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REVIEW

Evolving regulatory policies regarding food enzymes produced by recombinant microorganisms

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ABSTRACT. Bio-based industries rely extensively on the use of enzymatic biocatalysts. The global market for industrial enzymes, of which approximately half is used for food applications, is estimated at $5.5 billion. Most enzymes used in food production worldwide are produced by recombinant DNA techniques. Production and use of food enzymes are regulated by three main bodies: the Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives; the European Food Safety Authority; and the U.S. Food and Drug Administration. Regulation in the U.S. follows a largely product-oriented approach while the EU emphasizes production processes. Both systems have, or are developing, lists of approved enzymes to facilitate trade while protecting consumer health and welfare. This paper compares regulatory policies, and presents the growing food industry in Turkey as a case study of a national system responding to the food enzyme production and regulatory landscape.

KEYWORDS. food enzymes; policy; recombinant microorganisms; regulation; Turkey

INTRODUCTION

Enzymes, by their simplest definition, are biocatalysts. A catalyst is a substance which initiates or accelerates a reaction without being consumed and can continue to act repeatedly. Catalysts are important in industrial applications because by the help of catalysts it is possible to obtain the product at a much faster rate than the spontaneous reaction rate in nature. In that sense, enzymes are catalysts

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which are responsible for starting or accelerating the rate of a biochemical reaction in a living organism, without itself being consumed. In addition to their roles *in vivo*, enzymes can work *in vitro*, allowing them to be utilized in industrial processes. Common examples of enzyme-catalyzed reactions include the breakdown of proteins, carbohydrates and fats in foodstuffs[1,2] The breakdown reactions take a few hours when the enzyme is used, but take several years (>30 years) in the absence of enzymes.[3] The International Union of Biochemistry and Molecular Biology (IUBMB), an international non-governmental organization, has defined six main classes of enzymes based on the reactions they catalyze: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases.[3]

Microorganisms and enzymes have been used by humans unknowingly for thousands of years in the production of food such as beer, bread, and cheese. Today, enzymes are industrially produced from animals and plants by extraction, or from microbial sources, and are used for food production and food processing purposes (Table 1).[2] The two major commercially produced plant-based enzymes are papain from papaya and bromelain from pineapple; both of which are proteases (i.e. they are used to break down proteins). There are also enzymes such as rennet, which are traditionally produced from animal sources. While numerous other enzymes also could perform valuable food processing functions, only a few plants- or animal-based enzymes are on the market because of the insufficiency of the sources and lack of consistency between batches. Sanitary issues can also arise with animal tissues during enzyme production and extraction.[5] The difficulty and the cost of the purification processes applied for plant- or animal-derived

TABLE 1. Examples of uses of enzymes in the food industry [1.]

Application	Enzyme	Catalytic action	Technological effect
Baked goods	Lipases	Hydrolysis of lipids	Improving handling of dough, enhancing dough strength and stability, increasing bread oven spring and specific volume
	Hemicellulases	Hydrolysis of hemicelluloses (arabinoxylan backbone)	Improved distribution of flour and water; softer, flexible and stable dough which is easy to handle, improved sensory properties and volume of bread
Dairy	Proteases	Hydrolysis of proteins	Separation of milk into solid (curd) and liquid (whey) phases in cheese making
	Lysozyme	Hydrolysis of peptidoglycans in the cell wall of Gram-positive bacteria	Inactivation of bacteria causing spoilage in cheese products, causing “late blowing”
Fruit and vegetable juices	Pectinases	Hydrolysis of pectins (a polysaccharide in plant cell walls)	Decreased viscosity of the fruit juice, higher recovery yields and clarified juice
Meat and fish	Transglutaminases	Cross-linking of proteins	Improved texture, cohesiveness, and shelf life of meat products, harder fish protein paste
Sugar	Amylases	Hydrolysis of starch	Production of a large variety of sugars and sugar syrups from starch
Wine	Pectinases and hemicellulases	Hydrolysis of pectin and hemicelluloses [plant cell wall components)	Improved extraction and clarified grape juice for wine making
Beer	Amylases	Hydrolysis of starch	Increased concentration of fermentable sugars, hence, higher ethanol concentration in beers

[1]Adapted from 2, and 4

enzymes is another bottleneck. Therefore, today about 85% of industrial enzymes are produced from microorganisms (50% fungus and yeast, 35% bacteria) while the remainder is produced from plants. The high rate of use of microbial sources results from several advantages: developing and optimizing fermentation processes can allow well-characterized enzymes to be produced and purified at a large scale with high yields; moreover, enzymes derived from microbial sources are generally more active and stable than plant- or animal-based enzymes; and, microorganisms are more suitable for genetic modifications.[6]

Enzymes can be produced from wild-type (as exist in nature) or genetically modified (mutant) microorganisms.[7] Currently, most of the enzymes used in the food industry are produced from genetically modified (GM) microorganisms. This provides two advantages. First, it is possible to produce modified (genetically engineered) enzymes with improved properties for food manufacturing purposes. Improvements may include increased production yield and selectivity, and improved performance by taking into account the food matrix conditions, such as pH, temperature, salt concentration, and cofactor requirements.[8] Secondly, it is possible to express the enzyme-encoding gene in a microbial host that promotes higher yield, shorter time and lower process cost than the production based on a wild-type strain.[9,10]

The global industrial enzymes market value is about $5.5 billion and is expected to reach $7.0 billion by 2023.[11] Currently, North America (40% market share) and Europe (30% market share) are the largest consumers for industrial enzymes.[5] According to the report on Industrial Enzymes Market; United States (US) in North America occupies the top position in the global industrial enzymes market where one of the major factors driving the growth of the market is the increasing use of enzymes in the food and beverage industry.[12] Asia–Pacific is likely to register the highest growth rate in industrial enzymes market through the forecast period of 2019–2024 owing to the high prevalence of chronic disorders, increase in youth population with disposable incomes, and improvement in patient awareness about enzymes based pharmaceuticals and protein engineering techniques in the region.[12,13] The food enzymes market is also expanding, and is projected to reach $2.94 billion by 2021[14] The global enzyme demand is met by about 12 major and 400 minor enzyme producers. The top two companies for enzyme production are Novozymes (Denmark) and DuPont-Danisco (US), followed by DSM, Roche, Amano, AB Enzymes, BASF, and Chr. Hansen.[4,6] Currently, more than 500 commercially available products such as cellulosic ethanol, pharmaceutics, paper pulp, high fructose corn syrup, bread, cheese and fruit juices are obtained by the help of enzymes.[15] According to the report released by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) in 2015, there are more than 70 enzyme types which are commercially available. Considering that a given enzyme can be produced from multiple microorganisms, more than 200 commercial enzyme products are currently available.[16] The share of the enzymes produced from GM microorganisms in the total industrial enzyme market is about 50%.[10]

The global enzyme market is dominated by food and feed applications, which account for 55% to 60% of the market.[5] The number of industrial enzymes for food processing is continuously increasing based on research and development (R&D) efforts to discover novel enzymes.[17] The majority of the enzymes used in the food industry are hydrolyses which are used to break down proteins, lipids and carbohydrates, however, enzymes belonging to other enzyme classes are also used.[2] Some examples for the use of enzymes in the food industry are given in Table 1. Non-food enzymes, such as those used for detergents, textiles, pharmaceutical, and biofuel industries are beyond the scope of this article.

The distinction between enzymes as food processing aids vs. additives is not always clear, making it difficult to make common definitions. As a result, differences arise in the regulations on food enzymes. In the European Union (EU), there are currently two separate regulations on food additives and food

enzymes (which are discussed in Section 4). However, lysozyme and invertase, and any other enzyme regulated under the food additives regulation, will possibly be no longer classified as food additives once the EU list of food enzymes is established.[18] In the US, food enzymes are considered as a subgroup of food additives. Regulation of such enzymes has been in place since the beginning of United States (US) food safety laws that were enacted more 100 years ago.

The three main institutions regulating food additives globally are: Joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA); the European Food Safety Authority (EFSA) and the US Food and Drug Administration (FDA).[19] In this paper, international regulatory policies and systems of food enzymes have been compared by focusing on the U.S. and EU. In addition, the regulatory system in Turkey is provided as a case study of a national system that is responding to the evolving food enzyme production and regulatory landscape.

INTERNATIONAL STANDARDS

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) was established in 1956 to collect and disseminate information on food additives and to establish specifications of identity and purity for food additives.[20] In 1962, FAO and WHO jointly established the Codex Alimentarius Commission (CAC) to address safety and nutritional quality of foods, and to promote trade by developing international standards based on sound scientific evidence. JECFA serves as the expert risk assessment body on additives, contaminants and natural toxicants in food, and has produced many internationally accepted data and publications that are widely used by governments, industry and research centers. The Codex Committee on Food Additives and Contaminants (CCFAC) fulfills the corresponding risk management role, including making recommendations to the CAC regarding the adoption of JECFA specifications.[20] It should be noted, however, that the CAC has no regulatory authority. Enforcement of standards depends on adoption into national regulatory frameworks.[21,22]

Codex Alimentarius (CODEX) aims to harmonize food and commodity standards and to provide guidelines and codes to contribute to the safety and quality of food trade. Commitment to CODEX varies depending on the degree of development of the internal regulation of the countries. Countries with well-established internal regulations (e.g. EU) generally acknowledge CODEX or use it as a basis for new regulation. The Countries with less developed internal regulations generally refer to or adopt CODEX standards.[23]

Regulation Policies and Systems in the U.S

In the U.S., the US Food and Drug Administration (FDA) is the regulatory authority and the FDA Science Board is the advisory scientific body. In addition, the U.S. is a member of the World Trade Organization (WTO) and the Codex Alimentarius Commission (CAC). The primary mission of the FDA is to "protect the public health by ensuring the safety, efficacy, and security of human and veterinary drugs, biological products, and medical devices; and by ensuring the safety of the nation's food supply, cosmetics, and products that emit radiation".[24]

FDA publishes guidance and regulatory information documents for the related audiences such as food consumers and food production companies. Guidance documents are informative instruments that the FDA has prepared to express its current opinion about a -topic.[25] The number of published food guidance documents are regularly increasing. From 1993–1997, FDA averaged less than one guidance document per year, while in the past 20 years (1997–2017), the number of guidance documents increased more than 30-fold.[26] While guidance documents are not legally binding, regulations published in the Federal Register under the Code of Federal Regulations (CFR) are legally binding.[25] Each title (or volume) of the CFR is revised annually

and is accessible through the web.[27] Failure to comply with laws and regulations may result in a Warning Letter, seizure, injunction, or civil or criminal penalties. Companies in the food, beverage, dietary supplement, and other sectors are often issued Warning Letters by the FDA.[26]

FDA's efforts to implement rules of the Federal Food, Drug, and Cosmetic Act on pre-market evaluation of food additives began in the late 1960s. The FDA employs a science-based pre-market safety evaluation system that relies on objective and independent FDA scientists.[28] In the U.S., food enzymes are defined as "food additives" which are used to improve food processing and the quality of the finished food.[29] According to the Code of Federal Regulations (CFR) Title 21, food additives includes all substances, not exempted by section 201(s) of the act, the intended use of which results or may reasonably be expected to result, directly or indirectly, either in their becoming a component of food or otherwise affecting the characteristics of food. The word "direct" used in the definition refers to substances which are intentionally added into the food for a particular target. The word "indirect" covers substances which are intentionally added to the materials that come into contact with food and which, as a result, cause unintentional immigration into the food.[28] Section 201(s) of the Federal Food, Drug, and Cosmetic Act exempts the use(s) of a substance that is generally recognized as safe (GRAS) from the definition of a food additive.[30]

There is no specific regulation governing enzymes in the U.S. According to CFR 21, enzymes are regulated as direct or secondary direct additives, or GRAS, depending on their intended use and the method used to allow the substances in food.[21] The FDA's Office of Food Additive Safety (OFAS) evaluates food additive petitions and GRAS notices for enzyme preparations.[30] The assessment of GRAS status of food additives is based on the opinion of expert scientists, based on two approaches. First, the opinion could be based on the traditional use of additives in food, without the scientific procedures required for approval of a food additive. An additive used in food before January 1, 1958 (e.g., table salt) is considered to be safe on the basis of general use. Second, the opinion could be based on existing scientific data. The scientific procedures for GRAS evaluation should be based on generally available, accepted and published scientific data, information, or methods. The decision may also be supported by the unpublished scientific data, information, or methods.[31]

The technical information requested from the applicants includes (but is not limited to): identity, method of manufacture, specifications, use levels, dietary exposure, and data for toxicological studies. FDA recommends that summary information and data on food additives be provided in the GRAS notifications, and detailed data should be submitted to FDA upon request. GRAS status based on scientific assessment requires the same quantity and quality of scientific evidence as is required for a food additive which is regulated as a direct or secondary direct additive.[30] However, in the case of GRAS evaluation, the main safety data must be generally available to the scientific community, hence, cannot be kept confidential for the applicant.[32] In 1997, FDA also proposed a voluntary (and open to public) notification program (21 CFR 170.36) for GRAS additives. The applicant can inform the FDA that an additive has been designated as GRAS. If FDA is not concerned with the food safety data and information provided in the GRAS notice, a letter is issued to the notifier stating that the agency has no questions regarding the GRAS consideration of the substance under the intended conditions of use. In recent years, numerous food enzymes have been evaluated through the GRAS notification program.[30] Enzyme preparations affirmed as GRAS for specified or unspecified food uses are listed in the Part 184 of CFR Title 21.

If an enzyme is not considered GRAS, a food additive petition, which essentially covers the same technical information as a GRAS notice, should be submitted to FDA.[33] Independent of regulating as food additive or GRAS, the intended use of the enzyme preparation is taken into account in the safety assessment. Food enzyme petitions are required to cover five general areas of information: (i)

identity (identity of the enzyme, characterization of the enzyme source, composition of the enzyme preparation); (ii) proposed use; (iii) intended technical effect; (iv) analysis method for the presence of the enzyme in food; and (v) full reports of all safety investigations with respect to the enzyme. The petition should also contain information on manufacturing process, specifications for identity and purity, and an estimate of the dietary exposure to the enzyme preparation. In the case of microbial enzyme production, the process conditions and all materials used in fermentation and downstream processes also should be identified. Regardless of the source of the enzymes (microorganism, plant or animal), enzyme production should be carried out in accordance with the current good manufacturing practice (cGMP). If GM microorganisms are the source of the enzyme, required additional information includes: source(s) of the introduced DNA; the specific gene(s) encoding the enzyme(s) of interest; and any other genes and regulatory DNA sequences necessary for a gene.

According to FDA Federal Register,[34] "*The method by which food is produced or developed may in some cases help to understand the safety or nutritional characteristics of the finished food. However, the key factors in reviewing safety concerns should be the characteristics of the food product, rather than the fact that the new methods are used*". This statement reflects the product-oriented approach of FDA in safety assessments, rather than focusing on production processes. FDA has a similar ideology for GMOs. In Federal Register it is states that "*Any genetic modification technique has the potential to alter the composition of food in a manner relevant to food safety, although, based on experience, the likelihood of a safety hazard is typically very low*" and " … *has no basis for concluding that bioengineered foods differ from other foods in any meaningful or uniform way, or that, as a class, foods developed by the new techniques present any different or greater safety concern than foods developed by traditional plant breeding*".[34,35] These statements imply that there is no difference in terms of safety standards for GM and non-GM food products, according to FDA. The FDA's approach towards GM-foods is also valid for enzymes from GM and non-GM sources. In principle, the same safety considerations apply to enzymes derived from GM and non-GM microorganisms in the U.S. The most important subject for evaluating the enzyme is to examine the production strain and to determine the pathogenic and toxigenic potential of the strain.[9]

If a petition to FDA regarding an additive is accepted for review, FDA publishes a notice of the filing, the name of the petitioner, and a brief description of the proposal in the Federal Register within 30 days from the date of filing. The Commissioner may request detailed information regarding methods of production or a sample of the food additive. If not provided within 180 days, the petition is considered withdrawn without prejudice.[36] According to FDA, for a direct food additive, the average time between filing the petition until a final decision is published is about 24 months.[33] Enzyme preparations that are approved as food additives by a successful petition process, are listed in Part 173 of CFR Title 21.

Regulation Policies and Systems in the EU

Prior to 2008, the EU had not established regulations for food enzymes other than those used primarily as food additives. In some cases, food enzymes were regulated as processing aids under the legislation of the Member States. Differences between member countries complicated the evaluation process, prompting the establishment of a new EU framework legislation on food enzymes. The aim of this legislation was to establish an EU list of authorized enzymes. As the list has not yet been established, regulations governing the marketing and use of food enzymes and food products produced by food enzymes continue to follow individual national frameworks.[37]

The EU has been a member of the WTO since 1995 and a member of the CAC. The regulatory authority in the EU is the European Commission (EC) Directorate General for

Health and Consumers, and the advisory scientific body is the European Food Safety Authority (EFSA), which was established in 2002. The main aim of EFSA is to provide independent scientific advice with respect to food safety at all stages of food production and the supply chain. These findings can, in turn, provide a scientific basis for EU member states' legislation and policies impacting food and feed safety.[21] The regulation of EFSA for the review of food additives is similar to the US in terms of data required and the methods of review. However, unlike the US, most EU national frameworks and EFSA regional frameworks do not include processing aids in the definition of food additives.[19] The food additives and food enzymes are regulated by two different regulations in the EU (Regulations of the Food Improvement Agents Package, adopted 16 December 2008).

Regulation (EC) No 1333/2008 of The European Parliament and of The Council 16 December 2008 on food additives, describes food additives as substances that are not normally consumed as food itself, but are added to food intentionally for a technological purpose, such as the preservation of food [EC-1333][38] A food additive is a substance which remains functional in the final food product, e.g. lysozyme and invertase are considered as additives due to their activity in the final product. However, substances which may be used for a technological function and have no technical effect on the product, such as food enzymes, falls within the scope of Regulation (EC) No 1332/2008 of The European Parliament and of The Council 16 December 2008 on food enzymes. A 'food enzyme' is defined as a product obtained from plants, animals or microorganisms, or products thereof, including a product obtained by a fermentation process using microorganisms that: (i) contain one or more enzymes capable of catalyzing a specific biochemical reaction; and (ii) are added to food for a technological purpose during the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods. A 'food enzyme preparation' is defined as a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardization, dilution or dissolution [EC-1332][39]

As such, Regulation 1332/2008 only covers enzymes which are added to food to perform a technological function. For example, some enzymes used in bread making are in the scope of Regulation (EC) No 1332/2008. These enzymes are functional in the dough during the processing steps (fermentation and dough leavening), but are denatured by the heat during the baking process and so are not functional in the final product (bread). Another example is immobilized enzymes, such as lactase (beta galactosidase), which are used industrially to hydrolyze lactose to obtain lactose-free dairy products. Since lactase remains bound to the immobilization matrix, it is not present in the final food, hence it falls in the scope of Regulation (EC) No 1332/2008.[40]

Regulation (EC) No 1332/2008 provides rules for a Community list of approved food enzymes; conditions of use of food enzymes in foods; and the labeling of food enzymes. The intent is to facilitate trade, protect human health, and where appropriate, protect the environment. Food enzymes cannot be approved or sold if they do not fulfill the principles stated in Regulation (EC) No 1332/2008. As such, they must: (i) be safe when used, (ii) meet a technological need, and (iii) not mislead the consumer. In addition, food enzymes should be kept under continuous observation; even if the use of an enzyme has been approved, it can be re-evaluated, if necessary. Enzymes intended for human consumption (e.g., nutritional or digestive purposes), and microbial cultures traditionally used in the production of food (e.g., cheese and wine) but not specifically for enzyme production, are out of the scope of this Regulation.

The authorization procedure to establish, manage, and update a community list for food additives, food enzymes, and food flavorings was established by the Regulation (EC) No 1331/2008 of the European Parliament and of the Council of

16 December 2008 with the intent to facilitate free movement of food while guaranteeing the health and welfare of consumers. Inclusion on the list is based on a risk assessment by EFSA; only substances included in these lists are authorized on the Community market. According to Regulation (EC) No 1332/2008, this list should be supplemented by information regarding origin, allergenic properties and purity [EC-1331][41] The creation of the first EU list necessitated risk assessments of the enzymes already present in the market and those which will be marketed in the future. Enzyme producers were asked to provide dossiers containing the necessary information.[42] The application process for the submission of dossiers started in September 2011 and finished in March 2015 (Article 17 of the Regulation (EC) No 1332/2008). Due to the high number of the dossiers received (>300), the Union list of authorized food enzymes (Community list) is not established yet [as of October 2018][43] Prior to adoption of the Community list of food enzymes, Regulation (EC) No1333/2008 will apply to food enzymes falling within the scope of Regulation (EC) No 1332/2008 [[37], EC.-1333 [38]].

The EU classification of qualified presumption of safety (QPS) serves a similar purpose to GRAS in the US. Microorganisms which are assigned to the QPS group do not need to undergo full safety assessment and are listed on the EFSA website.[44] For a microorganism to be considered as QPS, the taxonomic identity must be well defined; the available information must be sufficient to establish its safety; lack of pathogenic properties must be established and substantiated; and its intended use must be clearly described. Microorganisms that do not fulfill those criteria must undergo a full safety assessment.

The Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 established rules for the contained use of genetically modified microorganisms (GMMs) in order to protect human health and the environment. In Directive 2009/41/EC, a genetically modified microorganism (GMM) is defined as "*a microorganism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination*". Genetic modification is defined to include: (i) rDNA techniques covering the insertion of nucleic acid molecules produced outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation, (ii) techniques involving the direct insertion of genetic material into a microorganism, including micro-injection, macro-injection, and micro-encapsulation, (iii) cell fusion or hybridization techniques which cover fusion of two or more cells by means of methods that do not occur naturally. The contained use is defined as "*any activity in which microorganisms are genetically modified or in which such GMMs are cultured, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment*". The Directive states that the development of biotechnology, involving the use of (GMMs), contributes to the economic expansion of the Member States. However, a case-by-case risk assessment is required as the nature and scale of risks associated with the contained use of GMMs are not yet fully known.[45] Industrial GM microorganisms (including those used for food enzyme production) fall into this directive.

Although there are international standards for the safety assessment of GMOs (including GMMs), many countries have also developed their own legislation. The regulatory system for GMO safety evaluation in the EU, which follows the main international principles developed by CAC and OEC, is one of the most comprehensive legislations in the world.[8] In the EU, genetically modified food and feed is regulated under the Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003. The Regulation covers food and feed produced 'from' a GMO but not food and feed 'with' a GMO. This is a similar distinction between the 'food additive' and 'processing aid'. Food and feed produced 'from'

a GMO covers materials derived from the GM source and is present in the final food or in the feed product. This type of food and feed are regulated under Regulation (EC) No 1829/2003. Food and feed produced 'with' a GMO refers to the materials which are used during processing and are not present in the final product [EC-1829][46] Food enzymes which fall within the scope of Regulation (EC) No 1829/2003 on genetically modified food and feed are also regulated according Regulation (EC) No 1331/2008 on food enzymes [EC-1332][39]

The first GMOs were introduced on the European market in 1996.[47] GMOs can be approved and marketed both as animal feed and food in the EU. In the EU, the risk assessment of GMMs which are involved in the production of a variety of food and feed is performed by EFSA through its scientific panels. The risk assessment process is a prerequisite that must be fulfilled before the products are commercialized and placed on the market.[48] For products obtained by fermentation of GMMs which fall under Regulation (EC) No 1829/2003 and/or Regulation (EC) No 1332/2008, EFSA has published a guidance document on the risk assessment of GMMs and their products intended for food and feed use.[49] Four categories of GMMs have been designated, with increasing levels of information required for safety assessment. Most enzyme preparations are considered under Category 2, which involves complex products in which both GMMs and newly introduced genes are no longer present. This is the 'produced with GMO' case and food enzymes in Category 2 fall under the scope of Regulation (EC) No 1332/2008.

Two aspects are considered in the risk assessment of products (i.e. food enzymes) with GMMs: characterization of the GMM; and the potential effects of its modification with respect to product safety, including cases when the GMM itself is the product. For the characterization of GMM, the recipient/parental organism, the donor(s) of the genetic material, the genetic modification, and the final GMM and its phenotype should be defined. For the product, the stages of the production process of the GMM (fermentation, cultivation) should be described and information relating to the product preparation process should be presented. The product should be described in terms of its identity, intended use and mode of action, composition, physical and technological properties. The GMM and/or its product for human health should be considered in terms of potential toxicity, allergenicity, nutritional value. Finally, exposure assessment/characterization related to food and feed consumption should be performed and the potential environmental impact of GMMs and their products should be evaluated. As a result of these investigations, the panel shares its scientific opinion on the safety of the product. This opinion serves as a basis for the different European regulatory authorities to make a decision on the commercialization of the product.[48,49]

Regulation (EC) No 1332/2008 also states that food enzymes that are QPS are subject to the general labeling obligations with respect to both traceability and labeling of genetically modified organisms, and the traceability of food and feed products produced from genetically modified organisms. In labels food enzymes should be designated by their technological function in food, followed by the specific name of the food enzyme. Labeling must be easily visible, clearly legible and indelible.

CASE STUDY: REGULATION POLICIES AND SYSTEMS IN TURKEY

Turkey is an example of a country with an expanding food sector that utilizes enzymes, including imported rDNA enzyme products, in food production. Due to its large and dynamic food industry capacity, Turkey exports food products to many countries. There is also an increasing trend of R&D expenditure in Turkey; the ratio of the gross domestic expenditure on R&D (GERD) to gross domestic product (GDP) rose from 0.69% to 0.96% in the decade from 2007 to 2017.[50] This acceleration is an indication of the increased importance given to R&D activities in Turkey; however, the biotechnology sector has not moved forward as rapidly as other sectors. Despite experienced researchers and

expertise in biotechnology, it has been difficult to achieve commercialization. To fill this gap, the Ministry of Industry and Technology published the "Turkey Biotechnology Strategy and Action Plan" for 2015–2018. The intent is to stimulate R&D and technology innovation capacity in the fields of health, agricultural, and industrial biotechnology, and to make Turkey a center for the development of innovative, high value-added products suitable for global competition.[51]

The domestic enzyme production is not currently sufficient for the needs of the Turkish food industry, making Turkey an importer of food enzymes. To ensure safety of the imported food enzymes for use in the food sector in Turkey, the enzyme products imported from other countries must pass through an intense and rigorous approval process. Turkey applied to join the European Economic Community in 1987, and was declared eligible to join in 1997. Turkey is still a candidate country for EU membership.[52] As a result of the harmonization process with the EU, regulations being implemented in Turkey are quite similar to the regulations of EU. However, there are also some differences, as described below, reflecting societal and legal considerations. The Turkish Food Codex Food Additives Regulation was prepared in parallel to the Regulation (EC) No 1333/2008 on food additives of the EU[53] Similar to Regulation (EC) No 1333/2008, the purpose of the Turkish regulation is to specify the list of food additives, food enzymes and food flavorings; conditions for their usage in foods; and labeling rules. Subsequently, food enzymes were removed from this regulation and are instead covered by the Turkish Food Codex Food Enzymes Regulation [24 February 2017].[54]

Similar to the EU, the purpose of the Turkish Food Codex Food Enzymes Regulation is to establish the list of permitted food enzymes; conditions for their usage in foods; and rules and procedures for labeling of food enzymes, including enzymes used as processing aids. The creation and updating of the list are carried out either by the General Directorate, or upon the application made by a food business operator or an organization that represents the relevant food business operators. The evaluation is carried out in accordance with the provisions set forth in the Regulation on the Joint Permission Procedure for Food Additives, Food Enzymes and Food Aroma Substances of Turkish Food Codex Food Enzymes Regulation (2017).[54] Due to the current laws and regulations applicable to food enzymes, the long and costly approval and implementation process will become easier with the establishment of these lists. For food enzymes imported, produced, processed and/or marketed before the date on which the Regulation enters into force, it is not necessary to comply with provisions of the regulation. However, after listing, it will be obligatory to obtain permission for each food enzyme not included in this list.[54]

The Turkish Food Codex Food Enzymes Regulation also stipulates that consumer and human health, consumer rights, fairness in the trade of food and, where appropriate, protection of the environment, are also taken into consideration. Similar to the EU Regulation (EC) No 1332/2008 on food enzymes, the Turkish Food Codex Food Enzymes Regulation defines a food enzyme as: a product obtained from plants, animals or microorganisms, or a product containing one or more enzymes capable of catalyzing a specific biochemical reaction and obtained by a fermentation process using microorganisms; or, a product obtained by fermentation using various microorganisms and added to food for a technological purpose at any stage of the production, processing, preparation, treatment, packaging, transport or storage of foods. A food enzyme preparation is defined as a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardization, dilution or dissolution.[54]

A food enzyme can be listed as permitted if: (i) the recommended amount of use of the enzyme-based on current scientific evidence does not pose a risk in terms of consumer

health; (ii) the enzyme is used due to a reasonable technological need; (iii) its use, including the structure, freshness, quality of the components used or naturalness of the product or production process and nutrition quality of the product does not mislead consumers; and (iv) the enzyme obeys other relevant legislation. For the listed enzymes, there should be information about the name of the food enzyme, the source of the enzyme, purity criteria and other necessary information, the foods to which the food enzyme can be added, and the conditions under which the enzyme can be used. Additional specifications may also restrict the sale of a food enzyme directly to the final consumer, or require labeling of the food enzymes used in the production of food to ensure that the final consumer is directly informed of the physical condition of the food or of the specific treatments to which it has been subjected.[54]

The Turkish Biosafety Law (No. 5977) [published in the Turkish Official Gazette (No. 27533), 26 March 2010] describes biosafety as the safe operation of GMOs and their products in order to protect human, animal and plant health and environment and biological diversity. Four types of GMO-related products are defined. Purified food enzymes fall into the fourth category: (iv) products obtained from GMOs - products that are partially or fully derived from GMOs but do not contain or are made from GMOs.[55] Provisions of the Regulation on Genetically Modified Organisms and Their Products [published in the Turkish Official Gazette No. 27671, 13 August 2010][56] are carried out by the Republic of Turkey Ministry of Agriculture and Forestry and cover: (i) application, evaluation, decision, processing, packaging, labeling, storage, transportation, placement on the market, import, export, transit, monitoring, inspection and control related to GMOs and their products for food and feed purposes; (ii) research, development and trial studies under controlled conditions of GMOs and their products which are imported or developed within the country; and (iii) application, evaluation, decision, import, export, processing, labeling, placing on the market, monitoring, inspection and control activities related to GMMs and closed area conditions such as laboratory and facility where indoor activities will be carried out. It should be noted that the GMO Regulation covers both commercial activities and research and development studies.

Applications, application documents, scientific evaluation reports, and decisions are announced to the public through the Biosafety Information Exchange Mechanism. The purpose of the exchange mechanism is to facilitate the effective sharing of the information and documents related to GMOs and their products at national and international levels, to inform the public and to ensure the participation of the public in the decision-making process (Turkey Biosafety Information Exchange Mechanism, 2019a).[57] While there is no obligation to apply for approval for R&D studies related to GMOs in Turkey, it is obligatory to inform the Ministry of Agriculture and Forestry about the subject and the result of the activity, and permission must be obtained from the Ministry for GMOs and their products to be imported for research, development and education purposes.

Similar to the concept of GRAS or QPS, a simplified decision-making process based on existing information and previous risk assessment that there is no risk of the GMO and its products, and no harm to human, animal and plant health, or environment and biological diversity, has been defined in the Turkish Biosafety Law (No. 5977).[55] Knowledge of the taxonomy and biology of the target organism and the gene source, and sufficient information on the effects of GMO on human, animal, environmental health and biological diversity must be submitted (Article 6 of the Turkish Biosafety Law). There should be information from previous risk assessments that there is no negative effect of the GMO. In addition, it is necessary to have detailed methods and data to identify the transferred genetic material and identify it in the target organism into which it was transferred.

In order to import products containing GMOs and the products obtained from GMOs (such as food enzymes), it is mandatory that the GMO

from which these products are obtained has been approved (under Article 22). GMOs and their products and products obtained from GMOs are sampled and analyzed according to Turkish Veterinary Services, Plant Health, Food and Feed Law (No. 5996 under Article 23).-[58]Similarly, imported food enzymes produced from GMMs must be evaluated in Turkey according to Biosafety Law No. 5977 and the Regulation on Genetically Modified Organisms and Their Products. However, there is a difference between a GM plant and an enzyme produced by a GMM with respect to the presence of rDNA in the final product. A GM plant is, itself, an rDNA product; in contrast, after successful bioseparation processes, a food enzyme produced by a GMM does not contain rDNA. Based on this, a recommendation was released by the Biosafety Board (11 April 2015) which implies that there is no need for approval of the Biosafety Board for processing aids such as additives and enzymes produced from microorganisms, since there is no DNA in these products. In accordance with this decision, the Ministry has decided that imported processing aids such as additives and enzymes produced from microorganisms will only be evaluated according to Veterinary Services, Plant Health, Food and Feed Law (No. 5996).[59] This decision has prevented GMO-related bottlenecks and difficult approval processes for the imports of rDNA-free products, including food enzymes.

When the Biosafety Law and the related Regulation were first published, the upper limit values for GMO content were not defined. This led to the identification of each product containing the GMO as illegal, regardless of its concentration. Article 2 of the Regulation was amended in 2014 to state that if the product had less than 0.9% GMO, it would be considered as a GMO contamination (e.g. caused by residues from previous transportation of a GM-product in the container) and such products may be used for approved purposes (Official Gazette, No. 29014, 29 May 2014). The GMO contamination limit of 0.9% is in accordance with EU regulations. It is important to note, however, that the upper limit of 0.9% for GMO contamination applies only to those genes which are previously approved by the Biosafety Board[60] As of April 2019, there are 36 GMOs (10 soy and 26 maize genes) approved by Biosafety Board in Turkey, all as animal feed.[61]

In 2018 the Biosafety Board was abolished (in accordance with Article 206 of the Turkish Official Gazette No. 30473, 9 July 2018)[62] and the duties and responsibilities transferred to the Ministry of Agriculture and Forestry (Presidency Circular, Turkish Official Gazette No. 30497, 2 August 2018).[63] Responsibility for evaluation of the applications related to GMOs and products, conducting the other duties mentioned in the Biosafety Law and related regulations, and the secretariat services of the Committees currently reside with TAGEM (Turkey Biosafety Information Exchange Mechanism, 2019a).

There are significant differences between attitudes towards GMOs in the EU and Turkey. Most importantly, GMOs are approved only as animal feed in Turkey, where GMOs can be registered either as feed and/or food in the EU. In addition, severe criminal penalties, such as imprisonment and fines, have been defined for violations of the Biosafety Law. According to Article 14 of the Law, those engaged in activities related to GMOs and their products are liable for damages to the protection of human, animal and plant health and the environment, biodiversity and sustainability, even if they have obtained permission under the Biosafety Law. This responsibility is valid even if no damage has occurred if the GMO and its products are found not to meet the requirements in the application and decision. For example, According to the Article 15 of the Biosafety Law, a person who imports, produces or releases GMOs and products in contradiction with the provisions of the Law shall be sentenced to imprisonment for a term of 5 years to 12 years and a judicial fine (Turkish Biosafety Law, 2010).[55]

The differences and similarities of the regulatory systems on food enzymes in the U.S., EU, and Turkey are summarized in Table 2. In the U.S., the FDA has a more product-oriented approach, while in the EU, the EFSA adopts a process-oriented approach. As Turkey is in the nomination process of the EU, regulations are based on EFSA's methods and are compatible with the CODEX.

TABLE 2. Comparison of the regulatory systems on food enzymes in the US, EU, and Turkey.

	US	EU	Turkey
Regulatory body	USDA (FDA]	EFSA	Ministry of Agriculture and Forestry
Regulation	Code of Federal Regulations (CFR) Title 21 (Part 170)	Regulation (EC) No 1332/2008	Turkish Food Codex Food Enzymes Regulation
Date of the Regulation	15 March 1977 (Revised annually)	16 December 2008	24 February 2017
Definition of food enzyme	Food additives which are used to improve food processing and the quality of the finished food	A product obtained from plants, animals or microorganisms or products there of including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalyzing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.	A product obtained from plants, animals or microorganisms or a product containing one or more enzymes capable of catalyzing a specific biochemical reaction and obtained by a fermentation process using microorganisms; or, a product obtained by fermentation using various microorganisms and added to food for a technological purpose at any stage of the production, processing, preparation, treatment, packaging, transport or storage of foods.
Assumption safety on the basis of general use/ reasonable evidence	GRAS	QPS	Simplified Procedure

CONCLUSION

Ensuring sustainable production and consumption of healthy and safe foods is the central objective of food regulations. To this end, individual countries and international agencies have established legal regulations on foods and food additives. The increasing worldwide utilization of recombinant DNA technologies for foods and food products has led to evolving political and regulatory approaches. The well-established systems in the U.S. and EU, along with the CODEX, frequently serve as a basis for the development of regulatory systems for food and feed in other countries. Production and processing of many food products rely on enzymatic activities. The use of such enzymes, depending on their presence in the final product, may be considered as processing aids (food enzymes) or food additives. Advantages of cost, quality, and consistency have led to rapidly increasing utilization of food enzymes that have been produced from GMMs. Regulations for food enzymes from GMMs are currently evolving in the EU and national agencies, including the establishment of lists of authorized food enzymes produced from such sources that may facilitate the application process for designated enzyme sources.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

LIST OF ABBREVIATIONS

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
CAC	Codex Alimentarius Commission
CCFAC	Codex Committee on Food Additives and Contaminants
CFR	Code of Federal Regulations
cGMP	Current good manufacturing practice
CODEX	Codex Alimentarius
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
FAO	Agriculture Organization of the United Nations
FAS	Foreign Agriculture Service
FDA	United States Food and Drug Administration
GDP	Gross domestic product
GERD	Gross domestic expenditure on R&D
GM	Genetically modified
GMM	Genetically modified microorganism
GMO	Genetically modified organism
GRAS	Generally recognized as safe
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
OECD	Organisation for Economic Co-operation and Development
OFAS	Office of Food Additive Safety
QPS	Qualified presumption of safety
R&D	Research and development
rDNA	Recombinant DNA
TAGEM	General Directorate of Agricultural Research and Policies
US	United States
USDA	United States Department of Agriculture
WHO	World Health Organization
WTO	World Trade Organization

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