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Foreign Body Contamination of Food – Scanning Electron Microscopy and Energy Dispersive Spectroscopy as Tools for Identification

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Abstract

Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) are powerful tools for the characterisation of foreign body (FB) or particulate contamination of food products. Whether the result of processing, packaging or interaction with consumers, once identified foreign body contamination needs to be characterised as rapidly and efficiently as possible in order to trace its source. An outline of the operation and capabilities of SEM/EDS is presented in order to highlight its application to FB identification. Three case studies are presented, one of FB contamination that was caused by agglomerated flour particles, one of a glass fragment reported by a consumer in a jar of baby food and one of a suspected dental implant in a food product.

KEYWORDS: Foreign body contamination, particulate contamination, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), light microscopy (LM), glass identification

1. INTRODUCTION

Detection and identification of foreign bodies (FB) in food compounds are essential activities contributing to food safety and quality assurance. The presence of FB's in food products can have a number of serious ramifications ranging from process down-time, to customer complaints detrimental to a company's reputation, to expensive product recalls and, ultimately, litigation.

In the USA the FDA monitors product recalls and classifies the severity of the risk. Foreign body contamination such as glass fragments or metal particles would warrant a Class II product recall. This is classified as: "A situation in which the use of, or exposure to, a violative product may cause temporary or medically reversible adverse health consequences or where the probability of serious adverse health consequences is remote." (CFR, 2002). Such recalls are not infrequent occurrences, one example being a recall in May 2001 when 5400 32oz jars of crispy sauerkraut were recalled due to the suspected presence of glass fragments (FDA Enforcement Report, 2001). Contamination with foreign bodies that are also hazardous allergens, such as peanuts, would require the higher level Class I recall, where there is a potential for serious threat to health, possibly causing death. Such allergen related recalls represented 36% of all recalled food products in 1999 in the US (Vierk, Falci, Wolyniak & Klontz, 2002).

In the UK in recent years up to half the prosecutions related to food defects have been connected to contamination with foreign matter (Lewis, 1993) and between the years 1988 and 1994 FB's represented the single largest cause of food defect prosecutions (Graves, Smith & Batchelor, 1998).

Particulate contamination of food can arise from numerous sources, prior to purchase these might include process problems such as wear particles from conveyors, or breakages within the processing plant. Packaging materials and interactions during storage are also significant vectors for contamination and contamination can occur with FB's that are part of the food product, such as bone chips in meat products. In recent years HACCP proposals have sought to identify vectors such as these and guidelines are produced by a number of regulatory authorities (FDA, 2001). Irrespective of QA measures within the manufacturing and retail environments contamination may occur subsequent to purchase in the consumer's home (Lewis, 1993). There is also the possibility of deliberate contamination for reasons of nuisance or sabotage (Platek, Ranieri & Wolnik, 1997 and Heitkemper, Platek & Wolnik, 1995).

Detection of FB's during production relies on a number of established techniques including metal detection and X-rays (Graves *et al*, 1998). Ultrasound is a technique finding increasing application in this field and great potential in the detection of organic contaminants such as teeth and bone fragments and polymeric materials (Hæggström & Luukkala, 2001 and Zhao, Basir & Mittal, 2003). Foreign bodies may also be detected and reported by consumers subsequent to purchase. However or whenever foreign matter is discovered in food, two fundamental questions must be answered; "what is it?" and "where did it come from?". The answers to these questions need to be found as rapidly as possible.

Identifying particulate contamination might involve a number of techniques ranging from light microscopy through a variety of more specialised techniques. Common particulate contaminants include metal, glass and insects, hence the correct

identification of such contaminants can call upon the expertise of materials scientists, food technologists, biologists and entomologists, amongst others, and can be a complex process. Once the material is definitely identified the source of the contamination may be deduced by the QA manager or operators familiar with the process. The list of possible sources of contamination can be extensive but it is frequently possible to speculate on the source of the contamination prior to analysis, thus narrowing the analysis options and speeding the results.

2. SOURCES OF CONTAMINATION

The sources of particulate contamination of food are extremely varied and are often process specific, glass fragments are more likely to be found in products packaged in glass containers, for example. When looking at the FB's that have resulted in prosecutions in recent years in the UK it is seen that nearly half of all FB's were either metal or insects (24% and 25% respectively), the next largest contribution coming from glass fragments (10%) (Graves *et al,* 1998).

Any list of possible sources of FB's can in no way be comprehensive. The value of such lists, however, is that they focus attention on various aspects of production processes and areas of food preparation that are prone to such contamination. Metal can enter foods from a large number of sources. Lead shot and fishhooks are readily identifiable objects, as are screws from spectacles. In the process plant metal filings, swarf, and grinder residue might conceivably be a result of maintenance activity and wear particles might be indicative of aging machinery. Glass particles can come from packaging processes or from more disparate sources such as light fittings, watch glasses or gauge dial glasses, amongst others. In all the above cases, procedures are almost always in place to prevent such accidental contamination during processing of food products, though occurrences are still possible.

Glass-like particles can often be polymeric in nature, possibly arising from packaging materials. Alternatively they can be naturally occurring crystalline substances that are not, in fact, foreign matter, but associated with the food stuff, such as struvite crystals that form from natural constituents of fish or shellfish, or calcium tartrate that can form naturally in fruit juices (Charbonneau, 1998). Glass fibres can appear due to contamination from insulation or composite materials such as glass-reinforced polymer (GRP or fibreglass).

Other fibres found in foodstuffs can be classified as natural or man-made. These might be traced to human hair or follicles of rodent fur (frequently found associated with rodent excreta (Stasny, Albright & Graham, 1981)). They might be synthetic or natural fibres associated with textiles, such as nylon, cotton or wood fibres (from paper based packaging).

Insects, parts of insects, and rodents can occur in a number foods resulting from improper storage prior or subsequent to purchase. Correctly identifying insects by species can lead to conclusions about when and where the contamination occurred. For example contamination with a species of beetle known only to survive in centrally heated buildings in the UK would obviously reduce the number of possible locations where contamination could have occurred (Lewis, 1993). With careful analysis it is often possible to determine whether the insect or rodent has

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been cooked and as such whether it passed through the complete food processing procedure.

3. IDENTIFYING THE CONTAMINANT

Each food manufacturer will have QA procedures that will be followed once foreign matter has been detected or reported in a food product. At the outset these procedures will involve optical examination. Documentation and, possibly, macro-photography should record the original form of the specimen, any adhering matter, obvious signs of wear or fracture and any sign of melting. This simple morphological analysis will provide valuable information that will dictate subsequent treatment, for example, whether the particulate appears metallic, glassy or fibrous. Following the initial examination it might be necessary to perform further analysis, if the nature and origin of the contaminant is still unclear. A test of physical properties, such as hardness or density might shed further light on the particle.

Following these basic tests if the contamination is still not conclusively identified further morphological and chemical analyses might be necessary. There are a variety of techniques available to the analyst, some more specialised than others, and the level of certainty necessary in categorising the particle dictates the level to which any analysis need be taken.

Light microscopy

Light microscopy (LM) is a technique available in many quality control labs and it is often the only technique that is necessary, after basic observation, to identify particles. Micrographs can provide instant identification of distinctive particulates; for example, pollen and insect parts, both of which can form a portion of windbourne dust and as such can become contaminant material in foodstuffs, more frequently post-purchase.

Further to simple morphology an experienced light microscopist can gather a great deal of additional information regarding particulate matter, sometimes far more information than might be gathered using more expensive and elaborate techniques. Polarised light microscopy is a powerful addition to regular light microscopy due to the birefringent properties of many particles. Many food constituents, such as starch granules, are bi-refringent and as such polarised light may be used to eliminate such particles from any given sample. Many minerals, too, are birefringent; coupling this knowledge with a good grounding in morphological identification can provide extremely accurate classifications of particles. An example would be particles of silica, SiO_2; chemical analysis would give the basic constituents but an examination using light microscopy would be able to provide information regarding the precise nature of the material, eg. quartz, opal, diatom etc. Even greater accuracy can be achieved by an experienced microscopist, drawing distinctions, for example, between the quartz particles typical in any sample of settled dust and quartz particles that are specifically sand (McCrone Draftz & Delly, 1967). Identification to this level of accuracy requires access to specialised references, and much experience on the part of the microscopist.

Light microscopy is particularly useful for identification of fibres and glass particles. Fibres such as asbestos are frequently most easily identified using their aspect ratio and birefringence (McCrone *et al*, 1967). Mammalian fibres, such as rodent fur, may be identified with some certainty as to species by examination of the cortex, cortical pigment, medullary cell shape and pigment distribution within medullary cells (Vazquez, 1961). Identifying fibres to this level of certainty, though, is a specialised and time-consuming technique.

Glass fragments are usually transparent, sometimes coloured, often curved and their fracture surfaces are frequently conchoidal, all of these properties can be characterised with the assistance of the light microscope. Measurement can be made of refractive index and this can be used to further identify the glass and its possible source, for example bottle and plate glass usually display a refractive index between 1.52 and 1.57, borosilicate glass (Pyrex^{TM}) has a lower index, at about 1.47 and lead glasses may be above 1.80 (McCrone *et al*, 1967). Identifying a glass by type and possible source requires additional expertise and information (such as density measurement) and is often best performed by specialist glass identification laboratories. These labs will often send the fragment to the nearest Scanning Electron Microscope lab for additional characterisation.

Scanning Electron Microscopy

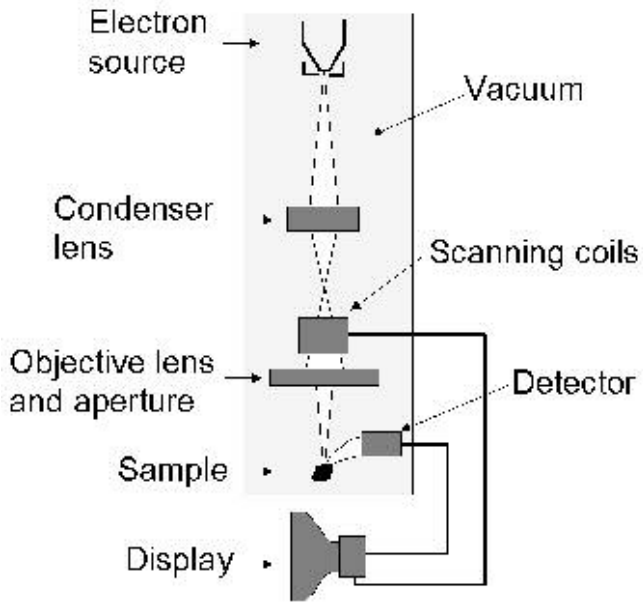

Figure 1: Basic components of a Scanning Electron Microscope. Note entire system is under vacuum conditions.

The scanning electron microscope (SEM) is often the next logical step after light microscopy. In fact, given the simplicity of sample preparation, the ease of

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interpretation of SEM images and the increasing accessibility to such instruments (through University labs offering commercial services), the SEM might even be considered the first step in some instances. It is essential to understand the operation of an SEM in order to prepare samples correctly and interpret results to the fullest possible extent.

In a scanning electron microscope a beam of electrons interacts with the sample in a number of ways. The generation of secondary electrons is highly dependent on topography and as such these can be used to image the morphology of the sample. Backscattered electrons can give additional information regarding atomic mass variations of the sample and the energy of the X-rays that are generated when an electron beam hits a solid specimen can be used to determine what elements are present (this is known as Energy Dispersive Spectroscopy and is discussed in the next section).

Due to the nature of the electron beam the depth of field of an SEM is far greater than that of a light microscope (LM), as such the whole of a particle can often be in focus at the same time. In addition the resolving power of an SEM is greater than that of an LM, allowing far greater magnification. Whereas a light microscope can resolve objects of approximately 0.0002mm a state-of-the-art SEM can resolve objects of approximately 1.5nm. Figure 1 shows the general configuration of a scanning electron microscope. When considering microscopy it is more accurate to discuss resolution rather than magnification. The human eye can resolve two points 0.2mm apart. If using LM with a maximum magnification of 1000x then two objects 0.0002mm apart can be resolved. If the resulting image is enlarged further than 1000x, eg. as a slide projection no additional information is provided even though the magnification has increased.

Sample size for the SEM can range from tens of millimetres downwards and the technique is well suited for analysing extremely small samples in a non-destructive manner. However, two distinguishing features of the technique have a significant impact on sample preparation. Firstly the sample chamber for all but the very latest “environmental” scanning electron microscopes (ESEM) is under vacuum. This means that only samples with very low vapour pressure can be analysed. Greases, liquids and oils cannot be imaged directly and can only be analysed if some additional sample preparation is performed (eg. drying or freezing). In addition to this, as the mechanism of sample imaging in an SEM involves striking the surface of the sample with an electron beam, the sample must be conducting. A standard technique for ensuring this is to coat the sample prior to analysis with a conductive material such as carbon, platinum or gold. The coating layer is only a few nanometres thick so does not introduce topographical artefacts.

Example SEM micrographs are provided in figures 2-4 showing particulate matter that can be distinguished by morphology with relative ease. Again experience has a role to play in the correct identification of particulate contamination. Most SEM operators will have sufficient experience to correctly identify many food contaminant particles; when there is doubt, or when very precise identifications are necessary, then further expertise, in the person of a materials scientist, entomologist or palynologist can be used.

The morphology of a sample can also reveal its processing route. A case reported in the literature relates to the presence of a mouse in a food product, the complainant suggesting that the mouse had been cooked with the product. An SEM

investigation of the morphology of cooked mouse meat showed clear breakdown of collagen. On investigation of the contaminant mouse the collagen was intact and the mouse was raw, therefore it had not been processed with the product (Charbonneau, 2001).

One of the biggest advantages of SEM, over conventional LM, is that the particle can be analysed for elemental composition simultaneously with being imaged, using energy dispersive spectroscopy (EDS).

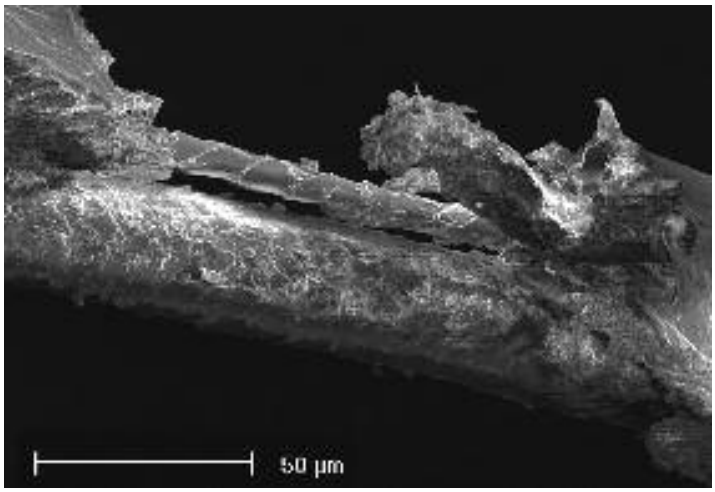

Figure 2: Mouse hair with adherent faecal matter. Fibres found in food can be from human or animal sources or synthetic in nature. Rodent hairs are often associated with rodent faeces due to the animals' method of grooming (Vazquez, 1961) .

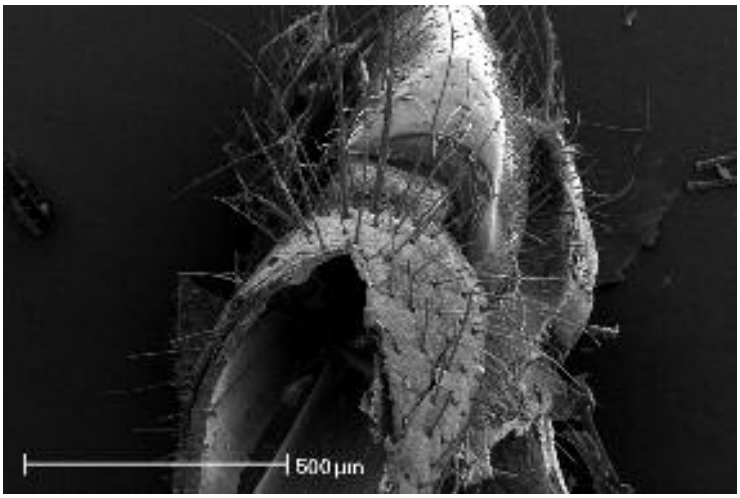

Figure 3: Portion of a fly. Insect parts can contaminate food after processing, during storage or use. As insects can be regional the presence of particular insects or insect parts can be indicative of where and when contamination occurs. This level of identification would require the expertise of an entomologist.

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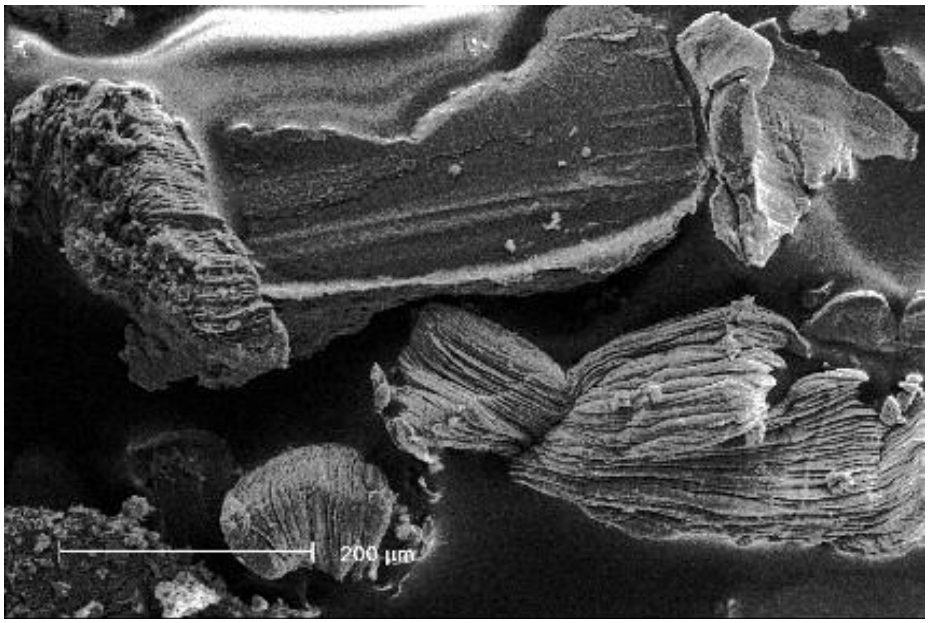

Figure 4: Aluminium "sawdust". The shape of sawn particles would indicate the cause of contamination in this case. Metal turnings, wear particles and grinding particles all have distinctive morphologies.

Energy Dispersive Spectroscopy

When an electron beam strikes a solid, as it does in an SEM, X-rays are generated. The energies of these X-rays are distinctive of the elements present in the solid. Measuring these energies using energy dispersive spectroscopy (EDS) enables the determination of the elemental composition of the specimen. The results are presented in the form of a spectrum, as shown in figures 5 and 6. As EDS is a frequent attachment to SEM's it is often possible to attain simultaneous images and spectra.

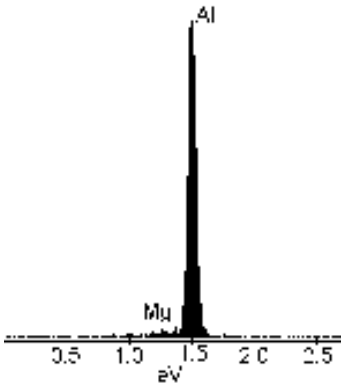

Figure 5: EDS spectrum obtained from the aluminium "sawdust" in figure 4. In this

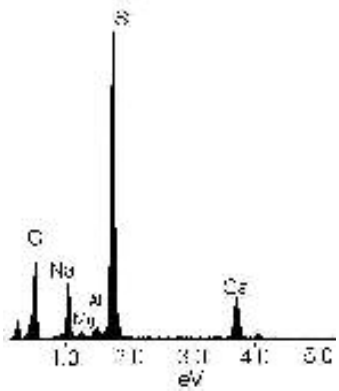

Figure 6: EDS spectrum from a glass particle. The elements present allow identification of

When identifying particulate contaminants of food EDS is a very powerful tool that has been employed with increasing frequency since the 1970's (Philips & Bertraud, 1976). If simple morphology is insufficient to identify a contaminant then it is desirable to identify its composition. However, by the very nature of particulate contamination the sample is likely to be so small that wet chemistry tests to determine composition would be impossible. Energy dispersive spectroscopy is a non-destructive technique that needs only a very small amount of sample.

When determining the source of particulate contamination it is often the trace elements that are important. The presence of iron, for example, might be important in terms of identifying a material as a steel, but the presence of low levels of chromium and nickel will then indicate a stainless steel. If molybdenum is present then the material is likely to be 316 stainless steel. Couple this information with knowledge about the materials making up the process-line equipment and the particle might be traced to a particular piece of machinery.

Similarly with glass particles, analysis of trace elements can narrow the identification of the source of the contaminant. As quality control in the glass manufacturing industry has improved over the last 4 decades variations between glass fragments of the same type (that is intended for the same purpose) have reduced. This means traditional measurements such as refractive index and density measurements may not be sufficient to discriminate between glasses in some cases (Curran, Hicks & Buckleton, 2000). As such elemental analysis using EDS may be employed. Table 1 shows some of the basic glass types and their nominal compositions. Only the latest EDS systems are capable of detecting boron, most older systems can detect elements down to sodium in atomic weight but not lower. As such the absence of calcium, rather than the presence of boron, is a prime indicator that a glass particle has come from a borosilicate, such as Pyrex™, this finding may be correlated by density measurements as borosilicates are significantly less dense than standard soda-lime glass. In addition light microscopy can be used to identify refractive indices and radius of curvature of glass fragments, along with surface markings that may be diagnostic. Light microscopy and EDS are highly complimentary techniques for the identification of glass fragments reported as food contaminants.

EDS is a technique that may be described as semi-quantitative. The intensity of each peak in the spectrum is related to the quantity of material present. When compared to a standard sample then the technique is fully quantitative. For accurate quantification samples should be polished flat. Rarely, with a food contaminant, is this possible due to the nature of many of the samples. However, this level of accuracy is not often necessary for identification of material type and source. If two samples are supplied and are suspected of being from the same source then this level of accuracy is necessary for a positive correlation. A negative correlation does not, necessarily, require quantification.

Sample preparation for EDS is the same as for SEM, adherent matter, such as retained foodstuff, is usually removed from the particle prior to analysis. The power of these two techniques, SEM and EDS, lies in their complimentary nature as will be exemplified in the case studies presented here.

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Table 1: Common glasses and their constituent elements (Smith, 1993)

Glass	Uses/Comments	SiO_2	Na_2O	K_2O	CaO	B_2O_3	Al_2O_3	Other
		Approximate amount						
Fused silica	Difficult to melt and fabricate but can be used up to 1000°C. Very low expansion and good thermal shock resistance.	99.5						
Soda-lime (plate glass)	Easily fabricated, used for windows, bottles, containers, light bulbs.	72	13		11		1	MgO 1-4
Lead silicate	Electrical applications, radiation windows, fluorescent lamp envelopes, TV bulbs	63	7.6	6	0.3	0.2	0.6	PbO 21 MgO 0.2
High lead	Low melting point, easily formed. Absorbs X-rays. Decorative uses ("cut glass")	35		7.2				PbO 58
Borosilicate (Pyrex™)	Low expansion, good thermal shock resistance and chemical stability. Widely used in chemical industry	80.5	3.8	0.4		12.9	2.2	

3. CASE STUDIES

Given here are examples of the way in which the techniques outlined above can be used in a complimentary fashion to investigate the nature of foreign bodies found in food. The case studies are typical of incidents investigated at the Research Centre for Surface and Materials Science, University of Auckland, though some details have been changed or omitted to protect client confidentiality.

Methods and materials

In all cases the foreign bodies were prepared for SEM analysis by mounting onto standard 10mm aluminium pin stubs using double-sided carbon adhesives tabs. Samples were coated with platinum using a Polaron SC7640 Sputter Coater. Analysis was conducted using a Philips XL30 S-FEG field emission scanning electron microscope and an EDAX Phoenix Energy Dispersive Spectroscope. Images were acquired using an accelerating voltage of 5keV and a spot size of 4. EDS analysis was performed using an accelerating voltage of 20keV and a spot size of 5.

Case study 1 – black particles in flour

A food product manufacturer discovered black particles in their flour during a visual QA inspection. A sample of flour containing obvious black flecks was submitted for analysis. The black particles were in the order of 1mm and were simple to extract from the flour using tweezers, during this process it was discovered that there were also white particles of equivalent size that had been overlooked in the original visual inspection. The particles were relatively hard; they could be handled with tweezers without disintegrating, and under the light microscope appeared mineral in nature, figure 7. The particles were soluble in water.

Figure 7: Macro-photograph of particles found in flour, stub is 10mm diameter.

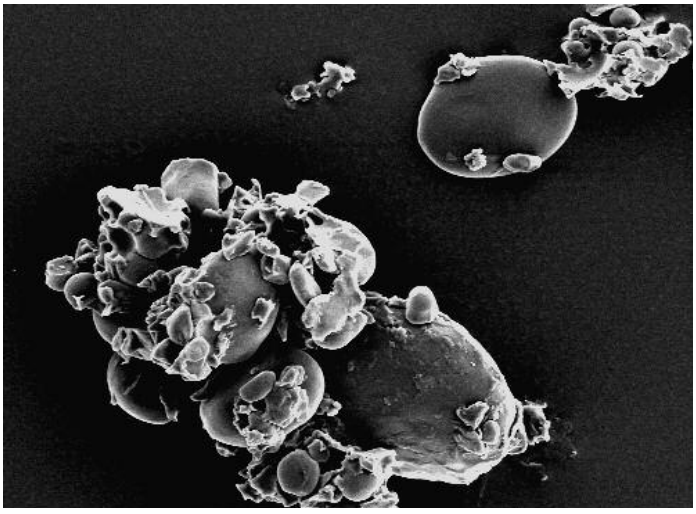

Figure 8: SEM image of flour particles showing distinctive morphology of starch granules.

Examples of two black and two white particles and a number of grains of flour were prepared for SEM/EDS analysis as outlined above. The morphology of flour particles is distinctive as shown in figure 8, comprising a high proportion of

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starch particles. The morphology of the contaminant particles was the same whether black or white, but did not correspond to their macroscopic mineral appearance. Rather the contaminant particles were clearly comprised of strongly agglomerated flour particles. This was most clearly shown in regions where obvious flour particles were also present, a transition to the more agglomerated morphology being apparent, as shown in figure 9. The solubility of the particles reinforced this conclusion, as did EDS analysis, finding the particles were predominantly carbon and oxygen. The food product manufacturer destroyed all the product possibly affected by the problem to avoid consumer complaints. The flour supplier cleaned the storage silos and installed a finer screen. Due to the SEM/EDS findings it was possible to locate the vector of the contamination and as such attribute liability.

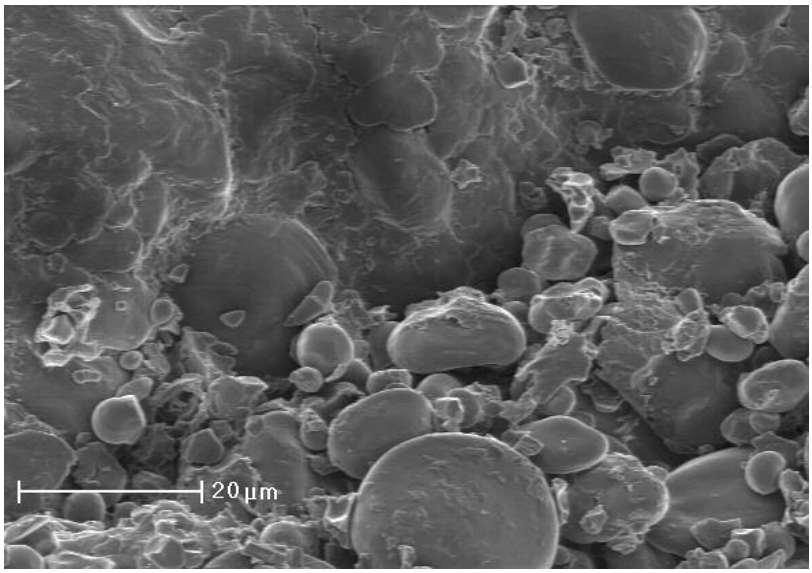

Figure 9: SEM image of "foreign body" particles showing a transition region where individual starch granules are showing signs of agglomeration.

Case study 2 – glass particle in baby food

A glass fragment, found in an imported jar of baby food, was reported by a consumer. Both the fragment and the jar were submitted for analysis. The fragment was exceedingly fine, only 0.3mm in thickness and retained only a single manufactured surface. As such it was impossible to determine either the radius of curvature of the original source of the fragment or its density for purposes of comparison with the baby food jar. The manufactured surface of the fragment, was smooth with no mould marks, consistent with the internal surface of a blow-moulded container. Examination of the baby food jar revealed no evidence of surface damage. The fragment, and a sample of glass from the jar, were analysed using EDS to ascertain whether it was likely they were the same material. The semi-quantified results of this analysis are presented in Table 2; the glass of which the jar was manufactured displayed significantly higher calcium and the presence of magnesium. This result indicated that the fragment was not sourced from the jar, or

jars on the packing line of the plant, and provided an increased level of confidence that other imported product was not at risk.

Case study 3 – suspected dental implant in confectionary product

A metal component was returned as a customer complaint to a confectionary manufacturer. The size and configuration of the component suggested it was a dental implant such as a crown, comprising two "pins", a "cap" and adherent white cement. The pin and cap alloys comprised predominantly gold and silver with additions of copper (in the cap alloy) and zinc (in the pin alloy), as shown in Table 3. Both the compositions are amongst those frequently used for dental alloys. The alloy in the cap matches the specification for high-gold alloys used in crowns and bridges, the alloy in the pins matches that used for solders (Reclaru & Meyer, 1995).

The surface of the cap was smooth and highly polished with evidence of some wear and porosity whereas the surface of the pins was pitted possibly by corrosion or by a deliberate etch prior to implantation to improve adhesion, figure 10; the higher oxygen content of this surface, Table 3, suggests corrosion. Some ductile damage clearly occurred after the surface pitting (as the pitting can be seen on folded back portion of the shear lip generated by the ductile damage) implying that this damage occurred as the implant was pulled from the foundation tooth, figure 11.

Table 2: Elemental composition of glass fragment and baby food jar.

	Fragment		**Jar**	
Element	**Wt %**	**At %**	**Wt %**	**At %**
Oxygen	31.9	45.7	24.7	37.4
Sodium	9.2	9.2	7.6	8.0
Magnesium	n/d	n/d	0.6	0.6
Aluminium	1.4	1.2	1.5	1.4
Silicon	45.5	37.1	49.4	42.7
Potassium	0.6	0.4	1.1	0.7
Calcium	11.3	6.5	15.1	9.2

Table 3: Semi-quantified EDS analysis (weight %) of suspected dental implant

	Cap	Pin
Carbon	n/d	n/d
Oxygen	n/d	9.8
Zinc	n/d	13.4
Palladium	4.5	3.1
Silver	25.3	18.6
Copper	12.8	n/d
Gold	57.4	55.2

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Taken together the SEM and EDS results confirmed the suspicion that this was a dental implant and strongly suggested that the implant had been pulled form the foundation tooth as the confectionary was consumed.

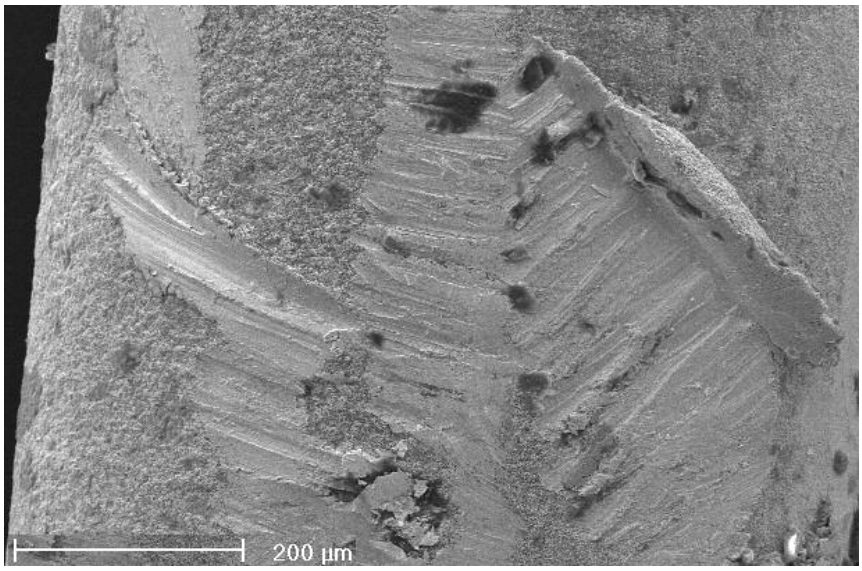

Figure 10: Surface of "pin" of dental implant showing surface pitting and ductile damage.

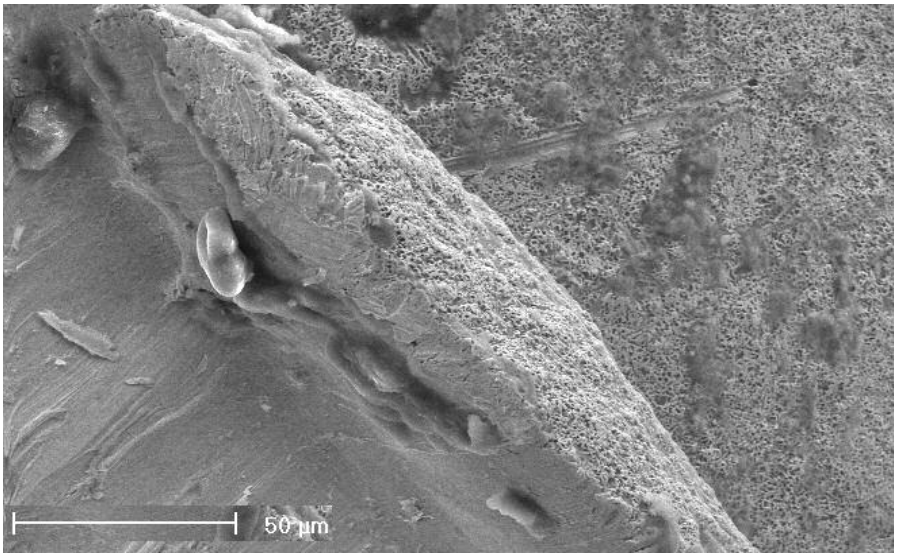

Figure 11: Higher magnification image of ductile damage showing that it occurred after surface pitting.

5. CONCLUSIONS

A methodical, systematic approach to analysis represents the fastest and most economic means of identifying foreign body contamination of food. This is an iterative process where tests are performed until the contaminant material is identified. The techniques outlined in this paper are complimentary and can be used in a variety of combinations to give the necessary information. The more exact the nature of the complaint (for example when a court case is at stake) the greater the necessary certainty of identification. Used in combination with more traditional QA techniques scanning electron microscopy, coupled with energy dispersive spectroscopy, is a powerful and highly efficient tool for identifying particulate contamination of food. Identifying particulate contamination as accurately, efficiently and economically as possible brings together the analysis techniques of the microscopist and the process knowledge of the QA manager. Possibly the most important quality of the analysts, though, is experience.

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