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Food & Allergy Consulting & Testing Services

ALLERGEN DETECTION INFORMATION SHEET

INTRODUCTION

The food industry is required by law to supply allergy advice on product labels that will arm allergic consumers with adequate information to make smart and safe purchasing decisions. This means that common food allergens must be managed effectively, to prevent cross contact and support 'free from' claims. It is therefore paramount that controls are validated and verified by means of suitable and reliable testing methods. Suitability means that a method should be as fast, sensitive (has a low limit of detection or LOD), accurate (provide accurate information) and specific (allow unambiguous identification of the allergens) as possible. [1, 2, 3].

Food products are highly complex biological samples due to the countless biomolecules that comprise them. The complexity of these samples demands careful consideration when selecting the methods used. The most suitable method ensures a high degree of confidence in detecting and confirming the absence or presence of trace amounts of allergens.

TRADITIONAL ALLERGEN-DETECTION METHODS

To date, the methods most widely used have been indirect: detecting allergen coding genes (by polymerase chain reaction or PCR) or allergenic proteins via antibody-antigen complexes (by enzyme-linked immunosorbent assay or ELISA).

PCR

Proteins are encoded by DNA, so it is reasonable to attempt the detection of an allergen using this approach. The difficulty, however, is that the absence of DNA does not guarantee the absence of allergenic proteins, as DNA must be intact to be detected, and it can be degraded readily by several natural and food-production processes. Furthermore, DNA quantity typically only correlates very loosely to protein quantity.

ELISA

Though it is the most widely used tool for quantitative allergen detection, ELISA suffers from several well-documented drawbacks, mostly relating to the immunological method of detection. Immunological techniques are based on the recognition and interaction of a target analyte and one or more antibodies. Antibodies recognise and bind to an'epitope' – a small portion of the whole molecule. The epitope of the allergen involved in the immunoglobulin binding is either linear or conformational. The linear epitope is a continuous string of amino acids, and recognition is specific to the amino acid sequence (the primary structure of the protein). The conformational epitope may be a continuous or a discontinuous string of amino acids, and recognition depends on the three-dimensional shape of the protein (protein tertiary structure). As antibodies only recognise epitopes, rather than the whole molecule, the specificity of an antibody depends on the uniqueness of the epitope. A lack of specificity may lead to false positives or negatives [4, 5].

False positives may cause additional expense, delay and irritation; but false negatives could mean the exposure of consumers to risk. Common food-processing methods can cause the partial or complete denaturation or hydrolysis of the target analyte, leading to an under-estimation of the presence of an allergenic protein at best, and at worst, its complete non-detection [6, 4, 7, 5, 8, 9].

The need to keep proteins in a particular conformation also precludes efficient extraction procedures, which can compound the risk of not detecting an allergen that is present in a sample. This is illustrated by Figure 1 below.

Further drawbacks with ELISA relate to its insufficient sensitivity for current reference doses (kit dependent) for food allergens [1], and a lack of transparency regarding calibrants and targets. In some cases, assumptions built into the method lead to questionable results [10].

ELISA is fast and sensitive, but sometimes this method may be inaccurate for some processed food samples [4, 5, 6], and can sometimes be non-specific [8]. However, it is very easy to use and requires minimal equipment, which has led to its widespread adoption.

AN ADVANCED ALLERGEN DETECTION METHOD

Mass spectrometry (MS) is familiar to many in the food industry as a widely used analytical tool. MS has long been used for the study of proteins, with early experiments designed for protein identification. Today, MS is routinely used in high-throughput proteomics, and can be similarly used for the detection of trace allergens in food matrices [11, 2, 1]. Hyphenated methods such as Targeted Proteomics (by LC-MS/MS), coupling a separation technique with MS, allow the direct and absolute identification and quantification of allergens. Mass spectrometric methods are routinely performed at the peptide scale, making detection independent of the tertiary structure of the allergen, extremely specific, and still detectable after food processing [1, 7, 5].

Previous challenges with the implementation of MS for the routine detection of allergens in food included the specialised knowledge required to operate the technology, and the lack of the background information about the target proteins that was required to detect them. These challenges have been overcome through widespread experimentation and the publication of a variety of methods and targets [12, 13, 11, 2].

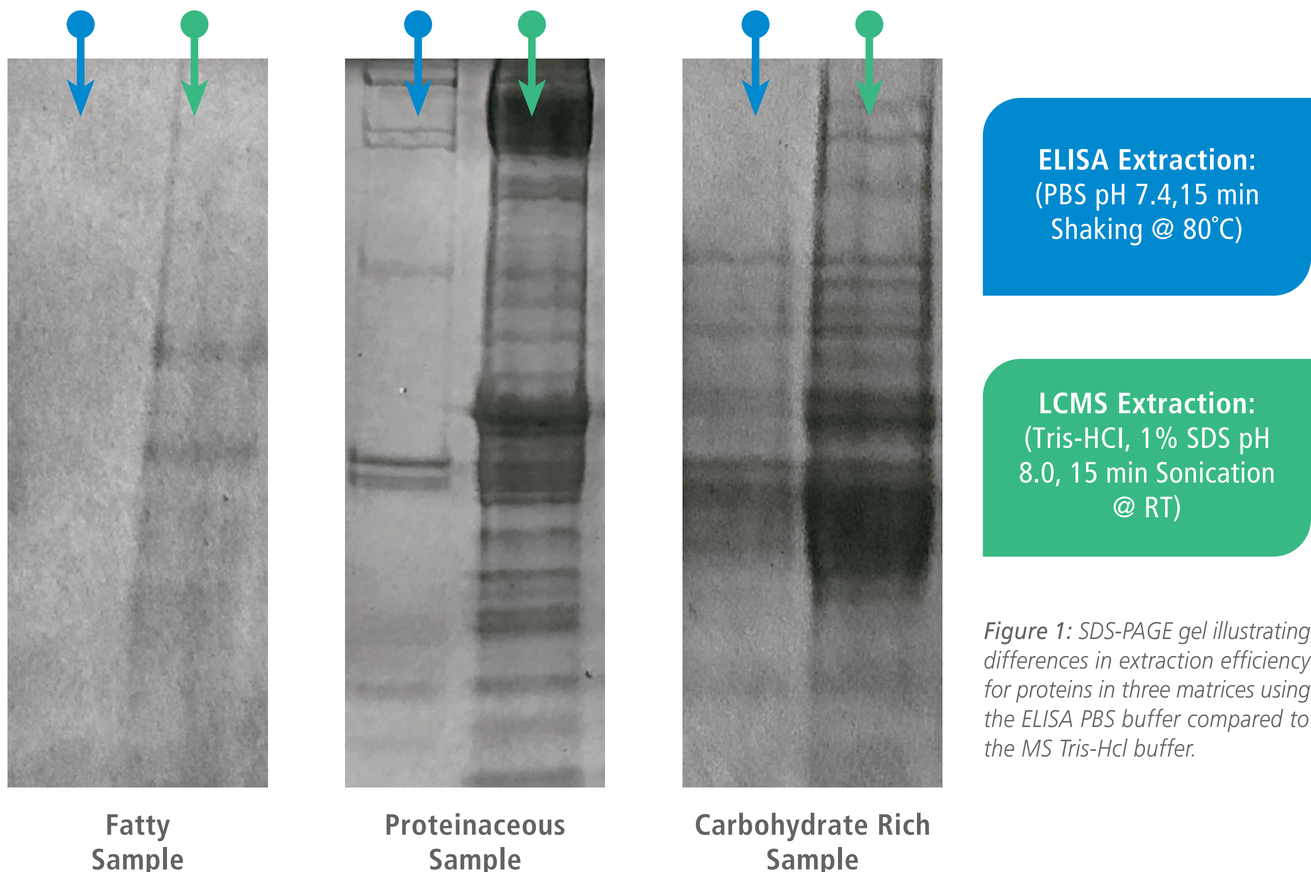

Figure 1: SDS-PAGE gel illustrating differences in extraction efficiency for proteins in three matrices using the ELISA PBS buffer compared to the MS Tris-Hcl buffer.

Given that the detection and quantification of trace amounts of allergens in food matrices is our primary goal, MS is our best tool using current technology. MS-based methods are fast, highly sensitive, accurate, and highly specific. This is demonstrated by the fact that the trusted method in comparative studies (even those conducted by ELISA kit manufacturers) is mass spectrometry [8, 7].

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