FIVE FUNDAMENTALS OF ALLERGEN ANALYSIS

by Martin Candia, Product Manager, Romer Labs

1 Allergen sources and allergen load

Food allergies have been on the rise worldwide in recent decades, in particular in western countries. This has brought along increasing concerns about food safety that have translated into stricter controls and regulations by official regulatory bodies. These controls demand that industries in the food sector incorporate appropriate allergen management plans that ensure either that food products do not contain allergens that are not part of the ingredient list, or, in some cases, that they are contained below defined concentrations. Allergen analysis, then, becomes an integral part of any allergen management plan.

In this series of articles, we tackle the most common issues and problems encountered by those conducting allergen analysis. In this first instalment, we will focus on the allergens we should test for and their concentrations.

Allergen management

One of the first steps in allergen management is to identify the allergens you would expect to find in a given production run or environment. With literally dozens of allergens that must be declared on product labels, it would not only be impractical but a waste of resources to test for all of them if there is no concrete reason to expect to find them. This requires a thorough knowledge of the products: allergens might not only be main ingredients but might also be unintentionally added, for example through processing aids or composite ingredients. Also, less direct routes of contamination should be taken into account, like how the ingredients have been processed, transported, stored or even grown (in the case of raw materials like crops).

Once the 'candidates' have been identified, it is necessary to analyse their relevance. In many cases, it is not necessary to manage all the allergens in one ingredient individually, but it is enough to focus on the most stable one or the one with the highest concentration. In order to do this, it is important to consider what the allergen load of the ingredient is. Because food allergens are almost exclusively proteins, the allergen load of an ingredient is related to the protein content of that ingredient and, in particular, to the amount of protein coming from the allergen source. Thus, ingredients rich in protein represent a higher danger than ingredients with low protein content, even if both derive from an allergenic source. Good examples of this are soy protein isolate (SPI) and soy lecithin. Both ingredients come from an allergen source, namely soybeans. However, while the first is almost exclusively composed of proteins (usually, at least 80% of SPI is made up of proteins), the second is mainly composed of glycerophospholipids and would contain proteins only as traces.

A second important point to consider is the form of the ingredient: is it a powder, a paste, a liquid? Allergens present in the same form can usually be managed and monitored together, while extra controls and testing may be required for allergens present in different forms. Consider, for example, the cleaning procedures: pastes and particulates are usually more difficult to get rid of than liquids. One practical way to deal with this is to manage the component with the highest allergen load in each ingredient form. All these considerations help us estimate the final concentration of each allergen in our sample.

Now that we have identified the critical ingredients and allergens in the product, it is easier to define the measures to manage it. We are then ready for the next step: testing the product or production line to confirm that our measures were effective and the allergen has been eliminated.

Check out the next issue for more on product and production line testing.

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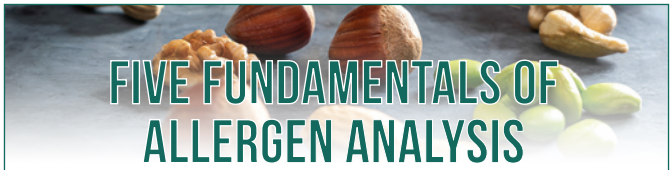

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2 Risk-based sampling

An important and often overseen part of allergen analysis is the process of sampling. Allergens that make their way into a product by cross-contact can be considered contaminants. Yet, unlike other 'contaminants', the distribution of the allergen contamination tends to be heterogeneous, a result of most cross-contact processes.

Furthermore, allergens do not spread once they are in the product (as would be the case of microbial contaminants). The sampling strategy is therefore of great significance in allergen control.

Determine high-risk areas

Some of the most advantageous sampling strategies are those based on previous risk-assessment. The best way to begin is to define the possible allergens, their presentation and allergen load, as well as the frequency and the points where cross-contamination is more likely to happen (and how).

It also helps to define the kind (for example swabs, rinsates, extrudates, finished product, etc), number and size of samples to take, as this allows for an initial, rough estimate of the amount of contaminant allergen to be expected. Thus, if the only possible cross-contact point in a product is the storage room, fewer samples will have to be obtained than if the production line itself is a critical point.

Similarly, powder-based allergens or allergen-containing materials can be spread through the air, necessitating the swabbing of surfaces other than only immediately adjacent ones.

No composite samples!

The nature of the sample itself and the process from which it derives also have an impact on the size of the sample to be taken. This also affects the degree of homogeneity that can be achieved when processing it. Most methods are able to test a minimum amount of sample (normally between 0.2-1g and, in some cases, up to 5g).

Therefore, these small amounts must derive from a thoroughly homogenised sample that is as representative as possible of the material being tested. This is of particular importance when this material is in itself heterogeneous (like most finished products). But beware! By no means does this mean that equivalent materials should be pooled to obtain a larger and more representative composite sample before homogenisation. In fact, the opposite is true.

Precisely because of the uneven distribution of allergens, the composition of samples tends to dilute the allergens, rendering them undetectable by regular methods of analysis. Furthermore, the pooling of samples leads to the loss of vital information regarding the exact time and origin of the cross-contact.

These are just some of the challenges that can come up during allergen analysis. In the next instalment, we address how to prepare the material for testing.

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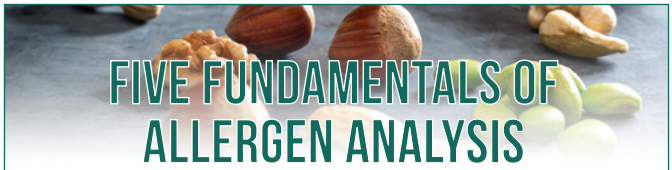

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3 Dealing with matrix effects

Allergen analysis is a powerful and indispensable tool to inform, confirm and evaluate every allergen management plan. Nevertheless, particular methods do come with their own sets of challenges. A particularly wide-ranging obstacle is the so-called 'matrix effect'. From an analytical point of view, a sample could be described as comprised of two components: the analyte (the allergen, in our case) and the matrix, which is everything in the sample that is not the analyte. The composition and physicochemical characteristics of the matrix can profoundly affect the response of the method of analysis, leading to an under-(suppression) or overestimation (enhancement) of the allergen in the sample.

The matrix can affect LOD and LOQ

One of the most commonly overseen aspects of the matrix effect is that it alters both the limit of detection and limit of quantification of the method. Therefore, unless the kit provider has already validated the method for the matrix we are analysing, the first thing to do is to check the LOD of the method in this matrix and to evaluate the matrix effect. This can be done by the method of standard addition (for quantitative methods), or by evaluating the ability of the method to recover defined amounts of an added allergen (in the case of qualitative tests).

The matrix and the sample extraction

The matrix can also affect other aspects of the method of analysis without directly contributing to the response itself. For example, the pH of the matrix and the presence of certain compounds can disturb the conformation of the allergen or cause precipitation when in the solution. A common example of this sort of interference is that caused by the high content of polyphenols in samples like chocolate or certain grains.

If the allergen of interest is rich in proline residues or –SH groups (as is the case for milk proteins like casein and β-lactoglobulin, respectively) the allergens tend to aggregate during extraction and are thus under-estimated. For these cases, the addition of a different protein (that itself does not interfere in the detection) also rich in proline or –SH groups is recommended. The interaction of the polyphenols with the extra protein prevents the allergen from reacting, normally improving the recovery of the method. There are also some commercially available polymers to help with this issue.

High sugar or fat content can also affect the efficiency of the extraction procedure before the analysis. The formation of very viscous solutions or colloids tends to interfere with extraction efficiency. In these cases, different approaches should be considered, such as warming up the sample before extraction, the extension of the extraction time or the centrifugation of the sample after this step. In some cases, further optimisation of the method could require collaboration with the kit developer.

Immunoassays are the most commonly used method in allergen detection in food. Unfortunately, they are vulnerable to low pH levels or high concentrations of alcohols, which could directly disrupt the structure of the detection antibody. In these cases, it is always useful to check the pH and to adjust it to the level for which the kit was validated. For alcohol content, the simple dilution of the sample is usually enough. One caveat: you will need to recalculate the LOD of the method (which will increase) to account for this extra dilution step.

As you can see, allergen analysis is more than just picking a kit off the shelf and using it! In the next issue, we will discuss more challenges that surround allergen analysis.

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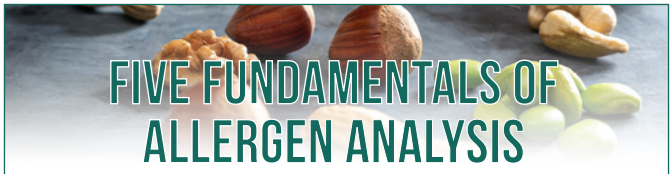

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4 Choosing the right method of analysis

Allergen surveillance plays a key role within any allergen management plan. Whether setting the plan in place or carrying it out, we need methods of allergen detection. This helps us not only to evaluate the measures we need to apply but also to verify the effectiveness of the plan and, of course, to monitor the allergen status of all ingredients, materials and products. It is therefore essential that the methods selected to check for allergens are well suited to our purposes.

It is tempting to think that choosing a method should be as simple as finding one that detects the allergen of interest and has a reasonably low limit of detection. No matter whether you intend to perform the method in-house or send your samples to an external laboratory, there is more to it than just picking one with a low LOD.

The first thing to determine is whether you need qualitative or quantitative results. This will depend on the purpose of the test. It is also important for the simple reason that qualitative methods are usually faster and more economical than qualitative ones. If you have set a certain value for the amount of allergen acceptable in a certain material, a qualitative method might be enough during routine testing. Both qualitative and quantitative methods can help you validate the procedure.

It is important to check what the actual analyte of the method is. Though the aim of a method may be to determine a particular allergen, the actual analyte is sometimes just part of that allergen; for example, instead of detecting milk, the method will actually detect only casein or beta-lactoglobulin. Make sure you understand the implications for your product and confirm that the detection of this analyte is appropriate.

Be sure to determine the actual LOD and LOQ of the method for your specific matrix. Some matrices present LODs significantly different from the ones stated in the method's instructions. This is because, in most cases, that value corresponds to the LOD in blank buffer.

If you are using allergen threshold levels as guidance, there is an extra layer of complexity: you first have to determine the LOD and LOQ for the portion size of your product. Leaving aside that there are no universal thresholds and that values might vary by 100-fold from guideline to guideline, a simple example will elucidate this problem: the portion size for some cookies is around 20g, while that for a cereal bar could be closer to 200g. If you were to check the egg content of these products, you might need LODs/LOQs of 215ppb or 21.5ppb, respectively. Thus, a method suitable for one product will not do the trick for the other.

Finally, and as previously described, the matrix can present challenges that affect the recovery of the allergen. Be sure you pick a method that provides a percentage of recovery within a previously defined, acceptable range for your matrix (usually 60-140% is recommended for food allergens), and adjust your acceptance criteria accordingly.

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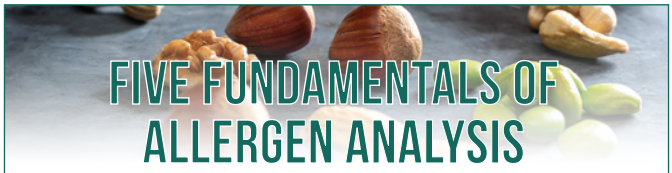

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5 Make it easier to keep your plant clean

Cleaning procedures are an indispensable component of every allergen management plan. Yet the story starts long before the cleaning does: you need to make sure the cleaning procedures you choose are easy to follow and fit for purpose. This raises several issues, many of which are not always considered by manufacturers. Here are a few of the most important ones.

Facility design, layout and equipment

Think about how your production facility is designed. Maintaining dedicated facilities or separate spaces for allergenic material can give you a head start, as such practices can help define different regimes for the different sectors of the plant. While such practices can reduce cross-contamination, it is not always possible to enact them, as they would affect the design of the food production facilities themselves.

Nevertheless, many other more practical adjustments can be made. For example, crevices, corners and grooves should be removed wherever possible. Particulate material tends to accumulate easily in crevices, such as where a wall and a floor converge, and is not always easily removed. Generally speaking, the fewer crevices and grooves a production line has, the better. They can be replaced, for example with rounded joints between floor and wall, which are more accessible to cleaning implements.

This is as true for the design of the plant as it is for the machinery used in the production line. Old equipment, in particular, is not designed with ease of cleaning in mind, adding yet another challenge. It is also important to assess the material (such as plastic, stainless steel or Teflon) and the texture of food processing surfaces. Why? Some surfaces should be avoided, as pastes and food soils can adhere to them.

The layout of the different sectors of the plant should also be taken into account. Equipment should be situated in a way that allows for easy access, not only to the equipment itself but also to any surrounding surface. Maintaining adequate space between the equipment and any walls is recommended.

An allergen cleaning method fit for purpose

But what about the cleaning methods themselves? As a general rule, any method that tends to spread material, such as the use of compressed air, should be avoided, in favour of the use of vacuum cleaners, for example. For the same reason, wet cleaning is preferable to dry cleaning, as the latter can disperse powders and particulates.

Furthermore, wet cleaning can be automated and uses chemicals that facilitate the elimination of proteins; food allergens, of course, are proteins. Relevant parameters to take into consideration include the temperature at which the cleaning is performed, the kind of mechanical interaction between the cleaning agent and the surface, the duration of the cleaning, and the kind of agent being used. Although water can be excellent for rinsing, it is poor at eliminating proteins. Other agents, such as detergents, proteases or chlorinated alkali detergents, are much more effective.

Finally, do not forget that there are two key components of any allergen management plan: clearly identifying target allergens and avoiding cross-contamination. This means using dedicated cleaning tools and materials, and if necessary, single-use, disposable tools as well.

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