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A study to investigate ways to reduce the dustiness of bakery ingredients and exposure to allergens

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Research Report

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A study to investigate ways to reduce the dustiness of bakery ingredients and exposure to allergens

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This study investigated whether changing the ingredients of bakery improvers would decrease their dustiness and, consequently, help to reduce the exposure of bakers to allergens in the bakery dust. The study was carried out in partnership with the Association of Bakery Ingredient Manufacturers (ABIM).

Typical ingredients in bakery improvers are wheat flour, fungal alpha amylase, soya flour, calcium sulphate, vegetable oil and emulsifier. Emulsifier is made from data ester (E472e) with a 'free flow agent', usually calcium silicate in the UK, to prevent sticking in bakery equipment. The combinations of ingredients that provided the biggest decrease in dustiness and exposure were identified using several tests including dustiness testing, particle sizing, user testing with simulated bakery tasks, and analysis for protein, allergens and calcium.

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List of abbreviations

ABIM: Association of Bakery Ingredient Manufacturers
ACTS: Advisory Committee on Toxic Substances
Ca: Calcium
FAA: Fungal alpha amylase
HSE: Health and Safety Executive
HSL: Health and Safety Laboratory
ICP-MS: Inductively coupled mass spectroscopy
MEL: Maximum exposure limit
STEL: Short term exposure limit
STI: Soya trypsin inhibitor
WEL: Workplace exposure limit
WFA: Wheat flour antigen

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Objectives and introduction

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Typical ingredients in bakery improvers are wheat flour, fungal alpha amylase, soya flour, calcium sulphate, vegetable oil and emulsifier. Emulsifier is made from data ester (E472e) with a 'free flow agent', usually calcium silicate in the UK, to prevent sticking in bakery equipment. The combinations of ingredients that provided the biggest decrease in dustiness and exposure were identified using several tests including dustiness testing, particle sizing, user testing with simulated bakery tasks, and analysis for protein, allergens and calcium.

Main findings

Initial dustiness testing revealed that:

- Addition of soya flour and oil decreased the dustiness of improvers;
- Addition of calcium sulphate and emulsifier increased the dustiness of improvers;
- Emulsifier was far dustier than either of its component parts (data ester and calcium silicate);
- The calcium silicate within emulsifier may be responsible for increasing dustiness.

On the basis of these results, three control measures were investigated to reduce dustiness of bakery improvers and exposure to allergens. The main findings are summarised below.

1) Increasing the vegetable oil content of improvers

This was the most effective method of decreasing the dustiness of bakery improvers and also decreased the amount of allergens that became airborne. This study investigated the effects of raising the oil from 2% to 4% of the improver mix. This change was associated with a 77% decrease in airborne allergens in the user test. Seasonal temperatures may affect how easily this improver could be blended, however it is otherwise thought that this control measure is the easiest for the baking industry to implement.

2) Reducing the amount of calcium sulphate in improvers

This method reduced the dustiness of the improver but the effect on the amount of allergens in the dust was less clear. Although the dust from the dustiness tests contained less allergen than standard improver dust, the dust from the simulated user tests did not.

3) Reducing the amount of calcium silicate within the emulsifier mix

This was the least effective of the three control methods tested. Dustiness testing showed a reduction in the amount of dust produced, but the difference in the simulated user test was marginal. Although the dust from the dustiness tests contained less allergen than standard improver dust, the dust from the user tests contained slightly more allergen.

1 INTRODUCTION

1.1 BACKGROUND

Bakers have one of the highest incidence rates of occupational asthma as reported by occupational and chest physicians; they are around 80 times more likely to develop occupational asthma than the average British worker.

Flour contains several allergens, a key one being wheat flour antigen (WFA); approximately 90% of sensitised bakers have antibodies to WFA. To improve the quality and the processing of dough, baking flour is supplemented with additives. Enzymes (for example, fungal and bacterial alpha-amylase) are added to release the sugars from starch and aid fermentation. These and other enzymes are known to increase risk of sensitisation and allergy amongst bakery workers.

Enzymes are added via an 'improver', which comprises several agents mixed into flour to improve the quality of the bread. Binding agents (such as soya flour and oil) and emulsifiers (such as E472e) are also used in improvers. This emulsifier is primarily data ester (E472e: diacetyltartaric acid esters of mono and diglycerides) mixed with a 'free flow agent' to prevent it from clogging within baking machinery. In the UK, the free flow agent is usually calcium silicate added at 5% of the emulsifier mixture. Improvers also contain calcium sulphate or calcium carbonate; used as cheap bulking agents, calcium supplements and they help the fatty materials to blend into the mix.

Table 1 Typical constituents of bakery improvers in the UK

Ingredient	**Notes**	**Known allergens**
Flour		Wheat flour antigen
Ascorbic acid		
Fungal (or bacterial) alpha amylase	Enzymes used to aid fermentation	Fungal (and bacterial) alpha amylase
Soya flour	Used as a binding agent	Soya trypsin inhibitor
Rapeseed oil	Used as a binding agent	
Emulsifier	Used as a binding agent Made from data ester (E472e diacetyltartaric acid esters of mono and diglycerides) and calcium silicate (free flow agent)	
Calcium sulphate (or calcium carbonate)	Used as bulking agents, calcium supplements and to aid blending	

1.2 CURRENT CONTROLS OF EXPOSURE AND PREVIOUS WORK

Traditionally, bakery worker exposure to flour has been reduced using mechanical control measures such as local exhaust ventilation, vacuuming to remove flour dust, respiratory protective equipment, safety and awareness training and adjustments to unsafe working practice. Previous work carried out by the Health and Safety Laboratory (HSL) has demonstrated that despite introducing these control measures, bakery workers continue to be exposed to allergens within flour dusts [(2)].

In 2001, the Advisory Committee on Toxic Substances (ACTS) and the Health and Safety Commission agreed some Occupational Exposure Limits (OELs) to drive down exposure to flour dusts. They agreed a Maximum Exposure Limit (MEL) for flour dust of 10 mg/m^3 for long-term exposure (the average amount of dust collected over an 8 hour time period), and a Short-Term Exposure Limit (STEL) of 30 mg/m^3 (the dust measured over a 15-minute reference period). These figures were incorporated into EH40/2001.

ACTS reviewed the effectiveness of this maximum exposure limit in 2004, three years after its implementation. This review included a study by HSE and HSL to evaluate the impact of the MEL and STEL. The study showed that the MEL had not had much impact and poor working practices were still being undertaken (such as dry brushing and flour dusting by hand). The study also found that only 27% of bakeries surveyed in the UK were aware of the exposure limits. For 1 in 5 people, the inhalable dust exposure exceeded the MEL and for 1 in 3 people, the levels were higher than 5 mg/m^3. On the basis of the review, the ACTS recommended that HSE work together with the industry to develop a strategy to reduce exposure to flour dust and help the bakeries to comply with the MELs.

Occupational Exposure Limits were replaced with Workplace Exposure Limits (WELs) in 2005; this WEL was reported in EH40/2005. The WEL for flour dust is 10 mg/m^3 for long-term exposure (8 hour time weighted average) and 30 mg/m^3 for short-term exposure (15 minute reference period).

1.3 AIMS AND OBJECTIVES OF THE STUDY

As flour and improvers are naturally dusty materials, there is a high risk of dust being created during handling and being inhaled by bakery workers. Improvers are available in a paste or liquid, however mixing the improver as a dry powder is regarded as the most effective way to ensure it is thoroughly and evenly dispersed throughout the flour.

A possible way of reducing the risk to bakery workers is to reduce the dustiness of the materials they are working with. This could potentially be done with the improvers by changing the proportions of the ingredients within them. The Association of Bakery Ingredients Manufacturers (ABIM) is currently working on this issue by developing improvers that contain binding agents. Binding agents that have been considered include fatty powders such as soya flour, emulsifiers such as E472e (diacetyltartaric acid esters of mono and diglycerides), and

rapeseed oil. If these agents help to reduce the dustiness of the ingredients, this would represent a simple, practical and cost effective method of reducing the exposure to bakery workers.

On the recommendation of ACTS, HSE commissioned this study to work in partnership with ABIM, with the aim of reducing the dustiness of bakery improvers. The main objective was to examine combinations of ingredients and binding agents and identify which generates the least dust and has the lowest allergens content.

2 METHODS AND STUDY PLAN

2.1 SUMMARY OF METHODS USED

The dustiness of the improvers and the potential exposure to bakery operators was measured using several methods: dustiness testing, particle size analysis, user testing and immunological analysis. These methods are summarised below, detailed descriptions are shown in Appendix 6.1.

2.1.1 Dustiness testing

Dustiness testing provides a measure of the potential of a powder or substance to produce dust. It can be measured by a number of methods; this study used European standard EN15051, which separates the airborne dust into three health-related fractions. The improver is placed into a rotating drum which lifts the improver and lets it fall, producing a dust cloud. Air is drawn through the drum and the airborne dust is collected. The air first passes through a foam with a large pore size to collect the inhalable fraction (dust that can enter the airways). The air is then drawn through a small pore size foam to collect the thoracic fraction (dust that can reach the chest area). Finally, the fine particles representing the respirable fraction or the dust that can reach deep into the lungs are collected on a filter. The foams are weighed and the dustiness is calculated as amount of dust per kilogram of improver. The collected dust was retained for allergen content analysis.

2.1.2 Particle size distribution

Particle size is important because it is one of the properties that contribute to the dustiness of the ingredient or mixture. The particle size distribution was established with an Aerosizer instrument, which can estimate the range of particle sizes within a powder from 0.2 to 700 microns. The particles within the powder are accelerated and forced through a nozzle at a very fast speed. The time it takes the particles to travel across the measurement region is measured using two lasers and, since smaller particles travel at a faster rate than larger ones, the size of the particle can be calculated.

2.1.3 User testing

User testing was performed to study how improvers behaved when handled manually. Bakery tasks (scooping and pouring) were replicated in an exposure chamber, an enclosed box where the air flow through the chamber can be controlled. The improver was handled through glove ports in the side of the chamber, so the operator was not exposed to the dust created from the improver. Inhalable and respirable dust air samplers and a respirable dust monitor were set up within the chamber to collect and measure the airborne dust produced by the scooping and

pouring activity. The samplers were placed at a height typical of the operators' breathing zone and so represent the amount of dust an operator could realistically be exposed to. The collected dust was retained for allergen content analysis.

2.1.4 Extraction of dust from dustiness and user test samples

The proteins and agents were removed from the filters and foams by soaking them in a mild detergent. The container containing the sample and detergent was constantly agitated in order to help dislodge the material from the filter or foam. The resulting liquid was filtered to remove any fibres or pieces of foam from the sample and stored in a freezer until analysis.

2.1.5 Immunological testing

The dust created in the dustiness and user testing was retained, extracted and analysed for several agents: wheat flour antigen, soya trypsin inhibitor, total soluble protein and calcium. These methods are summarised below, detailed descriptions are shown in Appendix 6.1.6.

Wheat flour antigen analysis

Wheat flour antigen (WFA) is a key allergen in the baking industry and 90% of sensitised bakers have antibodies to this allergen. The wheat flour antigen (WFA) was analysed using an immunological technique called a sandwich-linked immunoassay (ELISA). This technique is a standard immunological method to detect a specific protein and was used to quantify the amount of WFA in each sample.

The principle of ELISA uses antibodies that bind very specifically to the target molecule of interest (e.g. WFA). These antibodies are used to trap the molecule of interest (WFA) within the sample. Antibodies can also be labelled with enzymes, and when added to the trapped target molecule the enzymes cause an indicator substance to change colour. This colour change can be measured and quantified; the deeper the resulting colour, the more of the target molecule is present in the sample.

Soya trypsin inhibitor

Soya trypsin inhibitor (STI) is a respiratory allergen in soya flour and an ingredient in bakery improvers. STI was also analysed using a sandwich-linked immunoassay. The procedure is essentially the same as the method for measuring WFA, but the antibodies used are ones that will specifically bind to STI.

Again, this technique was used because it can specifically detect the amount of STI in the sample. STI is a potent allergen in soya flour, a common ingredient in bakery improvers. By collecting and analysing the dust created from the dustiness and user tests for STI, these results will not only show whether the improvers are more or less dusty but also whether the operators would potentially be exposed to more or less STI.

Total soluble protein

The total protein assays were used to determine the biological content of these dusts. These assays are not specific and detect all proteins of either animal or plant origin. Samples were added to a 'protein determination reagent', which contains copper that binds to the protein molecules to form a light blue complex. The reagent also contains bicinchoninic acid, which binds to the protein and copper complex to produce an intense purple colour. The greater the amount of protein in the sample, the more intense the colour produced.

Two methods of measuring the total soluble protein were used for this study, which differed in their sensitivity. The sensitive method was used to detect the protein in the filter and foam samples from the dustiness and user tests. The method used for the bulk improver samples was able to detect large amounts of protein. These methods of protein detection are essentially the same and both use the same chemical reaction and colour change described above.

The total soluble protein was measured in these samples, as this is a measure of the total biological material in the sample. This will include other proteins as well as allergens, and gives a measure of the total biological load.

Calcium analysis

The improver contained calcium from two ingredients (calcium sulphate and calcium silicate in emulsifier). These substances could potentially irritate the airways and so the calcium content was analysed in the samples.

The extracts from the foams and filters were analysed for calcium using inductively coupled mass spectroscopy (ICP-MS). This is a very sensitive and specific method of detecting calcium in samples.

2.2 OVERALL STRATEGY OF THE STUDY

ABIM provided six improver mixtures that were intended to range from very dusty to not dusty, depending on the binding agents that had been added to them. These improvers were tested for dustiness and the dust collected on the filters and foams was extracted and retained for immunological analysis. The dustiness of these improvers had some very unexpected results; two of the ingredients intended to decrease the dustiness actually had the opposite effect. This unexpected finding was therefore investigated and it was agreed that some additional testing would be performed.

In order to better understand the properties of improvers, the dustiness of each individual ingredient was tested and the particle size distribution established. One of the ingredients (emulsifier) was found to be very dusty when tested alone. This is actually composed of data ester (the ingredient itself) mixed with a 'free flow agent' to enable the ingredient to move through machinery easily. These components were also tested for dustiness and particle size and it was found that the emulsifier mixture was dustier than either component on their own, indicating that the ingredients were interacting.

Simple mixtures of flour and the additives were made at concentrations typical of those found in improvers. They were tested for dustiness and particle size to determine whether these ingredients could be interacting with the flour. The dust collected on the filters and foams was extracted and retained for immunological analysis. The effect of the following ingredients was investigated when mixed with flour: emulsifier, the calcium silicate within the emulsifier and calcium sulphate.

On the basis of these experiments, HSL found three possible methods of reducing dustiness in bakery improvers to try in real improver mixtures:

- Increasing the oil in the improver;
- Decreasing the calcium sulphate in the improver;
- Using emulsifier that contained less calcium silicate.

In order to test whether these methods would work in improvers, seven revised improver mixtures were made by ABIM. These improvers were designed to test the effectiveness of each method of reducing dustiness on its own and together. These improvers were tested using dustiness testing and the dust collected on the filters and foams was collected and retained for immunological analysis. These revised improver samples were then tested with user testing to find out whether these improvers and alterations would help reduce dustiness when they were handled manually. The dust produced from the user testing was collected, extracted and tested by immunological analysis.

The three potential methods of reducing dustiness were investigated with the revised improver samples, so the results and discussion of this report will focus on these key samples.

3 RESULTS

3.1 INITIAL FINDINGS

The results of the tests on the initial sets of improver samples are summarised briefly in this section; details are provided in Appendix 6.2.

Original ABIM improver mixtures

ABIM made six improver mixtures with various binding agents that were intended to range from not dusty to very dusty. These were analysed for dustiness and the results were as follows:

- The addition of soya flour and oil decreased the dustiness;
- The addition of emulsifier and calcium sulphate increased the dustiness;
- Analysis of allergens in the dust (STI and WFA) showed that samples containing emulsifier and/or calcium sulphate also had larger amounts of allergen in the dust.

It had previously been thought that all the additives in these improvers would decrease the dustiness of the improver, so these results were very unexpected. HSL, HSE and ABIM therefore agreed to look at the properties of the individual ingredients to determine how they were affecting the dustiness of the improvers.

Individual ingredients

Dustiness tests were undertaken for each of the ingredients of the improvers and the following order of dustiness was obtained (from most to least dusty): emulsifier, calcium sulphate and calcium carbonate, flour and soya flour.

Emulsifier is composed of two agents, the data ester (E472e diacetyltartaric acid esters of mono and diglycerides) and calcium silicate, a free flow agent added to enhance mixing of bulk materials. These two components were therefore dustiness-tested and it was found that neither of these components were as dusty on their own than when combined in an emulsifier.

Particle size measurements of the improver ingredients showed that the ingredient with the smallest particle size was the calcium silicate, followed by calcium sulphate.

Simple combinations of ingredients

It was then decided to examine how the different ingredients interacted with each other to cause the overall dustiness of the improver mixture. HSL looked at whether the added ingredients could be interacting with the flour in the improver. Simple mixtures of each ingredient with flour were made in order to investigate whether these ingredients would increase the dustiness of flour. These tests showed gave the following results:

- Flour was mixed with emulsifier and the resulting mixture was 18 times dustier than flour on its own. Particle size analysis showed that the emulsifier also reduced the particle size of the flour.

- Calcium silicate was mixed with flour at various concentrations; as the content of calcium silicate was increased there was a dose-dependent increase in dustiness. Particle size analysis showed that the presence of calcium silicate reduced the average particle size of the flour.

- Calcium sulphate was mixed with flour; the resulting mixture was nearly 10 fold dustier than flour alone and over 4 fold dustier than calcium sulphate alone. Particle size analysis showed that the calcium sulphate reduced the average particle size of the flour.

The airborne dusts from these mixtures were analysed for allergen and compared. All the additives mixed into the flour (emulsifier, calcium sulphate and calcium silicate) helped the allergen (WFA) in the flour to become airborne. Reducing the amount of calcium silicate in the flour also reduced the airborne allergen content.

Revised improver mixtures

On the basis of the above findings, HSL found three possible methods of reducing dustiness in bakery improvers:

- Increasing the oil in the improver;

- Decreasing the calcium sulphate in the improver;

- Using emulsifier that contained less calcium silicate.

These results were discussed with HSE and ABIM and it was agreed that new improver mixtures would be prepared based on the results of the initial tests.

3.2 REVISED IMPROVER MIXTURES

3.2.1 Aims and samples

The improver mixtures were revised in order to investigate these three potential ways of decreasing the dustiness of improvers:

1. Increasing the amount of vegetable oil in the improver to 4%, the maximum amount that could be realistically added to an improver.

2. Decreasing the amount of calcium sulphate in the improver to 5%, as the practical minimum.

3. Decreasing the amount of calcium silicate within the emulsifier to 3% (equivalent to 0.6% in the overall improver), as the minimum level of free flow agent.

Seven improver mixtures were prepared by ABIM to show the effect of these changes on the dustiness of improver and the allergen content of the dust. The contents of these samples are shown in detail in Table 6, Appendix 6.1.1. In brief, the sample contents were as follows:

Table 2 Summary of contents of revised improver mixtures

Sample code	Description of improver mixture
Red2	The control sample: a typical improver used in the baking industry. The modified improvers were compared to this sample.
Orange2	This sample does not contain emulsifier. When compared to Red2, this sample demonstrates the effect of removing the emulsifier completely.
Green2	This sample does not contain calcium sulphate. When compared to Red2, this sample demonstrates the effect of removing the calcium sulphate completely.
Black2	The emulsifier within this sample contains the minimum amount of calcium silicate that could be used (3%). When compared to the Red2 sample, this sample demonstrates the effect of reducing the free flow agent within the emulsifier.
Blue2	This sample contains the minimum amount of calcium sulphate that can realistically be used (5%). When compared to the Red2 sample, this shows the effect of reducing the calcium sulphate in the improver.
White2	This sample contains the maximum amount of oil that could be realistically be used in improver (4%). When compared to the Red2 sample, this shows the effect of adding extra oil to improvers.
Yellow2	This sample contains all the changes in the Black2, Blue2 and White2 samples, i.e. decreased calcium sulphate and calcium silicate in the emulsifier and increased oil. This sample therefore demonstrates the effect of implementing all three realistic changes to the improver.

The methods are summarised in section 2.1 and full details are shown in Appendix 6.1. In brief, the following methods were used:

- Standard dustiness testing: to measure the potential of each improver to produce dust.
- User testing: to examine the dustiness of the improvers when handled manually.
- Analysis of the allergen content of the dusts: to investigate whether the changes to the improver could also change the allergen content of the dust (WFA and STI).

The results for each of these tests are described below.

3.2.2 Dustiness testing

The dustiness of these improvers was tested and the results are shown in Table 18, Appendix 6.2.5. These dustiness results are also shown in Figure 1.

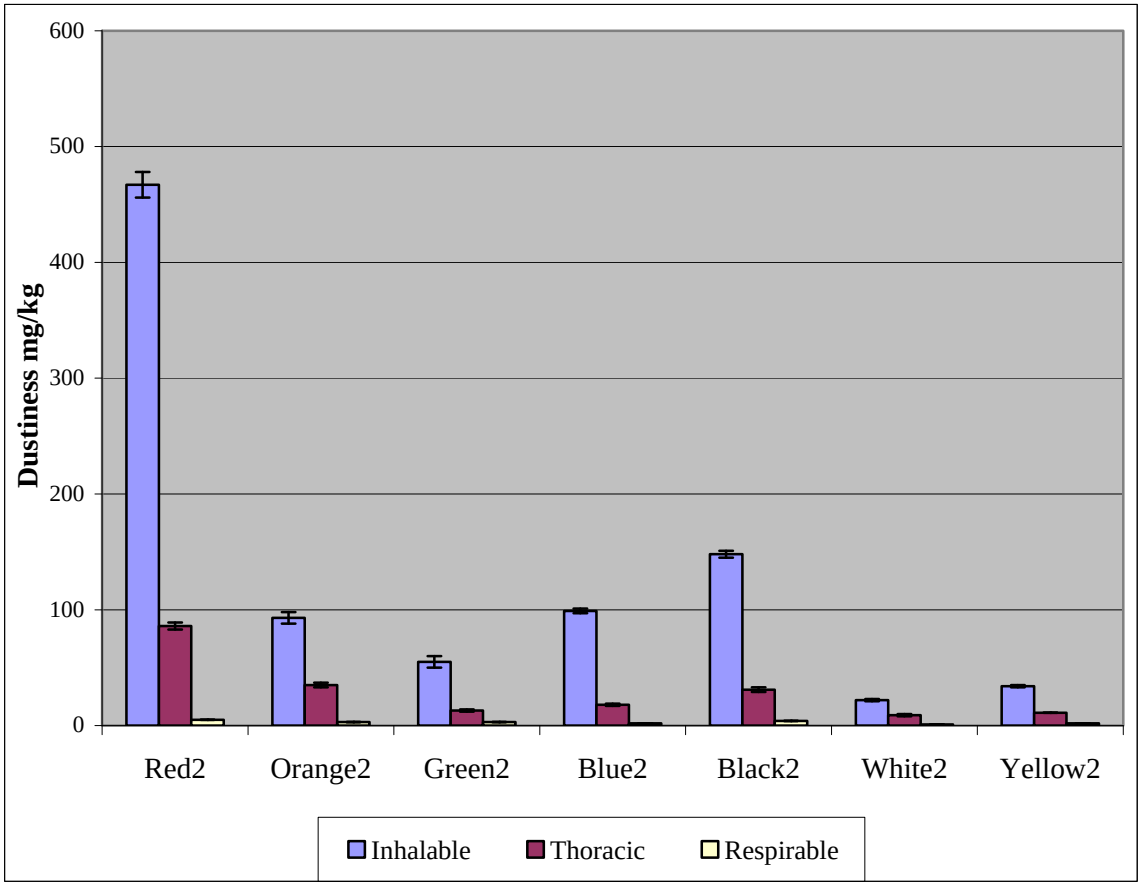


Figure 1 Dustiness testing of revised 2009 improver mixtures

Key to Figure 1 Sample descriptions and key ingredient changes

Sample	Description of change	Ca sulphate	Emulsifier	Oil
Red2	Typical Improver: control sample	25%	Contains 5% Ca silicate	2%
Orange2	No Emulsifier	25%	None	2%
Green2	No Ca sulphate	None	Contains 5% Ca silicate	2%
Blue2	Reduced Ca sulphate	5%	Contains 5% Ca silicate	2%
Black2	Contains emulsifier with reduced Ca silicate	25%	Contains 3% Ca silicate	2%
White2	Contains extra oil	25%	Contains 5% Ca silicate	4%
Yellow2	Contains all 3 realistic improvements	5%	Contains 3% Ca silicate	4%

The results of the dustiness tests in Figure 1 were as follows:

- The control sample (Red2, a typical improver used in the baking industry) was the dustiest of these improver samples.
- Increasing the oil to the maximum amount (White2) reduced the inhalable fraction of the dust by over 20 fold (to less than 5% of standard improver).
- Removing the calcium sulphate (Green2) decreased the inhalable fraction of the dust by over 8 fold (to 12% of the dustiness of normal improver). This is the third least dusty of the improver samples.
- Reducing the calcium sulphate to the minimum level (Blue2) reduced the inhalable fraction of the dust by nearly 5 fold (to 21% of standard improver).
- Removing the emulsifier (Orange2) decreased the inhalable fraction of the dust by 5 fold.
- Reducing the calcium silicate within the emulsifier (Black2) reduces the inhalable fraction of the dust 3 fold compared to the control sample (Red2); this reduction was not as great as that achieved by increasing oil (White2) or reducing calcium sulphate (Blue2).
- Implementing all three changes (Yellow2: reduced calcium sulphate and calcium silicate within emulsifier and increased oil) reduced the inhalable fraction of the dust by approximately 14 fold (to approximately 7% of standard improver) and comparable with that achieved with oil alone (White2).

Increasing the oil content had the largest impact on reducing the dustiness (quantified using standard dustiness testing).

3.2.3 Immunological analysis of the dust from dustiness testing

A summary of the wheat flour antigen (WFA), soya trypsin inhibitor (STI) and soluble protein results for the revised improver samples is shown below. The methods used are summarised in section 2.1 and full details are included in Appendix 6.1.

Bulk samples

The content of the total soluble protein, wheat flour antigen (WFA) and soya trypsin inhibitor (STI) in the bulk revised improver samples are shown in Table 19, Appendix 6.2.5. The results are expressed per gram of bulk improver and as a percentage of the bulk improver.

Airborne levels from dustiness testing

Total soluble protein, soya trypsin inhibitor and wheat flour allergen in airborne samples are expressed per gram of bulk improver and as a percentage of that component in the bulk improver (Table 20, Appendix 6.2.5). All of the bar charts and tables showing the results from the dustiness filters contain the mean results for three replicate samples.

Soluble protein

The results are described in detail with figures 38 and 39, appendix 6.2.5.

Soya trypsin inhibitor

Figure 2 shows the average results for the amount of Soya Trypsin Inhibitor in the collected dust (see Table 6 for sample details).

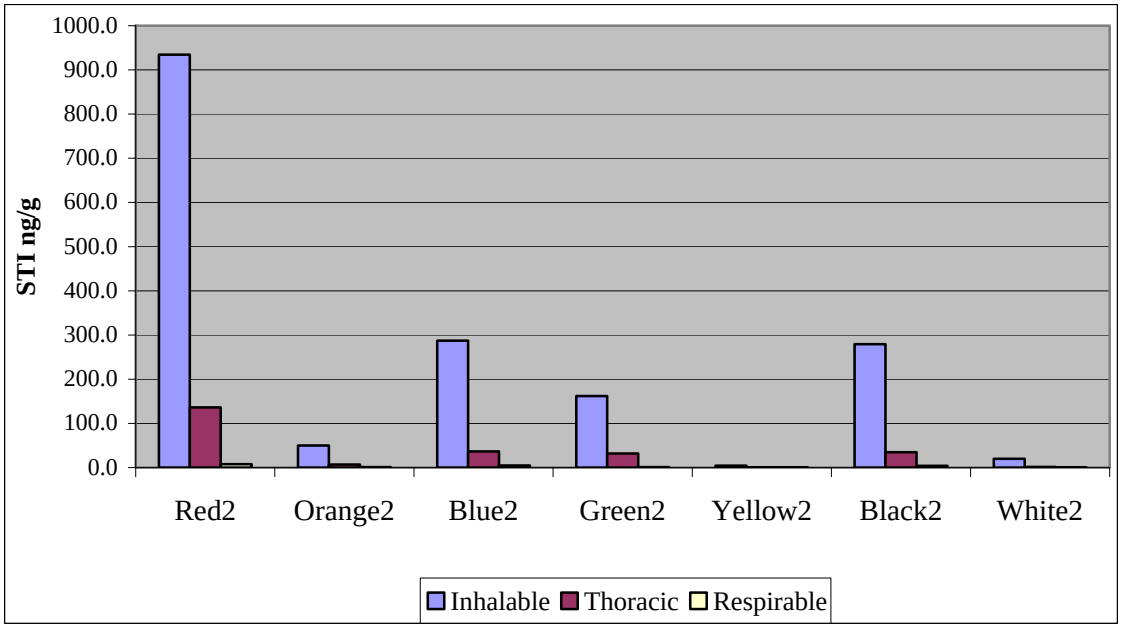


Figure 2 Average amount of soya trypsin inhibitor on the filters per gram of improver in the drum

Key to Figure 2 Sample descriptions and key ingredient changes

Sample	Description of change	Ca sulphate	Emulsifier	Oil
Red2	Typical Improver: control sample	25%	Contains 5% Ca silicate	2%
Orange2	No Emulsifier	25%	None	2%
Green2	No Ca sulphate	None	Contains 5% Ca silicate	2%
Blue2	Reduced Ca sulphate	5%	Contains 5% Ca silicate	2%
Black2	Contains emulsifier with reduced Ca silicate	25%	Contains 3% Ca silicate	2%
White2	Contains extra oil	25%	Contains 5% Ca silicate	4%
Yellow2	Contains all three realistic improvements	5%	Contains 3% Ca silicate	4%

Figure 2 shows the amount of soya trypsin inhibitor (STI) deposited on the filters and foams. The control sample (Red2) produced the highest amount of aerosolised STI. All of the changes made to these improvers decreased the amount of STI in the dusts, with the largest reductions in the samples that contained no emulsifier (Orange2: STI in the inhalable fraction was decreased by 19 fold), extra oil (White2: STI in the inhalable fraction was decreased by 47 fold), and when all three changes were implemented (Yellow2: STI in the inhalable fraction was decreased by 220 fold).

Wheat flour antigen

Figure 3 shows the results for the amount of wheat flour allergen in the dust samples from the revised improver samples (Table 6).

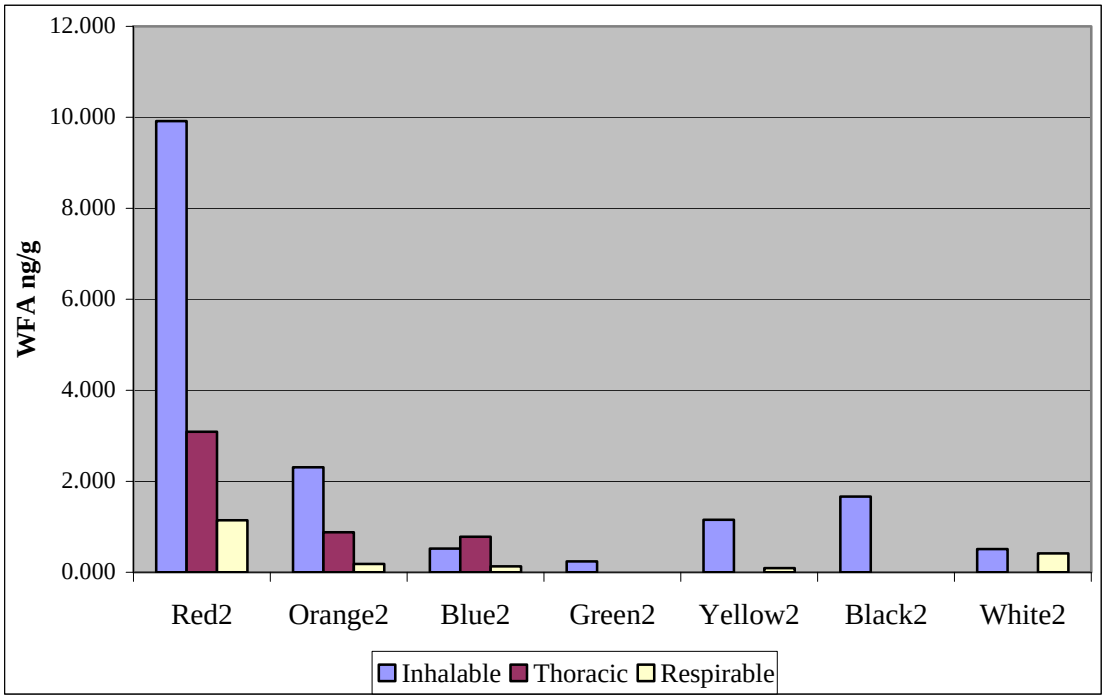


Figure 3 Amount of wheat flour allergen on the filters per gram of improver in the drum

Key to Figure 3 Sample descriptions and key ingredient changes

Sample	Description of change	Ca sulphate	Emulsifier	Oil
Red2	Typical Improver: control sample	25%	Contains 5% Ca silicate	2%
Orange2	No Emulsifier	25%	None	2%
Green2	No Ca sulphate	None	Contains 5% Ca silicate	2%
Blue2	Reduced Ca sulphate	5%	Contains 5% Ca silicate	2%
Black2	Contains emulsifier with reduced Ca silicate	25%	Contains 3% Ca silicate	2%
White2	Contains extra oil	25%	Contains 5% Ca silicate	4%
Yellow2	Contains all three realistic improvements	5%	Contains 3% Ca silicate	4%

Figure 3 shows that the amount of airborne wheat flour allergen from the control improver sample (Red2) was the highest. All the changes to the formulation of this improver reduced the content of WFA trapped on the filters. The largest reductions in airborne WFA were in the samples that contained: reduced calcium sulphate (Green2: WFA in the inhalable fraction was reduced by 42 fold) and the sample with additional oil (White2: WFA in the inhalable fraction was reduced by 19 fold).

3.2.4 User testing

User testing was performed in order to investigate whether the results from the dustiness testing were also seen in a work-task simulation. The user test was performed using the conditions for each improver sample of: sampling time of 45 minutes, scooping and pouring at a rate of 20 pours per minute, in still air conditions in an exposure chamber.

The user testing was performed on the control sample (Red2), the sample with extra oil (White2), the sample with the reduced calcium sulphate content (Blue2) and the sample with the reduced calcium silicate in emulsifier (Black2) for the revised improver samples (Table 6). Table 21 (Appendix 6.2.5) shows the details of the results and includes the inhalable and respirable dust concentrations for the user tests, as measured by the microdust monitor and gravimetric results.

The user tests produced less dust than the standard dustiness tests, and so the weight of the dust on the filters for the respirable fraction (PGP10 sampler, Table 21) was too low to be accurately measured by gravimetric analysis. The microdust monitor gave a more reliable measure of the respirable dust levels and so these results were used for the analysis and are shown in Figure 4. It should be noted that the microdust results are likely to differ from the true level of respirable dust since the instrument is calibrated in the factory with a ‘standard’ test dust, which will have different properties from the dust tested here. Only the inhalable sample (measured gravimetrically from CIS sampler) and the respirable fractions (measured using the microdust sampler) are reported in Figure 4.

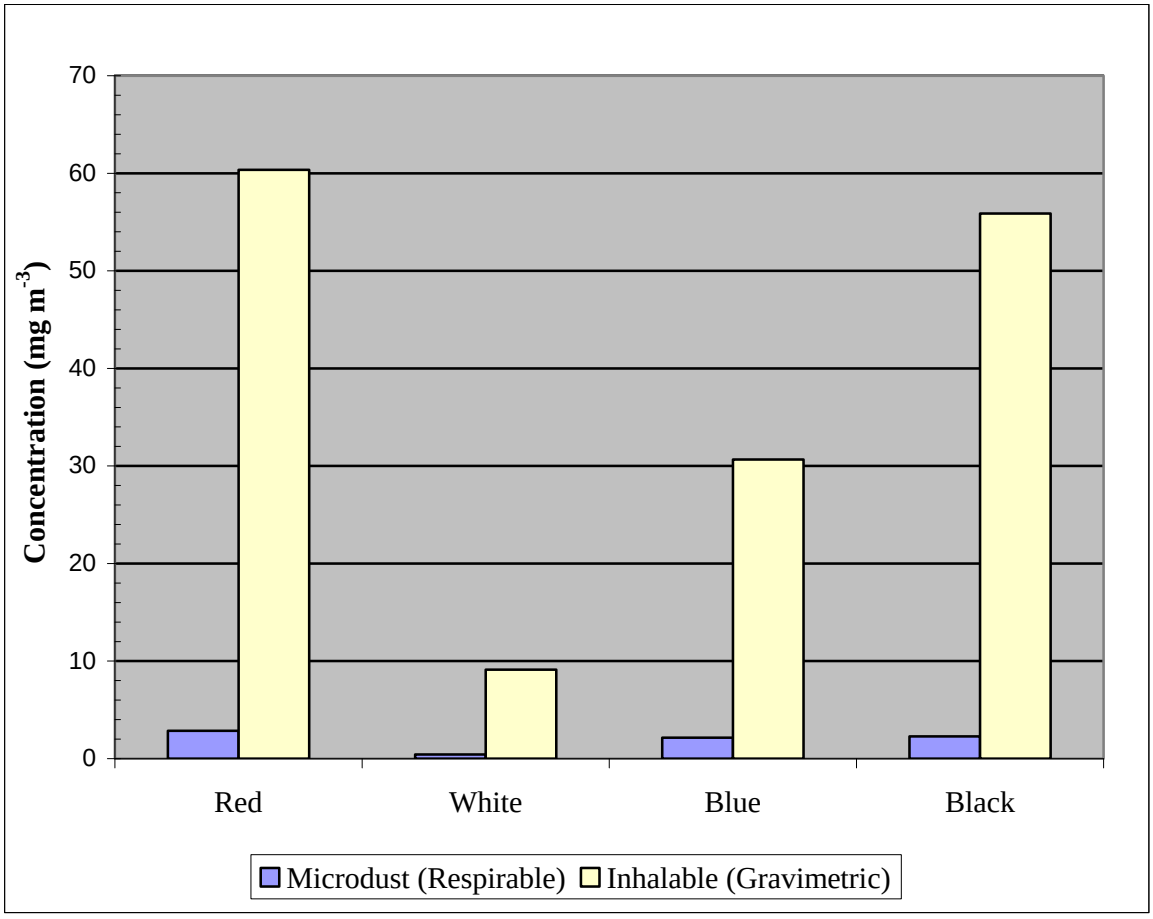


Figure 4 Exposure concentrations from user testing of inhalable and respirable dust

Key to Figure 4 Sample descriptions and key ingredient changes

Sample	Description of change	Ca sulphate	Emulsifier	Oil
Red2	Typical Improver: control sample	25%	Contains 5% Ca silicate	2%
Blue2	Reduced Ca sulphate	5%	Contains 5% Ca silicate	2%
Black2	Contains emulsifier with reduced Ca silicate	25%	Contains 3% Ca silicate	2%
White2	Contains extra oil	25%	Contains 5% Ca silicate	4%

The results in Figure 4 demonstrate that the inhalable dust levels for the control sample (Red2) were the highest (60.36 mg/m^3) and were also high for the Black2 sample containing the emulsifier with reduced calcium silicate content (55.87 mg/m^3). The inhalable dust was lowest for the sample containing additional oil (White2: 9.12 mg/m^3 a 6.6 fold decrease in dust compared to the control). Reducing the calcium sulphate concentration (Blue2) reduced the dustiness to approximately a half (30.67 mg/m^3) of the control.

3.2.5 Immunological analysis of dust from user test

The dust samples were analysed for wheat flour antigen (WFA), soya trypsin inhibitor (STI) and soluble protein.

Airborne levels from user testing

The user testing was performed on the control sample (Red2), the sample containing extra oil (White2), the sample with reduced calcium sulphate content (Blue2) and the sample containing emulsifier with reduced calcium silicate (Black2) for the revised improver samples (Table 6). The content of soluble protein, STI and WFA were expressed in micrograms per m^3 (for protein) or nanograms per m^3 (for allergens). They are shown in detail in Table 22, Appendix 6.2.5.

Soluble protein

These results are detailed in Figure 42, Appendix 6.2.5. In summary, all the control measures reduced exposure to soluble proteins; the most effective method by far was the addition of oil.

Soya trypsin inhibitor

Figure 5 shows the airborne soya trypsin inhibitor (STI) from the revised improver samples.

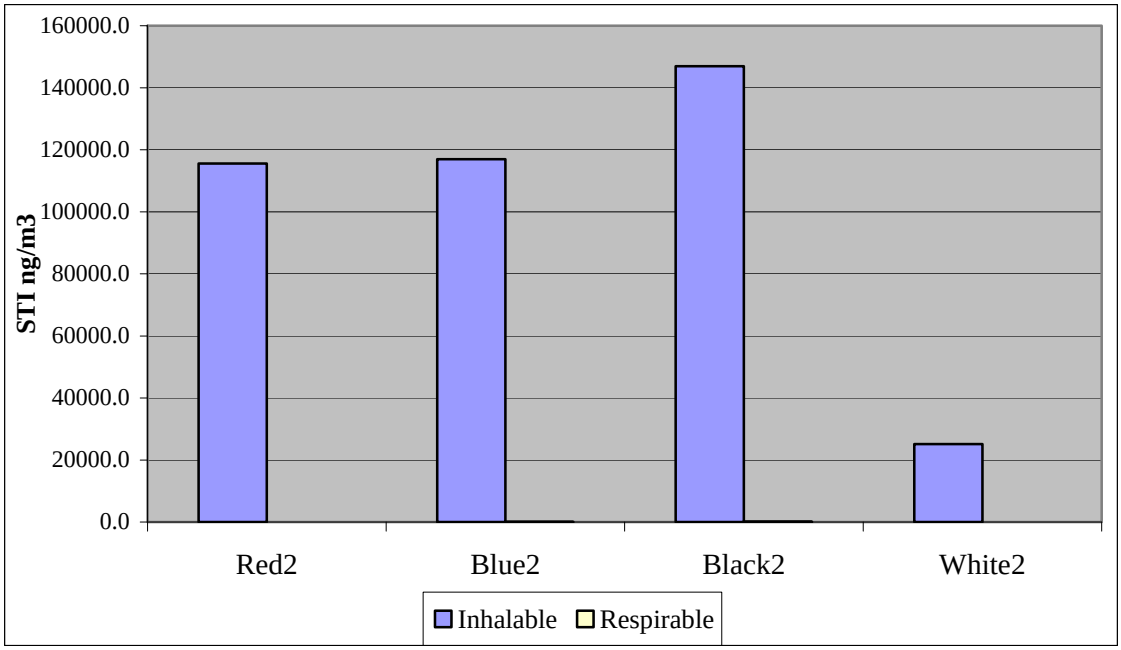


Figure 5 Concentration of soya trypsin inhibitor on the filters (ng/m3)

Key to Figure 5 Sample descriptions and key ingredient changes

Sample	Description of change	Ca sulphate	Emulsifier	Oil
Red2	Typical Improver: control sample	25%	Contains 5% Ca silicate	2%
Blue2	Reduced Ca sulphate	5%	Contains 5% Ca silicate	2%
Black2	Contains emulsifier with reduced Ca silicate	25%	Contains 3% Ca silicate	2%
White2	Contains extra oil	25%	Contains 5% Ca silicate	4%

Figure 5 shows that by adding extra oil to the improver (White2) the exposure to STI was reduced by nearly 5 fold compared to the control sample (Red2). For this allergen, reducing the calcium sulphate content of the improver (Blue2) did not reduce the aerosolised STI compared to the control. Reducing the calcium silicate in the emulsifier (Black2) appeared to have slightly increased the exposure to STI; this sample had approximately 27% more STI on the filter than the control, but this change may not be statistically significant as the analytical imprecision of the method is approximately 12%.

Wheat flour antigen

Figure 6 shows these results for airborne wheat flour allergen from the revised improvers (Table 6).

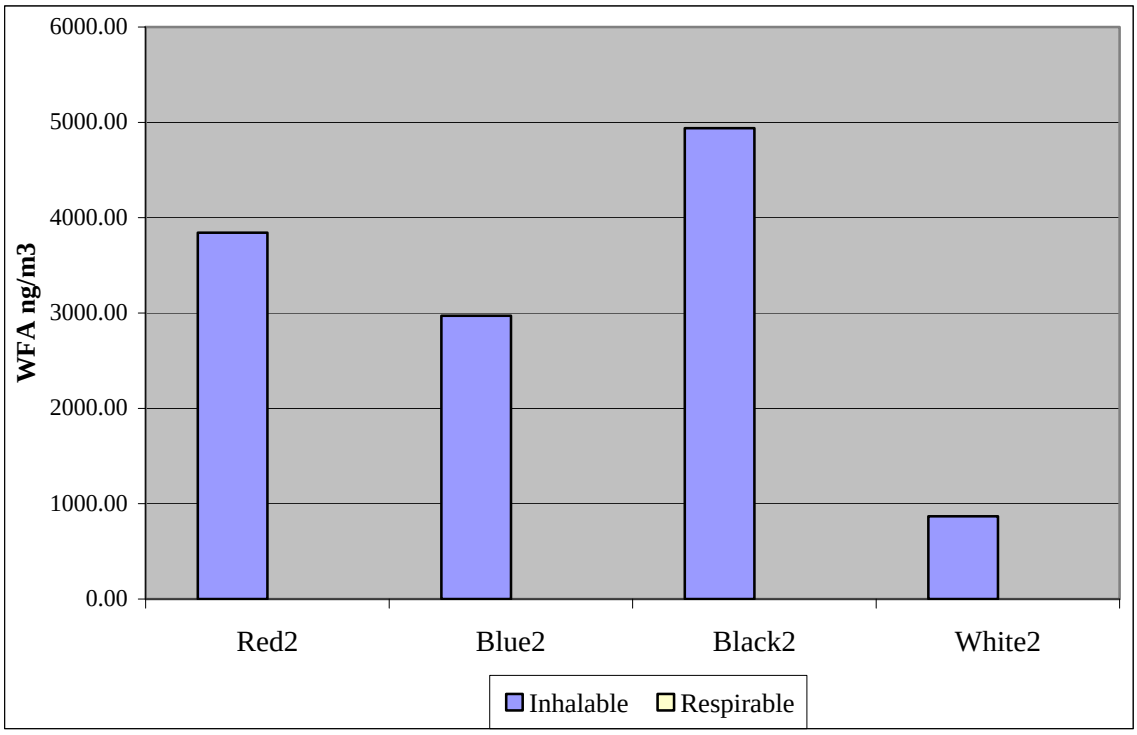


Figure 6 Concentration of wheat flour allergen on the filters (ng/m3)

Key to Figure 6 Sample descriptions and key ingredient changes

Sample	Description of change	Ca sulphate	Emulsifier	Oil
Red2	Typical Improver: control sample	25%	Contains 5% Ca silicate	2%
Blue2	Reduced Ca sulphate	5%	Contains 5% Ca silicate	2%
Black2	Contains emulsifier with reduced Ca silicate	25%	Contains 3% Ca silicate	2%
White2	Contains extra oil	25%	Contains 5% Ca silicate	4%

Figure 6 showed that the addition of extra oil to the improver (White2) reduced the aerosolised WFA in the inhalable fraction (adding oil reduced the WFA by 4.4 fold). Reducing the calcium sulphate in the improver (Blue2) slightly decreased the WFA in the dust to 77% of the WFA found in the control sample. The reduction of calcium silicate in the emulsifier (Black2) appeared to increase the content of WFA in the dust (the WFA in the dust for this sample was 28.5% more than the control). These differences in Blue2 and Black2 may not be statistically significant as the analytical imprecision of the method is approximately 12%.

3.3 MAIN FINDINGS

The ingredients of bakery improvers can have a dramatic effect on their dustiness and the allergens that may become airborne and inhaled by the bakery operative. Three control measures have been investigated that attempt to reduce the dustiness of bakery improvers and exposure to allergens. The main findings can be summarised as follows:

Adding extra oil to improvers

Increasing the oil content was found to reduce the dustiness of the improver by nearly 5 fold in the dustiness tests and by nearly 6 fold in the user tests. This was by far the most effective method of decreasing the dustiness of bakery improvers. Adding extra oil (to the maximum level that ABIM noted as practical) greatly decreased the amount of airborne allergens (both WFA and STI) and their tendency to become airborne. These effects were observed consistently in both the standard dustiness tests and the user test.

Reducing the amount of calcium sulphate in improvers

This change reduced the dustiness of the improver in the standard dustiness tests by nearly 5 fold and to a lesser extent (by half) in the user test. Calcium sulphate has a small particle size and when mixed with flour it reduced the mean particle size of the mixture. A mixture of calcium sulphate and flour is dustier than either flour or calcium sulphate on their own. Reducing the calcium sulphate in the improver was effective at reducing the amounts of

airborne allergens (STI and WFA) in the dustiness tests. However, in the user tests this measure did not appear to have an impact on the potential exposure to STI and the airborne WFA was only slightly reduced.

Reducing the amount of calcium silicate within the emulsifier mix

Emulsifier increased the dustiness of baking improvers. Calcium silicate is used in emulsifier as a free flow agent, and this agent within the emulsifier initially appeared responsible for increasing the dustiness; when mixed with flour only, there was a direct correlation between the amount of calcium silicate and the dustiness of the flour. Calcium silicate has a small particle size and by mixing it into flour, it reduced the mean particle size of the mixture.

In the dustiness tests, reducing the calcium silicate in the emulsifier decreased the dustiness of the improver. Reducing the calcium silicate within the emulsifier was also effective at reducing the amount of airborne allergens (STI and WFA). However these findings were not repeated in the user test, which only resulted in a small reduction to the total dust. The exposure to WFA and STI allergens was slightly increased, but this may not be statistically significant.

Of the measures described above, the most effective method of reducing dustiness of bakery improvers and reducing exposure to allergens was to add extra oil.

4 DISCUSSION

Three potential methods of reducing dustiness were investigated using revised formulations of the improver mixture. This discussion focuses on the key findings from this series of tests. The initial findings (original improvers and the examination of the ingredients) are discussed in Appendix 6.3.

4.1 REVISED IMPROVER SAMPLES

Three possible ways of decreasing dustiness and exposure to allergens were investigated.

1. Increasing the amount of vegetable oil in the improver to 4%, the maximum amount that could be realistically added to an improver.

2. Decreasing the amount of calcium sulphate in the improver to 5%, as the practical minimum.

3. Decreasing the amount of calcium silicate within the emulsifier to 3% (equivalent to 0.5% in the overall improver), as the minimum level of free flow agent.

Seven improvers were prepared to investigate the effect of these potential control measures. The mixtures were based on a typical baking improver recipe, with one modification to each formulation, so that each variation could be compared to the control improver. One improver contained all of the modifications.

4.1.1 Dustiness testing

The addition of extra oil

The improver containing the extra oil (White2, 4% oil) showed the biggest reduction in the dustiness tests (dustiness was reduced by over 20 fold). The improver with all the modifications (Yellow2) displayed similar dustiness (dustiness was reduced by 14 fold), showing that addition of oil is the modification that makes the most difference. The dust produced from the dustiness testing was analysed for two allergens (STI and WFA). The amount and percentage of airborne STI and WFA in the dust from these two modified improvers (White2 and Yellow2) was also low, demonstrating that extra oil reduced the potential of these allergens to become airborne. Both these modifications (shown by the sample with extra oil and the sample with extra oil plus the other changes) made a substantial reduction in the potential exposure compared to the standard improver mixture. Out of the control measures investigated, the addition of extra oil is thought to be the easiest to implement. However, extra oil could affect how easily the improver is blended and this effect on blending could vary depending on seasonal temperatures.

Reducing the calcium sulphate

Reducing the calcium sulphate in the improver to 5% also reduced the dustiness of the improver by nearly 5 fold but not as much as adding extra oil. Removing the calcium sulphate altogether further reduced the dustiness (by 8.5 fold).

This reduction was also seen for the airborne STI; reducing the calcium sulphate decreased the STI in the inhalable dust (by over 3 fold) but not as much as employing extra oil. However the percentage of the STI that was airborne was slightly higher for the sample without calcium sulphate than the sample with only reduced calcium sulphate. It is possible that the efficiency of STI recovery from the bulk sample has been altered by the absence of calcium sulphate. In fact, one of the reasons calcium sulphate is added to improvers is to help the fatty materials to blend.

Reducing the calcium sulphate in the improver reduced the airborne WFA by 19 fold; removing it altogether reduced the airborne WFA further (a 42 fold reduction in the inhalable dust). The percentage of airborne WFA was higher for the sample with no calcium sulphate compared to the sample with reduced calcium sulphate. The percentage of measured WFA in the bulk sample was approximately ten times higher than detected in the sample with reduced calcium sulphate; this is possibly for the same reason that calcium sulphate alters the efficiency of WFA extraction from the bulk material. It is also possible that the relationship between amount of calcium sulphate in the improver and the airborne release of allergens is not linear and could therefore be an optimum amount of calcium sulphate that would help to reduce the exposure to allergens.

Reducing the calcium silicate within the emulsifier

Reducing the calcium silicate within the emulsifier reduced the dustiness of the improver 3 fold in the standard dustiness tests, but was the least effective of the three methods. Removing the emulsifier reduced the dustiness even more, to approximately the same as an improver with minimum calcium sulphate (the dustiness of the inhalable fraction was reduced 5 fold).

Reducing the calcium silicate in the emulsifier also reduced the airborne STI by over 3 fold in the inhalable dust, approximately the same amount as the sample containing minimum calcium sulphate. Removing the emulsifier reduced the aerosolisation of STI even further and reduced the amount of STI in the inhalable fraction by over 5 fold. These reductions were not as large as those achieved by adding extra oil.

Reducing the calcium silicate in the emulsifier reduced the airborne exposure to WFA by 6 fold, but without any emulsifier the WFA in the inhalable fraction was only reduced 4 fold. The relationship between exposure to WFA, emulsifier and calcium silicate is not straightforward and does not appear to be linear. It is possible that there is an optimum amount of emulsifier and /or calcium silicate that would reduce the exposure to allergens, but this may need further clarification.

The dustiness testing of these improver samples showed that all three of these methods reduced the dust and airborne allergens. Of these, adding extra oil is the most effective way to reduce dustiness and exposure.

4.1.2 User testing

The user test was performed on four samples: the typical improver, the sample containing extra oil, the sample containing reduced calcium sulphate, and the sample containing reduced calcium silicate in the emulsifier. These were the three separate modifications that could realistically be performed, together with the typical improver.

The user test consisted of handling the improvers manually in a manner that simulated how the improvers would be handled in the industry. This was performed in an exposure chamber and samples were gathered on air filters to gain an idea of the type of exposure the operator would get for each of the improvers. This was performed in as controlled and repeated a manner as possible, but since this was performed manually the operations would not be duplicated exactly and there were obvious variations between tests. The tasks were performed continuously for 45 minutes during which period dust was collected by air samplers and measured using a microdust monitor (real time dust monitor). As previously described, the weight of the dust for the inhalable fraction was reported and the microdust results were reported instead of the respirable fraction.

The user test results showed the same trend as the gravimetric results for the dustiness tests. The typical improver had the highest levels of total dust, and there is a reduction in dust for all three modifications implemented. The most effective modification was the addition of oil, followed by the reduction of calcium sulphate, and lastly the reduction of calcium silicate in the emulsifier. This is the same pattern found with the dustiness measurements except that the reduction of calcium silicate in the emulsifier showed only a small reduction in dust in the user test. The extra oil reduced the dust for the user test by over 6 fold and the reduction in calcium sulphate reduced the dust to approximately half that seen in the control sample.

When handled manually, the reduction of calcium silicate in the emulsifier was less effective as a control measure than when these samples were tested in a rotating drum, as the dustiness was only reduced slightly in the user test and this may not be a significant change. Since the use of free flow agents is widespread in mechanised mixing of food, their potential effect on the dustiness of materials containing allergens may need to be considered alongside the impact of other ingredients. The addition of extra oil, however, produced a large improvement in the total dust and the reduction of calcium sulphate shows a reasonable drop in the total dust levels, so these modifications were still effective during the user test.

A possible reason why there are differences between the standard dustiness drum test and the user test may be due to the amount of energy imparted by these two procedures. The standard drum test applies a constant uniform rotational energy, whereas the user test reproduces more realistic handling conditions. The way that improvers are handled and the presence of air currents are important variables in the amount of material that becomes airborne. This may also suggest that certain mechanised processes that impart more energy to the improver may generate more dust than when the improver is handled manually.

The addition of extra oil was the only control measure in the user tests that reduced the STI on the inhalable filters. This sample had levels of STI that were nearly 5 fold lower than the control sample. In the inhalable fractions, the reduction of calcium sulphate had no effect at all on the level of STI, while the reduction of calcium silicate in the emulsifier increased the amount of

STI detected by 27%. The amount of STI on the respirable filters (Table 22, Appendix 6.2.5) showed that the most STI was detected for the improver containing the reduced calcium silicate in the emulsifier, and the least amount of respirable STI was in the improver with additional oil. The addition of oil was the only modification that did not increase exposure when compared to typical improver. A similar pattern was evident for the WFA in the inhalable fractions.

The user test investigated the behaviour of these agents when handled in a more realistic scenario and the results are reported in units that are comparable to those used in occupational hygiene surveys. The scooping and pouring task was based on observations that were made during a visit to a bakery, so these results are more applicable to the exposure found in bakeries than the dustiness results. However, these tasks were performed once only for a limited time period and variations in how the task is carried out in the workplace are not represented in the data, so these results cannot be taken as representative of actual exposure in the industry. Taking these limitations into account, the data showed that only the addition of extra oil consistently reduced exposure to improver dust and allergens.

5 CONCLUSIONS

The ingredients used in bakery improvers were studied in terms of their dustiness properties and behaviour when mixed.

Oil and soya flour reduced the overall dustiness of the improver. The presence of calcium sulphate and emulsifier increased the dustiness of the improver. The emulsifier contained calcium silicate as a ‘free flow agent’; the effect of calcium silicate on the dustiness of flour was examined. A direct correlation between the amount of calcium silicate and the dustiness of the flour was found.

Three measures to control dustiness of bakery improvers were investigated. These are shown below in order of their effectiveness on dustiness and exposure to bakery allergens.

1. Adding extra oil to bakery improvers reduced the dustiness of the material and the potential exposure to allergens; it was the most effective modification studied. This was demonstrated both in the dustiness tests (by over 20 fold) and in the user test (by over 6 fold). It is generally thought to be the easiest method to implement, however extra oil could affect how easily the improver is blended and so could be subject to limitations.

2. Reducing the amount of calcium sulphate in the improver reduced the dustiness of the material in both the dustiness test (by nearly 5 fold) and the user test (reduced by half). During dustiness testing, this modification reduced the levels of airborne allergens, however it did not in the user tests. This method is potentially difficult to implement due to the intellectual property rights of different bread manufacturers; however the results of this study provide an indication of the impact of calcium sulphate on the dustiness of the improver.

3. Reducing the calcium silicate in the emulsifier reduced the dustiness of the improver (3 fold) and the airborne allergens in the dustiness tests. However in the user test, this modification did not reduce the dust and the levels of airborne allergens were slightly elevated. Although the free flow properties of the emulsifier would need to be investigated, this method would be easier to implement than reducing the calcium sulphate.

This study has shown the potential for reducing dustiness of bakery improvers through altering the content of their ingredients. There are other controls that can be applied, such as local exhaust ventilation and containment, however changing the formulation of the improver represents a practical and inexpensive method of reducing the dustiness.

6 APPENDICES

6.1 METHODS APPENDICES

6.1.1 Samples tested

Original improver mixtures

The original six improver mixtures sent from ABIM were coded by colour so that the analysis could be blind. These improvers were intended to range from very dusty to not dusty in their properties depending on the binding agents added to the mixtures. Aliquots of each bulk material stored in sealed plastic bags at 4^0C. The ingredients contained in these original improver samples are shown in Table 3.

Table 3 Constituents of original 2008 improvers

Code	HSL ID number	Wheat flour (%)	Ascorbic acid (%)	Fungal alpha amylase (%)	Full fat soya flour (%)	Calcium sulphate (%)	Emulsifier E472e containing 5% carrier (%)	Rapeseed oil (%)
Green	00123/08	58.956	1	0.044	20		20	
Yellow	00124/08	78.956	1	0.044	20			
Red	00125/08	98.956	1	0.044				
Black	00126/08	33.956	1	0.044	20	25	20	
Orange	00127/08	31.956	1	0.044	20	25	20	2
Blue	00128/08	99	1					

Each of these improver mixtures underwent dustiness testing in triplicate. The inhalable, thoracic and respirable fractions of these samples were generated and extracted. For each of these bulk improver samples nine further samples (filter and foams) were generated from the dustiness testing and analysed for the allergens (WFA and STI). Three Blank filters were used to correct the results of the analyses. Therefore, a further 57 samples were generated from the improver mixtures in table 3, these filter and foam extracts were recorded on worksheet 08-0019/B as HSL sample numbers 00721/08 to 00777/08.

A portion of the bulk samples in Table 3 were also extracted and analysed for allergens as described.

Individual ingredients

ABIM provided the individual ingredients from the improver mixtures; again these were coded and stored in sealed plastic bags at 4^0C. The samples are shown in Table 4.

Table 4 Samples of individual ingredients

Code	HSL ID	Ingredient	Notes
A	01825/08	Flour 1	Filler flour – cheaper flour than bread making flour. Finer in texture.
B	01826/08	Calcium Sulphate	Used as a calcium supplement and filler in improvers. Was originally suggested as something to decrease dustiness.
C	01827/08	Soya Flour	Suggested as an ingredient that would decrease dustiness.
D	01828/08	Flour 2	Filler flour – cheaper flour than bread making flour. Finer in texture.
E	01829/08	Emulsifier E472e	This is the same emulsifier used in Table 3; it is composed of 95% data ester, 5% calcium silicate. This ingredient was originally suggested as a binding agent.
F	01830/08	Flour 3	Bread making flour.
G	01831/08	Calcium carbonate	Not used in the improvers in Table 3, however used in the same way as calcium sulphate.
No code	03195/08	Data Ester (E472e Emulsifier)	This is the emulsifier ingredient without the carrier / anti caking / free flow agent. This would be unusable on its own, as it would stick together.
No code	03196/08	Calcium silicate	Used as an anti-caking agent in emulsifier (free flow agent). This forms 5% of the emulsifier (E above).
No code	03197/08	Rapeseed oil	Suggested as an ingredient that would decrease dustiness.

Each of these individual ingredients were analysed for dustiness in triplicate. The filters and foams produced from the dustiness testing were not retained. Aliquots of the bulk samples in table 4 were extracted and analysed for the allergen content.

The following samples from table 4 were also subjected to particle size distribution testing: Data ester (E472e), Soya flour, Flour 2, Flour 3, Calcium sulphate and Calcium silicate (sample numbers 03195 / 08, 01827 / 08, 01828 / 08. 01830 / 08, 01826 / 08 and 03196 / 08 respectively).

Within sample variation and simple combinations of ingredients

Further investigations of the samples in Tables 3 and 4 were performed.

- Fractions from various areas of the "Black" and "Orange" (Table 3, Appendix 6.1.1) sacks to investigate whether the contents were distributed homogenously within the bulk.

- Simple combinations of the ingredients in Table 4 to investigate how these ingredients interact with each other when in combination versus the behaviour of the ingredients on their own.

Samples tested in these experiments are shown in Table 5.

Table 5 Fractions of improver mixtures and simple combinations of bakery ingredients

HSL ID	Ingredients	Description	Reason for testing	Dustiness samples generated
05355/08	Orange: Top	A sample selected from the top of "Orange" sack	To look at variation within the "Orange" sample. This improver sample contained all the ingredients so was the most likely to have heterogeneity in the sample.	Did not extract from these filters and foams.
05356/08	Orange: Middle	A sample selected from the middle of "Orange" sack		
05357/08	Orange: Bottom	A sample selected from the bottom of "Orange" sack		
07646/08	Black: Fraction 1	A sample selected from the top of "Black" sack	To look at variation within the "Black" sample. The "Black" sample contained all ingredients with the exception of oil.	These bulk fractions were analysed for allergens only.
07647/08	Black: Fraction 2	A sample selected from the middle of "Black" sack		
07648/08	Black: Fraction 3	A sample selected from the bottom of "Black" sack		
07649/08	Emulsifier (100g) + Flour 3 (400g)	A mix of 80% flour (F, Table 4) and 20% emulsifier (E, Table 4). This is same ratio as in improver samples – without other ingredients.	To compare with dustiness of flour and / or emulsifier on their own and look at the effect of mixing.	05358 /08 to 05366 / 08. 9 samples.
07650/08	Ca sulphate (100g) + Flour 3 (300g)	A mix of 75% flour (F, Table 4) and 25% Ca sulphate (B, Table 4). This is same ratio as in improver samples – without the other ingredients.	To compare with dustiness of flour and / or Ca sulphate on their own and look at the effect of mixing.	05376 / 08 to 05384 / 08. 9 samples.
07651/08	Ca silicate (10g) + Flour 3 (490g)	A mix of 98% flour (F, Table 4) and 2% Ca silicate (Table 4). This is the maximum amount of Ca silicate that could be used in improver.	To compare with dustiness of flour and / or Ca silicate on their own and look at the effect of mixing.	05367 / 08 to 05375 / 08. 9 samples.

07652/08	Ca silicate (5g) + Flour 2+ 3 (495g)	A mix of 99% flour (F+D, Table 4) and 1% Ca silicate (Table 4). This is the same amount of Ca silicate that would be in the improver above (as part of emulsifier).	Several concentrations of Ca silicate in flour were tried to look at whether there was a dose response – to investigate whether dustiness decreases with decreasing Ca silicate.	07654 / 08 to 07662 / 08. 9 samples.
07653/08	Ca silicate (3g) + Flour 2+ 3 (497g)	A mix of 99.4% flour (F+D, Table 4) and 0.6% Ca silicate (Table 4). This is the lowest amount of Ca silicate that could potentially be used in emulsifier (advised by ABIM)		07663 / 08 to 07671 / 08. 9 samples.

These samples were all analysed for dustiness in triplicate, with the exception of the "Black" fractions (07646 / 08 to 07648 / 08). These "Black" fractions were analysed for allergens only to investigate the 'within sample' variation. The bulk "Black" sample shown in Table 3 (00126 / 08) was re-analysed after 4 months to see if the initial dustiness data was reproduced in comparison with the "Orange" fractions (05355 / 08 to 05357 / 08) i.e. to investigate the effect of oil on the dustiness properties.

The inhalable, thoracic and respirable fractions of the dustiness samples were generated as described. Where indicated in Table 5, dustiness filters and foams were extracted and analysed for allergens. The bulk samples in Table 5 were also extracted and analysed for allergens.

The following mixtures of ingredients shown in Table 4 were subjected to particle size distribution testing:

- 25% calcium sulphate in flour 2: to investigate whether adding calcium sulphate to flour changed the particle size distribution.
- 20% emulsifier in flour 2: to investigate whether adding emulsifier (as provided by the manufacturer, sample E in Table 4) to flour changed the particle size distribution.
- 19% data ester in flour 2: to investigate whether the data ester within the emulsifier changed the particle size distribution of the mix when added to flour.
- 0.6% calcium silicate, 19.4% data ester in flour 2: The calcium silicate and data ester represent emulsifier with the minimum amount of free flow agent. This sample is to look at whether a modified emulsifier would have a different effect from adding standard emulsifier to flour on the particle size distributions.
- 1% calcium silicate in flour 2: to investigate whether adding calcium silicate to flour changed the particle size distribution. This proportion of calcium silicate was the same as in a 20% emulsifier mix.
- 0.6% calcium silicate in flour 2: The calcium silicate is the same as that contained in the proposed modified emulsifier with the minimum amount of free flow agent. This was to investigate whether reducing the calcium silicate changed the particle size distribution.

Revised improver mixtures

ABIM provided revised mixtures of the improver samples; again these were coded by colour so that the analysis was blind. These improvers were stored sealed in plastic bags at room temperature. The temperature of storage was changed to room temperature based on data obtained from within sample variation experiments and discussions with the ABIM members concerning realistic storage conditions. The ingredients contained in these samples are shown in Table 6.

Table 6 Constituents of revised 2009 improvers

Code	HSL ID	Wheat flour (%)	Ascorbic acid (%)	Fungal alpha amylase (%)	Full fat soya flour (%)	Calcium sulphate (%)	Emulsifier E472e containing 5% carrier (%)	Emulsifier E472e containing 3% carrier (%)	Rapeseed oil (%)
Red2	00846/09	31.956	1	0.044	20	25	20	-	2
Orange2	00847/09	51.956	1	0.044	20	25	-	-	2
Blue2	00848/09	51.956	1	0.044	20	5	20	-	2
Green2	00849/09	56.956	1	0.044	20	-	20	-	2
Yellow2	00850/09	49.956	1	0.044	20	5	-	20	4
Black2	00851/09	31.956	1	0.044	20	25	-	20	2
White2	00852/09	29.956	1	0.044	20	25	20	-	4

These supplied samples were designed to show the effect of manipulating the levels of particular ingredients that were thought to increase or decrease the dustiness of the improver as a whole. The samples in Table 6 represent the following:

- Red2 was the control sample, reflecting a typical improver used in the baking industry.
- Orange2 was a typical improver that does not contain emulsifier, so demonstrating the effect of emulsifier on the dustiness of improver.
- Green2 was a typical improver not containing calcium sulphate, so demonstrating the effect of calcium sulphate on the dustiness of improver.
- Black2 was a typical improver that contained emulsifier produced using the minimum amount of calcium silicate (“free flow agent”). This amount of free flow agent was discussed with the ABIM members and was thought to be the smallest amount that could possibly be used.
- Blue2 was a typical improver that contained the minimum amount of calcium sulphate that could realistically be used in an improver as discussed with the ABIM members. This sample demonstrated the change in dustiness that was obtained by reducing calcium sulphate in the improver mix.
- White2 was a typical improver that contains the maximum amount of oil that could realistically be used in improver as discussed with the ABIM members. This sample

demonstrated the change in dustiness that was obtained by increasing oil in the improver mix.

- Yellow2 was a typical improver and contained all the changes described in the Black2, Blue2 and White2 samples, i.e. decreased calcium silicate in the emulsifier, decreased calcium sulphate and increased oil. This sample demonstrated the dustiness of the improver when all three changes were implemented.

Five of the improver samples above differed by only one ingredient from the control, in order to ascertain the effect of that particular ingredient. One improver sample ("Yellow2") contained the three realistic changes in combination.

Each of these samples was analysed for dustiness in triplicate. The inhalable, thoracic and respirable fractions were generated and extracted. For each of the bulk improver samples listed above, nine further samples (filter and foams) were generated from the dustiness testing and analysed for the allergens as described. Three blank filters were used to correct the results of the analyses. Therefore a further 66 samples were generated from the dustiness testing of the improver mixtures in Table 6. These filter and foam extracts were recorded as HSL sample numbers 03881/09 to 03946/09.

Aliquots of the bulk samples in Table 6 were also extracted and analysed for allergens WFA and STI.

The user test was performed on the "Red2", "White2", "Blue2" and "Black2" samples. "White2", "Blue2" and "Black2" represented changes that could realistically be made to baking improver and "Red2" was the control. Filters for inhalable and respirable fractions were generated from the user test and analysed for their allergen content. Appropriate blanks for gravimetric and allergen analysis were carried out. Therefore a further 24 samples were generated from the user tests of the improver mixtures in Table 6. These filter extracts were recorded as HSL sample numbers 03947/09 to 03970/09.

A user test was initially trialled before any samples were analysed. This suggested an extension in sampling times in order to collect an accurately measurable dust weight. Samples were reanalysed using these new conditions. The results from this second analysis are used in this report.

6.1.2 Dustiness testing method

Dustiness can be measured by a number of instruments, but it is a relative term and the measurements obtained are dependent on the apparatus used, properties of the chemical tested, the influence of environmental conditions and the dust fractions measured. The European standard EN15051 was produced by the members of the CEN/TC137/WG3 as a means of providing standardisation in the measurement of dustiness of bulk materials. This standard establishes reference test methods that classify the dustiness in terms of health related fractions of the bulk material.

European standard EN15051 and HSE MDHS 81 describe standard apparatus to measure how dusty a powder or material is. The apparatus used for dustiness testing is a rotating drum with

longitudinal vanes to lift the material under test and let it fall, producing a dust cloud. Air is drawn through the drum and the airborne dust is collected on two size selective foams and finally onto a filter. From these, dustiness values for the three health related size fractions (respirable, thoracic and inhalable) are determined. These values give a measure of the propensity of the dust to become airborne. The bulk samples were analysed in triplicate using a rotating drum dustiness test as described in the European standard EN15051.

The Dustiness tester, 20ppi metal foams, 80ppi metal foams are available from J.S.Holdings manufactured by Dunlop. The GF/A Fibreglass filters are available from Whatman 1820 090. Two 20ppi foams collected the inhalable fraction of the dust, one 80ppi was used to collect the thoracic fraction of the dust and the respirable fraction was collected on a GF/A filter (80mm diameter). The filers and foams should not be subject to weight changes through change in temperature and humidity, however they were allowed to acclimatise overnight in the climatically controlled balance room before weighing.

The filters and foams were weighed in tins, both before and after exposure on a balance with 0.01 mg accuracy. The tins were used to prevent loss of dust from the filters and contamination of surfaces. The two 20ppi foams were treated as a single entity and weighed together to give a single combined weight. Control items were weighed alongside the foams and filters to give a reference weight; this was taken into account during the calculation of the results.

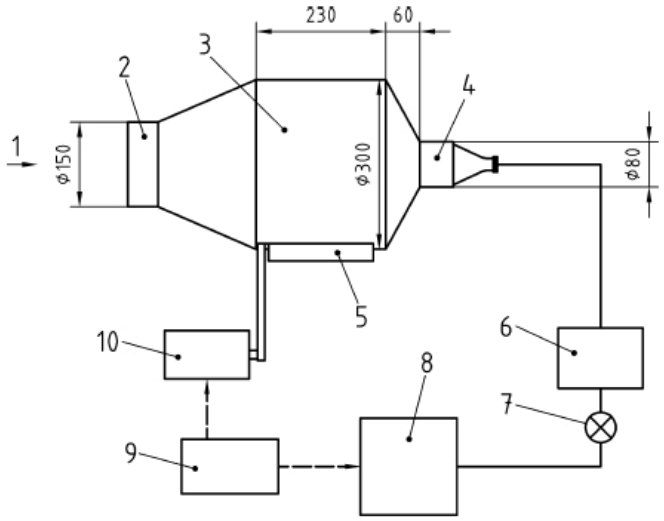
After weighing, the filters and foams were assembled into the dustiness apparatus such that the air was firstly drawn through the 20 ppi foams, then the 80 ppi foams and finally through the GF/A filter. This assembly was attached to the drum. 35 ml of the specified bakery dust sample (for example, bakery improver or ingredient) was loaded into the drum.
The drum rotated at 4 rpm and the air was drawn through the apparatus at 38L/min. It was operated on a timer and each test lasts approximately 1 minute.

The equipment was dissembled and the filters and foams were carefully replaced in their original tins. The test was repeated for a second and third run in order to analyse the sample in triplicate.

After testing, the filters and foams are conditioned in a climatically controlled balance room overnight before reweighing.

The foams and filters were reweighed in their original weighing tins alongside the same control items as used for the pre-exposure weighing. Results were recorded and entered into a spreadsheet that calculated the inhalable, thoracic and respirable dust fractions from the raw data.

Dimensions in millimetres



Key

1 air flow
2 inlet stage (protective filter)
3 dust generation section - rotating drum
4 dust collection stage (two particle size-selective foam stages and filter)
5 rollers
6 mass flow meter
7 control valve
8 pump
9 timer
10 drive motor

NOTE General tolerances according to EN 22768-1.

Figure 7 Dustiness testing rotating drum and equipment

Calculation of dustiness results

The raw results were entered into a spreadsheet that performed the calculations; these were as follows:

Weight of dust captured in the size selecting stages:

$$D_n = (T_{Fn} - T_{In}) - (C_{Fn} - C_{In})$$

Where: D_n = weight of dust captured on the *n*th stage;
T_{Fn} = final weight of the *n*th test stage;
T_{In} = initial weight of the *n*th stage;
C_{Fn} = final weight of the *n*th control stage;
C_{In} = initial weight of the *n*th control stage.

The *n*th stage corresponds to the 20ppi foam, the 80ppi foam or the GF/A filter in the foam / filter assembly. The process allowed for any weight changes in the foams and filters during the test to be corrected.

The Dustiness values were calculated using the following equations:

$$D_I = \frac{\text{weight gain on the test foams + weight gain on the backing filter}}{\text{weight of test sample}} \times 100\%$$

$$D_T = \frac{\text{weight gain on the 80ppi test foam and backing filter}}{\text{weight gain on the two test foams + weight gain on the backing filter}} \times 100\%$$

$$D_R = \frac{\text{weight gain on the backing filter}}{\text{weight gain on the two test foams + weight gain on the backing filter}} \times 100\%$$

where: D_I = the dustiness value of the inhalable dust fraction;

D_T = the dustiness value of the thoracic fraction; and

D_R = the dustiness value of the respirable fraction.

Three runs were carried out for each sample and the mean, standard deviation and coefficient of variation was calculated for each. If the coefficient of variation was more than 10% then further test runs would be performed until consistent values were obtained with a coefficient of variation of less than 10%.

The proportion of the dust that became airborne is the inhalable fraction, the proportion of that airborne dust that would be able to penetrate past the larynx is the thoracic fraction and the proportion of airborne dust that is able to penetrate past the non-ciliated airways is the respirable fraction.

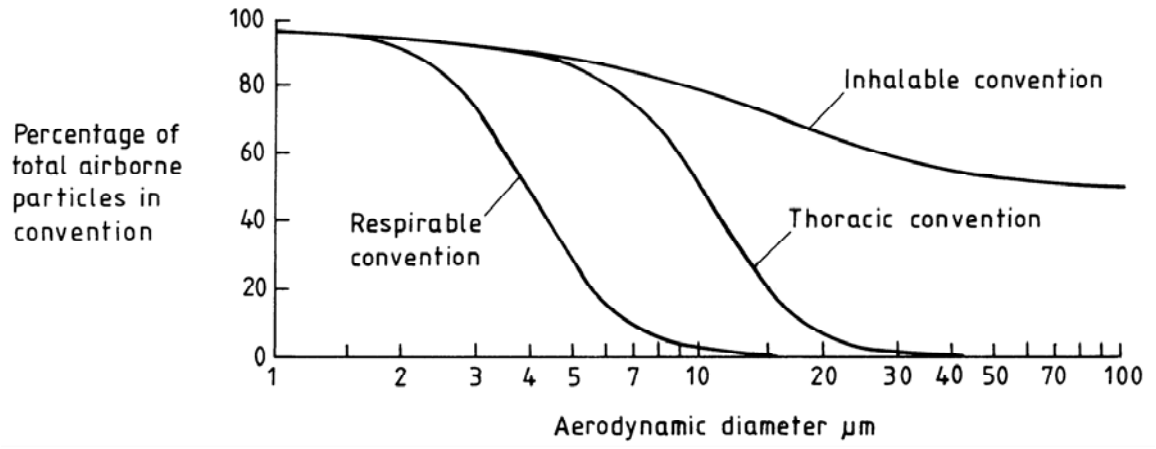


Figure 8 The inhalable, thoracic and respirable conventions as percentages of total airborne particles

The European Standard EN 15051 requires that dustiness of materials be classified into four different categories: very low, low, moderate and high. This scheme was based on data from a EU funded project and 12 different materials were tested. This classification is shown in Table 7.

Table 7 Classification of dustiness according to EN15051

Category of dustiness	Inhalable dustiness mass fraction (mg/kg)	Thoracic dustiness mass fraction (mg/kg)	Respirable dustiness mass fraction (mg/kg)
Very low	<200	<40	<10
Low	200 - 1000	40 - 200	10 - 50
Moderate	>1000 - 5000	>200 - 1000	>50 - 250
High	>5000	>1000	>250

6.1.3 Particle size testing method

Particle size distribution testing was performed on an Aerosizer (or aerodynamic particle inspector). This is an aerosol spectrometer that can be used to give near real-time measurements of the size-distribution of airborne particulate in the size range 0.20 - 700 microns. Additionally powders can be analysed by using an “aerodisperser” accessory. This introduces dry powders into the instrument at an optimum feed rate.

The Aerosizer operates on the principle of aerodynamic time of flight (TOF). The particles are accelerated by a constant, known force due to airflow and are forced through a nozzle at nearly sonic velocity. Smaller particles are accelerated at a greater rate than large particles due to a greater force-to-mass ratio. Two laser beams measure the time of flight through the

measurement region by detecting the light scattered by the particles. Statistical methods are used to correlate the start and stop times of each particle in a particular size range (channel) through the measurement zone. The time of flight is used in conjunction with the density of the particles and calibration curves.

In order to correctly size powders, the Aerosizer requires the density of each powder and so some preliminary work was carried out to measure this for each of the samples.
The density was calculated using standard density bottles. The density bottle was placed on a balance and tared. A sample of the powder was inserted and the bottle was reweighed to give the weight of the powder. This was then filled with a liquid (of known density) that the powder would not dissolve in and reweighed. From the increase in weight and density, the volume of the added liquid was calculated. This was then subtracted from the volumetric capacity of the bottle, to give the volume of the powder. Using this and the weight, the density was calculated.
The density of the single ingredients (listed in Table 13) was found in this way and the density of the mixtures was estimated from the proportions of these ingredients in the mix.

The Aerosizer was used to measure the "Aerodynamic diameter" of a particle, which is an indirect measure of particle size determined from measurements of the particles settling velocity. It is defined as the diameter of a unit density sphere with the same settling velocity as the particle being measured. Aerodynamic diameter is most widely quoted in aerosol studies.

6.1.4 User testing

Site visit to a bakery

In order to ensure that the user test was a realistic representation of tasks in a bakery, the team visited a bakery that contained both large-scale processes and an area with a smaller production line. The processes taking place in the bakery were observed and an insight was gained into how baking is performed.
How the ingredients were handled by the operators was observed and notes were taken of the methods of weighing / tipping / sieving and mixing of ingredients. The baking processes were also recorded using a handheld video camera.
Measurements of airflow and dust were taken in each area of the bakery using hand held monitoring equipment.
The data and the video were discussed with the members of ABIM and from this information the team was able to decide on which processes would be the most informative to replicate in the user tests and how the improver mixtures would realistically be used. It was decided that the processes in the preparation room, i.e. weighing out of improvers and ingredients, were the most relevant to replicate. This task involved tipping ingredients into a large container from a plastic scoop.

Calm air dust chamber

The test chamber illustrated comprises two galvanised steel sections of dimensions 1 x 1 x 1 m stacked one on top of the other, which is grounded to reduce any effects caused by charge on the aerosol. Typically the volume flow rate is set to approximately 400 l min^{-1}, which equates to a downward air velocity through the chamber of 0.4 cm s^{-1}.

Dust is usually introduced through the top of the chamber using some kind of dust generator / disperser. It passes over an ionising fan, which reduces the overall level of electrostatic charge on the aerosol. It then passes through a honeycomb section, which reduces any air turbulence that might be present. From there it passes to the sampling region, which may or may not contain a rotating turntable (depends on the application).

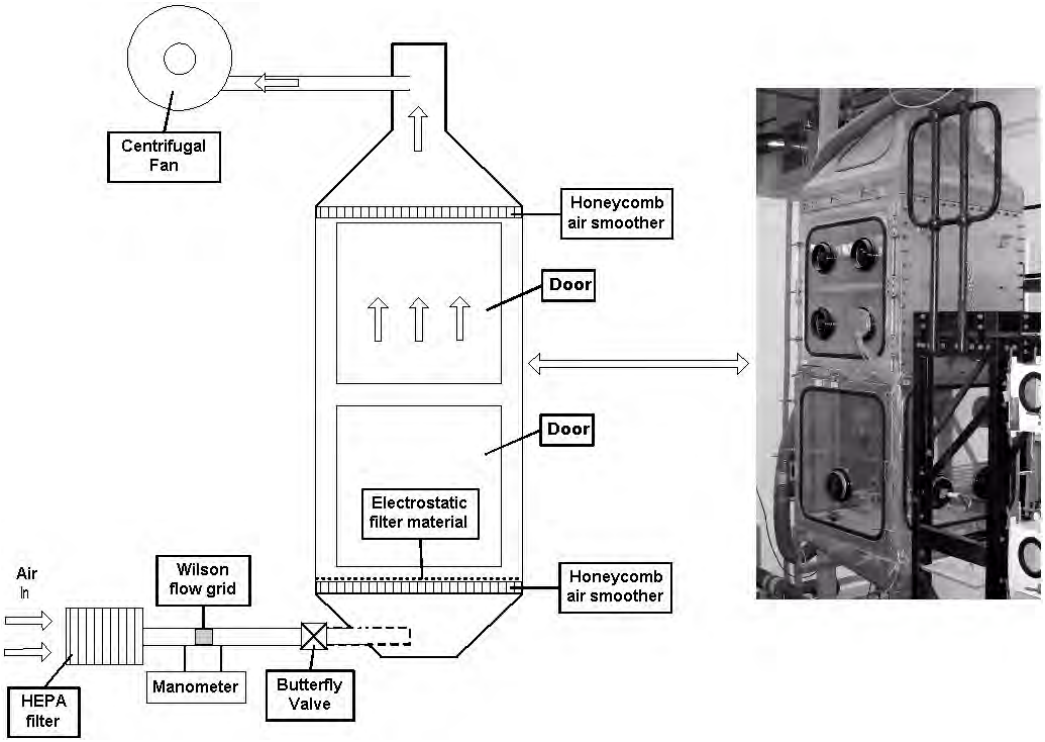


Figure 9 The calm air dust chamber

For the purpose of the ABIM tests it was initially intended to use the chamber with the air moving upwards at a velocity typical of that found in a bakery (25 cm s^{-1}). This entailed connecting a centrifugal fan to the top of the chamber with a HEPA filter attached to the bottom of the chamber to ensure that clean air entered (see figure 9).
The scooping and pouring of the improver was performed through glove ports in the side of the chamber and the measurement of airborne dust levels was carried out using CIS inhalable samplers that operate at a flow rate of 3.5 l/min and PGP10 Casella cyclone respirable samplers that operate at a flow rate of 10 l/min. Glass fibre filters were used in these samplers and gravimetric analysis was performed as described previously, by weighing the filters before and after sampling. A real-time dust monitor (Microdust) was also placed alongside the samplers to give an indication of temporal fluctuation. All of these were placed at heights typical of where they would be if they were attached within a workers breathing zone.

Trials and initial user tests

Firstly trials were performed with the red flour. These highlighted some technical issues about the sampling:
The background level of dust in the chamber was high, although the chamber had been thoroughly cleaned before use. It appeared that some of the dust remaining in the bottom of the

chamber was being re-entrained before entering the sampling region. This was reduced four fold by introducing a filter to the bottom of the chamber.
The initial user tests were performed for 30 minutes with the upwards ventilation switched on (as shown in Figure 9). The lifting and pouring of the improver was done at a regular height and the timing was monitored using of a metronome (12 pours per minute). It was found that the error for this sampling strategy was high; the weight of dust especially on the respirable filters was very low and the accuracy of the weight measurements was uncertain.

Final user test method

The results from the trials and initial tests indicated that the sampling technique needed to be changed. Therefore the user test was repeated using the following strategy:
The time of sampling was increased to 45 minutes, the ventilation in the chamber was turned off so the sampling was in still air conditions and therefore less diluted and the scooping and dropping of the improver was done from a greater height and with a greater frequency. Again this was monitored using a metronome and was an average of 20 pours per minute.
The weights on the filters were higher and this run was felt to be the most accurate of the user tests.
The reported results are from this set of user tests. The gravimetric results from the CIS samplers give a measure of the inhalable dust. Where concentrations of dust are low, the Microdust sampler can be used to give an indication of the respirable dust. The Microdust results were therefore used as the measure of respirable dust as the weights on the filters for the PGP10 samplers (respirable) remained low and the Microdust data would be more accurate. Due to the way that the Microdust sampler works, it should be noted that these results are likely to differ from the true level of respirable dust.

The respirable and inhalable airborne dust concentrations within the chamber were calculated from the measured deposit of dust on the filters (in mg) and from the total volume of air sampled measured in m^3 (calculated by multiplying the test duration by the sampler flow rate) The concentration was then estimated by dividing the mass of dust by the volume of air sampled.

6.1.5 Sample extraction

Extraction of dustiness samples and user test filters

Proteins were extracted from the foams and filters in extraction buffer (PBS, 0.1% Tween) for 2 hours with agitation. Samples of the buffer were retained to check for contamination. The volume of liquid the foams and filters were extracted in, is shown in the table below:

Table 8 Volumes of extraction buffer for dustiness and user tests

Sample	Type of sample	Volume for extraction (ml)
Dustiness: Inhalable	Foam: 20 pores / inch	60
Dustiness: Thoracic	Foam: 80 pores / inch	60
Dustiness: Respirable	80mm GF/A filters	15
User Test Filters	37mm GF/A filters	4

The liquid was recovered from the filters using serum filter plungers (Fisher Scientific); this was to remove any fibres from the sample. The tin containing the foams was tipped up and the majority of the liquid was recovered using a dropping pipette. The foams were very porous and in order to remove as much liquid as possible, the tin was tapped on the surface. Filter plungers (as above) were again used to remove any debris from the sample. The samples were capped and stored at -20^0C.

Extraction of bulk products

The proteins from the improvers and ingredients were extracted in extraction buffer (PBS, 0.1% Tween), using a 20% weight to volume solution, i.e. 10g flour in 50ml extraction buffer. The samples were extracted overnight with agitation at 4^0C. A sample of the extraction buffer was retained to check for any contamination issues.
The extract was extremely cloudy so the insoluble material was removed as follows: The liquid was centrifuged; the supernatant was removed and filtered using serum filter plungers (as above). The resulting liquid was stood upright for 1 hour at 4^0C to allow the remaining insoluble material to settle to the bottom of the tube. The supernatant was pushed through a 0.45 μm filter before use. Samples were stored at -20^0C.

6.1.6 Immunological analysis

Wheat flour antigen

To quantify wheat flour antigen (WFA), HSL used a sandwich enzyme linked immunoassay (ELISA) using an automated liquid handling system. The procedure is as follows:

- 96 well plates (Maxisorp plates from Nunc) were coated with the 4D9 wheat flour antigen monoclonal antibody (stock concentration 1.1mg/ml) diluted to 2 μg/ml in carbonate coating buffer. These were sealed and incubated overnight at 4^0C. Incubated plates were washed and blocked for 2 hours at room temperature in blocking buffer (1% Fish gelatine, 2% sucrose with preservative in PBS).
- The plate was inverted and tapped to remove the excess blocking buffer and placed in a 37^0C incubator overnight to ensure the plate was completely dry. Plates are stored in sealed bags with desiccant at 4^0C ready for use.
- The Wheat Flour Allergen standard (stock concentration 1.27ug/ml) has been affinity purified with 4D9 antibody. This was diluted in assay buffer (0.1% BSA, 0.05% Tween

with preservative in PBS), to the following concentrations: 63.5, 31.8, 15.9, 6.35, 3.175 1.27 and 0 ng/ml to produce a standard curve.

- The samples (extracted as in appendix 6.1.5) and a quality control sample were diluted 1 in 2 in assay buffer to produce matrix matching between samples and standards. This dilution is accounted for in the software's calculation.
- 100 µl of the standards, samples and the quality control sample were added to a dry coated plate in duplicate wells and incubated at room temperature for 1 hour.
- After incubation, the plate was washed 3 times and 100ul of 1A6 biotinylated wheat flour antigen monoclonal antibody, diluted to a concentration of 1.6 µg/ml in assay buffer, was added (stock concentration 0.9 mg/ml). The plate was incubated for 1 hour at room temperature.
- After incubation, the plate was washed 3 times and 100 µl of avidin horseradish peroxidase, diluted to a concentration of 200 ng/ml in assay buffer, was added (stock concentration 1mg/ml, Vector Laboratories). The plate was incubated for 1 hour at room temperature.
- After incubation, the plate was washed 3 times and 100 µl of TMB (3, 3', 5, 5' tetramethylbenzidine), SureBlue TMB Microwell peroxidase substrate, Insight Biotechnologies, was added. The plate was incubated for 10 minutes at room temperature. The reaction was stopped by adding 100 µl of 0.5M sulphuric acid. The absorbance of the plate was read at 450nm.
- The amount of wheat flour antigen in the samples was quantified against the standard curve and quality control samples are included to monitor the "inter" and "intra" assay performance.
- The sensitivity of the wheat flour antigen assay was calculated using QIVX ProQuant software to be 0.25 ng/ml.

Soya trypsin inhibitor

To quantify soya trypsin inhibitor (STI), HSL used a sequential addition sandwich assay using an automated liquid handling system. The procedure is as follows:

- 96 well plates (Maxisorp plates from Nunc) were coated with the Chemicon Ab 1239 monospecific antibody (stock concentration 10 mg/ml) diluted to 2 µg/ml in carbonate coating buffer. These were sealed and incubated overnight at 4^0C. Incubated plates were washed and blocked for 2 hours at room temperature in blocking buffer (1% fish gelatine, 2% sucrose with preservative in PBS).
- The plate was inverted and tapped to remove the excess blocking buffer and placed in a 37^0C incubator overnight to ensure the plate was completely dry. Plates are stored in sealed bags with desiccant at 4^0C ready for use.
- The STI standard (Sigma, stock concentration 1 mg/ml) was diluted in assay buffer (0.1% BSA, 0.05% Tween with preservative in PBS), to the following concentrations: 25, 10, 4, 1.25, 0.5, 0.2 and 0 ng/ml to produce a standard curve.
- The samples (extracted as in section 6.1.5) and a quality control sample were diluted 1 in 2 in assay buffer to produce matrix matching between samples and standards. This dilution is accounted for in the software's calculation.
- 100 µl of the standards, samples and the quality control sample were added to a pre washed coated plate in duplicate wells and incubated at room temperature for 1 hour.
- After incubation, the plate was washed 3 times and 100 µl of Chemicon Ab1239 biotinylated antibody, diluted 1 in 5000 in assay buffer, was added. The plate was incubated for 1 hour at room temperature.

- After incubation, the plate was washed 3 times and 100 µl of avidin horseradish peroxidase, diluted to a concentration of 200 ng/ml in assay buffer, was added (stock concentration 1mg/ml, Vector Laboratories). The plate was incubated for 1 hour at room temperature.
- After incubation, the plate was washed 3 times and 100 µl of TMB (3, 3', 5, 5' tetramethylbenzidine), SureBlue TMB Microwell peroxidase substrate, Insight Biotechnologies, was added. The plate was incubated for 10 minutes at room temperature. The reaction was stopped by adding 100 µl of 0.5M sulphuric acid. The absorbance of the plate was read at 450nm.
- The amount of wheat flour antigen in the samples was quantified against the standard curve and the quality control samples are included to monitor the "inter" and "intra" assay performance. The sensitivity of the STI assay was calculated using QIVX ProQuant software to be 0.11 ng/ml.

Total protein (sensitive method)

The filter and foam extracts were analysed for protein content using a sensitive colorimetric dye method that reports protein concentrations from 1.8 to 100 µg/ml. The protein assays are not specific but demonstrate the biological content of dust (proteins of animal and plant origin). This sensitive method of analysing total protein uses an automated liquid handling system, the procedure is for which is as follows:

- The protein standard (BSA, Sigma, 1mg/ml stock concentration) is diluted to 100 µg/ml in extraction buffer (0.1% Tween with preservative in PBS) to give the top standard. This was diluted to the following concentrations to create a standard curve: 80, 60, 40, 20, 10 and 0 ug/ml. 50 µl of the standards, samples and the quality control sample (BSA QC) were added to a 96 well plate (Maxisorp, Nunc) in duplicate wells.
- The protein determination reagent (Micro BCA kit, Pierce-Perbio) was made up as per the manufacturers instructions, i.e. 25 parts of Reagent MA, 24 parts of Reagent MB and 1 part Reagent MC. 50 µl of reagent was added to each relevant well and the plate was incubated for 2 hours. The absorbance of the plate was read at 560nm.
- The amount of protein in the samples was quantified against the standard curve and the quality control samples are included to monitor the "inter" and "intra" assay performance.
- The sensitivity of the sensitive total protein assay was calculated using QIVX ProQuant software to be 1.8 µg/ml.

Total protein (method for bulk samples)

The bulk product extracts were analysed for protein content using a colorimetric dye method that reports protein concentrations from 5 to 1000 µg/ml. This method of analysing total protein uses an automated liquid handling system (COBAS MIRA), the procedure is for which is as follows:

- The protein determination reagent was prepared; 200 µl copper II sulphate in 10 ml bicinchoninic acid solution, both from Sigma. The protein standard is BSA (Sigma, 1mg/ml stock concentration). The standard, samples and the quality control sample (BSA QC) were diluted 1 in 5 in ultrapure water and pipetted into cuvettes (25 µl total volume). 200 µl of

reagent was also added to each cuvette and these were incubated for 2 hours at room temperature. The absorbance of the liquid in the cuvettes was read at 550nm.

- The amount of protein in the samples was quantified against the standard and the quality control samples are included to monitor the "inter" and "intra" assay performance.
- The sensitivity of the sensitive total protein assay was calculated using QIVX ProQuant software to be 5 µg/ml.

Calcium analysis

The Calcium was analysed by ICP-MS as follows:

- Extracts were diluted five fold in 1% (v/v) nitric acid and the calcium concentration (as ^{48}Ca) determined by inductively coupled plasma-mass spectrometry (ICP-MS) (Thermo Fisher Scientific, Series 1 X7 ICP-MS) using direct nebulisation in normal mode.

- The 0-100 µg/L calcium standards (Primar multi-elemental ICP-MS standard, Fisher Chemicals, Loughborough, UK) were used for calibration and the limit of detection was 0.07 µg/L. Internal standards used were 10 µg/L of indium, yttrium and rhodium and the internal standardisation interpolated. No certified reference material was available so a 20 µg/L check standard was analysed at the start and end of the analysis and after every ten samples, this standard gave average results (n=17) of 20.5 ±1.7 µg/L. Sample extracts were also spiked with 50µg/L standard and gave a recovery of 98.5%.

Calculation of immunology results

The total protein, wheat flour antigen (WFA) and soya trypsin inhibitor (STI) were analysed in duplicate, calcium was analysed in triplicate. The mean was calculated from the duplicate and triplicate values and this was used in the calculations below. Any duplicates / triplicate values which had a large amount of variation were repeated. Raw results from the instruments for WFA, STI and calcium were reported in ng/ml. Total protein was reported in µg/ml, for the total protein results they will be reported as micrograms instead of nanograms, but otherwise the calculations are the same.

Calculations for bulk products

For the bulk products (improvers and ingredients), two calculations were performed:

$$\text{Allergen per gram of bulk material (ng/g)} = \frac{(C_P - C_B) * \text{Extraction Volume (ml)}}{\text{Weight of bulk sample extracted (g)}}$$

Where:
C_P = Average measured concentration of allergen / substance in the extract of bulk product (ng/ml, µg/ml for protein)
C_B = Average measured concentration of allergen / substance in buffer alone (ng/ml, µg/ml for protein)

This expressed allergen per gram of bulk product. This does not represent the content of a substance in the bulk material because the assay only measures a specific part of that substance. For example wheat flour contains wheat flour antigen, so it could be expected that the amount of WFA will increase with increasing wheat flour, but since the antigen is only a small part of the wheat flour, the total amount measured will not be the same as the amount of wheat flour in the sample. This equation demonstrated whether the relative amounts measured in the bulk product followed the same general trend as the content of the ingredients and whether the results from the filters were relevant to the bulk product used.

The percentage of measured allergen / substance in the bulk product was also calculated. This is the amount of measured allergen expressed as a percentage of the entire bulk product. This was used in further calculations for the filters and foams.

$$\% \text{ Active Substance in bulk} = \frac{(C_P - C_B) * \text{Extraction Volume (ml)}}{(\text{Weight of bulk sample extracted (g)} * 1 \times 10^9)} \times 100$$

This is essentially the same calculation as above, however the weight of the bulk sample extracted is converted into nanograms so that it is in the same units as the measured allergen. To express this as a percentage the proportion of measured allergen is multiplied by 100.

Calculations for the filters and foams from dustiness testing

For the extracts from the dustiness filters and foams, the following two calculations were performed:

$$\text{Allergen on filter per gram dust in drum (ng/g)} = \frac{(C_F - C_C) * \text{Extraction Volume (ml)}}{\text{Weight of sample in dustiness drum (g)}}$$

Where:
C_F = Average measured concentration of allergen / substance on filter (ng/ml, μg/ml for protein)
C_C = Average measured concentration allergen on blank (ng/ml, μg/ml for protein)

The above equation gives a measure of how much of the bulk dust is present on the filter as measurable allergen.

The amount of measurable allergen in the bulk product was calculated as a percentage (bulk equations, above). Therefore the percentage of the measured ingredient that reached the filter from the bulk product was also calculated. This provided a measure of what proportion of the allergen became airborne and whether the mixture of ingredients affected this.

% Allergen on filter from allergen in ingredient in drum =

$$\frac{(C_F - C_C) * \text{Extraction Volume (ml)}}{((\text{Weight sample in drum (g)} * 10^9) * (\text{Percentage measurable allergen in bulk (\%)} / 100))} \times 100$$

The amount of allergen on the filter in ng was calculated as before. For protein this was calculated in μg.
The weight of sample is converted into nanograms (for allergens) by multiplying by 10^9, so this was in the same units as the amount of allergen. In the calculation for protein, the weight was multiplied by 10^6 to express the values in micrograms.
The "percentage of measurable allergen in the bulk" is the percentage of measurable allergen / substance calculated in the bulk sample equation, above.

Calculation for the filters from user testing

For the extracts from the user test filters, the following calculation was performed:

$$\text{Allergen per volume air sampled (ng/m}^3\text{)} = \frac{(C_F - C_C) * \text{Extraction Volume (ml)}}{\text{Sample volume (m}^3\text{)}}$$

This is a standard measure of exposure for occupational hygiene surveys and shows the amount of allergen measured per m^3 air sampled. The volume of air sampled is calculated from the time of sampling (min) and the flow rate of the air sampler (L/min).

6.2 RESULTS APPENDICES

6.2.1 Original ABIM improver mixtures

Dustiness testing

Six improver mixtures were sent from ABIM that were intended to range from not dusty to very dusty in their properties, depending on their binding agents (Table 3). These were analysed for dustiness in triplicate; the mean, standard deviation and coefficient of variance for the inhalable, thoracic and respirable fractions are shown in Table 9.

Table 9 Dustiness testing of original 2008 improver mixtures

Code	HSL ID	Inhalable			Thoracic			Respirable			Moisture content
		Mean (mg/kg)	Std dev	COV %	Mean (mg/kg)	Std dev	COV %	Mean (mg/kg)	Std dev	COV %	Mean (%)
Green	00123/08	162	8	4.8	28	2	7.8	3	0	12.3	8.5
Yellow	00124/08	35	2	6.1	5	0	5.5	2	0	1.1	9.9
Red	00125/08	86	4	4.9	26	3	10.9	2	0	10.9	11.0
Black	00126/08	589	13	2.3	107	5	4.9	9	1	5.6	9.6
Orange	00127/08	485	40	8.2	82	6	7.5	3	0	9.8	9.4
Blue	00128/08	101	7	7.4	45	4	8.8	2	0	10.5	10.7

The dustiness results are represented in the following bar chart (Figure 10):

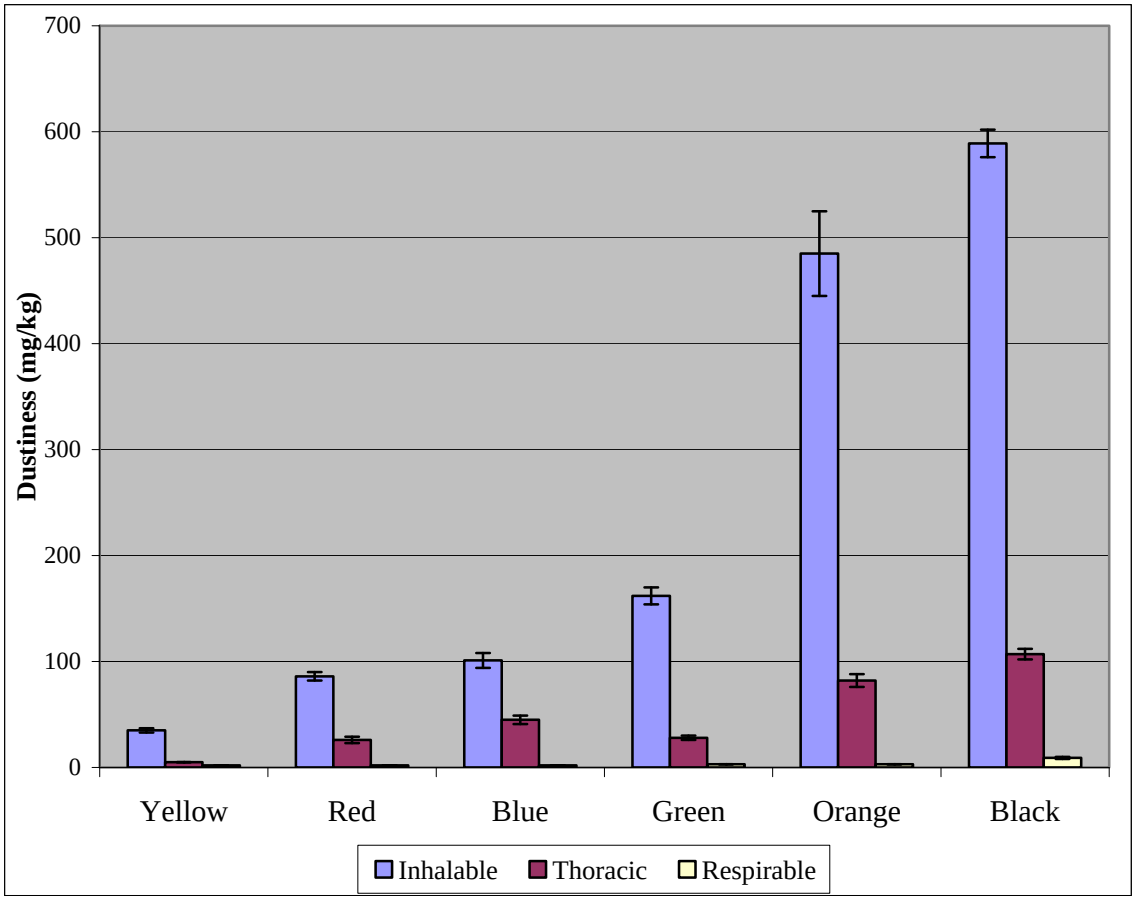


Figure 10 Dustiness testing of original improver mixtures

Key for figure 10 Sample descriptions and key ingredient changes

Sample	Description of sample	Soya	Emulsifier	Ca sulphate	Oil
Blue	Control: flour and ascorbic acid only	None	None	None	None
Red	Control: As blue, with fungal alpha amylase added	None	None	None	None
Yellow	Same as red with soya added	20%	None	None	None
Green	Same as yellow with emulsifier added	20%	20%	None	None
Black	Same as green with calcium sulphate added	20%	20%	25%	None
Orange	Same as black with oil added	20%	20%	25%	2%

Samples “Blue” and “Red” can be considered as control samples, as they contain flour and ascorbic acid only and flour, ascorbic acid and fungal alpha amylase respectively. While inspection across all the samples suggested very little difference between the “Blue” and “Red” samples, the inhalable and thoracic results for the blue sample were statistically larger ($p<0.05$) than for the respective “Red” samples.

"Yellow" contained the same ingredients as the "Red" sample with the addition of soya flour. The dustiness for the yellow sample (all fractions) is statistically lower ($p<0.01$) than the "Blue" and "Red" samples, indicating that the soya flour reduced dustiness.

The "Green" sample contained the same ingredients as the "Yellow" with the addition of emulsifier. Emulsifier was originally expected to reduce the dustiness, however this sample was dustier than the "Yellow" sample and even the "Red" and "Blue" samples.

The "Black" sample contained the same ingredients as the "Green" with the addition of calcium sulphate. This is the dustiest sample of all, and statistically different to the "Green" sample ($p<0.001$ for all three fractions).

The "Orange" sample contained the same ingredients as the "Black" sample with the addition of oil. The oil significantly decreased the dustiness of the improver ($p<0.01$), however this reduction does not compensate for the dustiness caused by addition of the other ingredients.

These results were very unexpected, as it had previously been thought that all the additives in the original improver samples would decrease the dustiness of flour. Only soya flour and oil seemed to have such an effect. Variation in moisture (Table 9) between samples does not appear to be the reason for the unexpected results.

It was at this point that the strategy of the study was revised in order to gain more information about which individual ingredients would modify the dustiness of the improver mix.

Immunological analysis

Filter and foam samples were generated from the dustiness testing and air sample filters were generated from the user test. These were analysed for wheat flour antigen, soya trypsin inhibitor and soluble protein. This was in order to investigate the allergen content of the airborne dust and therefore the potential exposure from the different ingredient combinations.

Bulk samples

Total protein, calcium, wheat flour allergen and soya trypsin inhibitor were analysed in bulk samples and results expressed as per gram of bulk improver (ng/g, μg/g for protein) and as a percentage of measured substance in the bulk improver (Table 10).

Table 10 Content of measured substances in the bulk improver samples from 2008

HSL ID	Sample code	Soluble protein		Calcium		Soya trypsin inhibitor		Wheat flour allergen	
		μg/g	%	ng/g	%	ng/g	%	ng/g	%
00123/08	Green	68484.8	6.848	3839.2	0.000384	77075.9	0.007708	5318.2	0.000532
00124/08	Yellow	101284.8	10.128	707.7	0.000071	14345.9	0.001435	51938.9	0.005194
00125/08	Red	71784.8	7.178	406.2	0.000041	ND	ND	97301.4	0.009730
00126/08	Black	78284.8	7.828	45210.0	0.004521	24625.9	0.002463	16463.9	0.001646
00127/08	Orange	72384.8	7.238	52980.0	0.005298	8472488.4	0.847249	15220.1	0.001522
00128/08	Blue	71384.8	7.138	429.6	0.000043	2.9	0.000000	164557.6	0.016456

The percentages in bulk samples were used to calculate the percentage that became airborne and collected on the filters.

Filters and foams from dustiness testing

Calcium, soya trypsin inhibitor and wheat flour allergen were measured and used to calculate (see calculation of immunology results Appendix 6.1.6):

a. The amount of the substance on the filter per gram of improver tested.

b. The percentage of the specific substance from the improver that has become airborne.

The results are shown in the Table 11.

Table 11 Average content of measured substances in the dustiness filters and foams from the original improvers

Sample	**Fraction**	**Soluble protein**		**Calcium**		**Soya trypsin inhibitor**		**Wheat flour allergen**	
		ng/g	**%**	**ng/g**	**%**	**ng/g**	**%**	**ng/g**	**%**
Green 00123/08	Inhalable	6.443	0.00941	1.424	0.03709	100.250	0.13007	4.160	0.07822
	Thoracic	11.036	0.01612	38.831	1.01143	31.861	0.04134	0.991	0.01864
	Respirable	4.239	0.00619	1.276	0.03325	5.738	0.00744	0.000	0.00000
Yellow 00124/08	Inhalable	9.259	0.00914	7.261	1.02602	16.774	0.11693	5.346	0.01029
	Thoracic	93.727	0.09254	38.231	5.40220	4.829	0.03366	0.000	0.00000
	Respirable	2.697	0.00266	3.784	0.53473	1.119	0.00780	0.000	0.00000
Red 00125/08	Inhalable	17.751	0.02473	54.145	13.32832	ND	0.00000	3.538	0.00364
	Thoracic	33.403	0.04653	161.397	39.72904	ND	0.00000	1.548	0.00159
	Respirable	6.085	0.00848	5.381	1.32450	ND	0.00000	0.000	0.00000
Black 00126/08	Inhalable	23.521	0.03005	56.975	0.12602	256.566	1.04186	30.754	0.18680
	Thoracic	21.069	0.02691	36.693	0.08116	46.011	0.18684	0.030	0.00018
	Respirable	7.579	0.00968	6.123	0.01354	10.790	0.04381	0.015	0.00009
Orange 00127/08	Inhalable	9.881	0.01365	62.904	0.11873	202.841	0.00239	5.961	0.03917
	Thoracic	1.089	0.00150	48.224	0.09102	53.587	0.00063	0.470	0.00309
	Respirable	0.021	0.00003	0.260	0.00049	6.052	0.00007	0.027	0.00018
Blue 00128/08	Inhalable	0.732	0.00103	3.310	0.77048	ND	0.00000	8.547	0.00519
	Thoracic	131.364	0.18402	30.068	6.99898	BLD	4.72797	0.176	0.00011
	Respirable	0	ND	1.251	0.29111	ND	0.00000	0	0.00000

Soluble protein

Figures 11 and 12 show the results of these calculations for soluble protein. Please refer to Table 3 for the contents of the improver samples.

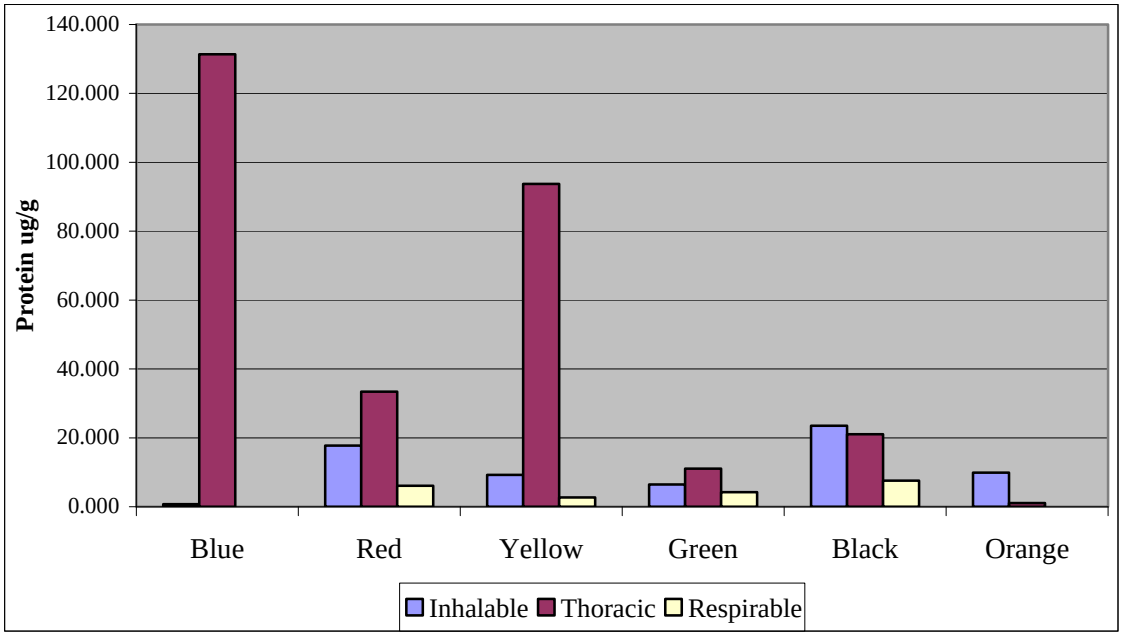


Figure 11 Amount of soluble protein on the filters per gram of improver in the drum

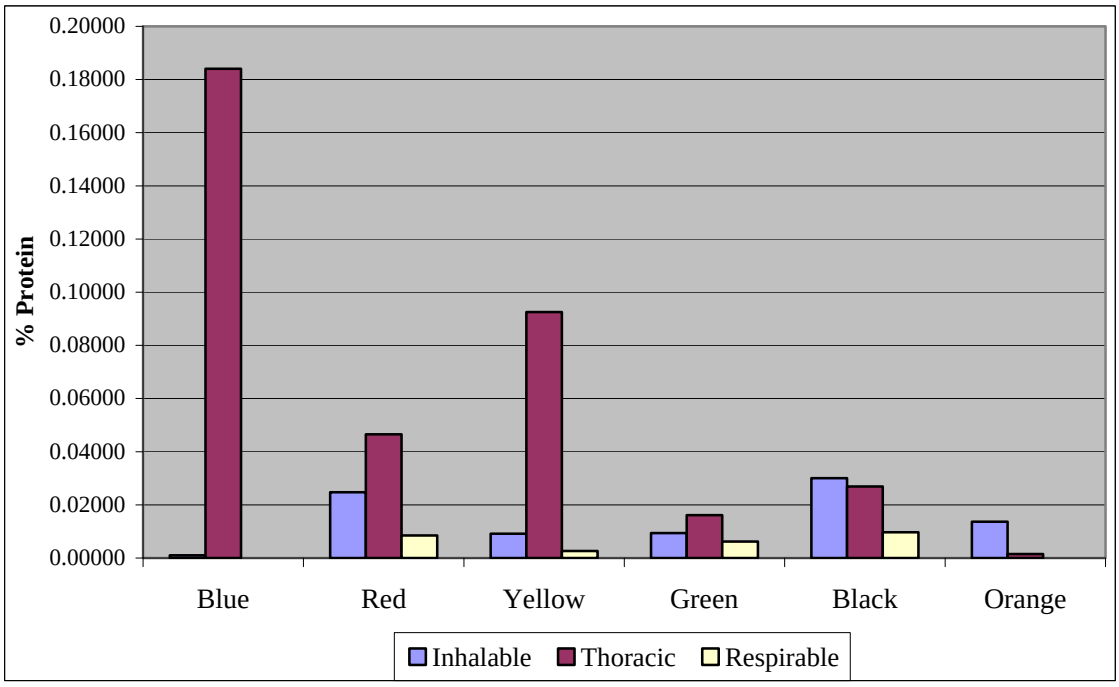


Figure 12 Percentage of protein from the bulk improver on the filters

Key for figures 11 and 12 Sample descriptions and key ingredient changes

Sample	Description of sample	Soya	Emulsifier	Ca sulphate	Oil
Blue	Control: flour and ascorbic acid only	None	None	None	None
Red	Control: Same as Blue, with fungal alpha amylase added	None	None	None	None
Yellow	Same as Red with soya added	20%	None	None	None
Green	Same as Yellow with emulsifier added	20%	20%	None	None
Black	Same as Green with calcium sulphate added	20%	20%	25%	None
Orange	Same as Black with oil added	20%	20%	25%	2%

Figure 11 shows that there was the highest amount of protein on the filters for the "Blue" sample, followed by the "Yellow" sample, then the "Red" sample. The "Green", "Black" and "Orange" samples had the least amount of total protein on the filters. This is probably because the "Green", "Black" and "Orange" samples contained less protein than the "Blue", "Red" and "Yellow" samples (see Table 3). The "Green", "Black" and "Orange" samples are 20% emulsifier and the "Black" and "Orange" samples also contain 25% calcium sulphate. So this pattern followed the protein content of the bulk samples. Protein in these samples could originate from agents such as wheat flour or soya flour; the "Blue" and "Red" samples contain the most wheat flour and the "Yellow" sample has less wheat flour but the addition of soya flour.

Figure 12 shows the same pattern is occurring for the percentage of protein in the bulk on the filters. These charts indicate that the amount of airborne protein is determined by the amount of protein in the bulk improver sample. Samples containing soya flour or a higher amount of wheat flour had more soluble protein on the filters.

Calcium results

Figures 13 and 14 show the results of these calculations for calcium. Please refer to Table 3 for the contents of the improver samples.

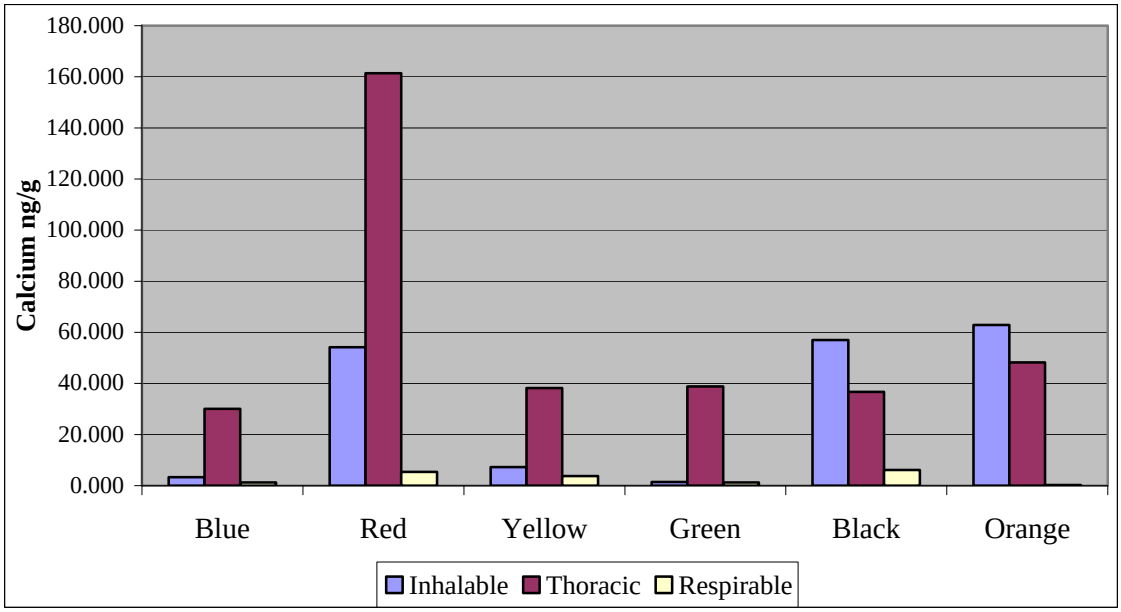


Figure 13 Amount of calcium on the filters per gram of improver in the drum

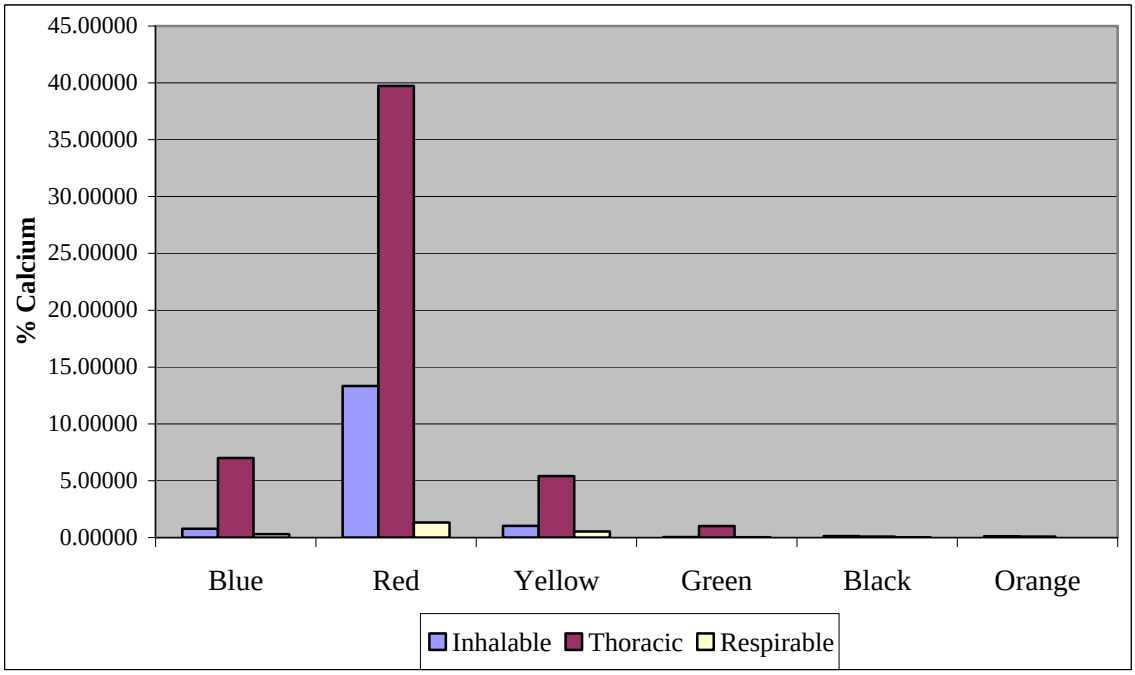


Figure 14 Percentage of calcium from the bulk improver on the filters

Key for Figures 13 and 14 Sample descriptions and key ingredient changes

Sample	Description of sample	Soya	Emulsifier	Ca sulphate	Oil
Blue	Control: flour and ascorbic acid only	None	None	None	None
Red	Control: As blue, with fungal alpha amylase added	None	None	None	None
Yellow	Same as red with soya added	20%	None	None	None
Green	Same as yellow with emulsifier added	20%	20%	None	None
Black	Same as green with calcium sulphate added	20%	20%	25%	None
Orange	Same as black with oil added	20%	20%	25%	2%

Figure 13 shows the amount of calcium detected on the filters in nanograms per gram of improver in the dustiness drum. This shows that the "Red" sample had the highest amount of calcium detected in the thoracic sample. The "Red" improver contained only flour, ascorbic acid and fungal alpha amylase, so this result could be due to cross reactivity, contamination or calcium in the flour. The "Black" and "Orange" samples contained calcium sulphate; these had the next highest calcium results indicating that this does get onto the filters. The amount of calcium extracted from the bulk material of these samples was between 10 to 100 fold more than the other improvers (Table 10). However the amount of calcium detected on the filters was approximately a third higher for these samples than the majority of the improvers. Therefore the proportion of calcium in the bulk that became airborne was low; it did not appear to become airborne easily (Figures 13 and 14).

Soya trypsin inhibitor

Figures 15 and 16 show the results of soya trypsin inhibitor for the original improver mixtures (Table 3).

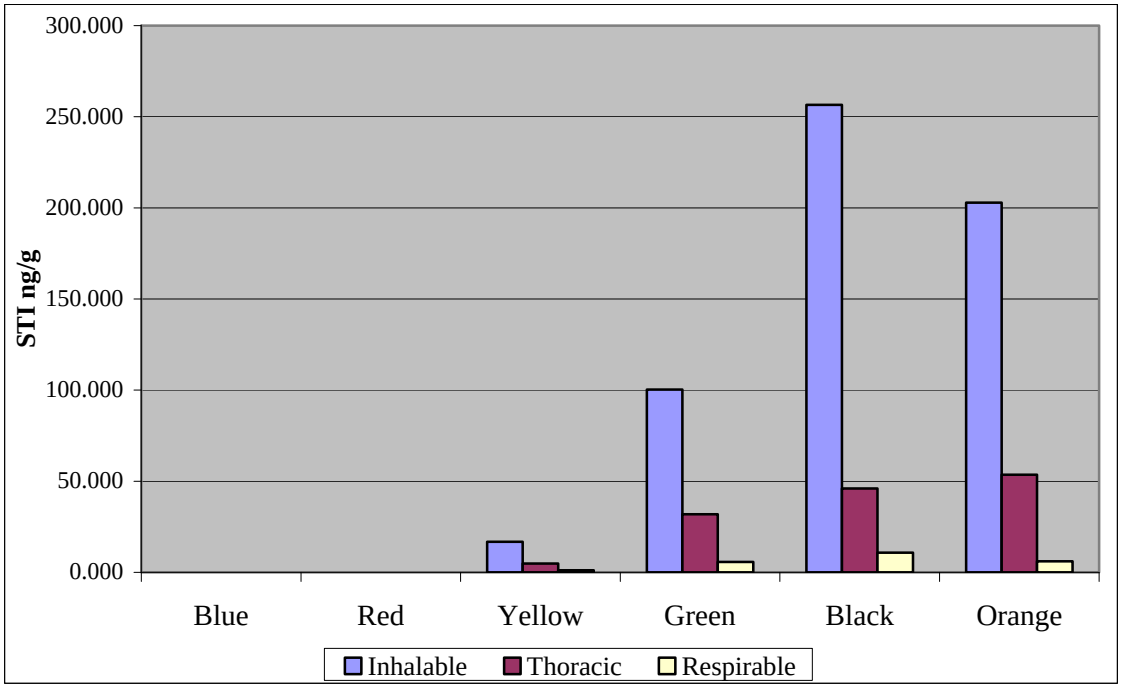


Figure 15 Amount of soya trypsin inhibitor on the filters per gram of improver in the drum

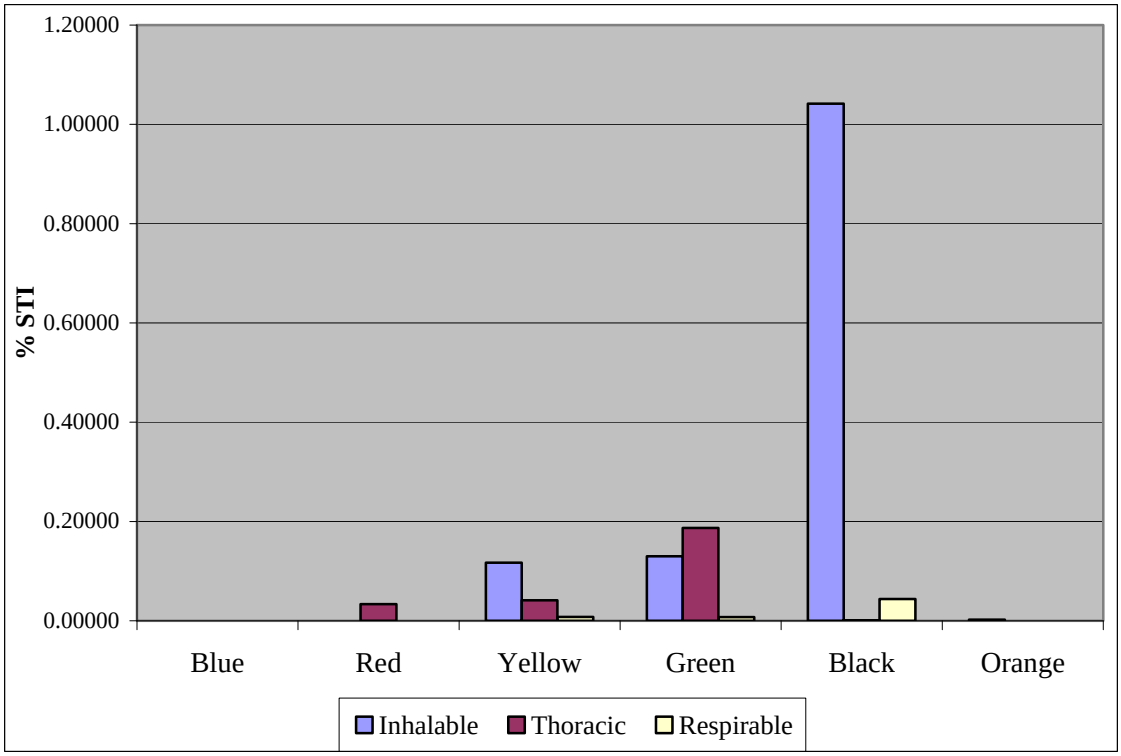


Figure 16 Percentage of soya trypsin inhibitor from the bulk improver on the filters, excluding the “blue” improver

Key for figures 15 and 16 Sample descriptions and key ingredient changes

Sample	Description of sample	Soya	Emulsifier	Ca sulphate	Oil
Blue	Control: flour and ascorbic acid only	None	None	None	None
Red	Control: As blue, with fungal alpha amylase added	None	None	None	None
Yellow	Same as red with soya added	20%	None	None	None
Green	Same as yellow with emulsifier added	20%	20%	None	None
Black	Same as green with calcium sulphate added	20%	20%	25%	None
Orange	Same as black with oil added	20%	20%	25%	2%

Figure 15 shows the amounts of airborne soya trypsin inhibitor (STI). Results for the "Blue" and "Red" samples were consistent with lack of soya in the samples. The "Yellow", "Green", "Black" and "Orange" samples all contained 20% soya flour and the amount of airborne soya follows the same trend as the dustiness experiments (Figure 10, Table 9). The results for the "Black" improver had the largest airborne STI; this improver contained all the ingredients with the exception of oil. In order of decreasing airborne STI; (a) the "Orange" improver contained all the same ingredients as the "Black" sample with the addition of oil, (b) the "Green" improver did not contain oil or calcium sulphate, but does contain emulsifier and (c) the "Yellow" improver does not contain emulsifier, calcium sulphate or oil.

Figure 16 shows the percentage of STI in bulk improver that became airborne. The percentage for the "Blue" sample has been removed from this graph: this sample did not contain soya but some contamination was present on the filter, giving an erroneous result. The highest percentage of the airborne allergen STI was for the "Black" improver, which was the dustiest sample (Figure 11). In decreasing order of airborne STI, (a) "Green" improver that contained emulsifier but not calcium sulphate, (b) the "Yellow" improver without emulsifier or calcium sulphate and finally (c) the "Orange" sample containing both emulsifier, calcium sulphate and oil.

Adding oil reduced the amount of airborne STI, as shown by the "Orange" sample in Figure 15. The STI detected in the bulk "Orange" sample is approximately 100 fold higher than that detected from the "Black" sample (Table 10). This suggests that the aerosolisiation of STI in the "Orange" improver has been altered with the addition of oil.

Wheat flour antigen

Figures 17 and 18 show the results for airborne levels of wheat flour antigen from the 2008 improver samples (Table 3).

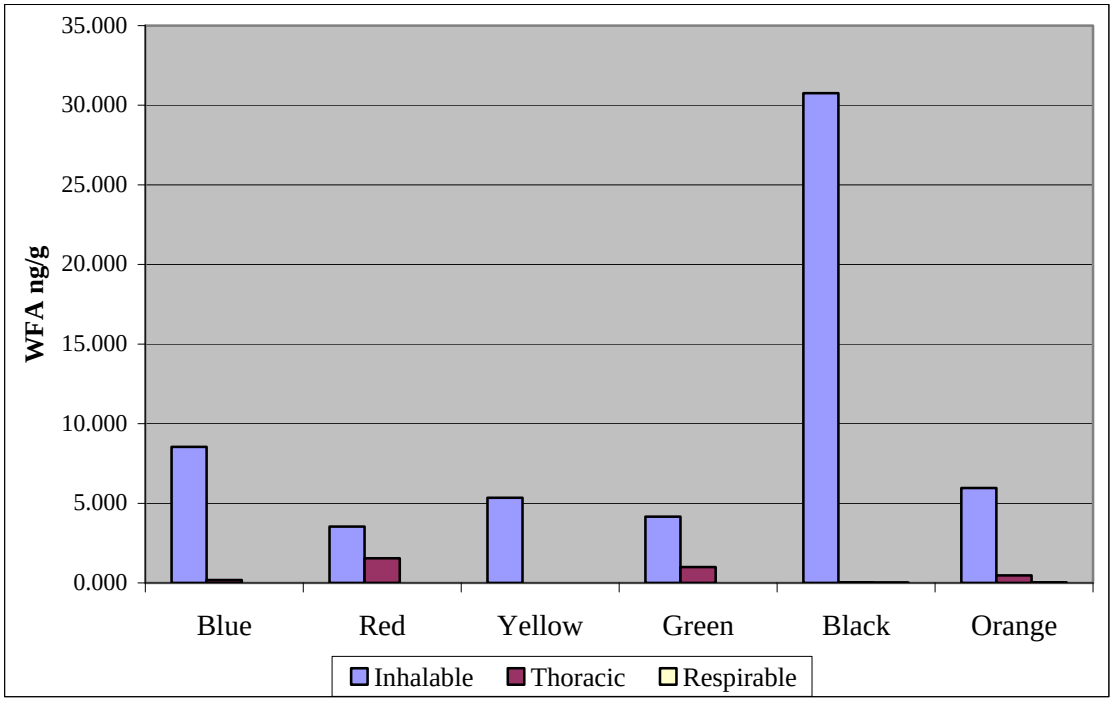


Figure 17 Amount of wheat flour allergen on the filters per gram of improver in the drum

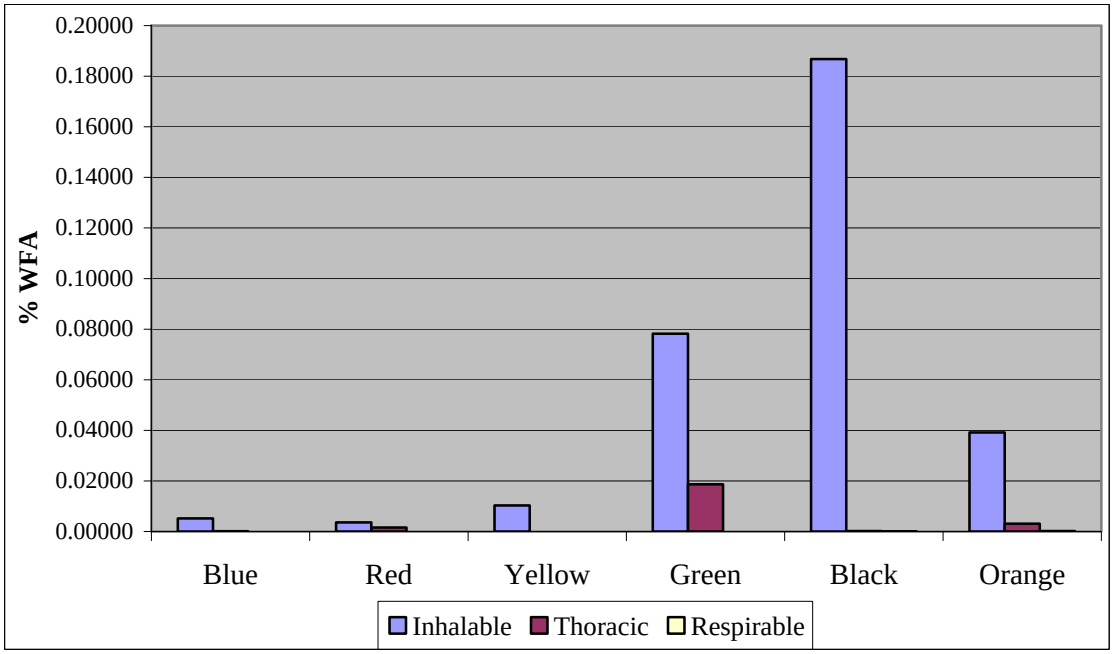


Figure 18 Percentage of wheat flour allergen from the bulk improver on the filters

Key for figures 17 and 18 Sample descriptions and key ingredient changes

Sample	Description of sample	Soya	Emulsifier	Ca sulphate	Oil
Blue	Control: flour and ascorbic acid only	None	None	None	None
Red	Control: As blue, with fungal alpha amylase added	None	None	None	None
Yellow	Same as red with soya added	20%	None	None	None
Green	Same as yellow with emulsifier added	20%	20%	None	None
Black	Same as green with calcium sulphate added	20%	20%	25%	None
Orange	Same as black with oil added	20%	20%	25%	2%

The largest amount of wheat flour allergen that became airborne expressed either as per gram of bulk or as a percentage of WFA in the bulk, was from the black improver that contained all the ingredients with the exception of oil (Figures 17 & 18).

Expressed per gram of improver, Figure 17 suggested that the differences between other improvers were not very large. However, when expressed relative to the amount of WFA in the bulk improver (Figure 18) both the “Green” and the “Orange” improvers showed large airborne levels compared to the “Blue”, “Red” and “Yellow” improvers. Therefore ingredients in the “Green”, “Black” and “Orange” samples increased the percentage of aerosolised allergen. These samples had the same ingredients as the other three with the addition of emulsifier. The “Black” and “Orange” improvers also contained calcium sulphate. However, the “Orange” improver also contained oil, which reduced the dustiness (Figure 10) and also appeared to reduce the relative amount of the allergen (wheat flour allergen) that became airborne.

6.2.2 Individual ingredients

Dustiness testing

In order to better understand the properties of the ingredients in baking improvers, the individual ingredients were supplied by ABIM (Table 4) and analysed for dustiness in triplicate. The mean, standard deviation and coefficient of variance for the inhalable, thoracic and respirable fractions are shown in Table 12.

Table 12 Dustiness testing of the individual ingredients

HSL ID Number	Ingredient	Inhalable			Thoracic			Respirable			Moisture content
		Mean (mg/kg)	Std dev	COV %	Mean (mg/kg)	Std dev	COV %	Mean (mg/kg)	Std dev	COV %	Mean (%)
01825 / 08	Flour 1	133	8	6.1	63	4	5.9	26	2	9.3	9.41
01826 / 08	Calcium sulphate	456	36	7.9	224	20	8.8	67	4	5.7	16.09
01827 / 08	Soya flour	86	6	7.0	29	2	6.6	5	1	13.0	5.71
01828 / 08	Flour 2	169	12	7.1	34	2	6.7	4	0	6.1	10.93
01829 / 08	Emulsifier	6850	224	3.3	2389	126	5.3	592	37	6.3	3.27
01830 / 08	Flour 3	339	18	5.4	83	6	7.8	23	2	8.5	11.66
01831 / 08	Calcium carbonate	317	4	1.1	166	6	3.4	39	2	5.6	0.03
03195 / 08	Data ester (E472e)	1093	29	2.6	351	17	4.7	20	1	4.3	3.13
03196 / 08	Calcium silicate	3827	70	1.8	1001	32	3.2	76	1	1.5	2.39

The individual ingredients (samples 01825/08 to 01831/08) are graphed in Figure 19.

From Figure 19 the dustiest of the baking ingredients was the emulsifier, being several orders of magnitude more dusty than any of the other ingredients (there was 15 times more dust in the inhalable fraction than that for calcium sulphate). From the rest of the ingredients, the next dustiest were the calcium sulphate and calcium carbonate. These were followed by the flour samples, of these the dustiest flour was Flour 3, this was a higher quality baking flour while Flours 1 and 2 were filler flours. Soya flour is a fatty material and is the least dusty of the ingredients used.

However, emulsifier is not just a single component; this ingredient is data ester (E472e: diacetyltartaric acid esters of mono and diglycerides) mixed with a carrier or free flow agent. In the UK this is typically calcium silicate in the following amounts: 5% calcium silicate and 95% data ester. Since the emulsifier was the dustiest of the ingredients, the dustiness of these components were analysed individually. The data is shown in the bottom of Table 12 and Figure 20.

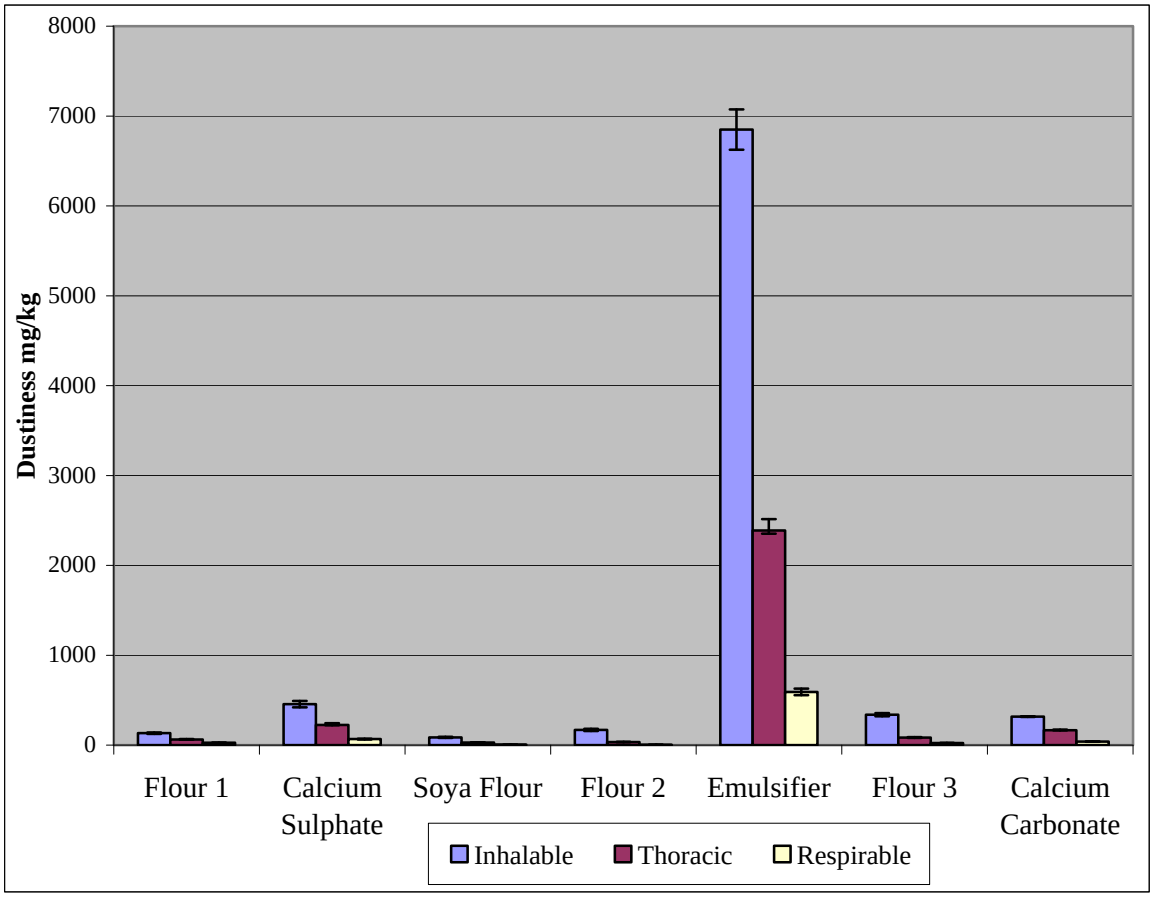


Figure 19 Dustiness testing of individual ingredients

Figure 20 shows the dustiness fractions from emulsifier (as on Figure 19), data ester (the emulsifier without a free flow agent) and calcium silicate (the free flow agent).

The mixed emulsifier was 6 fold dustier than the data ester and nearly twice as dusty as the calcium silicate. The action of a carrier or free flow agent is to prevent the ingredient (in this case data ester) from sticking in machines and pipes used for automated baking processes. HSL hypothesized that it does this by separating the aggregated data ester into smaller particle sizes, thus making the material dustier overall. The results of the particle size distribution testing are in the section below.

The calcium silicate is only a small component of the emulsifier (5%), however it appeared to have a large impact on the dustiness of the emulsifier as a whole. This result is consistent with the dustiness of the original improver mixtures (Figure 10); the addition of emulsifier increased the dustiness of the improver samples (as shown by the “Green”, “Black” and “Orange” samples).

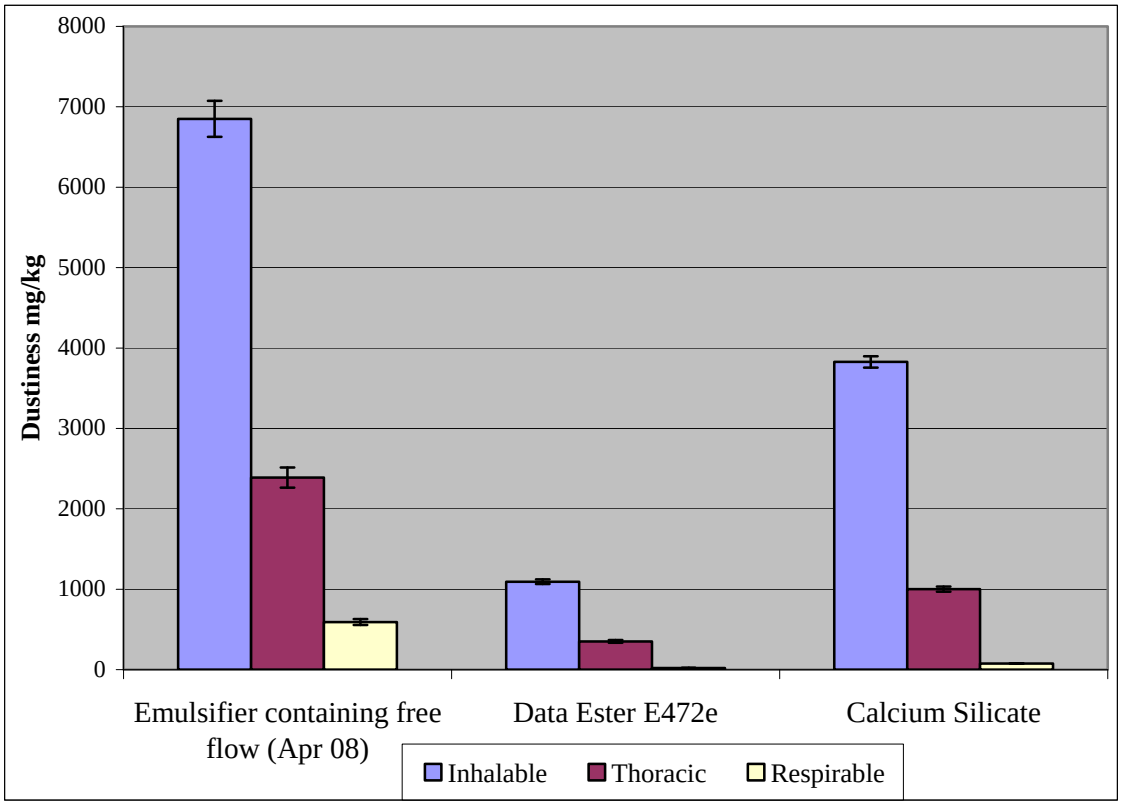


Figure 20 Dustiness testing of the components of emulsifier

Particle size distribution testing

It was thought that the dustiness of ingredients might be influenced by their particle size. Therefore some individual ingredients were analyzed for particle size distribution (see Table 4). The mean and standard deviation values for the particle size distribution are shown in Table 13.

Table 13 Particle size distribution testing for single agents and simple mixtures of ingredients

HSL ID	Ingredient / mixture	Density (g/cm^3)	Particle size (µm)			
			Aerodynamic diameter			
			Volume		Number	
			Mean	S.D.	Mean	S.D.
03195 / 08	Data ester	0.75	76.93	1.6	12.27	2.68
01827 / 08	Soya flour	1.01	31.76	1.61	11.91	2.02
01830 / 08	Flour 3	0.95	39.73	1.77	9.86	2.02
01828 / 08	Flour 2	1.16	29.58	1.64	9.62	2.01
01826 / 08	Calcium sulphate	1.36	27.42	1.58	6.51	2.43
03196 / 08	Calcium silicate	1.2	8.33	1.82	1.96	1.94

The values reported in Table 13 are the mean aerodynamic particle size for each given substance. The number distributions are the number of particles of that given size (diameter). The volume is the volume of that particular size of dust. These results would be affected by moisture content. In order to compare the particle sizes of the ingredients, they were analysed individually, as shown in Table 13. The density for these substances was determined and the particle size distribution was measured.

Figure 21 shows the means and standard deviations of the aerodynamic diameter number distributions for the individual ingredients. This measurement is used, as it is the figure most frequently quoted in aerosol studies. This shows the average particle size for the ingredients commonly used in bakery improvers.

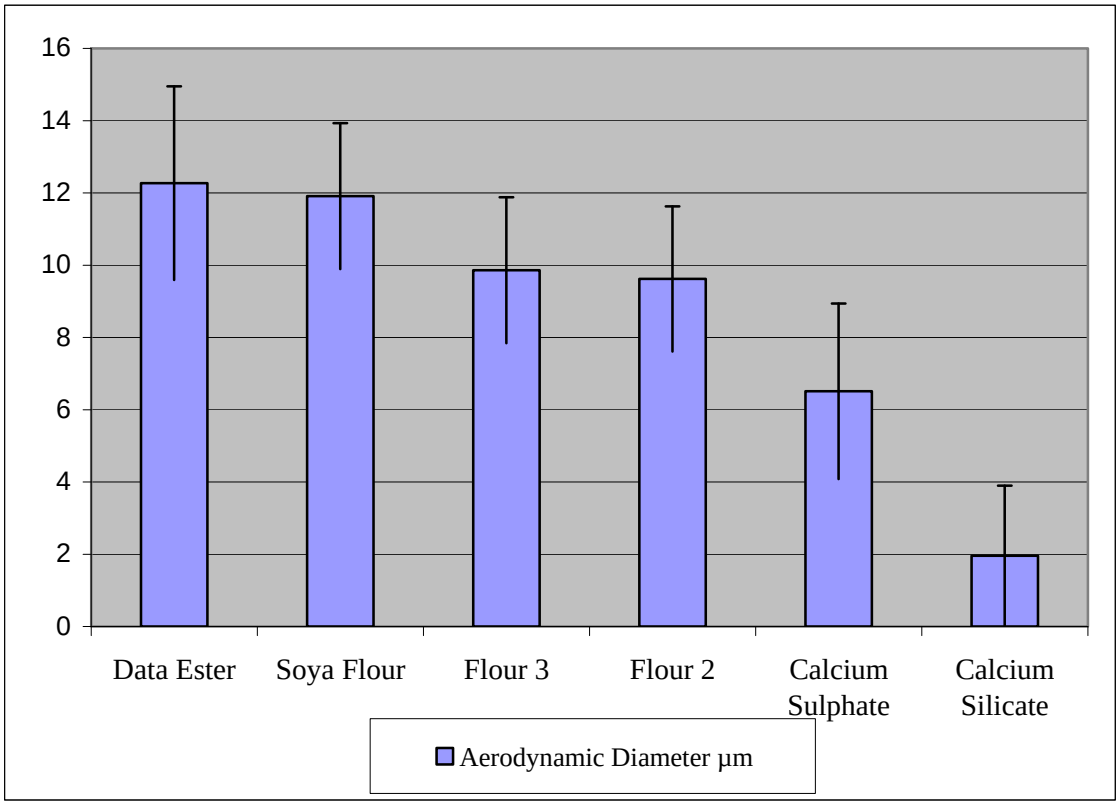


Figure 21 Mean aerodynamic particle size of the individual ingredients

Figure 21 suggests that calcium silicate and calcium sulphate had the smallest particle sizes and these ingredients increased the dustiness of improver mixtures in the dustiness testing. The ingredients with the largest particle sizes were the soya flour and the data ester (emulsifier without the added free flow agent). Soya flour has been shown (Figure 10) to reduce dustiness in the improver, which could be related to its large particle size. The data ester was a fatty material that had a tendency to stick together and form aggregates, so necessitating the addition of a free flow agent when used in improvers to ensure it did not become aggregated in the machines. The two types of flour tested were of medium particle size.

6.2.3 Within sample variation

Dustiness testing

The results gained from the original improver mixtures were very unexpected (Figure 10). In order to ensure that this was not due to heterogeneity in the samples, various fractions within the bulk sack of the "Orange" (Table 3) mixture were analysed. These results are shown in Figure 22.

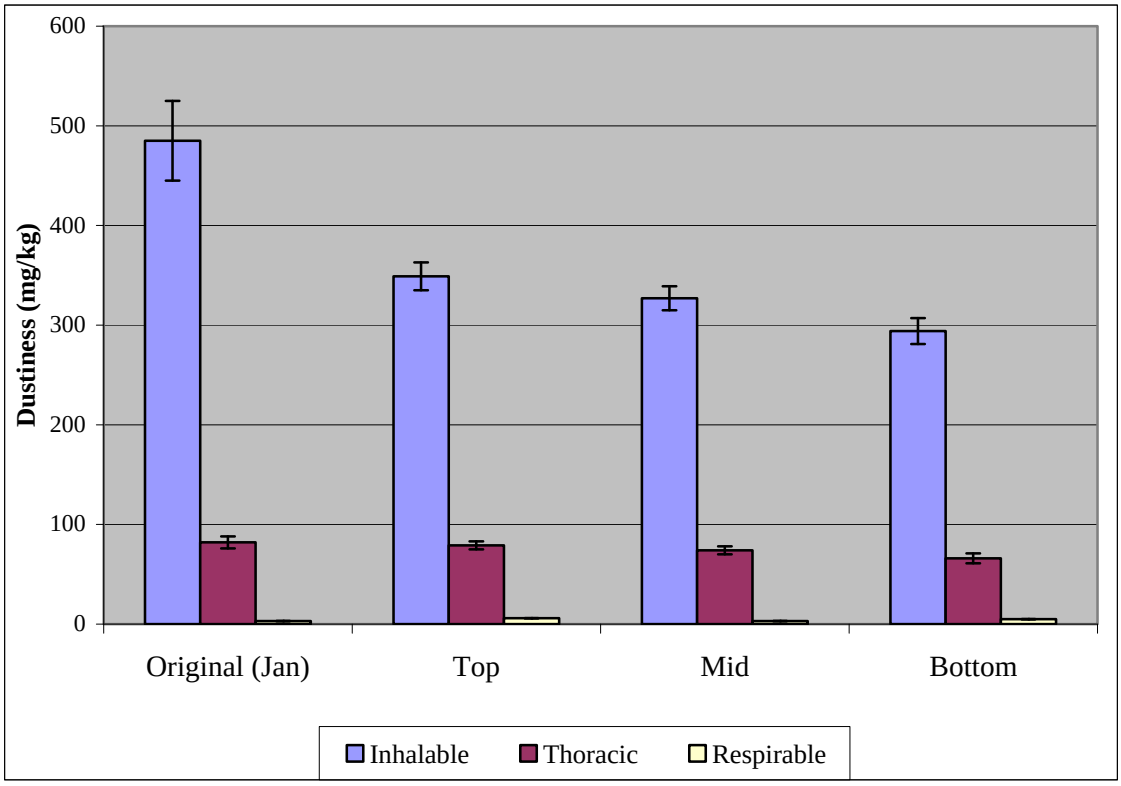


Figure 22 Dustiness of the fractions from the "Orange" sack, compared to dustiness testing of the "Orange" sample from Figure 3

From Figure 22 it can be seen that the samples from the top, middle and bottom of the sack are not much different from each other. However all three of these fractions from the "Orange" sack are lower in dustiness than the initial dustiness testing performed 3 months prior (left hand bar). It appeared that the sample does not have much variation within the sack, however it had changed in its properties over time. The "Orange" improver sample contained all the added ingredients including oil. All the samples had been stored in the refrigerator during this time. It was considered that the oil may have solidified within the improver and caused the sample to become less dusty.

These results were discussed with ABIM and in order to investigate whether this was the case, the "Black" improver sample was repeated for dustiness testing and compared to the initial result gained in Table 9 and Figure 10. The repeat dustiness testing of the "Black" sample is

shown in Figure 23 below and compared to the initial dustiness testing of the “Black” sample, 4 months prior.

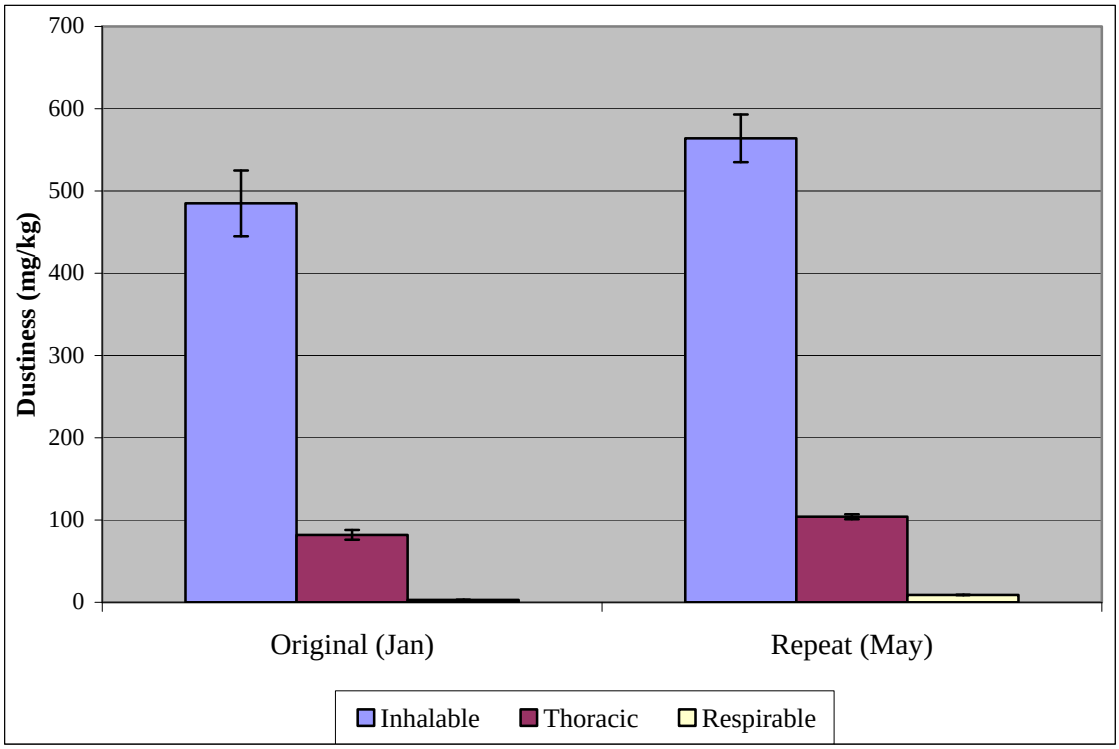


Figure 23 Repeated dustiness testing of the “Black” sample, compared to dustiness testing of the “Black” sample from Figure 4

Figure 23 shows the dustiness testing of the “Black” sample from the initial test (labelled “Original (Jan)”, also in Figure 10) and the repeated sample 4 months later. Considering the error bars, the dustiness of the “Black” sample has not changed during this time period.

The “Black” improver sample was the same as the “Orange” improver sample without the oil; the other contents were the same. Therefore the difference between the “Black” and “Orange” samples is due to the oil only.

The results from Figures 22 and 23 would indicate that the oil in the “Orange” sample has solidified in the refrigerator possibly causing some changes in the dustiness over time. The “Black” sample only contained dry ingredients and did not appear to have altered in its properties. Further improver mixtures were kept at room temperature.

6.2.4 Simple combinations of ingredients

Dustiness testing

It was hypothesized that the ingredients of the improvers were interacting and that these could be having an effect on the dustiness and behaviour of the improver as a whole. Simple combinations of the ingredients in Table 4 were made to investigate how these ingredients interacted with each other in combination, versus the behaviour of the ingredients on their own. Flour was mixed with emulsifier only and calcium sulphate only in order to investigate whether these ingredients would increase the dustiness of flour without any other agents present. Since calcium silicate appeared to increase the dustiness of the data ester in the emulsifier, whether calcium silicate would also increase the dustiness of flour was investigated by combining calcium silicate only with flour at various concentrations. The dustiness testing results for these combinations of ingredients are shown in Table 14.

Table 14 Dustiness testing of fractions of improver mixtures and simple combinations of bakery ingredients

HSL ID	Ingredient	Inhalable			Thoracic			Respirable			Moisture content
		Mean (mg/kg)	Std dev	COV %	Mean (mg/kg)	Std dev	COV %	Mean (mg/kg)	Std dev	COV %	Mean (%)
05355 / 08	Orange: Top	349	14	3.9	79	4	4.5	6	0	6.1	9.2
05356 / 08	Orange: Middle	327	12	3.5	74	4	5.0	3	0	9.8	9.2
05357 / 08	Orange: Bottom	294	13	4.6	66	5	7.4	5	0	5.9	9.1
00126 / 08	Black: Repeat sample	564	29	5.2	104	3	3.4	9	0	1.2	9.82
07649 / 08	Emulsifier (100g) + Flour 3 (400g)	3910	247	6.3	1013	5	0.5	71	4	5.2	10.67
07650 / 08	Ca sulphate (100g) + Flour 3 (300g)	2073	96	4.6	701	19	2.7	38	3	7.8	12.7
07651 / 08	Ca silicate (10g) + Flour 3 (490g)	7086	403	5.7	2145	124	5.8	270	23	8.4	12.37
07652 / 08	Ca silicate (5g) + Flour 2+ 3 (495g)	1452	34	2.3	455	21	4.5	38	1	3.5	12.71
07653 / 08	Ca silicate (3g) + Flour 2+ 3 (497g)	772	13	1.7	234	18	7.6	18	1	4.6	12.81

Table 12 and Figures 19 and 20 showed that the emulsifier was the dustiest individual ingredient. It was hypothesized that in turn the emulsifier could be mobilising the flour and other particles in the improver to make the mixture dustier as a whole. This was investigated by making a simple mixture of emulsifier and flour that did not contain any other ingredients. The emulsifier was added at the same concentration as a normal improver (20%) to show the effect of emulsifier on flour. This mixture was analysed for dustiness and is shown in Figure 24 below compared to flour and emulsifier alone.

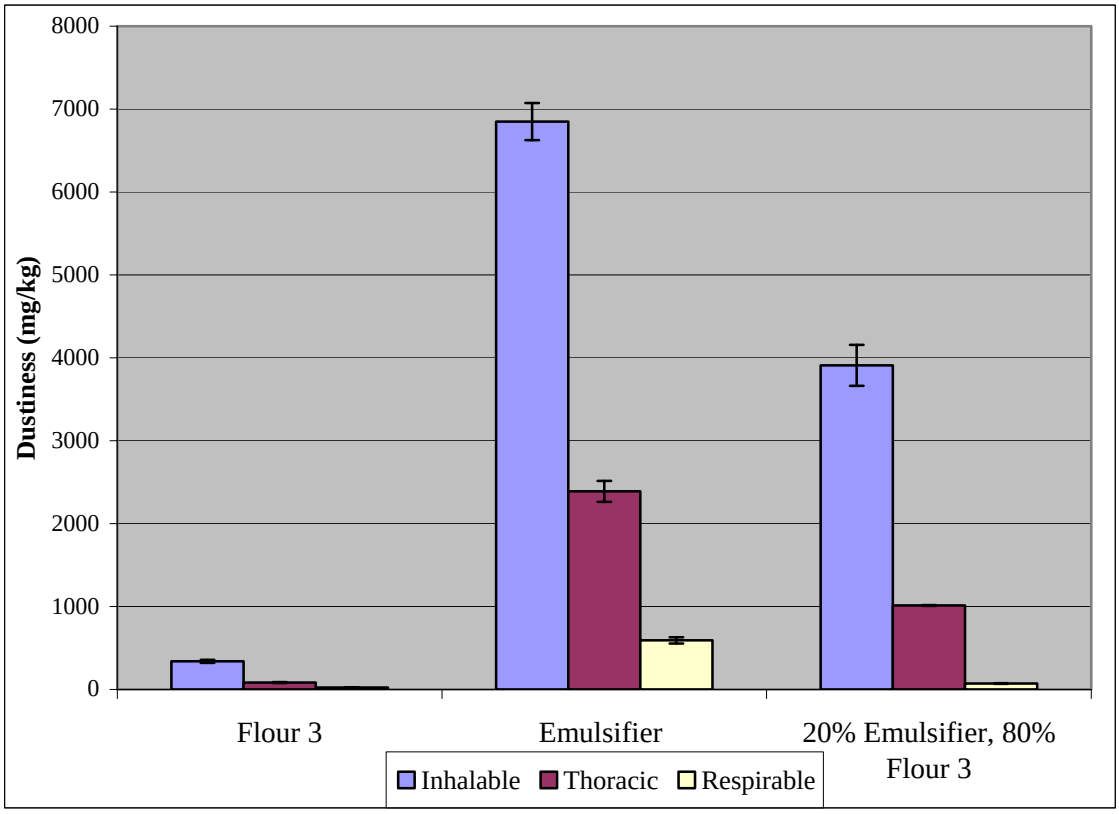


Figure 24 Dustiness testing of emulsifier and flour mixture compared to emulsifier and flour separately

Figure 24 clearly shows that when emulsifier is added to flour at 20%, the dustiness of the material was between that of emulsifier and flour. Therefore adding emulsifier at this standard concentration increased the dustiness of the flour. It was possible that the calcium silicate (free flow agent) in the emulsifier was breaking the aggregated flour into smaller particles, potentially making the flour dustier. This was investigated further in the particle size analysis and by assessing allergen content of the generated dusts to establish whether the dusts contained only the free flow agent or more allergen.

The first tests assessed whether the calcium silicate in the emulsifier was the agent responsible for increasing the dustiness of the flour. Calcium silicate is typically added to emulsifier at a standard concentration of 5%, with the emulsifier added to improvers at 20% of the improver mixture, i.e. calcium silicate would be 1% of the whole improver.

A dose response experiment was performed to study the effect of calcium silicate using concentrations of 2%, 1% and 0.6% calcium silicate added to flour. 0.6% was chosen as the lower concentration of calcium silicate because advice from both ABIM and DANISCO (an emulsifier manufacturer) was that the lowest concentration of calcium silicate that could realistically be used in an emulsifier was 3%. This equates to 0.6% of the improver mixture as a whole when emulsifier is added at 20% of the mix. 2% represented the maximum calcium silicate in an improver (10% of the emulsifier) that could be used and 1% is the standard amount of calcium silicate that would be in UK improver mixes (5% of the emulsifier).

The three concentrations of calcium silicate in flour were dustiness tested and compared to the dustiness of two flours (Flours 2 and 3) and emulsifier alone (Figure 25).

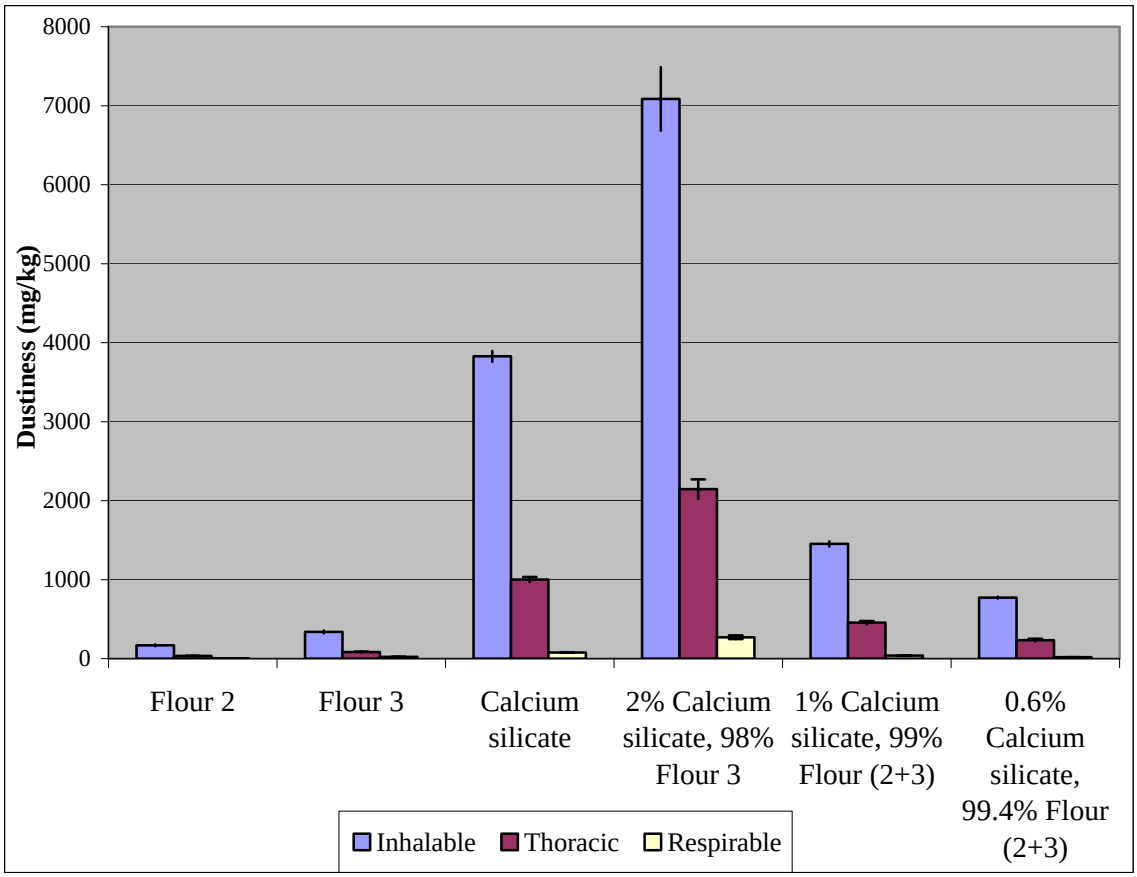


Figure 25 Calcium silicate dose response on the dustiness of flour, also compared to flour and calcium silicate separately

Figure 25 shows that flour alone is not a very dusty ingredient, in comparison with calcium silicate. When combined, the calcium silicate increased the dustiness of the mixture. Figure 25 shows a clear but non-linear dose response in dustiness caused by increasing calcium silicate concentration. Figure 25 indicates that the emulsifier increases the dustiness of improver because of calcium silicate (free flow agent). The amount of calcium silicate added to the mixture overall was very small, however its effect on the dustiness of the flour was considerable (flour increased in dustiness 4 fold when 1% calcium silicate was added). Therefore small

changes in the amount of calcium silicate in emulsifier could potentially have a dramatic effect on the dustiness of baking improvers.

These amounts of calcium silicate in flour are compared with standard emulsifier in Figure 26.

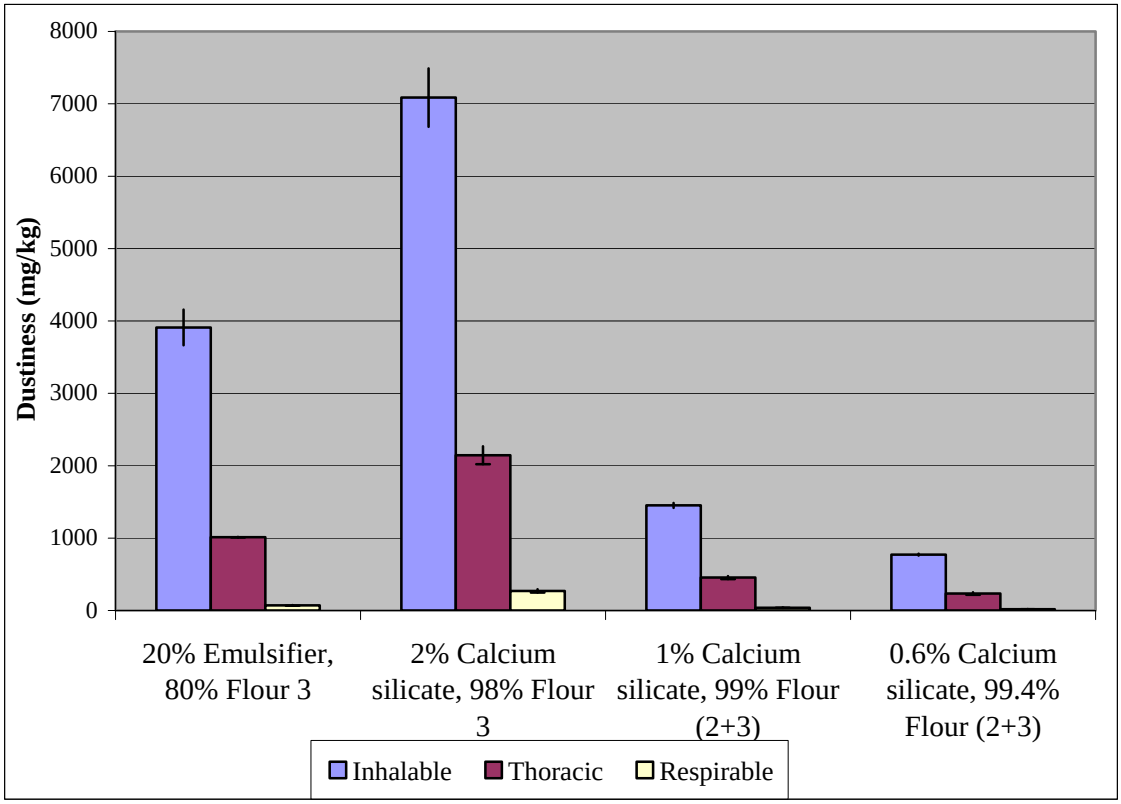


Figure 26 Calcium silicate dose response compared with dustiness of emulsifier in flour

From Figure 26 it can be seen that the dustiness of emulsifier in flour was much higher than that for 1% calcium silicate in flour. The amount of calcium silicate in these two samples is the same, so this difference was due to the data ester. The emulsifier in flour is approximately double the dustiness of the calcium silicate in flour mixture. This indicates that either the data ester was also acting to further increase dustiness of the improver or the combination of data ester and calcium silicate was particularly efficient at promoting dustiness.

Data shown in Table 9 and Figure 10 indicated that calcium sulphate was also an ingredient that increased the dustiness of the improver. Therefore this was investigated further by mixing calcium sulphate with flour at the concentration of 25% calcium sulphate, 75% flour. This is a typical amount of calcium sulphate used in improvers. The dustiness was tested and is shown in Figure 27 below, compared to flour and calcium sulphate alone (from Figure 19).

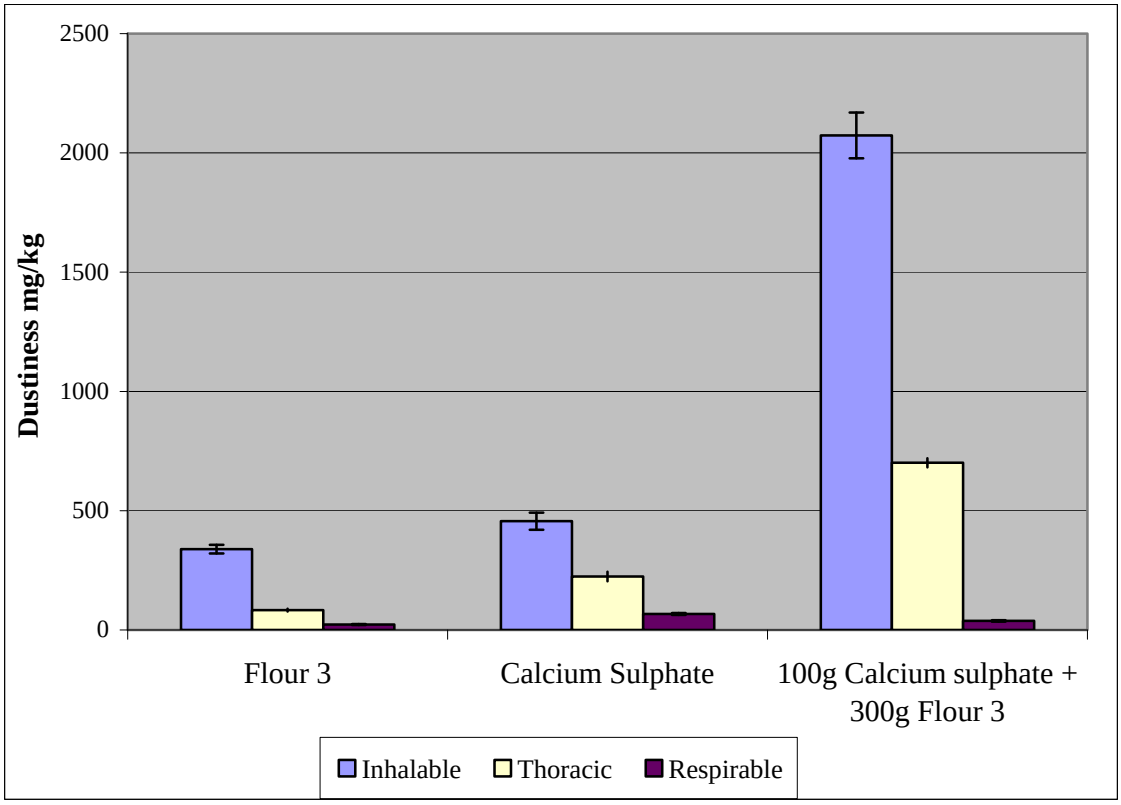


Figure 27 Dustiness testing of calcium sulphate and flour mixture compared to calcium sulphate and flour separately

Figure 27 shows that the mixture of calcium sulphate and flour is 6 fold dustier than flour alone and over 4 fold dustier than calcium sulphate alone. It is suggested that the calcium sulphate is acting to break up aggregated flour to produce smaller particles that are more easily made airborne. Calcium sulphate could potentially have this effect on the improver mixture as a whole and act to make baking improvers dustier than they would otherwise be.

Particle size distribution testing

The particle size distribution results for the simple mixtures are shown in Table 15. These results are graphed in Figures 28 to 30.

Table 15 Particle size distribution testing for simple mixtures of ingredients

HSL ID	Ingredient / mixture	Density (g/cm^3)	Particle size (µm)			
			Aerodynamic diameter			
			Volume		Number	
			Mean	S.D.	Mean	S.D.
Mixture	25% Ca sulphate + Flour 2	1.2	27.63	1.69	7.75	2.08
Mixture	20% Emulsifier + Flour 2	1.05	40.46	1.74	7.92	2.29
Mixture	19% Data ester + Flour 2	1.05	29.51	1.73	8.6	1.99
Mixture	0.6% Ca silicate + 19.4% data ester + flour 2	1.04	31.47	1.54	11.38	2.06
Mixture	1% Ca silicate + Flour 2	1.16	41.59	1.71	6	2.52
Mixture	0.6% Ca silicate + Flour 2	1.16	37.84	1.73	7.47	2.39

The values reported in Table 15 are the mean aerodynamic particle size for each given substance. The number distributions are the number of particles of that given size (diameter). The volume is the volume of that particular size of dust. These results would be affected by moisture content.
These mixtures were made to investigate the change in particle size distribution when the ingredients were combined. Densities for mixtures were estimated from the densities of the single ingredients and the proportions in which these were combined.
The means and standard deviations of the aerodynamic diameter number distributions were plotted to show the effect of combining the ingredients on the average particle size (see Figures 28 to 30).

A combination of 25% calcium sulphate, 75% Flour 2 was mixed in order to look at the effect of combining these ingredients on particle size. In the dustiness experiments it was found (Figure 27) that when flour and calcium sulphate were mixed together, they became far dustier than either ingredient on their own. The mean aerodynamic particle size is shown in Figure 28 with the flour and calcium sulphate results from Figure 21 included.

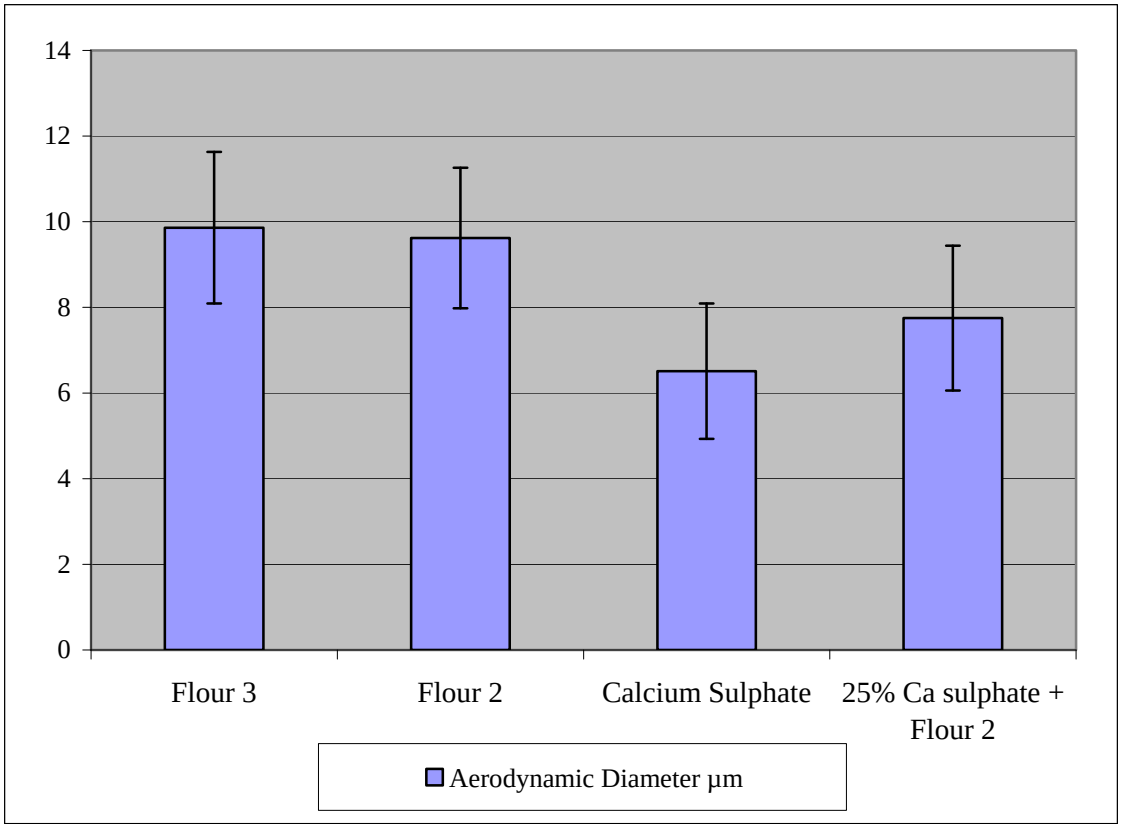


Figure 28 Mean aerodynamic particle size of calcium sulphate and flour mixture compared to calcium sulphate and flour separately

It can be seen from Figure 28 that the smallest particle size was calcium sulphate and the largest was for the two types of flour. When combined, the particle size is in between the sizes gained for the ingredients individually.

Two mixtures of calcium silicate and flour were made in order to look at the effect of a very small amount of calcium silicate on the particle size of the mix. A mixture of 1% calcium silicate and 99% flour and a mixture of 0.6% calcium silicate and 99.4% flour were made and the particle size distribution was analysed. The mean sizes are shown in Figure 29 together with the particle sizes of flour and calcium silicate from Figure 21 for comparison.

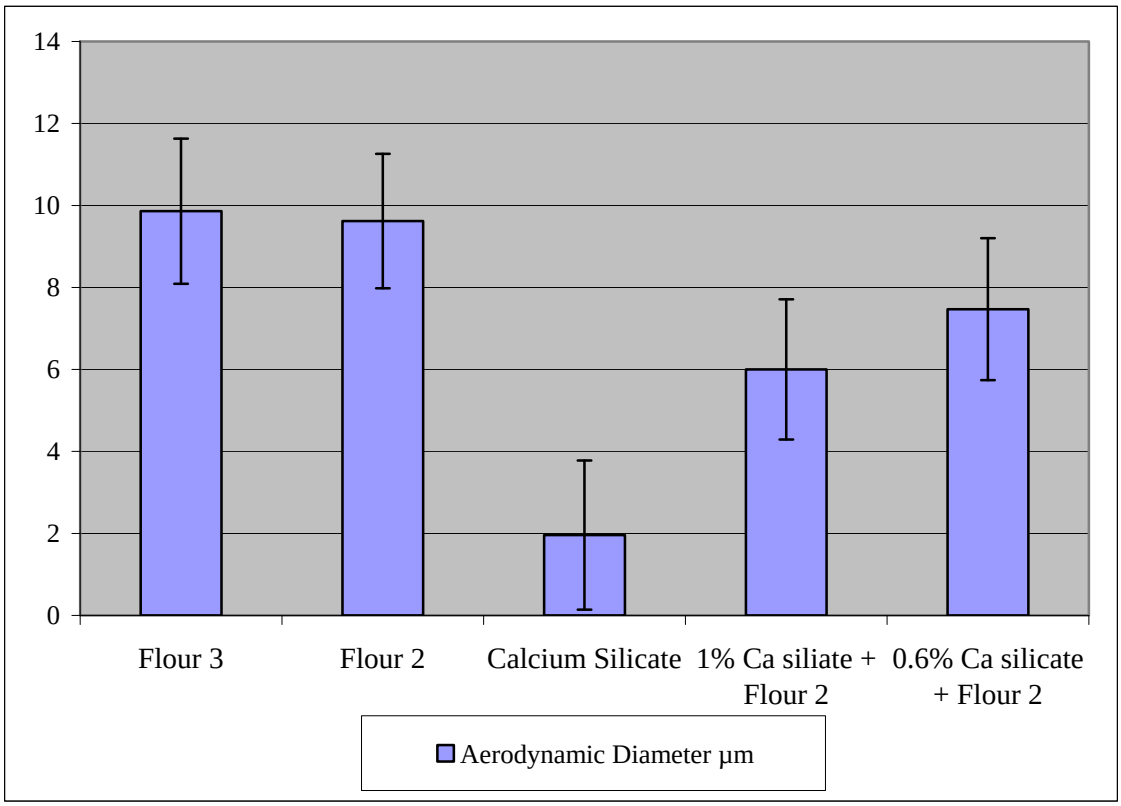


Figure 29 Mean aerodynamic particle size of calcium silicate and flour mixture compared to calcium silicate and flour separately

From Figure 29 it can be seen that adding calcium silicate reduced the mean particle size of flour. The mean aerodynamic diameter for the two mixtures was between the particle size for flour and calcium silicate alone.
The 1% calcium silicate and flour sample reflects the level of calcium silicate typical in bakery improvers (i.e. calcium silicate added as 5% of emulsifier and emulsifier added at 20% of the improver, gives an overall calcium silicate content of 1% in the improver). This sample demonstrated that the particle size of the flour could be reduced by the action of the free flow agent within the emulsifier.

The lowest possible amount of calcium silicate was considered to be 3% of the emulsifier; this would typically be 0.6% of the overall improver. When compared to the 1% calcium silicate, the particle size has increased. From Figures 25 and 26 it was shown that this change in the amount of free flow agent also decreased the dustiness of the flour by nearly half.

This effect on particle size was investigated when the calcium silicate was in an emulsifier as well as just in the flour alone. 20% emulsifier and flour was mixed and tested to compare the particle size with flour and emulsifier alone. As previously indicated, the lowest amount of calcium silicate in an emulsifier would be 3% (0.6% in the overall improver), so a mixture containing 0.6% calcium silicate, 19.4% data ester in flour was made to test whether an emulsifier containing less calcium silicate would have less of an effect on the particle size than standard emulsifier. As an extra control, data ester was mixed with flour only (no calcium silicate). The mean aerodynamic particle sizes for these combinations are shown in Figure 30 compared with flour, data ester and calcium silicate individually.

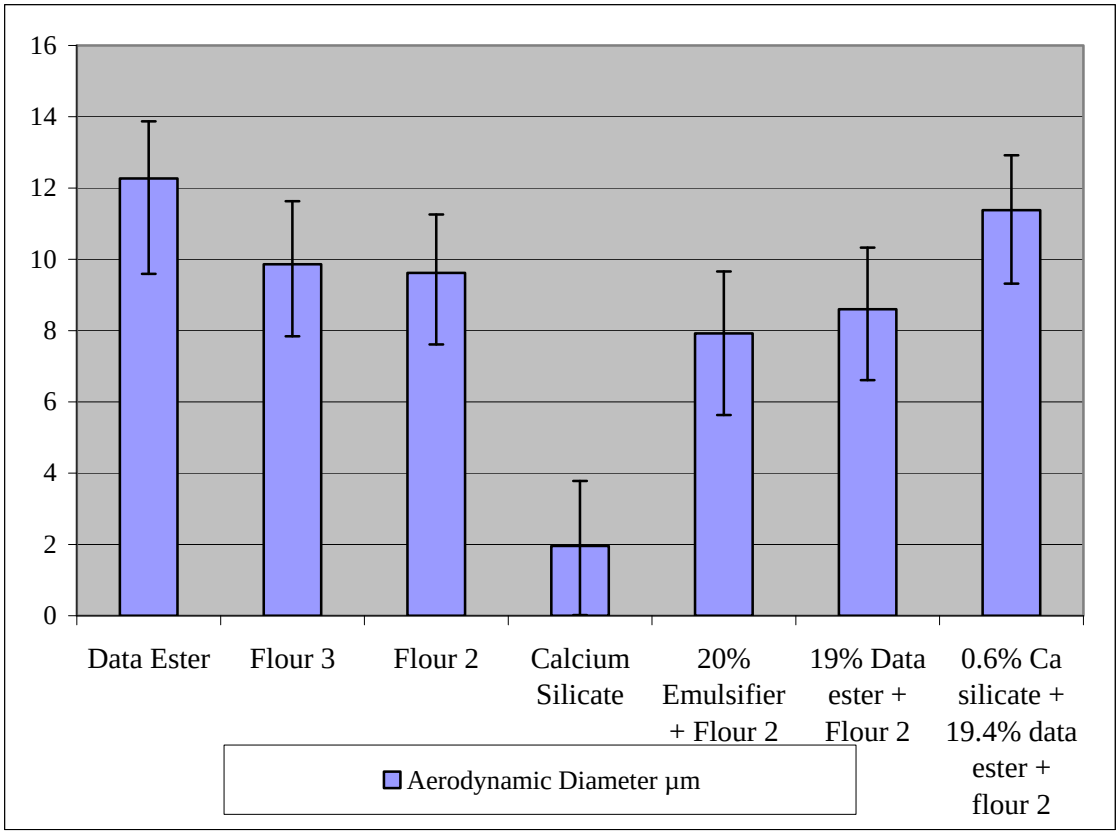


Figure 30 Mean aerodynamic particle size of emulsifier and flour mixture and test emulsifier and flour mixture compared to data ester, calcium silicate and flour separately

The results for data ester, two types of flour and calcium silicate are shown on the left hand side of Figure 30 for comparison. If the mixture of 20% emulsifier with flour and the 0.6% calcium silicate, 19.4% data ester and flour are compared it can be seen that decreasing the calcium silicate in the emulsifier appeared to increase the particle size. The data ester and flour mixture had a very similar particle size distribution as the standard emulsifier and flour mixture. Samples with smaller particle size distributions appeared to correlate with increased dustiness.

These samples were polydispersed powders and as such they had a wide range of particle sizes within them. This is the nature of the samples and is why the standard deviations are large for the particle size data. Therefore the particle size data should not be viewed as exact numbers, but more to show the general trend of the effect on particle size when certain ingredients are mixed together.

Immunological analysis

Bulk samples

Total protein, calcium, wheat flour allergen and soya trypsin inhibitor were analysed in bulk samples and results expressed as per gram of bulk improver (ng/g, µg/g for protein) and a percentage of measured substance in the bulk improver (Table 16).

Table 16 Bulk samples of simple combinations of ingredients - content of measured substances

HSL ID	Sample code	Soluble protein		Calcium		Soya trypsin inhibitor		Wheat flour allergen	
		µg/g	%	ng/g	%	ng/g	%	ng/g	%
07649/08	Emulsifier (100g) + flour 3 (400g)	9509.8	0.951	7243.4	0.000724	11.5	0.000001	3968.9	0.000397
07650/08	Ca sulphate (100g) + flour 3 (300g)	19809.8	1.981	7700.0	0.000770	19.8	0.000002	1044495.1	0.104450
07651/08	Ca silicate (10g) + flour 3 (490g)	23034.8	2.303	886.0	0.000089	99.2	0.000010	796995.1	0.079700
07652/08	Ca silicate (5g) + flour 3 (495g)	24334.8	2.433	2487.7	0.000249	357.9	0.000036	1074995.1	0.107500
07653/08	Ca silicate (3g) + flour 3 (497g)	26684.8	2.668	2209.2	0.000221	375.1	0.000038	1293995.1	0.129400
07649/08	Emulsifier (100g) + flour 3 (400g)	9509.8	0.951	7243.4	0.000724	11.5	0.000001	3968.9	0.000397

Airborne samples from dustiness testing

Airborne levels of calcium and wheat flour allergen (these samples do not contain soya flour) were calculated (see calculation of immunology results: Appendix 6.1.6) and expressed per gram of the combination or as a percentage of the specific component. The results of these calculations are shown in Table 17.

Table 17 Average content of measured substances in the dustiness filters and foams from the dustiness experiments

Sample	Fraction	Soluble protein		Calcium		Wheat flour allergen	
		ng/g	%	ng/g	%	ng/g	%
25% calcium sulphate 07650/08	Inhalable	17.130	0.08647	406.177	5.27500	212.747	0.02037
	Thoracic	49.415	0.24944	425.141	5.52129	110.660	0.01059
	Respirable	ND	0.00000	38.895	0.50513	8.808	0.00084
20% emulsifier 07649/08	Inhalable	84.158	0.88496	52.584	0.72596	630.977	15.89822
	Thoracic	111.999	1.17771	117.075	1.61631	491.650	12.38772
	Respirable	1.327	0.01395	4.184	0.05776	109.865	2.76819
2% calcium silicate in flour	Inhalable	121.946	0.52940	117.875	13.30383	2870.252	0.36013
	Thoracic	81.836	0.35527	111.393	12.57220	1508.268	0.18924

07651/08	Respirable	7.700	0.03343	37.250	4.20412	20.049	0.00252
1% calcium silicate in flour 07652/08	Inhalable	45.092	0.18530	92.263	3.70879	119.722	0.01114
	Thoracic	85.943	0.35317	112.352	4.51630	184.457	0.01716
	Respirable	0.432	0.00178	9.127	0.36688	15.307	0.00142
0.6% calcium silicate in flour 07653/08	Inhalable	33.472	0.12543	30.051	1.36027	139.100	0.01075
	Thoracic	30.559	0.11452	85.091	3.85167	118.977	0.00919
	Respirable	ND	0.00000	2.977	0.13475	6.384	0.00049

Soluble protein

Figures 31 and 32 show these results for soluble protein.

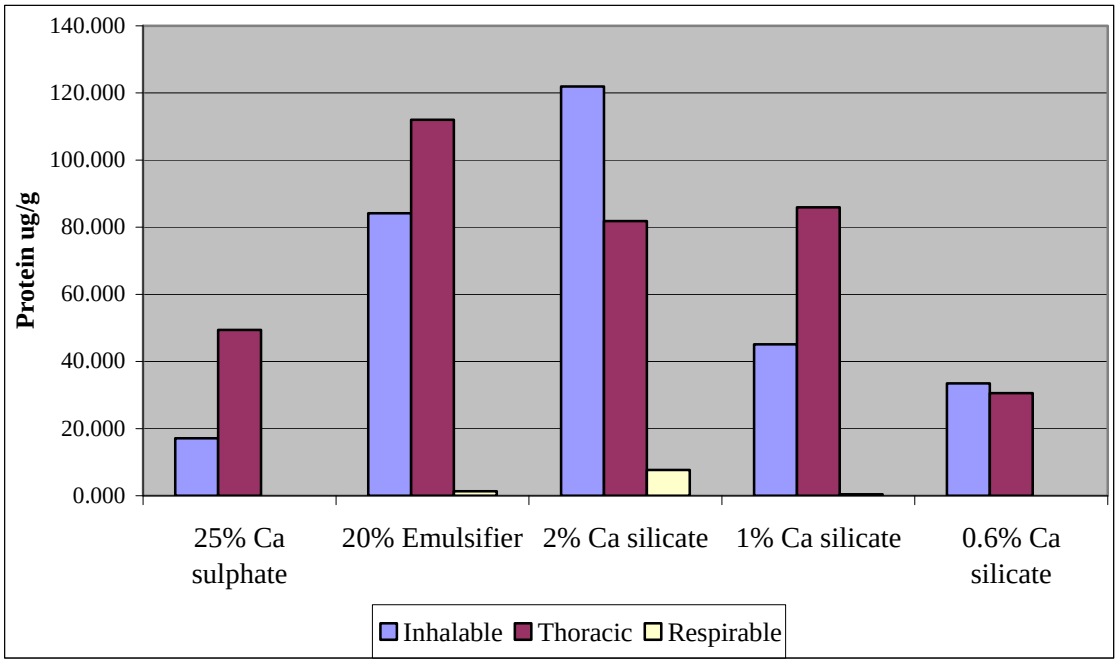


Figure 31 Amount of soluble protein on the filters per gram of ingredient in the drum

From Figure 31 it can be seen that the highest amount of soluble protein on the filters and foams was obtained from the emulsifier and flour mix and the 2% calcium silicate and flour mixture. The samples from the experiments with calcium silicate in the flour are on the right hand side of the chart and these show that as calcium silicate decreases in the mixture, the amount of protein that becomes airborne also decreases.

Standard emulsifier from the manufacturer only contained the same amount of calcium silicate at 1% overall, so this indicated that the data ester in the emulsifier was also having an effect. The airborne protein also increased in the sample containing 25% calcium sulphate, however this effect was not as large as that produced by the addition of emulsifier to flour.

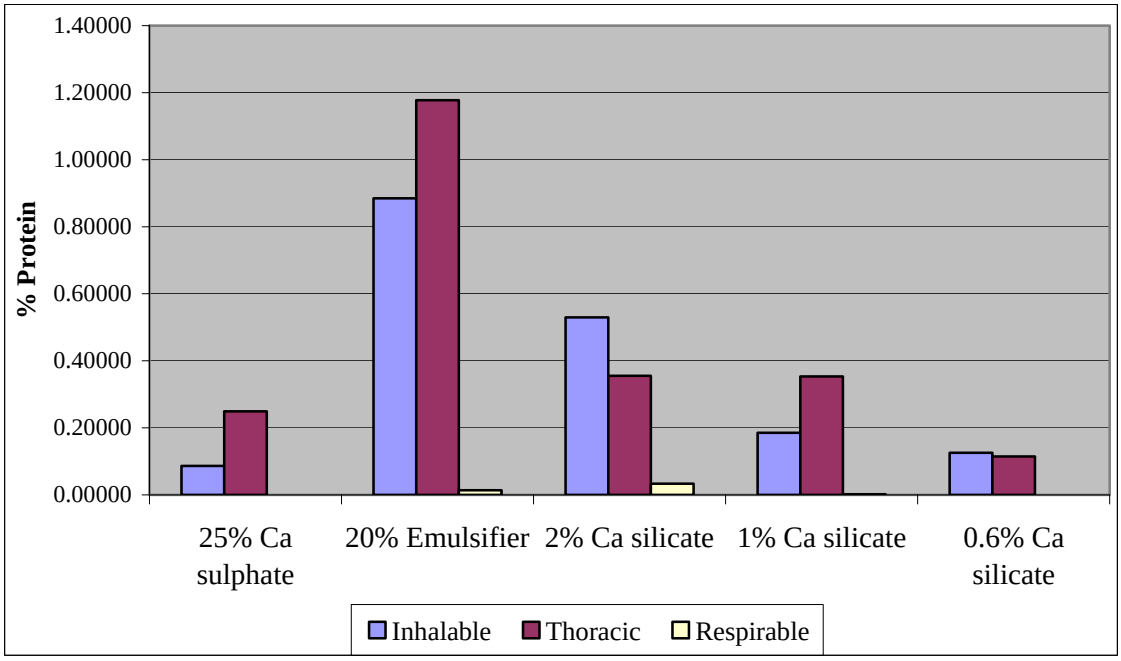


Figure 32 Percentage of protein from the bulk ingredients on the filters

Figure 32 shows the percentage of the protein mobilised from the bulk mixture onto the filter or foam. The calcium silicate and flour mixtures showed the same trend as Figure 31, so proportionally the amount of airborne protein decreased with decreasing calcium silicate. The emulsifier had a much larger effect on the amount of protein on the filters as this result has a higher percentage of protein than the 2% calcium silicate sample, again suggesting that the emulsifier mixture of data ester and calcium silicate had a large effect on exposure to proteins. The calcium sulphate had less of a potent effect on the exposure to soluble proteins; proportionally less protein was airborne than the emulsifier, 2% or 1% calcium silicate mixtures. However since calcium sulphate is typically added in larger amounts, this may become an important factor when dealing with large volumes of improvers.

The results showed that the airborne protein increased with increasing calcium silicate and for mixtures containing emulsifier and calcium sulphate. Emulsifier was particularly effective at aerosolising protein.

Calcium results

Figures 33 and 34 show the results of these calculations for calcium.

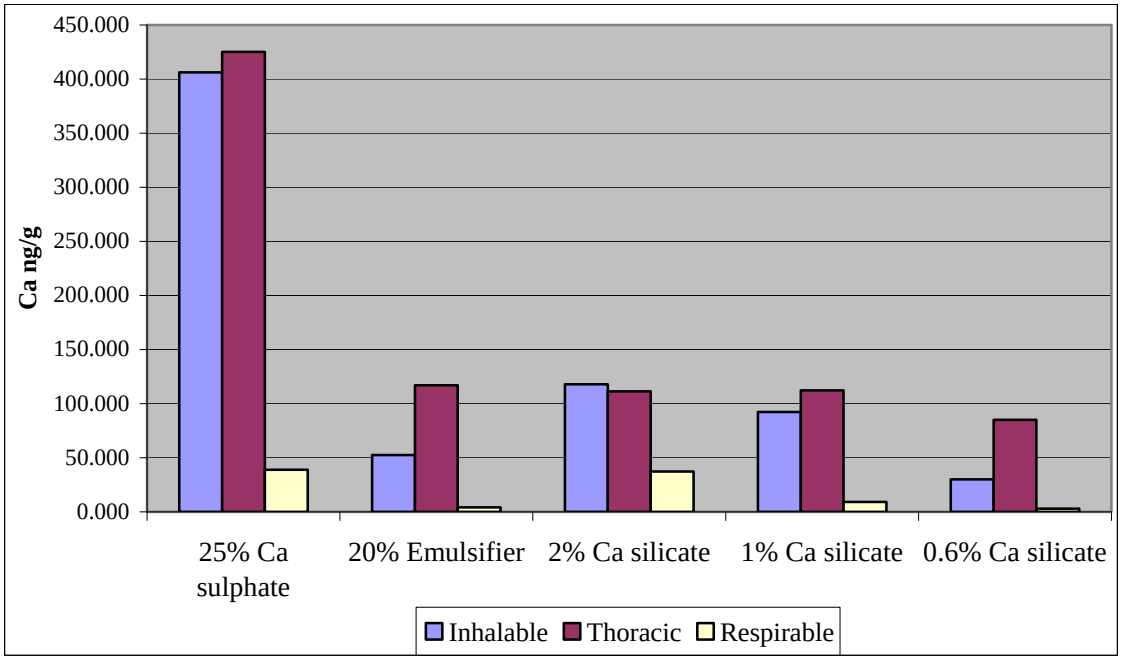


Figure 33 Amount of calcium on the filters per gram of bulk ingredients in the drum

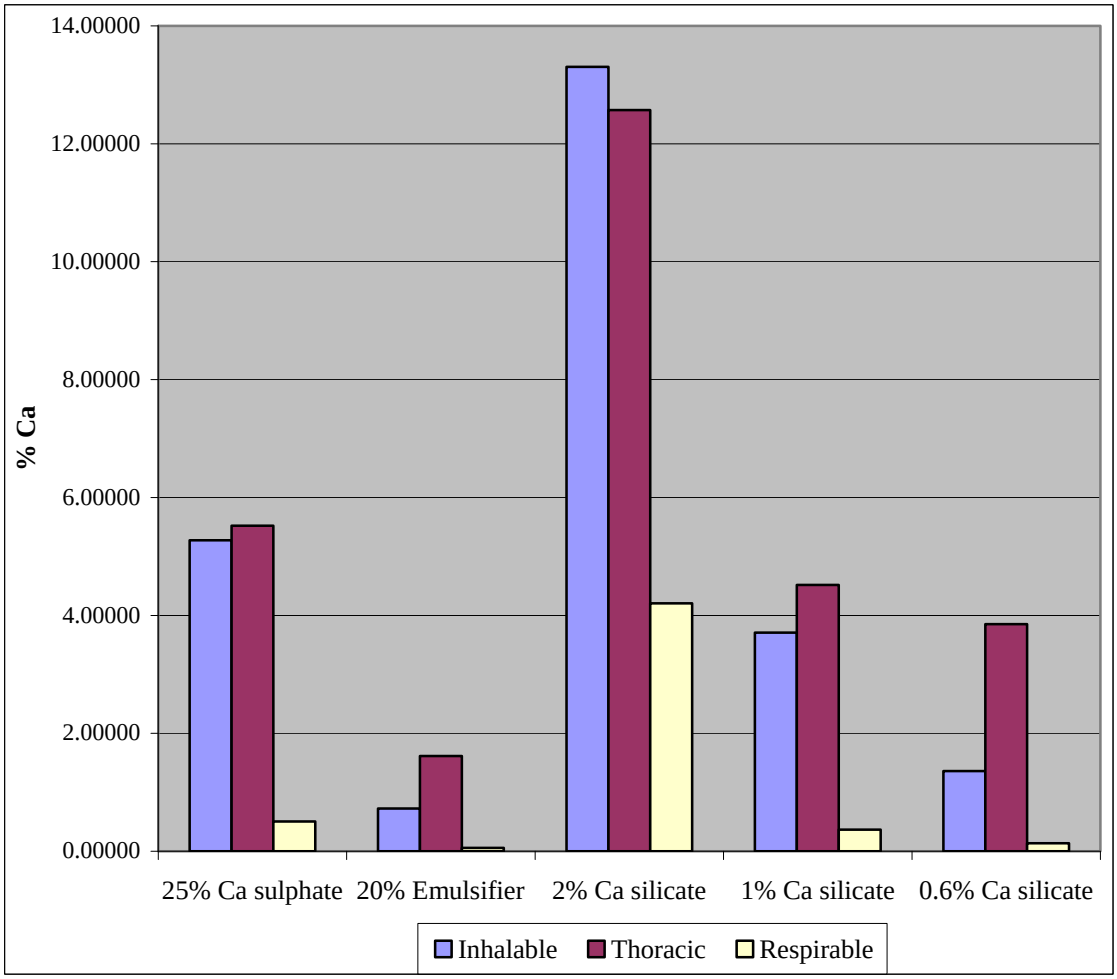


Figure 34 Percentage of calcium from the bulk ingredients on the filters

Figure 33 shows that the largest amount of calcium was found in the filters from the 25% calcium sulphate and flour mixture. This result is not surprising since this sample contained a large amount of calcium sulphate. The emulsifier and 2% calcium silicate mixtures appeared to make a similar amount of calcium airborne. There was a slight decrease in the airborne calcium with decreasing calcium silicate in the mix.

Figure 34 shows that calcium was made airborne in a dose dependant manner when calcium silicate was added. The 2% calcium silicate sample made the largest percentage of calcium airborne and was higher than the 25% calcium sulphate sample. The least calcium becoming airborne was for the sample containing emulsifier. There may be an interaction with data ester that reduced the proportion of the calcium that became airborne or there could be differences in the extraction efficiencies. Calcium sulphate was not as efficient at making calcium airborne but is handled in larger quantities in bakery improvers.

The proportion of calcium that became airborne therefore increased with increasing calcium silicate; calcium sulphate was less efficient at making calcium airborne, but is added to improvers in larger quantities so the airborne amounts were high.

Wheat flour antigen results

Figures 35, 36 and 37 show the results for wheat flour antigen.

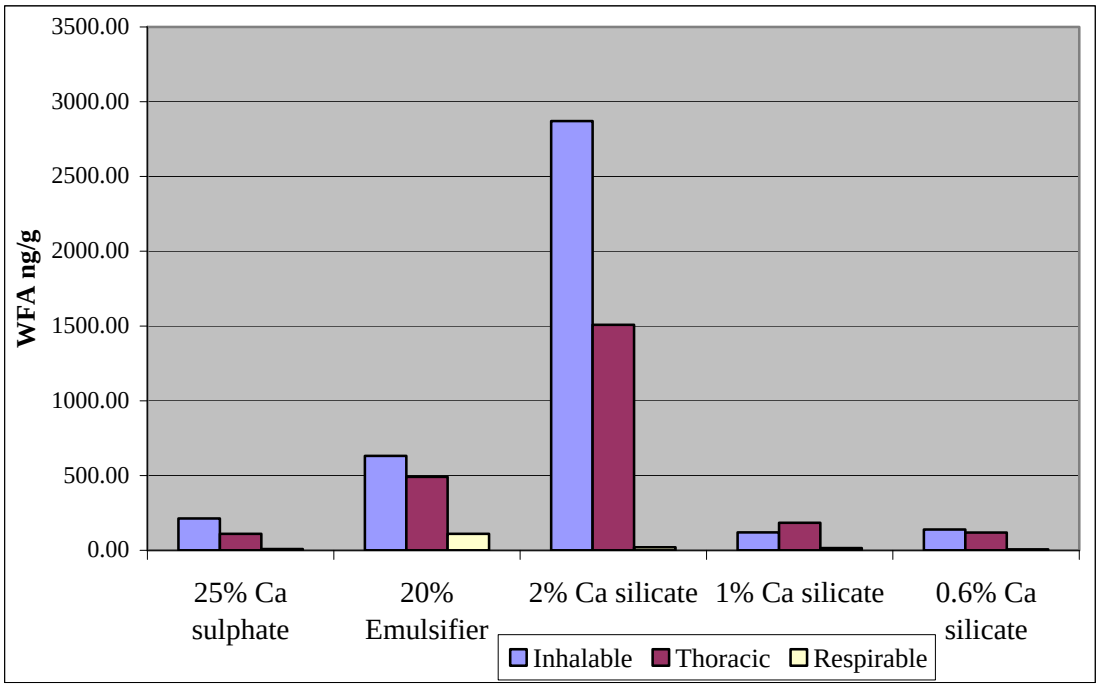


Figure 35 Amount of wheat flour antigen on the filters per gram of bulk ingredients in the drum

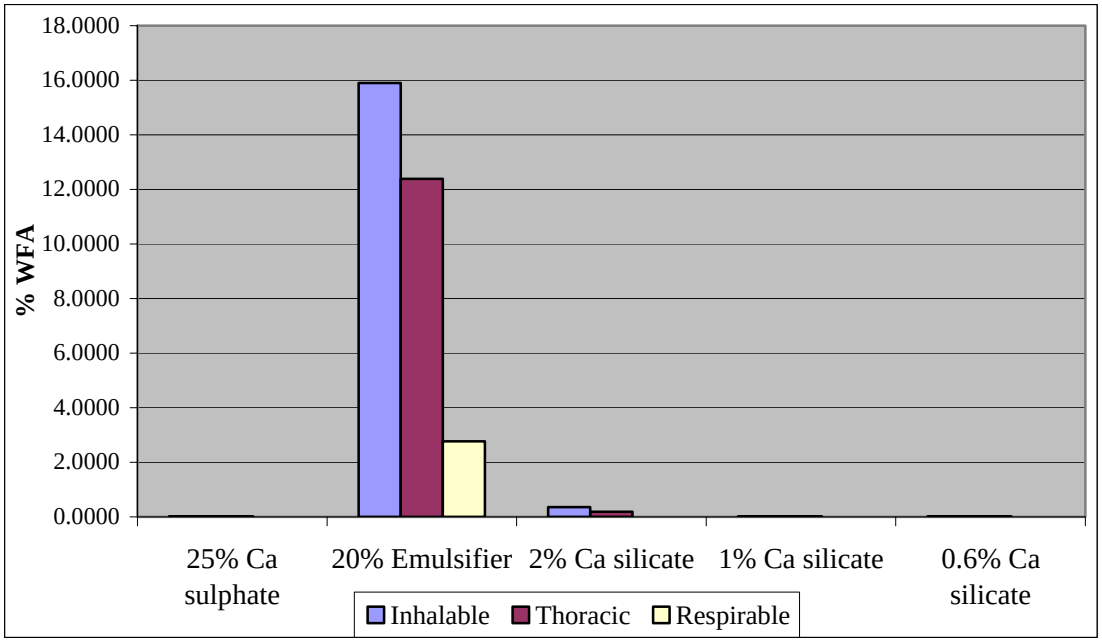


Figure 36 Percentage of wheat flour antigen from the bulk ingredients on the filters

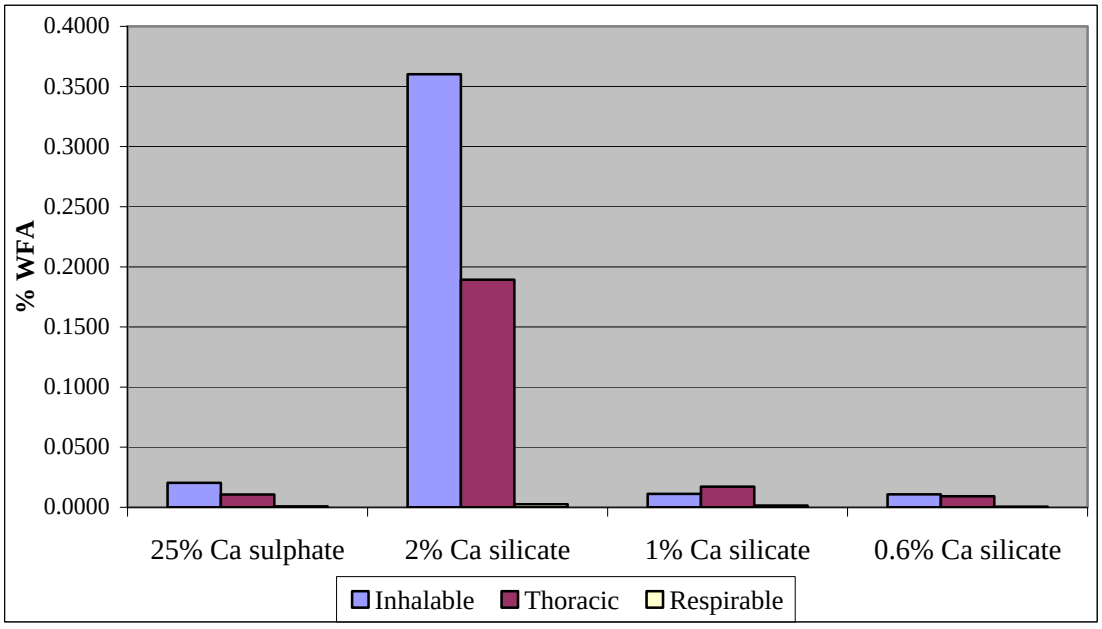


Figure 37 Percentage of wheat flour antigen from the bulk ingredients on the filters without emulsifier

Figure 35 shows that the largest deposit of wheat flour antigen on the filters and foams was gained from the mixture containing 2% calcium silicate, followed by the emulsifier and flour

mixture. The 1% calcium silicate and 0.6% calcium silicate samples had reduced amounts of wheat flour antigen on the filter, suggesting that decreasing the calcium silicate would help to decrease exposure to wheat flour antigen. The 25% calcium sulphate mixture did mobilise the wheat flour antigen, but not as effectively as the emulsifier. Figure 36 shows the percentage of wheat flour antigen that was airborne relative to its concentration in the bulk samples. The emulsifier had the most potent effect at aerosolising wheat flour antigen.

Figure 37 shows that a calcium silicate dose response was observed; the greater the amount of calcium silicate in the mixture the more wheat flour antigen became airborne. The relationship was non-linear and reduction in calcium silicate to less than 1% had little effect.

The percentage of the WFA that was mobilised due to calcium sulphate was approximately the same as the 1% calcium silicate and flour mixture. This indicated that calcium sulphate is not as efficient as calcium silicate in aerosolising WFA, but since it is used in much larger quantities, approximately the same amount of WFA would be found on the filter.

6.2.5 Revised improver mixtures

Dustiness testing

Table 18 Dustiness testing of revised improver mixtures

Code	HSL ID	Inhalable			Thoracic			Respirable			Moisture content
		Mean (mg/kg)	Std dev	COV %	Mean (mg/kg)	Std dev	COV %	Mean (mg/kg)	Std dev	COV %	Mean (%)
Red2	00846 / 09	467	11	2.4	86	3	3.5	5	0	5.4	9.36
Orange2	00847 / 09	93	5	5.5	35	2	5.1	3	0	0.5	11.5
Blue2	00848 / 09	99	2	2.4	18	1	3.9	2	0	0.5	8.88
Green2	00849 / 09	55	5	9.3	13	1	5.6	3	0	8.1	8.62
Yellow2	00850 / 09	34	1	4.2	11	0	3.7	2	0	11.1	7.90
Black2	00851 / 09	148	3	2.0	31	2	6.6	4	0	6.0	9.71
White2	00852 / 09	22	1	3.0	9	1	7.7	1	0	0.3	8.88

Immunological analysis of the dust from dustiness samples

Table 19 Content of measured substances in the revised bulk improver samples

HSL ID	Sample	Soluble protein		Soya trypsin inhibitor		Wheat flour allergen	
		µg/g	%	ng/g	%	ng/g	%
00846 / 09	Red2	101484.8	10.148	78213.4	0.007821	101557.6	0.010156
00847 / 09	Orange2	133234.8	13.323	22493.4	0.002249	28855.1	0.002886
00848 / 09	Blue2	111734.8	11.173	114138.4	0.011414	51545.1	0.005155
00849 / 09	Green2	101734.8	10.173	44350.9	0.004435	5131.9	0.000513
00850 / 09	Yellow2	119484.8	11.948	345188.4	0.034519	33517.6	0.003352
00851 / 09	Black2	103734.8	10.373	104988.4	0.010499	50820.1	0.005082
00852 / 09	White2	118734.8	11.873	1439988.4	0.143999	109282.6	0.010928

Table 20 Average content of measured substances in the dustiness filters and foams from revised improvers

Sample	Fraction	Soluble protein		Soya trypsin inhibitor		Wheat flour allergen	
		ng/g	%	ng/g	%	ng/g	%
Red2 00846/09	Inhalable	11.076	0.01091	934.276	1.19452	9.915	0.00976
	Thoracic	20.309	0.02001	136.052	0.17395	3.088	0.00304
	Respirable	1.815	0.00179	7.694	0.00984	1.144	0.00113
Orange2 00847/09	Inhalable	48.754	0.03659	50.122	0.22283	2.309	0.00800
	Thoracic	5.642	0.00423	6.744	0.02998	0.879	0.00305
	Respirable	0.009	0.00001	1.253	0.00557	0.183	0.00063
Blue2 00848/09	Inhalable	0.000	0.00000	287.333	0.25174	0.520	0.00101
	Thoracic	32.073	0.02870	36.535	0.03201	0.780	0.00151
	Respirable	0.000	0.00000	4.656	0.00408	0.129	0.00025
Green2 00849/09	Inhalable	2.960	0.00291	162.029	0.36533	0.238	0.00463
	Thoracic	60.594	0.05956	32.028	0.07221	0.000	0.00000
	Respirable	0.363	0.00036	0.980	0.00221	0.000	0.00000
Yellow2 00850/09	Inhalable	111.992	0.09373	4.240	0.00123	1.154	0.00344
	Thoracic	48.227	0.04036	0.366	0.00011	0.000	0.00000
	Respirable	2.619	0.00219	0.292	0.00008	0.092	0.00028
Black2 00851/09	Inhalable	3.084	0.00297	279.121	0.26586	1.665	0.00328
	Thoracic	0.000	0.00000	34.725	0.03307	0.000	0.00000
	Respirable	0.000	0.00000	4.066	0.00387	0.000	0.00000
White2 00852/09	Inhalable	16.485	0.01388	19.881	0.00138	0.512	0.00047
	Thoracic	59.842	0.05040	1.600	0.00011	0.000	0.00000
	Respirable	8.558	0.00721	0.391	0.00003	0.415	0.00038

Soluble protein

Figures 38 and 39 show these results for soluble protein. Please refer to Table 6 for the contents of the improver samples.

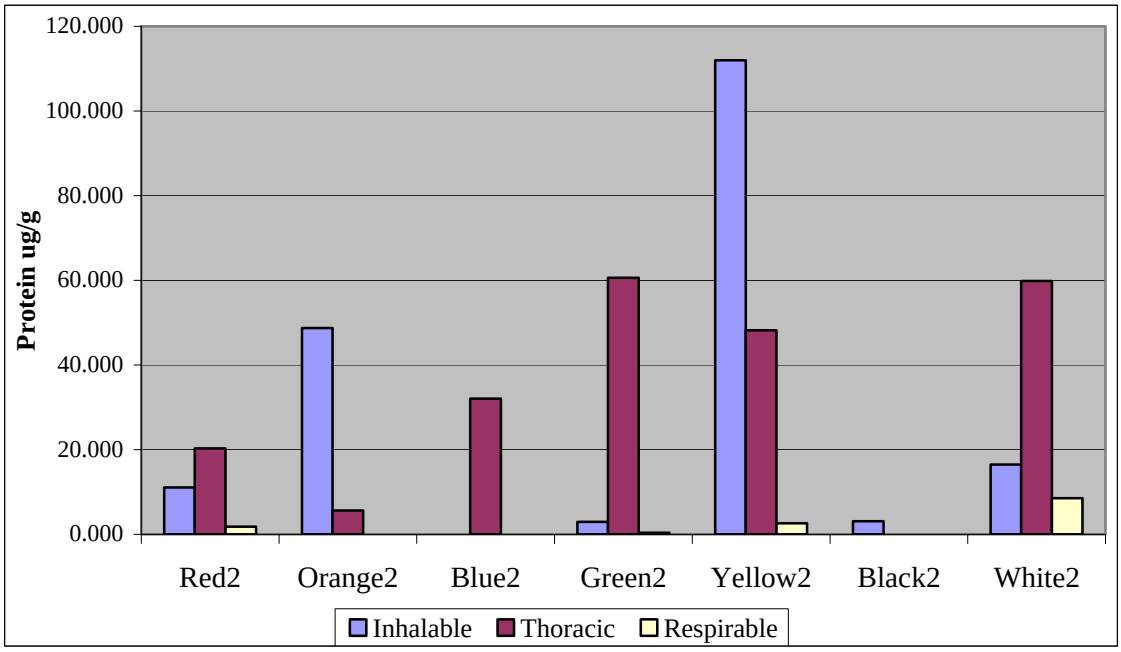


Figure 38 Amount of soluble protein on the filters per gram of bulk improver in the drum

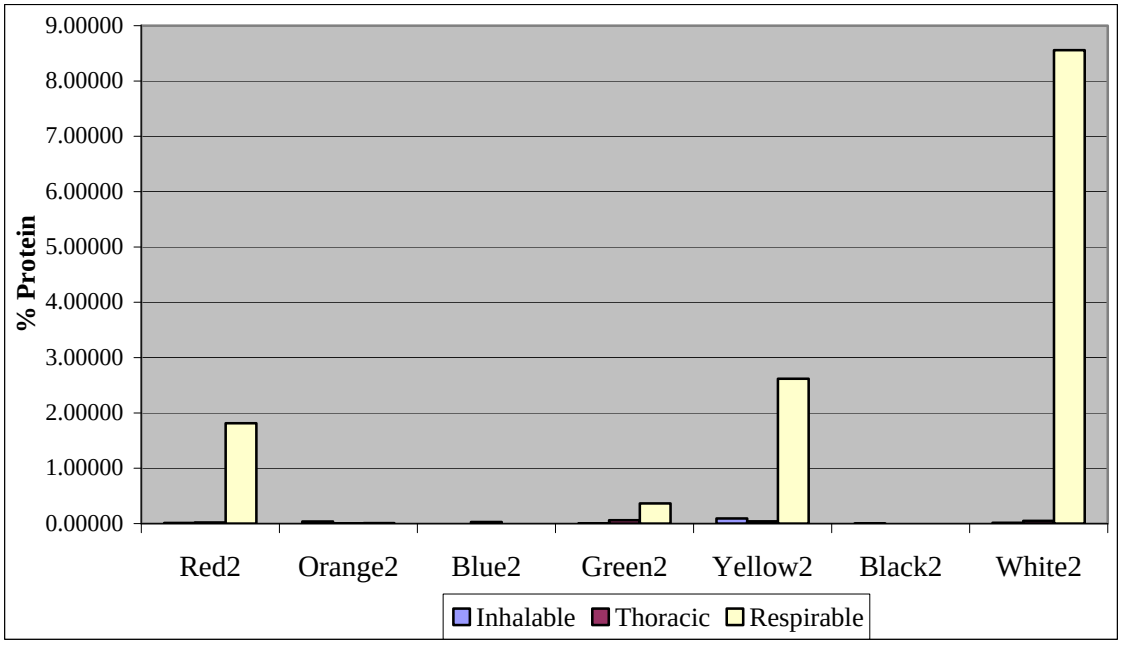


Figure 39 Percentage of protein from the bulk improvers on the filters

Key to figures 38 and 39 Sample descriptions and key ingredient changes

Sample	Description of change	Ca sulphate	Emulsifier	Oil
Red2	Typical Improver: control sample	25%	Contains 5% Ca silicate	2%
Orange2	No Emulsifier	25%	None	2%
Green2	No Ca sulphate	None	Contains 5% Ca silicate	2%
Blue2	Reduced Ca sulphate	5%	Contains 5% Ca silicate	2%
Black2	Contains emulsifier with reduced Ca silicate	25%	Contains 3% Ca silicate	2%
White2	Contains extra oil	25%	Contains 5% Ca silicate	4%
Yellow2	Contains all 3 realistic improvements	5%	Contains 3% Ca silicate	4%

Figure 38 shows the amount of soluble protein that was deposited on the dustiness filters per gram of bulk improver in the drum. The most protein was detected on the filters for the "Yellow2" sample (10 fold more protein in the inhalable fraction than the control); containing typical improver with all of the changes, i.e. decreased calcium sulphate, decreased calcium silicate within the emulsifier and increased oil. The total protein is the total amount of biological material and this could be from animal, microbial or plant material including flour, soya or rapeseed oil. The "White2" sample had a fairly high level of total protein in the thoracic fraction (3 times that of the control thoracic fraction); this sample was typical improver with additional vegetable oil; so this could have contributed to the protein concentration. The protein content of the "Green2" sample was also high on the filters (3 times that of the control thoracic fraction); this is typical improver that does not contain calcium sulphate. This is typically added at 25% of the mixture, so consequently this sample contained more wheat flour. The amount of total protein for the "Red" sample was low; this is the control sample i.e. typical improver without any changes to the formulation. When this compared to Figure 39, a larger percentage of protein was found in the respirable fraction for these samples. The "White2" sample had a high amount of protein in the respirable fraction compared to the other samples; this sample contained extra oil.

Soya trypsin inhibitor

The percentage of STI from the bulk improver on the filters was also calculated; this is shown in Figure 40 below.

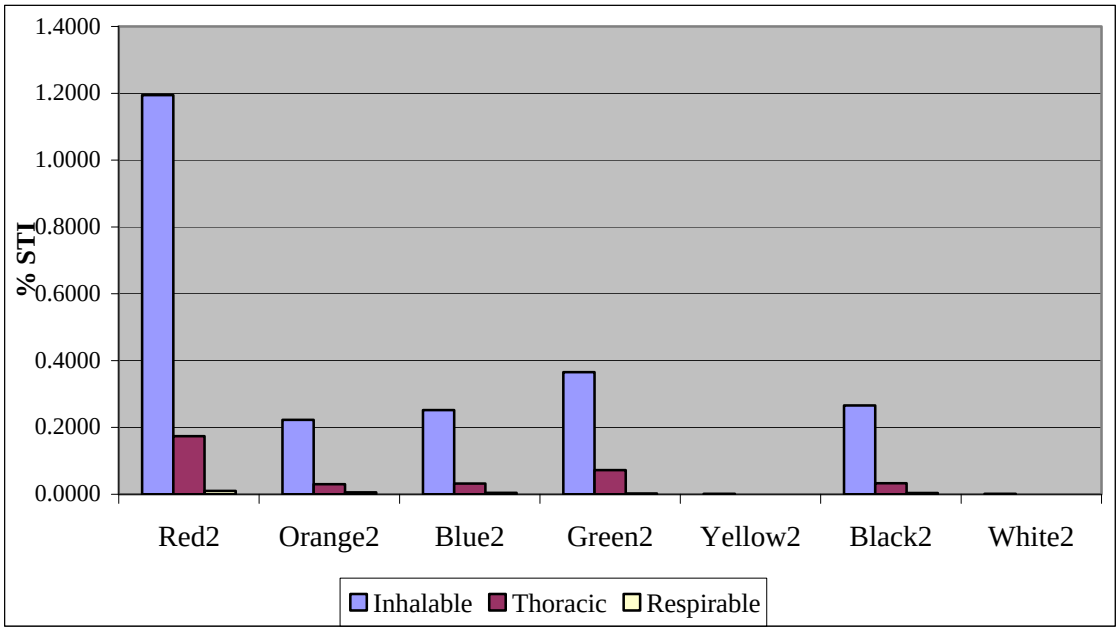


Figure 40 Percentage of soya trypsin inhibitor from the bulk improver on the filters

Key to figure 40 Sample descriptions and key ingredient changes

Sample	Description of change	Ca sulphate	Emulsifier	Oil
Red2	Typical Improver: control sample	25%	Contains 5% Ca silicate	2%
Orange2	No Emulsifier	25%	None	2%
Green2	No Ca sulphate	None	Contains 5% Ca silicate	2%
Blue2	Reduced Ca sulphate	5%	Contains 5% Ca silicate	2%
Black2	Contains emulsifier with reduced Ca silicate	25%	Contains 3% Ca silicate	2%
White2	Contains extra oil	25%	Contains 5% Ca silicate	4%
Yellow2	Contains all 3 realistic improvements	5%	Contains 3% Ca silicate	4%

Figure 40 showed a very similar pattern to Figure 2; the percentage of STI aerosolised from the bulk sample is greatly reduced by implementing any of the changes in the improver mix. The samples that had the largest decrease in their percentages of STI aerosolised were: the sample with the increased oil content ("White2": the percentage STI in the inhalable fraction was reduced by 853 fold) and the sample in which all three changes had been made ("Yellow2": the percentage of STI in the inhalable fraction was reduced by 995 fold).

Wheat flour antigen

The percentage of WFA from the bulk improver on the filters was also calculated; this is shown in Figure 41 below.

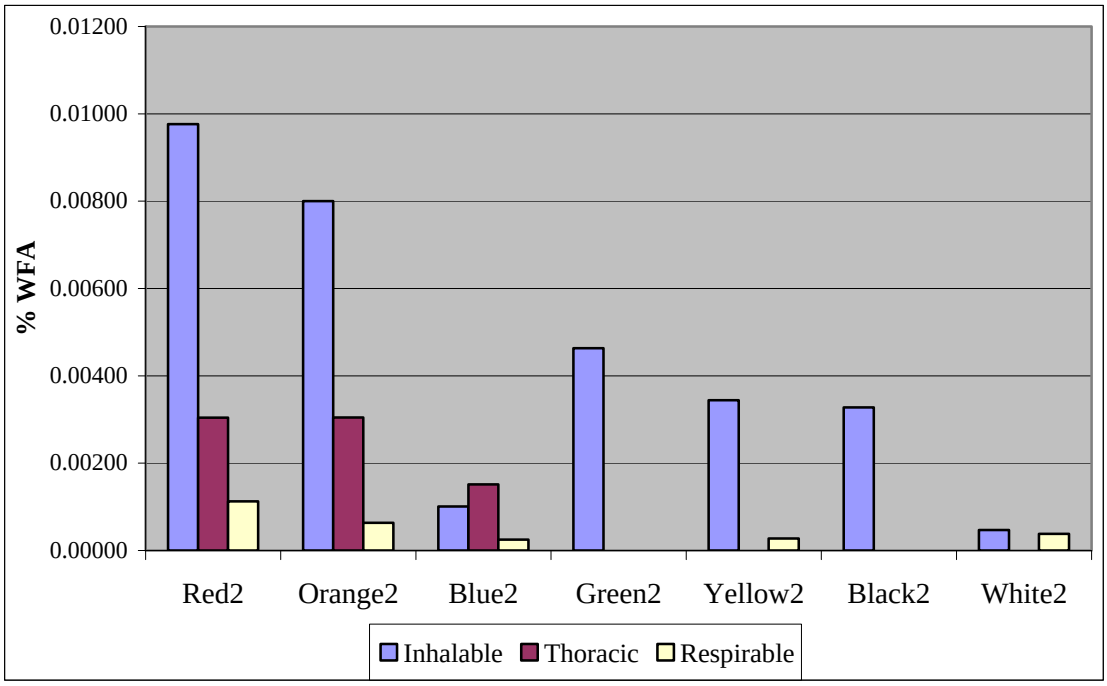


Figure 41 Percentage of wheat flour allergen from the bulk improver on the filters

Key to figure 41 Sample descriptions and key ingredient changes

Sample	Description of change	Ca sulphate	Emulsifier	Oil
Red2	Typical Improver: control sample	25%	Contains 5% Ca silicate	2%
Orange2	No Emulsifier	25%	None	2%
Green2	No Ca sulphate	None	Contains 5% Ca silicate	2%
Blue2	Reduced Ca sulphate	5%	Contains 5% Ca silicate	2%
Black2	Contains emulsifier with reduced Ca silicate	25%	Contains 3% Ca silicate	2%
White2	Contains extra oil	25%	Contains 5% Ca silicate	4%
Yellow2	Contains all 3 realistic improvements	5%	Contains 3% Ca silicate	4%

Figure 41 shows the percentages of airborne WFA relative to the amount of WFA in the bulk improver. The control sample ("Red2") had the highest percentage of aerosolised WFA. The greatest reduction in the percentage of WFA in the dust was obtained for the samples containing: reduced calcium sulphate ("Blue2": the percentage of WFA in the inhalable dust was reduced by nearly 10 fold) and increased oil ("White2": the percentage of WFA in the

inhalable dust was reduced by 20 fold). The percentage of WFA was reduced in all three health related fractions of the dust (inhalable, thoracic and respirable).

User testing

Table 21 Summary table of inhalable and respirable exposure from the user tests

HSL ID	Sample code	Microdust (Respirable) mg/m^3	Gravimetric concentration mg/m^3	
			Respirable (PGP10)	Inhalable (CIS)
00846 / 09	Red2	2.85	0.06	60.36
00852 / 09	White2	0.42	0.06	9.12
00848 / 09	Blue2	2.15	0.07	30.67
00851 / 09	Black2	2.28	0.09	55.87

Immunological analysis of the user testing filters

Table 22 Concentration of WFA, STI and protein in the user test filters

Sample	Fraction	Soluble protein ng/m^3	Soya trypsin inhibitor ng/m^3	Wheat flour allergen ng/m^3
Red2 00846/09	Inhalable (CIS)	1641.19	115568.70	3842.79
	Respirable (PGP10)	ND	15.53	ND
Blue2 00848/09	Inhalable (CIS)	862.78	116940.09	2971.43
	Respirable (PGP10)	ND	44.75	ND
Black2 00851/09	Inhalable (CIS)	1399.03	146946.44	4939.68
	Respirable (PGP10)	2.97	121.45	BLD
White2 00852/09	Inhalable (CIS)	253.64	25117.87	867.30
	Respirable (PGP10)	ND	13.06	ND

Soluble protein

Figure 42 shows these results for soluble protein. Please refer to Table 6 for the contents of the improver samples.

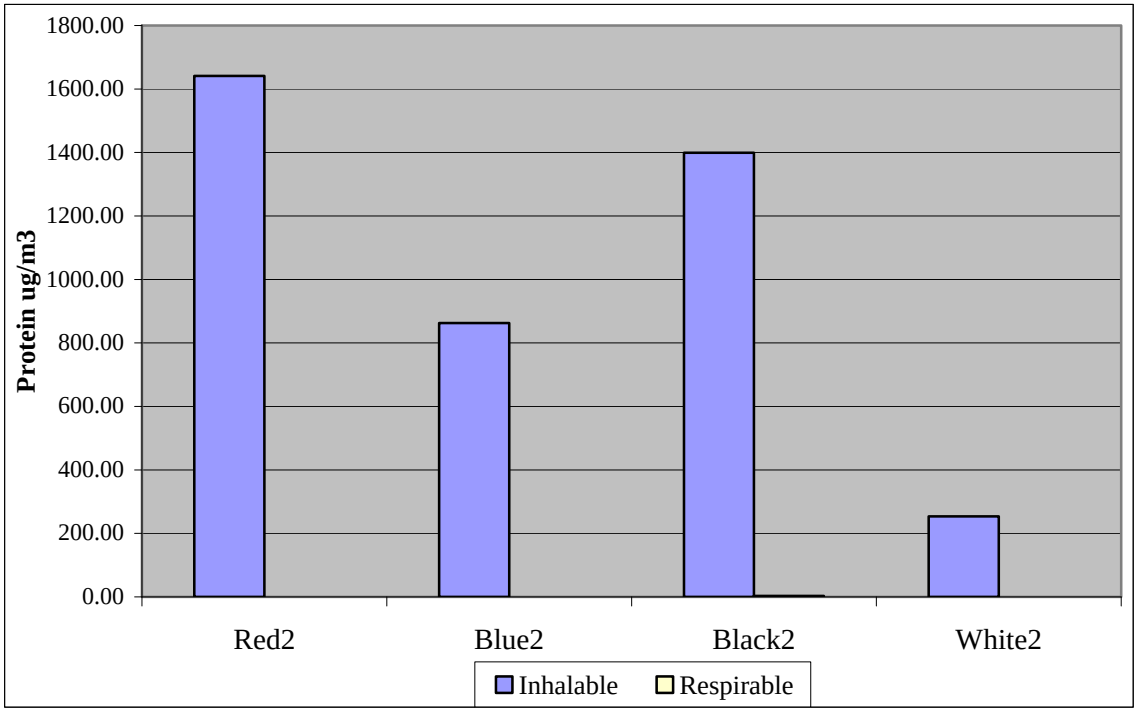


Figure 42 Concentration of soluble protein on the filters (μg/m3)

Key to figure 42 Sample descriptions and key ingredient changes

Sample	Description of change	Ca sulphate	Emulsifier	Oil
Red2	Typical Improver: control sample	25%	Contains 5% Ca silicate	2%
Blue2	Reduced Ca sulphate	5%	Contains 5% Ca silicate	2%
Black2	Contains emulsifier with reduced Ca silicate	25%	Contains 3% Ca silicate	2%
White2	Contains extra oil	25%	Contains 5% Ca silicate	4%

The highest concentration of soluble protein on the user test filters was for the "Red2" sample, this was the typical improver control. The largest reduction in exposure to soluble protein was for the sample containing extra oil ("White2"; over 6 fold reduction in soluble protein in the inhalable fraction). The second biggest reduction in protein on the filter was produced when the amount of calcium sulphate was reduced in the improver ("Blue2"; the inhalable soluble protein was reduced to approximately half that of the control). Reducing the calcium silicate in the emulsifier ("Black2") gave only a small reduction (to 85% of the soluble protein in the control inhalable sample). These results corresponded with the gravimetric and microdust results for the user testing.

6.3 DISCUSSION APPENDICES

6.3.1 Original improver mixtures

The initial results for dustiness testing on the first set of improvers sent to HSL in 2008 were extremely surprising. It had previously been thought within the baking industry that all the ingredients added to the flour would help to reduce dust and thus exposure to allergens. The initial dustiness tests showed that the addition of oil (2%) and soya flour would help to reduce the dustiness of the improver, however neither of these were sufficient enough to counteract the increased dustiness that was gained from calcium sulphate and emulsifier. The changes in the levels of dustiness were marked and the results that followed were consistent with these findings.

The samples reflecting airborne contaminants from dustiness testing of these “original improvers” were tested for the presence of soya trypsin inhibitor, wheat flour allergen, soluble protein and calcium. These results gave a measure of the amount of allergens that are made airborne for each of the improvers and in effect the amount of each substance the operator would be exposed to. In addition, the amount of airborne allergen was related back to the amount of allergen in each bulk improver. This gave a measure of the propensity of that specific allergen to become airborne.

The amount of airborne STI followed the same trend as the general dustiness, i.e. the dustier the material; the more STI became airborne. With the exception of one potentially contaminated sample, the percentages of airborne STI from the bulk material followed a very similar pattern. However the sample containing oil demonstrated that this single ingredient has a dampening effect and reduced the aerosolisation of STI.

The highest amount of airborne wheat flour antigen (WFA) on the filters and foams occurred with the sample that contained all the ingredients with the exception of oil. The percentage of WFA that became aerosolised follows a similar trend as the dustiness results. The exception to this was the disproportionally lower percentage of airborne WFA for the sample containing oil. This indicates that 2% oil lowered the proportion of WFA that was aerosolised, but did not lower the dustiness as much as was initially expected. The sample containing emulsifier and calcium sulphate (and therefore less wheat flour) had the highest propensity for releasing WFA into the air, showing that these agents have the potential to mobilise allergens. The sample containing emulsifier but not calcium sulphate gave the second highest level of airborne WFA.

6.3.2 Individual ingredients

The properties of the individual ingredients were examined, based on the data obtained for the original improvers. In the dustiness tests, the dustiest ingredient was the emulsifier, followed by calcium sulphate. Historically flour has been thought to be a dusty material, however in

comparison with emulsifier and calcium sulphate, three flour samples were found to be much less dusty than these other ingredients. The least dusty was soya flour; this could be expected since it is a fatty material. The addition of soya flour was found to reduce dustiness, however it should be recognised that soya flour contains allergens and by adding this ingredient the allergenic potential of the mixture is increased.

Since emulsifier was the dustiest of these ingredients, the separate constituents of it were examined (data ester and calcium silicate). Neither of these components were as dusty as the complete emulsifier mixture, demonstrating that the ingredients were interacting. Calcium silicate is added to emulsifier to improve mixing and enable emulsifier to flow freely through machinery, so it was hypothesized that calcium silicate was acting to break up the aggregated data ester into smaller particles, creating a dustier material overall. Particle size distribution demonstrated that calcium silicate had the smallest average particle size. Calcium sulphate is used as a bulking agent and calcium supplement and this had the next smallest average particle size. This ingredient also promoted dustiness so it was also thought that calcium sulphate could also be disrupting aggregates of other ingredients.

6.3.3 Simple combinations of ingredients

To examine how the interactions between the ingredients affected the dustiness and exposure to bakery improvers, additional tests were undertaken. Firstly the dustiest ingredient, emulsifier, was mixed with flour only. The concentration of emulsifier used was the same as that found in typical improvers (20%). The dustiness of this mixture was between the level of emulsifier alone and flour alone, so it appeared that the emulsifier was mobilising a flour dust. The particle size distribution for this sample was lower (but not significantly) than that for flour alone. The airborne samples from the dustiness testing were analysed for WFA and it was found that WFA was present on the filters, indicating that the emulsifier also mobilised a flour allergen.

Different concentrations of calcium silicate was mixed with flour to investigate its effect on dustiness and a dose response was found. The more calcium silicate that was present in the flour, the dustier the combination became. The mean particle size was also reduced with increasing calcium silicate in the flour. This suggests that the calcium silicate was breaking up aggregates of flour. The levels of airborne WFA also increased with increasing calcium silicate dose, however this relationship did not appear to be linear and reduction of the calcium silicate to less than 1% appeared to have little effect. When this lower level of calcium silicate was mixed with data ester and flour the average particle size of the mixture increased, compared to standard emulsifier in flour. This indicated that lowering the content of calcium silicate within emulsifier could be a potential measure to reduce the propensity of improvers to form dusts.

The dustiness tests showed that when mixed with flour, emulsifier created a dustier mixture than the equivalent amount of calcium silicate (1%). The content of airborne WFA was also higher for emulsifier (data ester and calcium silicate) mixed with flour than calcium silicate with flour. This indicates that the presence of data ester also increased the dustiness and allergen exposure and that there are interactions between the different components, which influence the dustiness of the mixture and the propensity for allergens to become airborne. The mean particle size of either the data ester and flour mix or emulsifier and flour mix is smaller than the data ester or flour alone. This would indicate that combining data ester and flour decreases the particle size of both components and that both calcium silicate and data ester act to break up the flour particles. However it should be noted that the particle sizes should be viewed as a general trend

and not exact numbers as these samples were polydispersed powders and as such had a wide range of particle sizes within them.

The second dustiest of the single ingredients was calcium sulphate and when added to the initial improver mixtures, increased the overall dustiness and the airborne WFA and STI. Therefore the same experiment as above was performed with this ingredient to further identify its effect on flour. A mixture of calcium sulphate and flour at the same concentrations as used in typical improver (25%) was compared to the dustiness of flour or calcium sulphate alone. The mixture was 6 fold dustier than flour or 4 fold dustier than calcium sulphate on its own. The mean particle size of the mixture was found to be in between the particle sizes of calcium sulphate and flour. Since calcium sulphate was 25% of the mixture, this would indicate that it might break up the flour aggregates into smaller particles. The amount of airborne WFA from this mixture was approximately the same as that for 1% calcium silicate in flour. Since the calcium sulphate is added at 25% and calcium silicate is 1% overall this indicates that calcium sulphate is not as efficient as calcium silicate at aerosolising the WFA.

6.3.4 Revised improver samples

For the discussion about the revised improver samples, including dustiness testing, user testing and related immunological analysis, please see the Discussion, Section 4.

7 REFERENCES

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HSE

Health and Safety Executive

A study to investigate ways to reduce the dustiness of bakery ingredients and exposure to allergens

This study investigated whether changing the ingredients of bakery improvers would decrease their dustiness and, consequently, help to reduce the exposure of bakers to allergens in the bakery dust. The study was carried out in partnership with the Association of Bakery Ingredient Manufacturers (ABIM).

Typical ingredients in bakery improvers are wheat flour, fungal alpha amylase, soya flour, calcium sulphate, vegetable oil and emulsifier. Emulsifier is made from data ester (E472e) with a 'free flow agent', usually calcium silicate in the UK, to prevent sticking in bakery equipment. The combinations of ingredients that provided the biggest decrease in dustiness and exposure were identified using several tests including dustiness testing, particle sizing, user testing with simulated bakery tasks, and analysis for protein, allergens and calcium.

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