière (1993). Proteins immunoreactive with serum IgE from two patients with severe baker's asthma were detected and their relative amounts evaluated using chemiluminescence as previously described (Laurière *et al.*, 2006). To compare the IgE reactive proteins to the total proteins submitted to the analysis and evaluate the efficiency of the transfer, the PVDF membrane was then post-stained using Indian ink staining as already described (Eynard and Laurière, 1998). No remaining proteins were detectable in the transferred gel, using Coomassie Blue staining.

RESULTS

Particle size distribution of the different wheat and rye flours used

The particle size distribution of the different flours used to generate aerosols was analysed using diffractometry. The results are presented on the left panels of Fig. 1, for whole meal (a), type 55 (b) wheat flours and type 130 (c), type 85 (d) rye flours. Each panel represents the differential and cumulative distributions of the volume of flour particles counted, as a function of the equivalent diameter measured by the laser diffractometer. These distributions revealed a wide spread range of the particle size values. Most of them largely exceed 10 μm . As a consequence, these particles are eliminated by the cyclone separator and could not enter the inhalation chamber. The particles with diameter $<10 \mu\text{m}$ represent between 2 and 10%, depending on the flour. Almost all the particles of the wheat flour type 55 (left panel b) were in the range of the diffractometer, between 1 and 600 μm . On the contrary, the whole wheat meal and the two rye flours (left panels a, c and d) displayed a larger spread of particle diameter. Consequently, particles $>600 \mu\text{m}$ were not counted. Experimental data of all samples were fitted to a cumulative log-normal bimodal law, using a non-linear Gauss–Newton regression method. The adjusted values of the median diameters (d_{50}) and the geometric standard deviations (s_g) of the two modes, as well as

the coefficient of proportion R relative to the first population, are reported in Table 1.

Particle size-distribution of the aerosols generated in the SAGAS, from the different wheat and rye flours

Two different techniques were used for each flour type. The particle size distributions were determined using either processed optical images of particles collected on cellulose ester membranes or an online optical particle counter based on light scattering. The results are reported in Table 1 and Fig. 1 (right column). The median of the diameter values, expressed in volume of particles ($d_{50,v}$) only accessible by the two methods, was in good agreement for the two methods. They show values of $d_{50,v} \sim 7 \mu\text{m}$ except for rye type 85; the expression in number of particles ($d_{50,n}$) shows median values $\sim 4 \mu\text{m}$ for all aerosols (Table 1). In addition, the size distribution profiles of the different cereal flours were quite similar and followed a log-normal distribution in all cases (Fig. 1).

Comparison of aerosols and flour particles from the different wheat and rye flours using SEM

The particles from the different flours and the corresponding aerosols generated, observed at the same magnification, are presented in the two first columns of Fig. 2, for whole meal (a), type 55 (b) wheat flours and type 130 (c), type 85 (d) rye flours. Flour particles mainly corresponded to endosperm tissue fragments of various sizes. Among them, embedded large (A-) and small (B- or C-) starch granules (Bechtel and Wilson, 2003) could be distinguished. The aerosol particles from the different flours displayed more homogeneous size distributions and high similarities. They are presented in the two last columns of Fig. 2. The third column is a 10 times magnification of the middle one. It shows that aerosols corresponded mainly to small starch granules and protein matrix fragments associated or not. Small starch granules were in the diameter range 2–5 μm . They displayed a roughly round or polygonal shape, characteristic of B- and C-starch granules (Bechtel and Wilson, 2003). Protein matrix fragments were identified by their irregular shape with remaining cavities imprinted by detached starch granules. Few other cell fragments like cell wall fragments were detected. The sizes of the particles observed were in accordance with the values found in the particle size distribution of aerosols.

Calculation of the deposition of inhaled aerosols particles in the airways

From the above data of size distribution, the repartition of aerosols deposited in the different parts of the respiratory tree was calculated. The calculation followed the recommendations of the ICRP, using the LUDEP software (Jarvis and Birchall, 1994). It

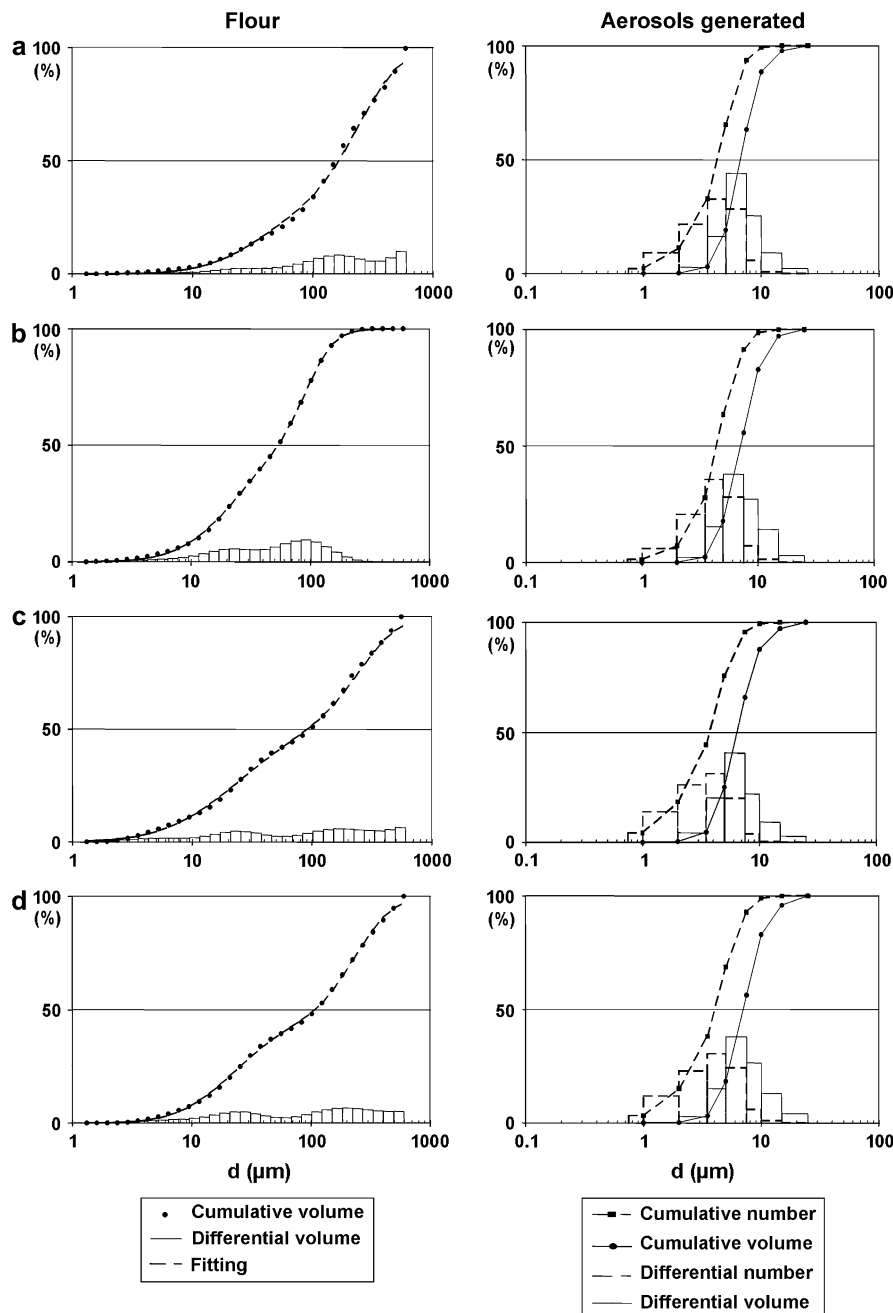


Fig. 1. Distributions of the volume of flour particles as a function of their diameter of respectively (a) whole wheat meal sieved at 1 mm, (b) wheat flour type 55 and (c) and (d) rye flours types 130 and 85, using laser diffractometry (left column) and similar distributions of volume and number of corresponding aerosol particles generated by the SAGAS apparatus, using an optical particle counter (right column).

was performed, assuming that the percentage deposited at a point was a function of the median aerodynamic diameter of an aerosol whose volume or mass distribution is log normal with a geometric standard deviation of 1.5. These values were chosen to correspond to the characteristic parameters of the distribution of the aerosols produced by the SAGAS apparatus. The other physiologic parameters cor-

responded to a normal man, at rest, breathing 20 l min^{-1} . The results are shown in Fig. 3. The calculated curves of deposition in the extrathoracic, tracheobronchial and alveolar parts of the airways all cross at $\sim 25\%$ of the mass inhaled for a median diameter of particles $\sim 7 \mu\text{m}$. This last value corresponded to the main type of particles generated by the SAGAS apparatus (Table 1, Fig. 1).

Table 1. Particle analyses of wheat and rye flours used and of corresponding aerosols, using different methods

		Wheat whole meal	Wheat type 55	Rye type130	Rye type 85
Flour particles					
Laser diffractometry					
First population	$d_{50,v} (s_{g,v})$	47.89 (2.75)	28.71 (2.59)	26.57 (3.28)	23.83 (2.44)
Second population	$d_{50,v} (s_{g,v})$	254.97 (1.89)	96.77 (1.47)	256.11 (1.82)	232.99 (1.80)
	R	0.39	0.64	0.57	0.47
Aerosol particles					
Optical particle counter					
	$d_{50,n} (s_{g,n})$	4.30 (1.70)	4.43 (1.61)	3.77 (1.82)	4.08 (1.80)
	$d_{50,v} (s_{g,v})$	6.75 (1.43)	7.13 (1.47)	6.53 (1.49)	7.08 (1.48)
Optical microscopy					
	$d_{50,v} (s_{g,v})$	7.69 (1.53)	7.34 (1.50)	6.58 (1.53)	8.79 (1.57)

Flour particle distributions fitted to bimodal laws, revealing two major particle populations (see Fig. 1). Aerosols were from the SAGAS apparatus. $d_{50,n}$, $d_{50,v}$, median diameters (μm) in the distributions of the number and volume of the flour and aerosol particles; $s_{g,n}$, $s_{g,v}$, corresponding geometric standard deviations and R , coefficient of proportion of the first determined flour populations relative to the total population.

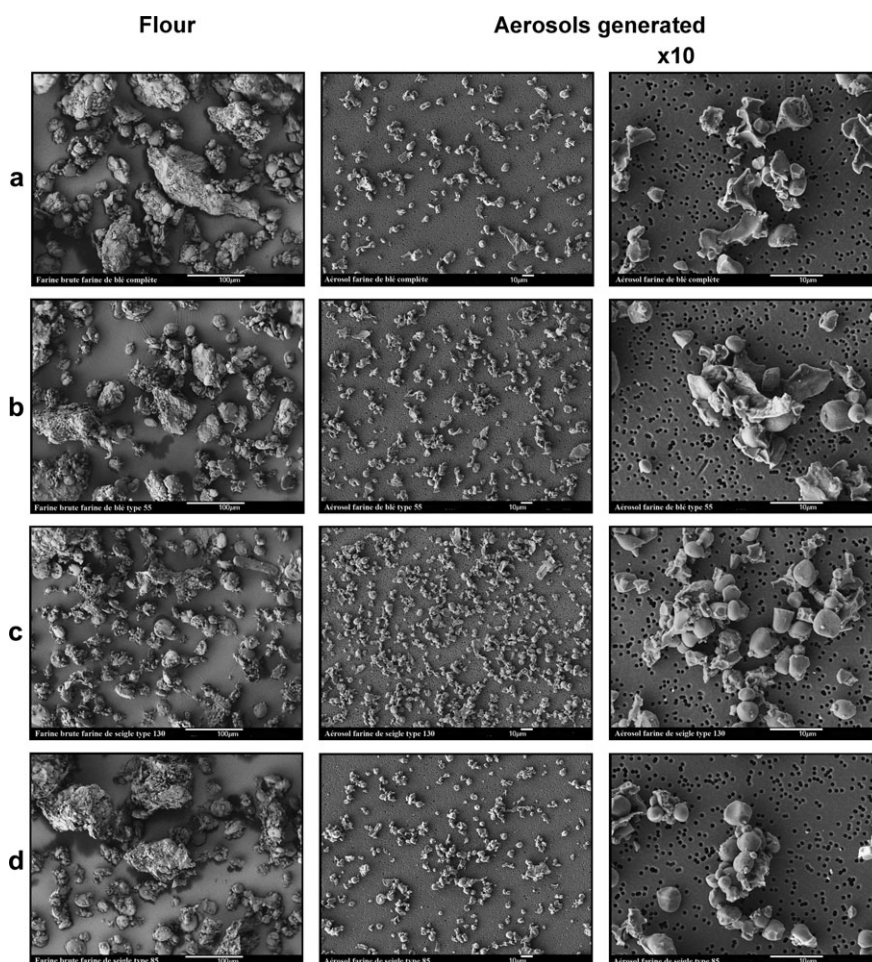


Fig. 2. Scanning electron microscopy of respectively (a) whole wheat meal sieved at 1 mm, (b) wheat flour type 55 and (c) and (d) rye flours types 130 and 85 (left column) and of corresponding aerosols generated by the SAGAS apparatus (middle and right column). The right column is a 10 times magnification of the middle column.

Protein content of flours and aerosol particles

Protein content of the different wheat and rye flours, the corresponding residual flours after use in the SAGAS apparatus and the aerosol particles produced by the apparatus and trapped on glass filters are presented in Table 2. It was estimated from the total proteins extracted using the Laemmli's sample buffer (Laemmli, 1970). An important protein enrichment of the aerosol particles was observed when compared to the original flour. This was verified for all samples analysed. These results were reproduced independently five times. The residual flour recovered after passing through the SAGAS apparatus showed also a slight enrichment in proteins. This was due to a drying phenomenon produced by the circulation of dry air in the cyclone of the apparatus. These differences disappeared upon freeze drying the flours (results not shown).

Electrophoretic analysis of proteins

The protein contents of the different samples of wheat and rye flours and the corresponding aerosol particles, and residual flours, obtained from the SAGAS apparatus, were compared. Proteins were extracted using SDS and analysed using SDS-PAGE

and densitometry. The Fig. 4 shows the observed protein patterns for flours (F), residual flours (R) and aerosol particles (a). Two samples of aerosol particles obtained independently (a1, a2) are presented for comparison and to show the reproducibility of the analyses between samples. Flour and airborne particles displayed typical patterns of endosperm gluten proteins characterized by a predominance of gliadins and glutenins easily identified in the electrophoregrams by the typical distribution of the protein bands. Due to their high abundance (70–80% of total proteins), they generally hide the albumins and globulins in flour protein patterns (see Figs 4–6). Differences however were observed between the aerosols, and the corresponding protein patterns of the whole wheat meal and rye type 85 and 130 flours, which are characterized by the presence of bran fractions rich in albumins and globulins (see arrows in Fig. 4). Under the same conditions, no significant differences could be detected between the protein patterns of the more refined wheat flour type 55, which is enriched in endosperm tissue, and the corresponding aerosols. These results were confirmed using densitometric analyses of all these protein patterns stained using Coomassie Blue (results not shown).

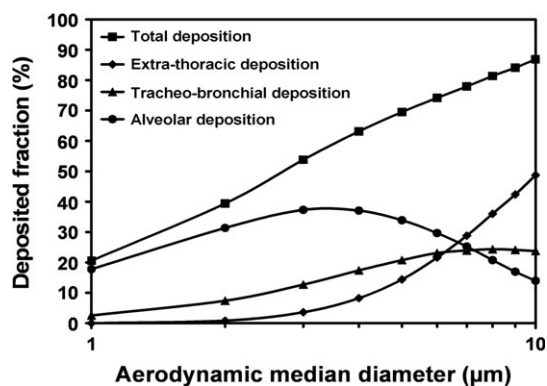


Fig. 3. Computerized calculation of the matter deposited in the different parts of the human airways, following the inhalation of a polydisperse aerosol from the mouth, as a function of mass median aerodynamic diameter. ICRP model, LUDEP calculation (ICRP, 1994; Jarvis and Birchall, 1994), with a geometric standard deviation of 1.5.

Analysis of airborne particles collected in a bakery

The same experiments were reproduced on dust particles collected from the atmosphere of an industrial bakery during bread making from normal white bread flour type 55. Atmosphere sampling was at different fixed places of the room or directly on three workers. The atmosphere concentration and the amounts of dust particles collected varied strongly, depending on where the particles were collected (Table 3). The collected particles were analysed for their protein content using both protein dosage (Table 3) and SDS-PAGE (Fig. 5). As for aerosols generated by the SAGAS apparatus and except for two samples, the protein content of dust particles exceeded that of the original flour. Also no differences were observed in the electrophoretic patterns of normal flour and the dust particles. Gluten proteins were clearly identified.

Table 2. Comparison of the protein content, recovered after protein extraction in the presence of SDS and 2-mercaptoethanol of samples of flours and of aerosol particles collected on membranes

	Protein amount extracted from flour and aerosol particles (%)			
	(Protein mass extracted/mass of the sample) × 100			
	Whole wheat meal	Wheat flour type 55	Rye flour type 130	Rye flour type 85
Aerosol particles	17.4 ± 2.4	14.8 ± 1.9	12.7 ± 1.4	10.2 ± 0.8
Original flour	8.0 ± 0.1	8.3 ± 0.2	4.5 ± 0.2	3.5 ± 0.1
Residual flour	10.0 ± 0.3	9.3 ± 0.2	5.5 ± 0.1	4.6 ± 0.2

Each value is the mean of five independent determinations.

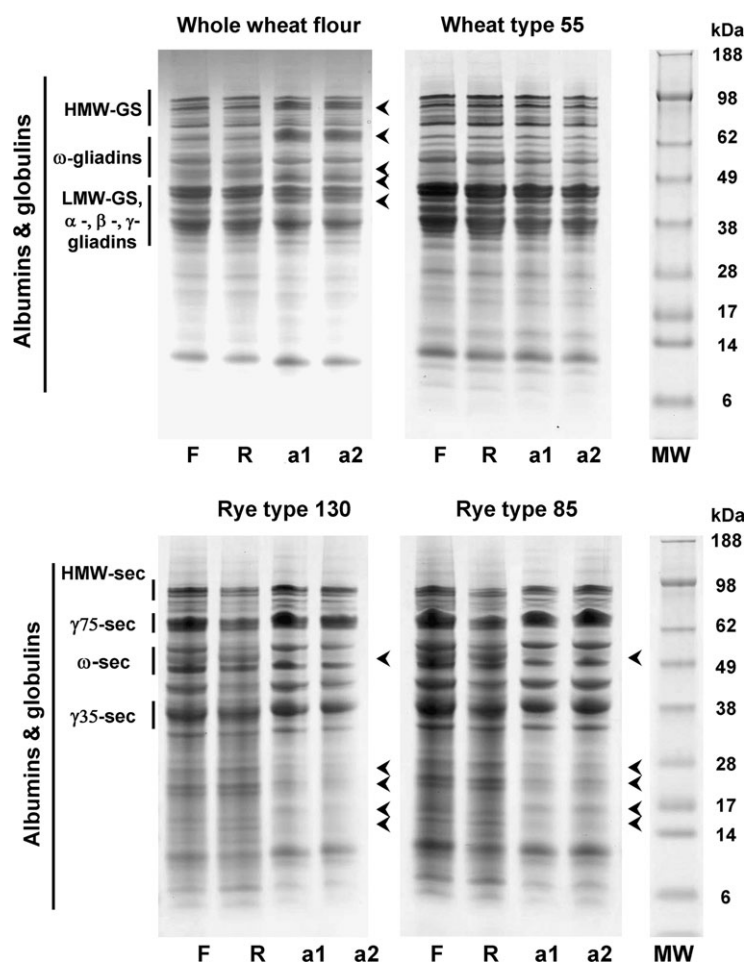


Fig. 4. SDS-PAGE analysis of total proteins from the different flours used to generate aerosols (F), the corresponding residual flours (R) collected and two samples (a1 and a2) of the corresponding aerosols generated by the SAGAS apparatus. MW, standard molecular weights.

Characterization of IgE immunoreactivity of proteins from flours and aerosols

The proteins immunoreactive with IgE from two patients suffering with severe baker's asthma and reacting strongly when challenged with the SAGAS apparatus were characterized using immunoblotting. In preliminary experiments, the IgE from these patients were shown to react with proteins which almost exclusively belong to the albumin and globulin fraction (results not shown). To allow comparisons, the same amounts of proteins from wheat and rye flours and from the corresponding aerosols collected from the SAGAS apparatus were analysed. For a given patient, small differences in the relative IgE reactivity of the revealed components was observed between the original flour and the residual flour and also between the whole meal flour (whole wheat flour or rye type 130) and the corresponding white flour (wheat type 55 or rye type 85). On the

contrary, large differences were observed between the original wheat or rye flours and the corresponding aerosols (Fig. 6). In all cases, the aerosol samples gave weaker reactive bands. Not all the bands detected in the flour were detected in the aerosol in the conditions of the analysis. The more reactive component had a relative molecular weight ~ 25 kDa, in both wheat and rye aerosols.

DISCUSSION

Generation of particles entering deeply in the airways

Samples of airborne particles of wheat flour collected at different places of a bakery showed a large heterogeneity in terms of amounts and protein compositions. Due to the mode of collection on filters, no size distribution could be obtained. Furthermore,

Table 3. Analysis of airborne particles collected directly on three workers (S1–S3) and at different places in the atmosphere of an industrial bakery (S4–S9)

Samples	Particle air concentration (mg m ⁻³)	Particle amounts collected (mg)	Extractable proteins in particles (w/w × 100)
Bread wheat flour (F) —	—	—	5.1
Samples collected on individuals			
S1	5.0	1.0	3.9
S2	5.1	1.0	7.7
S3	2.3	0.9	10.1
Samples collected at fixed places			
Dough making area			
S4	1.4	0.6	7.9
S5	1.6	0.5	6.3
S6	10.0	0.1	9.2
S7	4.6	3.5	3.3
Dough resting area			
S8	0.8	1.6	7.0
Baking area			
S9	0.4	0.3	8.4

Workers stood mainly at the different places of dough making area. Proteins, extractable using SDS, are expressed in percent of the weight collected (w/w × 100); values are the mean of three determinations on the same sample.

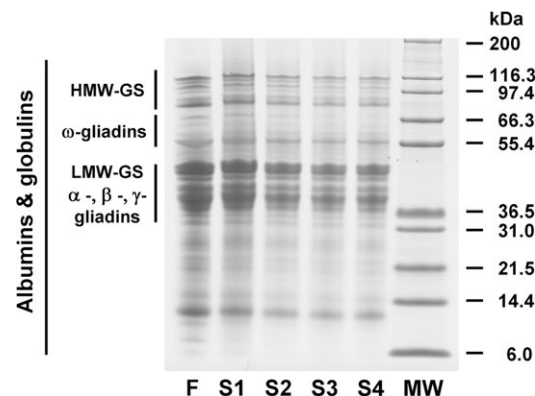


Fig. 5. SDS-PAGE analysis of the flour used (F) and samples of aerosols collected in the bakery (S1–S4). Samples are those listed in Table 3; MW, standard molecular weights.

it is known that only parts of these natural airborne particles (~20%) were able to penetrate the human airways (Sandiford *et al.*, 1994a). This suggests that only parts of them are involved in the triggering of rhinitis or asthma. For these reasons, we choose to artificially produce aerosols using the SAGAS apparatus. This allowed producing larger quantities of material with designed particle sizes. Wheat and rye aerosols were produced independently without cross-contaminations that would occur in a bakery atmosphere. They were produced from flours without

any additives, in order to detect only true wheat and rye allergens.

Physical analysis of the aerosol particles

Whereas flours display a large distribution of particle sizes, aerosols particles produced using the SAGAS apparatus displayed narrow size distributions centered at ~7 µm. These distributions were moderately influenced by the type of flour used (Table 1, Fig. 1). This was remarkable considering the differences of milling properties of wheat and rye flours. These results were the consequence of the good computerized control of the performances of the apparatus which allowed the reproducible delivery of aerosol particles over long periods, with adjustable characteristics. The ability of the produced aerosols to penetrate deeply the airways was confirmed by calculation from the observed particle distribution. The particles produced correspond to a maximum homogeneous deposit of flour material in the different parts of the airways (Fig. 3). For these reasons, we considered these aerosols as representative of the flour particles triggering the allergic reactions that we observed during the specific challenge tests (Choudat *et al.*, 1999, 2002; Bensefa *et al.*, 2004).

Biochemical characterization of the flour particles produced using the SAGAS apparatus

SEM observation of the particles delivered by the SAGAS apparatus, allowed the identification of some features of their composition and of their origin in the grain. Small starch granules were easily identified from their size and their round or polygonal shape. They were B- or C-starch granules more or less embedded into an amorphous material whose aspect was characteristic of the interstitial storage protein matrix present in the cells of the grain endosperm tissue. Some isolated cell wall fragments were also observed (Fig. 2, right column). B- and C-starch granules are known to be synthesized in the later stages of the grain maturation after the A-starch granules which are larger and lenticular shaped (Bechtel and Wilson, 2003). They are synthesized exclusively in the starchy endosperm tissue. These observations were consistent with the electrophoretic analyses which revealed a predominance of gluten proteins in the aerosols. These proteins are also exclusively synthesized in the starchy endosperm tissue (Evers and Bechtel, 1988; Gianibelli *et al.*, 2001). The estimation of the protein content of aerosols based on extractable proteins also showed a 2- to 3-fold increase compared to the original flour. This lowered their density and favored the formation of aerosols. Proteins have an average density of 1.32 to be compared to 1.5 for starch. Most of the samples of airborne particles collected in a bakery also showed a higher content of proteins, when compared to the corresponding

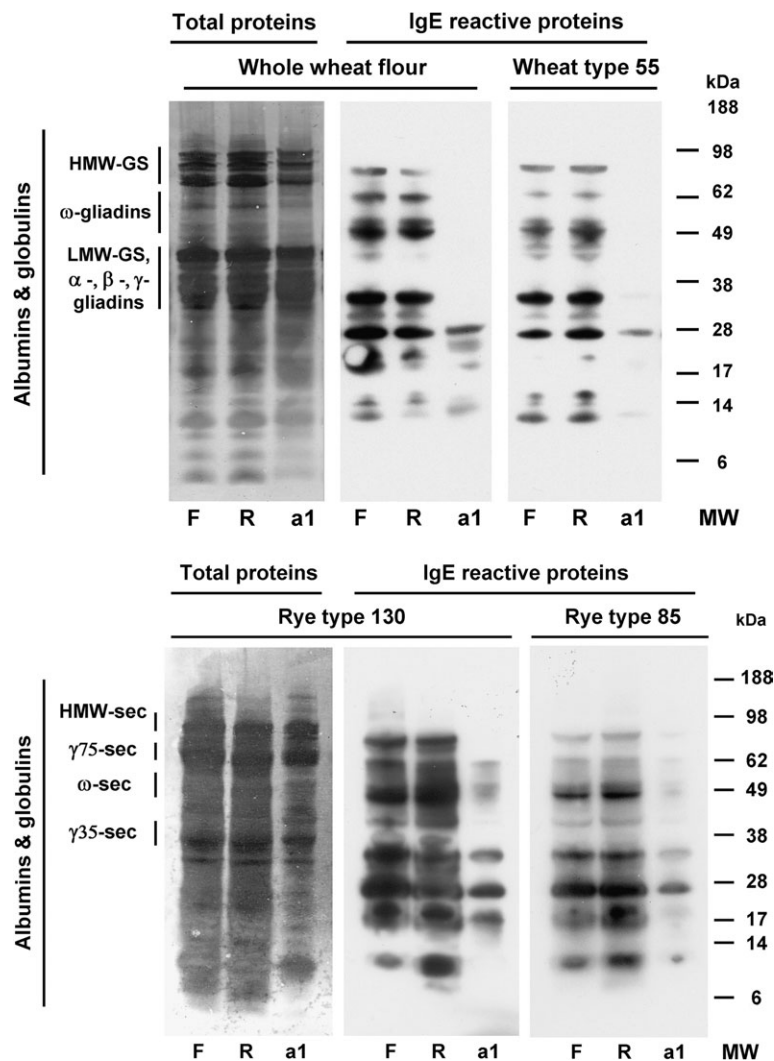


Fig. 6. IgE reactivity of proteins from wheat and rye flours (F), the corresponding residual flours (R) collected and one sample (a1) of the corresponding aerosols generated by the SAGAS apparatus. Proteins were separated using SDS-PAGE and immunoblotted.

Left panel, total proteins present on the membrane; middle and right panels, immunoblotting analysis using the serum of an asthmatic baker and MW, standard molecular weights.

flour. The increased protein content depended on the spot of collection (Table 3). Three samples (S3, S6, and S9) displayed values comparable to samples of wheat 55 aerosols produced by the SAGAS apparatus. The other samples of flour dust showed lower protein contents, probably due to the presence of larger particles. The exclusion of grain tissues other than the starchy endosperm in the aerosols was confirmed by the electrophoretic comparison of the whole wheat flour and rye flours, with their corresponding aerosols. Bands were modified or absent in the aerosols (arrows in Fig. 4). These differences can be explained by the exclusion of germ and aleurone tissues from the aerosols, which are known to form larger fragments and which are rich in albumin and globulins. Differences were less visible with

white wheat flour type 55 which is more enriched in starchy endosperm tissue (Evers and Bechtel, 1988).

IgE reactive protein content of aerosols and flours

The amount of IgE reactive proteins detectable in aerosols produced by the SAGAS apparatus was lower than that of the corresponding flour. Not all the components present in the flours were detectable in the corresponding aerosols. Furthermore, the balance between the detected components differed from that observed in the flour. The absence of signal in the aerosols for the other components detected in the corresponding flour was interpreted as a too low concentration to be detected by the method. These observations confirm that aerosols which penetrate the airways correspond to specific fractions

originating mainly from the starchy endosperm. As a consequence, they have a higher content in gluten proteins and a lower content in albumins and globulins, among which most of the allergen triggering asthma are found.

CONCLUSION

The particles which penetrate deeply in the human bronchial airways and which are responsible for rhinitis and asthma symptoms in sensitized bakers correspond to a selected part of the grain flour. They were specifically produced using an aerosol generator and analysed. The results showed that these aerosols can be characterized not only by their size <10 µm but also by their biochemical composition and their antigenicity for IgE from asthmatic patients. Their microscopic observation showed mainly intracellular fragments of protein matrix more or less associated to B- and C-starch granules. Electrophoretic analyses revealed also a strong enrichment in endosperm gluten proteins and the partial disappearance of some protein bands present in the flour when it contained bran fractions. Aerosols displayed also a lower content in IgE reactive proteins. All these observations strongly suggest that the aerosols generated derived mainly from the starchy endosperm and that tissue fragments containing allergenic albumins or globulins were less present in the aerosols, compared to the flour.

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