

EXPERT OPINION STATEMENT  
FOOD ALLERGY RESEARCH & RESOURCE PROGRAM  
UNIVERSITY OF NEBRASKA

**Testing of Microbially Derived Enzymes for Potential Allergens from  
Fermentation Media Raw Materials**

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Microbially derived enzymes are used by food processors as additives and processing aids in a wide variety of foods. Enzymes obtained from microbial fermentation are directly derived from microorganisms fed on sterilized media<sup>1</sup> that may include protein sources obtained from one or more of the recognized commonly allergenic foods (e.g., milk, soybean) or from a cereal source of gluten (e.g., wheat, barley). This paper addresses the relevance of testing microbial enzymes for allergenic material from the fermentation growth media.<sup>2</sup>

It has been the long-standing position of the Food Allergy Research & Resource Program (FARRP) at the University of Nebraska that testing of the products of fermentation (with limited exceptions), including microbially derived enzymes is unreliable using enzyme-linked immunosorbent assays (ELISAs).

While various fermentation media may contain one or more of the major food allergens, the biochemical reactions that occur during fermentation result in the breakdown of the fermentation media proteins. The extent of proteolysis is dependent upon the fermentation culture and the resultant enzyme (e.g., some enzymes are proteases). As proteins are digested, the resulting amino acids, along with other nitrogenous material, are consumed by the microorganisms for cell growth, cell maintenance, and production of enzyme protein.

<sup>1</sup> Aunstrup, K., O. Andresen, E.A. Falch, and T.K. Nielsen (1979) *Microbial Technology*. (Perlman and Peppler, eds.) Academic Press, pp. 281-309.

<sup>2</sup> For this paper, FARRP's analysis is limited to microbially derived enzymes that are intended for additive and processing aid applications in food.

Upon completion of fermentation, remaining fermentation media that are not consumed by the microorganism are typically separated and/or purified from the enzyme in the recovery process. Enzymes are recovered from the fermentation broth by standard chemical engineering operations, such as filtration and centrifugation, broadly used in enzyme production.<sup>3,4</sup> (See Appendices for further information.) The recovery steps result in separation of microbial biomass and other fermentation solids from the enzyme, concentration of the enzyme, and removal of impurities prior to final formulation with food-grade ingredients.

Any potential residual fragments from the food allergen would be difficult to measure as there is no reliable assay. Commercial ELISAs are able to detect only intact proteins in most cases. Any peptides, even larger ones, would not likely be detected, although this possibility has not been well investigated. Results would typically be reported as below the limit of quantitation for the enzyme preparation. Further, if any residual but undetected fragments of the food allergen remain, the relevance of any such residual material to food allergenicity is unproven. Accordingly, testing of fermented product does not result in reliable or useful data.

In addition, due to the specific catalytic nature of enzymes, only very small amounts of enzymes are generally required and used by food processors to make the desired modifications to the property of a food, and therefore any *de minimis* amount of fermentation media protein that may survive the fermentation process will not pose a significant public health risk to the consumer.<sup>5</sup>

FARRP also notes that regulatory agencies in the European Union and Japan do not require allergen labeling of enzyme preparations for the raw materials used in the fermentation process.

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<sup>3</sup> Atkinson, B. and F. Mavituna (1991) *Biochemical Engineering and Biotechnology Handbook*. (Atkinson, B. and Mavituna, F., eds.) Stockton Press, Hampshire, pp. 1146-1158.

<sup>4</sup> Kroschwitz, J.I. (1994) *Enzyme Applications in Encyclopedia of Chemical Technology*. 4<sup>th</sup> edition, Volume 9. (Kroschwitz, J.I., ed.), pp. 567-620.

<sup>5</sup> To the extent the enzyme producer uses an allergen as diluent to formulate the final product, labeling for such allergen is appropriate and required under Food Allergen Labeling and Consumer Protection Act.