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Safety and Regulatory Aspects of Food Enzymes: An Industrial Perspective

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Abstract
Linkages between diet habits and the quality of life continue to surface on numerous fronts. Safety concerns associated with food enzymes in general are possible allergenic, irritative and otherwise toxic properties .Oral toxicity is especially relevant to consumers of food enzymes**.** The regulation of enzymes internationally is quite varied between countries, with specific country either requiring a full approval process, a notification of enzyme or no requirement thereof. Pre-market approval may depend on whether an enzyme is classified as processing aid or a food additive, though the point of consideration regardless of classification is that the safety of the enzyme must be assured. Challenges to regulators and industry arise from unresolved issues and from lack of harmonization of both legislation and safety evaluation**.** The regulation of enzyme for Australia and New Zealand are contained within horizontal standards of processing aids. This standard regulates the use in food manufacture, stopping them from being used in food unless there is a specific permission in standard. In US enzymes classified as food additives or as GRAS, while certain enzymes are regulated as secondary direct food additives under the regulation. Finally the act permits an individual or company to decide on its own that based on the appropriate data whether an ingredient is GRAS for a particular intended use, and to market the ingredient for that use without any prior contact with the agency. In the EU, most food enzymes are not covered by food safety regulations neither on community nor on national level. On top of this availability of enzymes with new and unusual properties raises questions of safety. There seems to be a chance that these challenges will be tackled in the course of establishing a harmonized legislation on food enzymes.

Key words- food enzymes, regulations, health concern, market regulators, food additives, processing aids.

Introduction
Food processing through the use of biological agents is historically a well-established approach. The use of food biotechnology dates back to thousands of years ago (6,000 B.C) to the time of the Sumerians and Babylonians. They then used yeast to make fermented beverages such as beer, baking bread, and cheese and wine, The science along the Nile (around 2500 B.C) includedthe ancient Egyptians made wine using fermentation techniques based on an understanding of the microbiological processes that occur in the absence of oxygen, whereas the first purposeful microbial oxidation dates from 2,000 BC, with vinegar production.The use of plant enzymes such as malts were also used millennia ago even before the actual understanding of enzymes. Columbus, beginning with his first visit to the Americas in 1492, he along with other explorers introduced corn which then was native to the America, but now to the rest of the world, and Europeans adapted the plant to their unique growing conditions. Spanish navigators also returned with potatoes, which were then native to the Andes in South America.

Two centuries after their European introduction, potatoes became the staple food in Ireland, Germany and other European countries .Further advancement in food biotechnology occurred with the invention of the microscope by Anton van Leeuwenhoek, which led to the discovery of microorganisms which would then be used in food production. In 1871 when Louis Pasteur discovered that heating juices to a certain temperature would kill off bad bacteria which would affect wine and fermentation, food biotechnology enhanced. The same process was then used in milk production, heating milk to a certain temperature to improve the hygiene thereof.[1]

In the mid-1800s, **Gregor Mendel** carefully studied the principle of heredity. Experimenting with garden peas, he cross-bred the traits such as pea color, plant height and pod size. Mendel showed that differences, such as a plant's height or color, could be attributed to the passing of traits and genes — the basic building blocks of life. In the early 20th century, agricultural expert **Henry Wallace** applied the principles of hybridization to develop new, higher-yielding seeds.[2]

Coming to modern days, in the late XIX, century **Christian Hansen** found the use of rennet (a mixture of Chymosin and Pepsin) in production of cheese and bacterial amylases started at Genencor. Pectinases were used for juice clarification in the 1930s, during World War II,Invertase was also used for the production of invert sugar syrup in a process that pioneered the use of immobilized enzymes in the sugar industry. Still, the large-scale application of enzymes only became really established in the 1960s, when the traditional acid hydrolysis of starch was replaced by an approach based in the use of amylases and amyloglucosidases (glucoamylases), a cocktail that some years later would include Glucose (xylose) isomerase.[3]

From then on, the trend for the design and implementation of processes and production of goods anchored in the use of enzymes has steadily increased. Enzymes extracted from edible plants and the tissues of food animals, and those produced by microorganisms (bacteria, yeasts, and fungi), have been used for centuries in process of food manufacturing. Rennet is an example of a natural enzyme mixture from the stomach of calves or other domestic animals that has been used in cheese making for centuries. It contains a protease enzyme which is responsible for the coagulation of milk, causing it to separate into solids (curds) and liquids (whey). Alternatively, for centuries enzymes produced by yeast have been used to ferment grape juice in order to make wine. [4]

In the twentieth century, enzymes began to be isolated from living cells, leading to a large-scale commercial production and with wider application in the food industry.Microorganisms being the most important source of commercial enzymes today. Although they do not contain the same enzymes as plants or animals, a microorganism can usually be found to produce a related enzyme that will catalyze the desired reaction. Enzyme manufacturers have optimized microorganisms for the production of enzymes through natural selection and classical breeding techniques. Food Biotechnology has grown to include cloning of plants and animals, as well as more development in genetically modified foods in more recent years.

Scientists first discovered that DNA can transfer between organisms in 1946.The first genetically modified plant was produced in 1983, using an antibiotic-resistant tobacco plant. In 1994, the transgenic FlavrSavr tomato was approved by the FDA for marketing in the US - the modification allowed the tomato to delay ripening after picking.In the early 1990s, recombinant chymosin was approved for use in several countries, replacing rennet in cheese-making. In US in 1995, the following transgenic crops received marketing approval: canola with modified oil composition(Calgene), Bt potatoes (Monsanto), soybeans resistant to the herbicide glyphosate (Monsanto), virus-resistant squash (Monsanto-Asgrow), and additional delayed ripening tomatoes (DNAP, Zeneca/Peto, and Monsanto).In 2000, with the creation of golden rice, scientists genetically modified food to increase its nutrient value for the first time. As of 2011, the U.S. leads a list of

multiple countries in the production of GM crops, and 25 GM crops had received regulatory approval to be grown commercially.As of 2013, roughly 85% of corn, 91% of soybeans, and 88% of cotton produced in the United States are genetically modified.[5]

REGULATORY FRAMEWORK REGARDING FOOD ENZYMES

Food enzymes are a growing business worldwide worth about 1 billion US$. While both of the number available food enzymes and their annual turnover have been steadily increasing for many years so has the number and the kind of applications. As of April 2014 the major industry association AMFEP (Association of manufacturers and formulators of enzyme products) lists about 247 enzymes manufactured for the use in food industry. As enzymes are often used to replace steps in food processing encompassing harsh chemical or physical conditions they are perceive to be in line with both sustainable industrial production and careful processing of food in order to maintain nutritionally important ingredients such as vitamins, etc.

Enzymes have never been a focal issue either for regulators, consumer group or general public. In the beginning of 1990s this started to change when consumer and environmental groups were alarmed by increasing use of genetically modified microorganism for enzyme production. This eventually led to reviews of safety concerns and regulatory aspects, especially in Germany, Switzerland and Austria.

The fundamental aspects whether a pre-market approval is needed or what particular information manufacturers have to provide in the course of safety evaluation. In food legislation on food enzymes there has been a distinction on the basis of food additives and processing aids. Food additives are the substances not normally consumed as food in itself or as a characteristic ingredient with or without nutritive value instead used for technological purpose or to obtain some reasonable results whereas processing aids do not have any technological effect on finished food product moreover no health risks are related. In Canada, USA and japan for instance all food enzymes are regulated as food additives. In Australia food enzymes are considered as processing aids.

The joint FAO/WHO Expert committee on Food Additives (JECFA), has laid voluntary safety reviews on food enzymes since 1971 and does not differentiate on the basis of these categories. In the EU food legislation the food enzymes are considered as processing aids. Though this differentiation is not followed by the European commission’s own scientific committee SCF (Scientific Committee on Food) which is responsible for evaluation of food additives and its toxicity.[6]

FDA requires most detailed information on enzymes including technical data, structural modifications of GMMs and also the manufacturing and purification process and takes long term experience with certain enzymes; a GRAS status may be assigned to such enzymes. The JEFCA guidelines also require more detailed technical data on enzymes from GMM, whereas the SCF asks greater details on the vectors, data on manufacturing process, the usage and stability in food. JEFCA has laid down general principles for toxicological testing in separate publication. AMFEP guidance does not include detailed description of requirements; however it acknowledges the guidelines from JEFCA, SCF, COT.

Food enzymes and their cited uses in:

Table 1: Dairy Industry

Table 2: Baking Industry

Table 3: Juice industry

Table 4: Starch Industry

Table 5: Brewing Industry

Table 6: Animal Feed Industry

GOVERNMENT POLICIES TOWARDS FOOD IN BIOTECHNOLOGY

National Government .have embraced biotechnology as being an emerging technology similar in potential to microelectronics and informatics technology. Just as the development of very large scale integrated computers, so as in popular perception biotechnology holds out the promise of cure for almost every disease and manufacture of high value products at low cost.

Enzyme Regulation in Australia & New Zealand

The enzymes that are used in food processing are regulated as food additives. An enzyme is defined as a food additive, as prepared in proposal P276 of the *Food Standards Australia New Zealand (FSANZ),* to reviewthe regulation of enzymes as processing aids in clause 15, 16 and 17 of standards 1.3.3 processing aids. A separate proposal, P277 –Review of Processing Aids (other than enzymes), was finalized and gazette on 15th February 2007.

Proposal P276 focuses on the review of enzymes, apart from the other processing aids since FSANZ considers the assessment of safety, managing risk and technological issues were different for enzymes as distinct from other processing aids. Therefore matters considered as a part of this review include:

- The safety assessment of recently approved enzymes and the guidelines for the same and also the by-products of enzymatic reactions.
- The naming of the enzymes and source organism.
- Enzymes not being used in Australia and New Zealand currently.
- Other issues raised by submitters.

FSANZ has reviewed clauses, and has proposed a number of draft variations bringing changes to maintain health and safety, update nomenclature of enzymes and their sources, correction of errors, removing duplication and anomalies, enhancing the consistency and thus improving the function of standard [7].

REASONS FOR DECISION

- The proposed amendment strongly deals with the protection of public health and safety since no safety concerns were identified during the safety assessment.
- The proposed amendments also ensure consistency within the code and improvements with other international food standards.
- The proposed amendments have included and submissions on issues received, as well as advice from an expert advisory group, made up of experts external to FSANZ.
- These proposed amendments would not lead to any expected added costs to food manufacturers, consumers or regulatory agencies.
- The proposed amendments have made the regulations more cost effective.

TABLE 7: **SAFETY ASSESSMENTOF PERMITTED ENZYMES**

EUROPEAN UNION REGULATION

Regulation (EC) No 1332/2008 on food enzymes - the so called 'Framework regulation', for the first time harmonizes rules on food enzymes in the EU and fixed a deadline of 2 years for the submission of applications for authorization.

So far only two enzymes, **lysozyme** and **Invertase,** are considered as additives. The majority of about 160 food enzymes produced and marketed are however considered as processing aids, the regulation of which is still governed by national legislation. The EU member states differ in regulations like in France, Denmark, Poland and Hungary these enzymes are subjected to an authorization procedure, in the United Kingdom a voluntary approval system is in place.

In EU food additives are regulated by council directive **94/34/EEC** on the approximation of the laws of the member states concerning food additives authorized for use in food stuffs intended for human consumption. Most food additives may only be used in limited quantities in certain food stuffs. To achieve the technological effect, they must be according to the **GMP** (good manufacturing practice), where there is no health hazards and fulfill the purity criteria of the commission directives.

In 2003, European Food Safety Authority (EFSA) took over the responsibility from SCF for the evaluation of food additives for their safety with a scientific panel AFC. SCF considers enzymes from (edible) plant and animal sources species as posing no health problems, but the microbiological source is a concern of toxicological evaluation. Exposure deals with the quantity of enzyme consumed.

Till 2006 only five food enzymes had been evaluated by SCF and one by AFC:

- Thrombin
- Fibrinogen
- Urease
- Papain
- Inveratase
- Lactoperoxidse with glucose oxidase

The experienced gained in the meantime showed that the initial deadline for submitting applications was insufficient in order to allow stakeholders and in particular small and medium size enterprises to produce all necessary data within that period. Therefore, insteadof 24-month the period extends to 42 months by Commission Regulation (EU) No 1056/2012 thus amending the Regulation (EC) No 1332/2008.

Industry has now three years and a half to make available the information for the risk assessment of food enzymes and submit applications on existing and new enzymes starting from 11 September 2011 (Regulation (EC) No 234/2011). These food enzymes will undergo a safety evaluation by EFSA and will be approved by 'comitology' procedure (establishing the EU list).[8]

Till the time an EU list of food enzymes is drawn up, national rules on the marketing and use of food enzymes and food produced with food enzymes will continue to prevail in EU countries.

Other pieces of EU legislation relevant to food enzymes are the following:

- **Regulation (EC) No 1331/2008** - Establishes the common authorization procedure for food additives, food enzymes and food flavourings.

- **Regulation (EU) No 234/2011** - The common authorization procedure was implemented and came into force from 11 September 2011. That Regulation was amended by Commission Implementing Regulation (EU) No 562/2012 which laid derogation from submitting toxicological data in some specific cases and the possibility of grouping food enzymes under one application under certain conditions.

Scope of Enzyme Regulation

The food enzymes added for some technological purpose in the manufacturing, processing, preparing, treating, packaging, transporting or storing of food, including enzymes used as processing aids.

Limitations

- Enzymes intended for nutritional or digestive purposes of human consumption;
- Food enzymes used in the production of food additives under Regulation EC 1333/2008 and in the production of processing aids.

Microbial cultures traditionally used in the production of food (cheese, wine), which may incidentally produce enzymes but are not specifically used to produce them, are not considered food enzymes.

A food enzyme is included in the EU list if:

- It is not the reason of health concern to the consumer;
- There is a technological need;
- Its use does not mislead the consumer.

TABLE 8: Permitted enzymes in EU

US REGULATIONS

Enzymes used in food industry are regulated by US Food and Drug Administration (FDA) under the Food, Drug and Cosmetic (FDC) Act. Food ingredients may be "food additives" that are approved by **FDA** for specific uses or **GRAS** (generally recognized as safe) substances. A substance may be GRAS only if its general recognition of safety is based on the views of experts qualified to evaluate the safety of the substance.

GRAS status may be based either on a history of safe use in food prior to 1958 or on scientific procedures, which require the same quantity and quality of evidence as would be required to obtain a food additive regulation. Because GRAS status may be either affirmed by FDA or independently by qualified experts therefore FDA's regulations do not include all GRAS ingredients and the specific uses described in the GRAS regulations may not be comprehensive for the listed ingredients.

The FDC Act requires the approval of food additives prior to marketing. GRAS substances in contrast are not subjected to approval or notification to the FDA prior to marketing. The extent of toxicological testing of food additives depends on the assignment of a concern level on the structural features and on an estimation of exposure.

In order to evaluate the safety of an enzyme, FDA compares the enzymes to be assessed with the enzymes which are already approved or with those that have been safely consumed as a part of diet throughout human history.[9]

There is a list on FDA's regulations in **Title 21** of the **Code of Federal Regulations** (21 CFR), includes approved food additives and substances whose GRAS status has been affirmed by FDA. In addition, enzyme preparations may be the subject of a GRAS notice.

TABLE 9: Enzyme preparations approved as food additives listed in 21 CFR 173 and **affirmed as GRAS** in 21CFR 184

OTHER CONSIDERATIONS

An overall safety assessment of each enzyme preparation intended for use in food or food processing must be performed. This assessment should include an evaluation of the production organism, the enzyme component, side activities. The manufacturing process, and the consideration of dietary exposure. Guidelines for safety assessments of food enzyme preparations derived from microbial strains have been developed**[10].**Further, several internationally recognized scientists and expert groups have prepared guidelines for the safety assessment of food and food ingredients developed through biotechnology [11]which are applicable to enzyme preparations derived from recombinant sources**.**

FAO/WHO

Joint FAO/WHO Expert Committee on Food Additives (**JECFA**) has been conducting safety evaluations of enzymes since ten years before the SCF guidelines. It serves as a scientific advisory body to FAO, WHO, their member states, and the Codex Alimentarius Commission, primarily through the Codex committee on Food Additives and Contaminants and the Codex Committee on Residues of Veterinary Drugs in Foods [12]. The committee meets to

- Elaborate principles for evaluating their safety
- Conduct toxicological evaluations and establish acceptable daily intakes (ADI) or tolerable intakes
- Prepare specification of purity for food additives
- Assess intake

The first guidelines, General Specification for Enzyme Preparations Used in Food Processing was issued in 1981. It described requirement for technical data, source material, additives and processing aids used in enzyme preparation as well as hygiene and contaminants. The guidelines including all amendments and supplements until 1999 were published in an updated version of the Compendium of Food Additive Specifications published as FAO Food and Nutrition Papers. The evaluation procedure by the JEFCA is neither mandatory nor does an approval or rejection have any legal status.

REVIEWS OF BUSINESS COST CALCULATORS ON REGULATIONS

The regulation is set to improve the functioning of the internal market by removing disparities among member states and bringing more legal certainty to the market.

Only authorized food enzymes will be allowed to be commercialized and /or used in foods sold in the EU- irrespective whether they are used as processing aids or ingredients. This also applies to the imported food**. [13]**

TABLE 10: List of Business Regulators

TABLE 11: Identity: The characteristics of commercial food enzymes actually on the market have insufficiently been taken into account

FUTURE PROSPECT

From the above review several challenges for both further development of food enzyme regulation and for safety evaluation can be identified. These challenges can be attributed to differences in both existing legislation and in requirements for safety evaluation**.**Being foods, functional foods need to be safe according to all criteria defined in the current regulations. But in many cases, new concepts, new procedures will need to be developed and validated to assess functional food risks.The provision of safe, nutritious, high quality and affordable food to consumers is the central objective of EU policy, which should cover all stages of the food supply chain, "from farm to fork". Its standards and requirements should aim to ensure a high level of food safety and nutrition within an efficient, competitive, sustainable and innovative global market.

However, a series of emerging challenges and risks could put the currently successful food system under severe stress. These challenges include demographic imbalances, climate change, resource and energy scarcity, slowing agricultural productivity, increasing concentration of the supply chain, price volatility, changing diet trends.

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8) European Parliament and Council Directive No 95/2/EC on food additives other than color and sweetners,(1995)(http://ec.europa.eu/food/food/fAEF/enzymes/legislation_en.htm)

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11) OECD,1993: Health Canada,1994; FAO/WHO,1996; and Jonas et al, 1996

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13) Ta manakoungakal ;Food Standards Australia and New Zealand :Final Assessment Report Prosposal P276 Review of processing Aids (enzymes) (2007) (pg 63- 70)

TABLE 1 Dairy Industry

S.NO	INDUSTRY	ENZYME	USE
1.	DAIRY INDUSTRY	lipases	Cheese flavor, in-situ emulsification for dough conditioning, support for lipid digestion in young animals, synthesis of aromatic molecules
		lysozymes	Prevents the growth of clostridia in hard cheese, which contributes to flavor and structural defects such as late blowing
		Chymosin, papain	Protein hydrolysis, milk clotting, low-allergenic infant-food formulation, enhanced digestibility and utilization, flavor improvement in milk and cheese, meat tenderizer, prevention of chill haze formation in brewing
		Cyclodextrin	Boosts flavor in milk
		Glycosyltransferase	Removes cholesterol from milk fat
		Amino Peptidase	Hydrolysis of proteins (namely, soy, gluten) for savoury flavors, cheese ripening
		β-galactosidase	Removal of lactose from milk
		lactases	Lactose hydrolysis, whey hydrolysis
		L-arabinose Isomerase	Tagatose production
		Esterases	Flavouring of milk products, fat modification, cocoa butter, fat emulsion
		Catalases	Sterilization of milk, butter often in conjugation with glucose oxidase
		Bovine	**Cheese** manufacturing
		rennet	As a coagulant

TABLE 2 Baking Industry

S.NO	INDUSTRY	ENZYME	USE
2.	**Baking Industry**	Alpha amylase (bacterial and fungal)	Bread softness and volume, flour adjustment, ensuring uniform yeast fermentation Bacterial- starch conversion, alcohol & glucose production Fungal-acid resistant& maltogenic saccharification
		β-xylanase	Viscosity reduction, enhanced digestibility, dough conditioning
		Glucose oxidase	Dough strengthening(removal of oxygen)
		Laccases	Clarification of juices, flavor enhancer (beer)
		Lipoxygenase	Dough strengthening, bread whitening
		Transglutaminase	Modification of viscoelastic properties, dough processing, meat processing
		Galactosidase	Viscosity reduction in lupins and grain legumes used in animal feed, enhanced digestibility
		Glucanases	Viscosity reduction in barley and oats used in animal feed, enhanced digestibility
		Phospholipase	In-situ emulsification for dough conditioning
		Beta amylase	Maltogenic sacharification, supplement to bread

TABLE 3 Juice industry

S.NO	INDUSTRY	ENZYME	USE
3	**Juice Industry**	Amylase	Juice treatment
		Glucoamylases	Saccharification
		pectinases	Mash treatment, juice clarification
		cellulases, Laccase,maltase Naringinase Limoninase Hemicellulose Catalases	
		ALDC, (glucose oxidases)	Liquid coffee concentration, flavor, color extractor, juices

TABLE 4 Starch Industry

S.NO	INDUSTRY	ENZYME	USE
4	**Starch Processing**	Alpha amylase	Starch liquefaction and sacharification Sucrose hydrolysis, production of invert sugar syrup
		Invertase	Saccharification
		Pullulanses	High fructose corn syrup
		Glucoisomerase, β-amyalse, glucoamylase, Isoamyalse, Glycotransferases. pancreatin	

TABLE 5 Brewing Industry

S.NO	INDUSTRY	ENZYME	USE
5	**BREWING INDUSTRY**	Fructosyltransferase (levansucrase,inulase)	Tequila processing, sweetner
		β-glucanase	Wine production
		Pullunases Protease	
		Amyloglucosidases	Light beer
		Xynalase	Viscosity reduction, enhanced digestibility, dough conditioning
		ALDC(Acetolactate decarboxylase)	Beer maturation
		Cellulose Glucose oxidase	Wine industry Removal of oxygen from wine

		Pectinase	Wine clarification and pectin removal
		Anthocyanase	Decolorizing grapes
		Ficin	Beer ,ale
		Bromelain Maltase Pentosanase Pepsin urease	

TABLE 6 Animal Feed Industry

S.NO	INDUSTRY	ENZYME	USE
6	**Animal Feed**	**Phytase** **Amyloglucosidase**	

TABLE 7: **SAFETY ASSESSMENTOF PERMITTED ENZYMES**

Alpha Acetolactate decarboxylase	**Enzyme of low toxicity (2500 mg/kg bw/day) (14 day study)**
Alpha amylase	**Low oral toxicity**
Hexose oxidse	**Low oral toxicity**
Invertase	**No toxicological or other safety concerns**
Maltogenic amylase	**No toxicological or other safety concerns**
Xynalases	**No toxicological or other safety concerns**
Beta glucanase and xylanase	**Enzyme of low oral toxicity**

TABLE 8: Permitted enzymes in EU

ENZYMES	USES
E1103 Invertase	Food additives other than colors & sweeteners
E1105 Lysozyme	Food additives other than colors & sweeteners
Ureases, Beta glucanase, Lysozyme	Wine subject
Rennet	Milk coagulating
Pectolytic, Amlolytic, Proteolytic Enz	Processing of fruit juices

TABLE 9: Enzyme preparations approved as food additives listed in 21 CFR 173 and **affirmed as GRAS** in 21CFR 184

Section in 21 CFR	Description of Enzyme Preparation
173.110	**Amyloglucosidase** for use in degrading gelatinized starch into constituent sugars.
173.120	**Carbohydrase** and cellulose for use in clam and shrimp processing
173.130	**Carbohydrase** for use in the production of dextrose from starch
173.135	**Catalase** for use in the manufacture of cheese
173.140	**Esterase-lipase** as a flavor enhancer in cheeses, fats and oils, and milk products
173.145	**α-galactosidase** for use in the production of sucrose from sugar beets
173.150	**aspartic proteinase** as a milk-clotting enzymes, microbial for use in the production of cheese
137.105	**α-amylase** in flour
184.1012	**Alpha-amylase** is used to hydrolyze edible starch to produce maltodextrin and nutritive carbohydrate sweeteners.
184.1024	**Bromelain** is used to hydrolyze proteins and polypeptides.
184.1027	Mixed **carbohydrase and protease enzyme** for use in hydrolyzing proteins and carbohydrates in the preparation of alcoholic beverages, candy, nutritive sweeteners and protein hydrolysates
184.1034	**Catalase** , used to decompose hydrogen peroxide
184.1316	**Ficin** (peptide hydrolase) to hydrolyze proteins and polypeptides
184.1372	**Insoluble glucose isomerase enzyme**
184.1387	**Lactase** for use in hydrolyzing lactose to glucose and galactose
184.1388	**Lactase** for use in hydrolyzing lactose in milk.
184.1415	**Animal lipase** (triacylglycerol hydrolase) used to hydrolyze fatty acid glycerides
184.1420	**Lipase** used in the interesterification of fats and oils
184.1443	Malt (**α-amylase and β-amylase**) to hydrolyze starch
184.1583	**Pancreatin** (peptide hydrolase) used to hydrolyze proteins or polypeptides
84.1585	**Papain**
184.1595	**Pepsin** (peptide hydrolase) used to hydrolyze proteins
184.1685	**Rennet** (animal derived) to coagulate milk in cheeses and other dairy products
184.1914	**Trypsin** used to hydrolyze proteins
184.1924	**Urease** enzyme for use in the production of wine
184.1985	**Aminopeptidas** used as an optional ingredient for flavor development in the manufacture of cheddar cheese

TABLE 10: List of Business Regulators

COMPANIES	NAMES
Genencor International Inc	*Alice Caddow*
Association of Manufacturers and Formulators of Enzyme(AMFEP)	*Karolien De Neve*
Australian Food and Grocery Council	*Tony Downer*
Enzyme Solutions Pty Ltd	*Geoff Bearzatto*
Food technology Association Victoria	*David Gill*
Department of Agriculture, Fisheries and forestry	*Trent Brday*
New Zealand Food Safety Authority	*Carole Inkster*
AMFEP	*Hubb Scheres*
Queensland Health	*Gary Bielby*
F&N	*Haydn Vesty*

TABLE 11: Identity: The characteristics of commercial food enzymes actually on the market have insufficiently been taken into account……

AMFEP :	a) Supports the option of maintain the status quQ b) Suggests that food enzymes have shown from history to be inherently safe and thus there is no need to evaluate enzymes not examined since 1996, except those that have been identified to have a toxicological concern c) Suggests there is no good reason to delete any enzyme. d) Suggests only where a new food use of a current enzyme or the food use is proposed should the by-product of enzyme reactions be considered. e) Suggests using the current enzyme commission of the international union of biochemistry nomenclature be used for the standard. f) Recommend the concept of Total Organic solids, should be used throughout the identity and safety sections. g) Proposes the strain deposition to be unnecessary. h) Enzyme dosage is very low (not more than 50mg TOS/kg body weight/day). A calculation of the consumer exposure using the budget method normally suffices to define a very high safety margin- even using an exaggerated maximum intake approach, supposing that all processed food was manufactured with the enzyme under consideration and that all enzymes remains in the final food. Thus to use this method and resort to more refined calculation only in the case where it would not provide a high enough safety margin**.**
GENENCOR	Supports the maintaining of status quo of the standard to ensure consistency in enzyme and their source. Same as AMFEP They favour specific attention for enzymes derived from genetically modified sources and the right consumers to be made aware when enzymes from a genetically modified

QUEENSLAND HEALTH	sources are used in production of food. Proposes proper classification of enzymes and supports inclusion of enzymes that AMFEP has previously classified as safe and similar to nomenclature as far as possible.
ENZYME SOLUTIONS	Proposed that the routine amendments will not have an impact under the Imported Food Control Act 1992.
DEPARTMENT OF AGRICULTURE, FISHERIES & FORESTRY	Supports the safety assessment of enzymes, sources and by-products of enzyme reactions.
NEWZEALAND FOOD SAFETY AUTHORITY	It cautions against deleting the use of any enzyme not currently used in Australia and New Zealand to include not currently used in any country to ensure any deletions would not inhibit the international trade.
AUSTRALIAN FOOD AND GROCERY COUNCIL	Only the enzymes that all stakeholders agree are obsolete should be removed.
FOOD TECHNOLOGY ASSOCIATION OF VICTORIA	Submits that the enzymes are reclassified as ingredients requiring labeling that the GM status be declared on the label to enable consumers to make an informed choice.
FOOD AND NUTRITION AUSTRALIA	