Poster

Validation of new matrices to detect gluten using sandwich ELISA

Siglez Granado, Miguel Ángel / Chief Laboratory Officer / BIOMEDAL S.L.,Polígono Ind. Parque Plata, Calle Calz. Romana,40,41900 Camas, Sevilla

González Colchero, Jesús / Scholar / Universidad Pablo de Olavide, Sevilla

Tutor académico: Lopes Da Costa, Tania Isabel, Profesor Titular, Departamento de Sistemas Físicos, Químicos y Naturales,Edificio 22, 3ª Planta, despacho 9, Universidad Pablo de Olavide, Sevilla

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ABSTRACT

Celiac disease is a chronic inflammatory bowel disease that has an important genetic component. It is induced by wheat gluten and rye and barley prolamines, which generate an abnormal immune response that causes inflammation and damage to the lining of the small intestine and reduces the absorption of nutrients such as iron. calcium, vitamins A , D, E, K and folic acid [1].
Celiac disease affects about 1% of people in the world and a strict gluten-free diet for life is the only treatment available [1].
Nonceliac gluten sensitivity syndrome (NCGS) is gluten intolerance in people who do not have any wheat allergy or celiac disease [2].
Gluten is a mixture of proteins present in cereals. Alpha-gliadin stands out, a multimeric protein that presents a peptide toxic to celiac people. This peptide is called 33-mer and triggers the activation of the immune response, generating antigliadine antibodies (AGA) [3].

Our project has the objective of validating aromas, colorings and preservatives as matrices for the detection of gluten. To achieve this validation, a series of tests regulated by the ISO 17025 law must be carried out and will be supervised by the National Accreditation Entity through an audit. The objective of the validation activity is to confirm the reliability of the test information. One of the purposes of validation is to provide confidence to the recipient of information, such as administrations, consumers, businesses, etc.

The validation process consists in repeatability and reproducibility.
Repeatability consists of analyzing for each matrix three replicates of each fortification range: low (5ppm), medium (10-20 ppm) and high (50 ppm). Each replicate shall be analysed in the ELISA sandwich R5 test in triplicate.
On the other hand, reproducibility consists in analyzing for each matrix a replica of each fortification range in two different days. Also, each replicate will be analyzed in triplicate in ELISA sandwich R5.
Fortification consists of doping, for a certain concentration, a white matrix with an analyte of interest that we want to quantify. This allows us to obtain reliability in the quantification made during a test.

The ELISA sandwich R5 involves two antibodies with epitopes for the same antigen. R5 is fixed to the microplate and binds by complementarity to the 33-mer peptide of gliadin (antigen). The conjugate antibody has a HRP substrate binding region, which by oxidation causes color change to be quantified [4].

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