A BELGIAN STUDY ON THE PURITY OF FOOD ENZYMES SOLD ON THE EUROPEAN MARKET

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INTRODUCTION

Food enzymes (FE) that are commonly present in the food chain are often produced with microorganisms, including genetically modified microorganisms (GMMs). In order to increase consistency for FE approval in the EU, Regulation (EC) 1332/2008 was established with the intention of producing a list of authorised FE. Around 300 FE applications were submitted to the EU Commission, and EFSA is performing safety evaluation on the basis of confidential information received from the applicants. The food industry is responsible for the quality control of FE preparations sold on the market and nearly no analyses are performed by control laboratories. Consequently, no monitoring data is available on the quality of commercialised FE. This absence of control and information is mainly due to the lack of an interdisciplinary consortium that would enable development of relevant analytical methods and strategies to control FE purity. Therefore, a research project was financed by the competent Belgian (BE) authorities to provide appropriate analytical methods and strategies for efficiently controlling FE purity and monitoring the purity of FE preparations available on the EU market, with a focus on BE.

METHODOLOGY

• FE data were collected from various publicly available sources, such as the public parts of the submitted FE dossiers, the EFSA portals, published scientific opinions, patents and NCBI databases. These data were used to construct a database and a list of chemical and biological impurities potentially present in FE preparations (metals, antibiotics, toxins, allergens, fungi, spoilage/hygiene indicators, pathogens, producer organisms (including GMMs)).
• Several platforms, technologies and methods such as LC-MS, LC-MS/MS, UPLC-MS/MS, ICP-MS, AMA-254 (Advanced mercury analyser), MALDI-TOF MS, ELISA, classical microbiology, PCR, Real Time PCR, Sanger sequencing, Whole Genome Sequencing (WGS) (MiSeq - Illumina) were developed and/or tested to measure the selected impurities.

• FE model matrices were artificially contaminated with the selected impurities in order to confirm that the analytical methods used in this project are fit-for-purpose when it comes to detecting such impurities.
• 51 FE preparations sold on the Belgian market were collected and subjected to analysis.

RESULTS

• An FE database, FEDA (https://feda.sciensano.be), is publicly available and allows publicly available information from various sources to be collected and searched in one centralised web application.
• The performance of the various methods was successfully assessed on FE preparations.
• Methods of detecting microorganisms producing FE were developed, including 16S rDNA-based screening and viability assessment of target bacterial contaminations, as well as qPCR and PCR methods to detect GMMs and to assess the full length of AMR genes.
• The pilot monitoring provided an overview of the level of impurities in commercialised FE preparations. In 1/51 contamination by chloramphenicol was measured (above 0.3 µg/kg) (RASFF). In 4/20 FE preparations, an allergen (not labelled) was detected (RASFFs). B. cereus with enterotoxins and emetic toxin genes were detected in 17/39 FE preparations. Viable bacterial and fungal strains, as well as DNA, were observed in more than 50 % of samples. The presence of bacterial GMM DNA with full-length AMR genes was proven in 15/51 FE preparations (RASFFs) and was suspected in 23/51 additional ones. Living GMMs were isolated from 2 samples and characterised with WG.

DISCUSSION

The tools and methods developed in the project are useful for enforcement laboratories and competent authorities for future control plans targeting FE preparations present on the market. In addition, the pilot monitoring has enabled the identification of mislabelled allergens and contaminants potentially problematic for the security of the food chain, such as the presence of the antibiotic chloramphenicol, the presence of Bacillus cereus with toxin encoding genes, and viable strains or DNA from producer organisms, including GMMs with full-length AMR genes. Importantly, several FE preparations were found to contain recombinant GMMs including full-length AMR genes and were therefore found not to be compliant with GMO Regulations (EC) (1829/2003 and 1830/2003). These evidence-based results will assist in refining risks assessment by determining whether the detected impurities and the levels detected may impact the health of consumers. This study also paved the way for determining which types of impurities are more likely to be found in FE preparations at potentially problematic levels and should therefore be targeted with priority (risk management) in a future FE control plan.