



Food Analysis: Environmental Effects, Application of Enzymes, Mass Spectrometry and Enzyme Applications in the Food Industry

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Abstract:

Mass spectrometry has recently come to light as an efficient and specific tool for evaluating and analysing many different types of food. We showcase the current state of the art in food safety and quality determination to show how this method can classify and grade products, detect defects and diseases, distribute and visualise chemical attributes, and evaluate the overall quality of meat, fish, fruits, and vegetables. There was a synopsis of mass spectrometry's characteristics for each grouping in terms of the quality and safety attributes that were studied, the systems that were used (orbitrap, quadrupole time-of-flight, triple quadrupole, feature extraction, multivariate calibration, variables selection), and the performance (correlation, error, visualisation). It is clear that mass spectrometry may help with a lot of common inspection jobs, given its success in various food safety and quality applications. The biological catalysts known as enzymes are essential in the food industry, where they catalyse both good and bad chemical processes. These substances can be intentionally added to foods or occur naturally in them. Enzymes and subenzymes from various sources can be used in food in various ways, including as microbe inhibitors, aroma adders, physico-chemical property improvers, candy crystallisation inhibitors, meat tenderizers, antioxidants, heat treatment indicators, and many more. The optimal parameters for the activity of an enzyme are pH, temperature, concentration, and water activity. In an effort to reduce the stress that enzymes endure throughout food processing, research on enzymatic immobilisation strategies has been conducted. Enzymatic active packages are one kind of immobilisation technique; these allow the enzymes to migrate to the product or be trapped within, extending the products' shelf life; this method is novel for the enzyme market as well as the packaging market.

Keywords: Food, Environmental Effects, Application, Mass Spectrometry, Food Industry

Introduction:

Food quality and food safety are not always the same thing, says the World Health Organisation (WHO). When we talk about food safety, we're referring to all the potential risks that consumers face, whether they're long-term or short-term. No wiggle room here. Everything else that affects the value a product has to the buyer is considered part of its quality. Included in this category are both positive and negative qualities of the food, including its origin, colour, flavour, texture, and processing method, as well as impurities, contamination, discoloration, and off-odors.

With 500 million customers, the food and drink industry is a cornerstone of the EU economy, making the significance of safe and high-quality food all the more apparent. As of 2011–2012, the industry employed 4 million people across over 280,000 businesses, and its turnover was 1016 million euros, placing it among the top three manufacturing industries in a number of Member States. France, Spain, the United Kingdom, Denmark, and Belgium all rank it #1 [2]. In order to make the EU food and drink industry more competitive, we need to find ways to turn the problems and demands of today into opportunities. For example, we need to ensure that products are of higher quality and safety while still being affordable for consumers. We also need to manage crops in a sustainable way and ensure that they are environmentally compatible.

Exporters, importers, and government agencies all rely on reliable analytical results when it comes to food safety and quality testing. Rising levels of globalisation have put unprecedented pressure on the food supply chain, including producers, manufacturers, and regulatory agencies, to test a wider variety of foods for a wider range of contaminants and attributes, to accurately quantify the presence of these contaminants at lower levels, and to do so in shorter amounts of time [3, 4]. Mass spectrometry's reputation as a highly targeted and sensitive method for analysing food items with more precision and throughput has led to its fast adoption in the industry. Throughput is substantially enhanced by the capacity to

simultaneously monitor a range of pollutants and/or natural components, and more streamlined sample preparation techniques are made possible by increasing levels of sensitivity.

Mass spectrometry (MS) has several potential uses in the food industry, and various reviews highlight the complexity of these uses. The most current uses of metabolomic techniques based on MS for testing food safety, quality, and traceability were discussed by a few of them. [5]. A review of the recent advancements and uses of capillary electrophoresis-mass spectrometry (CE-MS) as a tool for foodomics analysis was published by Ibañez et al. The area of food analysis and food safety that has the most reviews is liquid chromatography-mass spectrometry (LC-MS). In order to guarantee that food-based products are free of chemical pollutants, Mohamed et al. assessed the potential and contribution of MS-based approaches. Ambient ionisation MS for direct food analysis and matrix-assisted laser desorption ionisation coupled to time-of-flight mass spectrometry (MALDI-TOF-MS) were among the most recent advancements in MS used to assess food safety and quality [6, 7]. To better comprehend biological systems and their dynamic evolution, the "omic" sciences revolutionised MS by using high-throughput methods. Also covered were the uses of current MS-based methods in food safety and traceability, glycomics, proteomics, ergonomics, metabolomics, and lipidomics [8]. Recent developments in MS have made it possible to screen for a wider number of analytes at lower concentrations, with more precision, and in less time than before. Chemical analytical processes used in the field, as well as the general characteristic steps of MS-based techniques, are introduced. In addition, we go over some of the possible uses for the various methods and ionisation sources.

Under physiological circumstances, enzymes—which are protein catalysts—are able to exhibit extremely high levels of specificity and reactivity. One way to tell how a biological system, including food, is doing its daily activities is by an enzyme analysis, which involves measuring substances with the help of added enzymes or

measuring endogenous enzyme activity [9]. Enzyme catalysis is able to measure unstable chemicals that would be difficult or impossible to quantify using other methods since it can occur at mild settings [10]. Enzyme reactions are also very specific, which means they can be used to test components of intricate mixtures without the need for expensive and time-consuming chromatographic separation methods.

In the field of food science and technology, enzyme analyses have multiple applications. Enzyme activity is a helpful indicator of proper food processing in a number of contexts. There has been a lot of reliance on enzyme thermal stability as a heat treatment metric; for instance, peroxidase activity is used to gauge how well vegetable products have been blanched. In order to determine how effective enzyme preparations are as processing aids, food technologists frequently use enzyme activity assays. The food scientist can also measure the components of foods that are substrates for enzymes using commercially available enzyme preparations. As an example, easily accessible enzymes can be used to evaluate the glucose level in a complex dietary matrix that also contains other monosaccharides [11, 12]. Measuring enzyme activity in relation to the concentration of enzyme inhibitors in food is an additional application of commercially available enzymes. An indicator of the content of organophosphate insecticides in food is the activity of the enzyme acetylcholinesterase, which is strongly inhibited by organophosphate pesticides. Measuring enzyme activity in relation to food quality is another area of study. As an example, milk produced by mastitic udders has significantly higher catalase activity. The number of bacteria in milk is correlated with catalase activity. The estimate of protein nutritive value is another application of enzyme assays in food quality determination. This is achieved by measuring the action of added proteases on dietary protein samples.

It is possible to monitor the presence of trimethylamine and other breakdown products in stored fish using enzymes. Food analysis also

makes use of enzymes as preparative techniques. Amylases and proteases are used in fibre analysis, and thiamine phosphate esters are hydrolyzed enzymatically in vitamin analysis [13]. One must be familiar with the fundamentals of enzymology in order to do accurate enzyme studies in food.

Impact of Concentration of Substance:

When an enzyme is used to catalyse a reaction, the substrate concentration and velocity form a connection. Regarding the concentration of the substrate, the reaction is of zero order, while with regard to [E], it is of first order. The enzyme-catalyzed reaction exhibits mixed order with regard to substrate concentration at concentrations ranging from first-order to zero-order.

Impact on the Environment:

The Impact of Temperature on the Activity of Enzymes In several ways, temperature can influence the enzyme activity that is being examined. First and foremost, the rate of an enzyme-catalyzed reaction and the enzyme's stability are both affected by temperature. When thinking about enzyme-catalyzed reactions, it's also possible to take into account how temperature affects the solubility of gases involved in the reaction (either as products or substrates) and how temperature affects the pH of the system [13]. One species that exhibits the latter is Tris (tris [hydroxymethyl] aminomethane), a common buffering agent, whose pKa fluctuates by 0.031 for every 1°C change.

Potentially Reversing:

An enzyme inhibitor is a chemical that, when introduced to a reaction medium that is catalysed by an enzyme, reduces the activity of the enzyme. There is a difference between irreversible and reversible enzyme inhibitors. The term "enzyme inhibitor" can refer to a variety of substances, some of which are inorganic ions (e.g., Pb^{2+} or Hg^{2+}), others that mimic substrate, and still others that are naturally occurring proteins (e.g., protease inhibitors in legumes) that bind particularly to enzymes. Firstly, inhibitors that cannot be reversed. The drop in enzyme activity observed will be precisely proportional to the

inhibitor supplied when the dissociation constant of the inhibitor enzyme complex is relatively modest. To guarantee a thorough enzyme-inhibitor response, it is necessary to ascertain the impact of time on the decrease of enzyme activity caused by the addition of inhibitor, as the irreversible combination of the two may respond at a slow pace [14]. For an accurate estimation of inhibitor concentration, it is necessary to preincubate the amylase inhibitor, which is present in many legumes, under defined conditions with amylase before measuring residual activity (1). With a decrease in total active enzyme quantity, irreversible inhibitors lower V_m . II. Inhibitors that can be reversed. With a dissociation constant greater than one, the majority of inhibitors allow the enzyme and inhibitor to remain free in the reaction mixture. We know about competitive, noncompetitive, and uncompetitive reversible inhibitors.

Approaches to Quantification:

Being familiar with the methodologies of reaction measurement is essential for practical enzyme analysis. You can track an enzyme reaction using any system physical or chemical property that has to do with substrate or product concentration. Enzyme reactions can be monitored using a broad range of techniques, such as mass spectrometry, chromatography, isotope measurement, titration, fluorimetry, manometric approaches, and absorbance spectrometry. The pyridine coenzyme NAD(H) and NADP(H) spectra show a clear shift in absorbance at 340 nm during oxidation-reduction, making them an excellent candidate for spectrophotometry as a tool for monitoring enzyme activities. When these coenzymes are either substrates or products in a coupled reaction, many methods rely on the change in absorbance at 340 nm. The α -amylase activity assay (3) exemplifies the use of multiple approaches to quantify enzyme activity. An endoenzyme known as α -Amylase cleaves starch at α -1,4 linkages. The internal connections of a polymer substrate are cleaved by an endoenzyme. Several approaches can be used to observe this reaction: polarimetry, a decrease in starch iodine complex colour [13], an increase in reducing groups upon hydrolysis, a

decrease in viscosity, and so on. Using just one assay to distinguish between α -amylase and β -amylase activity is challenging.

At the nonreducing end of starch, α -Amylase separates maltose. Although α -amylase activity is expected to induce a significant drop in starch viscosity or a lessening of iodine colour, β -amylase, when present in large concentrations, can also alter both of these properties [14, 15]. The analyst needs to find out the change in the number of reducing groups to compare in order to ascertain if α -amylase or β -amylase is being tested. Since α -amylase is an endoenzyme, it hydrolyzes a small number of bonds close to the polymeric substrate's centre, resulting in a significant drop in viscosity. In contrast, the exoenzyme β -amylase hydrolyzes the same number of links, but it has less impact on viscosity. An exhaustive, balanced equation for the specific enzyme-catalyzed reaction should be written out before beginning to build an enzyme test. In many cases, the best way to track the reaction in the lab is to look at the products and substrates [16] for physically and chemical qualities that can be measured using the tools we have.

Analysing Food using Mass Spectrometry:

Food has a very complicated chemical composition. Traditional nutritional classifications include macro- and micronutrients. The macronutrients are the building blocks of food and the primary sources of energy for the human body. Proteins, carbs, and fats are the three main categories into which they fall. Phytonutrients, vitamins, and minerals are all examples of micronutrients. A group of tiny chemicals known as vitamins [17] is incredibly diversified. There is no way around their essential role in the human body. C. J. Bates¹ compiled a mountain of data on vitamins and their research methods in a 2000 review article. According to Bates, one promising method for vitamin analysis is the use of stable isotopes in conjunction with mass spectrometry. In order to understand their metabolic fate and structural makeup, substances like fat-soluble vitamins A and K necessitate the use of nearly all of mass spectrometry's analytical capabilities [18].

Mass spectrometry has become an indispensable tool in the nutrition and food science laboratories, and vitamins A and K are a prime example of this. The body uses vitamin A and vitamin K, which are actually groupings of related chemicals, for metabolism. Vitamins are a common name for some related compounds. Hence, the metabolic activity, and not merely the chemical structure, defines these vitamins. It used to be impossible to distinguish between newly consumed exogenous vitamins and endogenous vitamins stored in the body prior to the development of stable isotope techniques, making it difficult to ascertain the metabolic activity of vitamins. Additionally, the concentrations of these compounds in bodily fluids are usually quite low. Preventing blindness, especially in youngsters, requires vitamin A (retinol) in the body [19, 20]. Both the whole molecule and provitamin A carotenoids are dietary sources of retinol. Plant-based foods contain β -carotene, the most important provitamin A carotenoid, which the body can convert to retinol through the action of a symmetric cleavage enzyme. Apo-carotenals can be generated via asymmetric cleavage of β -carotene as well [21]. Retinol is a member of the retinoids class of chemicals, which includes several others with significant physiological roles; for instance, retinaldehyde inhibits the production of fat and diet-induced obesity. 6 The ability to form blood clots is dependent on vitamin K. Hemostasis, bone metabolism, tissue calcification, and cell cycle control are all governed by a class of proteins that are dependent on vitamin K [22, 23]. The greatest quantities of phyloqui-none, the principal dietary supply of vitamin K, are found in certain plant oils and green, leafy vegetables. 10 Both of these vitamins are fat-soluble, and their analysis has made extensive use of mass spectrometric tools. The initial step in mass spectrometry for vitamin A was derivatization, usually using silylation reagents to create retinol trimethylsilyl ethers, followed by gas chromatography-mass spectrometry (GC-MS). The retinol is protected from degradation as it passes through the GC column by this trimethylsilyl group. Additionally, the retinol (M-H) ion is generated by cleaving the

group under electron capture negative chemical ionisation (ECNCI) conditions [24, 25]. To minimise degradation and maximise sensitivity, cool on-column injection was frequently employed; ECNCI yielded the best detection values, in the picogram range. Atmospheric pressure chemical ionisation (APCI) allowed for the analysis of retinoids in meals, nutritional supplements, fish eggs, and cell extracts using liquid chromatography-mass spectrometry (LC-MS). As of late, GC-MS and LC-MS analyses of vitamin K isomers following APCI have been conducted. Phylloquinone typically has on-column detection limits in the picogram range by LC-MS, and the best separations were usually achieved with C-18 or C-30 stationary phases. Since carotenoids breakdown at GC-MS temperatures, they necessitate the use of gentler technologies like LC-MS for analysis, which presents an intriguing analytical challenge. The most effective separations are usually achieved using C-30 stationary phases, which allow for the dis-tilting of cis and trans (double bond) isomers; however, either conventionally packed or capillary columns can be utilised. Atmospheric pressure chemical ionisation is significantly more sensitive than electrospray ionisation (ESI) for detecting carotenoids [26, 27]. Dietary supplements, green and yellow veggies, kale, spinach, carrots, fish eggs, tomatoes, golden rice, thermally processed veggies, and red palm oil are just a few of the many food samples that have had carotenoids measured. In order to learn more about vitamin absorption, metabolism, and excretion in vivo without the risks of radioactivity, nutrition research has looked into the human body's vitamin fate using stable isotope labelled standards (usually ^{13}C) and "conventional" mass spectrometry or isotope ratio mass spectrometry. Vitamins with stable isotope labels have been manufactured chemically for scientific purposes for many years. Intrinsically labelled foods, which are 36 foods cultivated with either $^{13}\text{CO}_2$ or D_2O , have recently emerged as potent new sources for administering labelled vitamins to research participants.

Fourier transform mass spectrometry, gas chromatography-trained LC-MS, and accelerator:

Have demonstrated that previous vitamin A use impacts human b-carotene absorption and its retinol equivalency. In addition, GC-MS has demonstrated that cooking b-carotene-containing vegetables in oil can provide people with substantial amounts of vitamin A, even if the conversion factor of b-carotene to retinol is quite weak [28, 29]. One method to quantify total body storage of vitamin A is to utilise a deuterated-retinol-dilution approach, which involves ingesting deuterated retinol and then detecting it using GC-MS.

Additionally, GC-MS has been used to investigate the kinetics of vitamin K metabolism and absorption using stable isotope-labelled phyloquinone as an ingested tracer. Researchers can distinguish between phyloquinone consumed through food and that which is stored in the body using isotope-labeled vegetables. This allows them to calculate the bioavailability of phyloquinone in food. There appears to be a strong correlation between changes in triglyceride concentrations and changes in deuterated phyloquinone concentrations in plasma and triglyceride-rich lipoprotein, suggesting that the two processes are closely related to fat absorption. The determination of the ion abundances of deuterated and endogenous phyloquinone was accomplished using GC-MS, while the first step was to assess the quantities of phyloquinone in plasma and lipoprotein sub-fractions using high performance liquid chromatography (HPLC). These brief examples show the difficulties of analysing food components in the food matrix or in post-consumption biological samples. In order to overcome the analytical challenges caused by the compounds' low volatility, high reactivity, low abundance, or thermal and light instability, the full spectrum of MS methods has been utilised for the majority of macronutrient and micronutrient classes [30, 31]. Proteins (Claudio Corradini, Lisa Elviri, and Antonella Cavazza), carbohydrates (Daniel Kolarich and Nicolle Packer), and certain phyto-micronutrients (Jean-Luc Wolfender, Aude

Violettea, and Laurent Fay) are some of the macronutrients analysed using mass spectrometry in this portion of the book. Arnis Kuksis delves deeply into the topic of mass spectrometric analysis of lipid oxidation products in meals and biological samples due to the biological significance and complexity of in vivo reactions from consumed or in situ oxidised foods. Secretions like milk contain both free oligosaccharides and protein-bound carbohydrates, which can be difficult to analyse even with state-of-the-art mass spectrometry.

A metabolomic evaluation of the biological effects and lipidomic investigations of dietary and tissue lipid oxidation were the last stages of this new discovery. An overview of the process of lipid autoxidation and the primary forms of autoxidized fatty acids found in dietary oils and fats is provided by Kuskis in the first section of his essay. The analysis continues by thinking about how the water-and lipid-soluble items are absorbed after consumption and the dietary oxo-lipid transformation [30, 31]. The main agents of disease and ageing, including unsaturated fatty acids, acylglycerols, glycerophospholipids, and cholesteryl esters, are being discussed in relation to lipidomics, which aims to detect these compounds as secondary autoxidation products. Endogenous and exogenous antioxidant mechanisms are discussed by Kuksis in his final section. Mass spectrometric analysis of phytonutrients—non-essential food components found in fruits, vegetables, spices, and traditional ingredients [32, 33]—is highlighted in the last chapter of this section by Jean-Luc Wolfender, Aude Violettea, and Laurent Fay. The authors provide a concise overview of the most important mass spectrometric methods used to detect, quantify, and identify natural compounds with health claims in food and biological fluids.

MALDI spectrometry for the measurement of carbs in food:

Among the many bio-macromolecules found in living things are carbohydrates, which can be categorised into three main classes according to their chemical structures: monosaccharides,

oligosaccharides, and polysaccharides. At the same time, these are the most important bioactive ingredients in diet. Chemical structure characterization proved challenging owing to the diversity and complexity of the structures, in contrast to proteins and nucleic acids that have been extensively researched. Chemical methods and instrumental analytical technologies such as high performance anion exchange chromatography (HPAEC), nuclear magnetic resonance (NMR), methylation with gas chromatography-mass spectrometry (GC-MS), etc. were previously used to characterise them; however, these analytical procedures typically took a long time and necessitated a large number of samples.

Since the 1990s, mass spectrometry has grown into a respected field and useful technique for studying both organic and inorganic chemical reactions. Mass spectrometric analysis of carbohydrates is another area that has found successful use [34]. When it comes to directly determining the intact mass of high molecular weight polysaccharides, MALDI MS shows encouraging advantages over conventional mass spectrometry approaches. Synthetic carbohydrates with high m/z values, around 24,600, were detected by MALDI MS, according to a recent study. Decomposition into fragments reveals information about monosaccharide, linkage type, and sequence, enabling rapid deduction of carbohydrate compositions and structures; furthermore, MALD MS spectra are relatively easily explicable, with mostly singly-charged ions. David J. Harvey has released nine editions of his comprehensive assessments on the topic of carbohydrates and glycoconjugates analysis by MALDI MS from 1999. The most recent one covers literature up until the end of 2016. (Harvey, 2021). According to searches conducted in databases such as Scopus, Web of Science, and PubMed, the number of papers published on the topic of MADLI MS applications in the realm of dietary carbs has surged, particularly in the last five years [35–38]. Since the ionisation efficiency of carbohydrates has been steadily improving, this review aimed to summarise the most recent

advances in MALDI MS detection for carbs in food. In addition, they discussed how they can be used in quantitative studies, food quality control analyses, and imaging using mass spectrometry, as well as for studying the structure of polysaccharides in food.

Application of Enzymes to the Food Sector:

Enzymes are organic compounds derived from proteins that are used to accelerate biological reactions without actually being a reagent in those reactions. The apoenzyme is the protein component, and the cofactor, which is either an organic molecule or a metallic ion, is the non-protein component. The two parts combine to form the holoenzyme, the fully functional enzyme (Figure 1). Enzymes' specificity—one of its defining features—is the basis for the enzymatic catalysis process. Some chemical reactions likely couldn't take place without enzymes [34, 35]. Because reactions occur at different rates, its presence alone cannot catalyse them. Enzymes have a wide range of uses in the food industry, which can derive them from microbes, plants, or animal tissues. The most common applications of enzymes include baking, dairy products, and beverages including wine, beer, and fruit juices. Enzymes are of great interest to the food industry for several reasons, including the ongoing search for longer-lasting foods, the desire to decrease waste and improve product quality, the processing of raw materials into the final product, and the use of enzymes as additives to alter desired properties like flavour, texture, and machinability. As a result, studies on enzymes that aid in food product stability and quality have been conducted. Also, deactivating enzymes that speed up bad things like food deterioration has been the subject of multiple investigations [36, 37]. In addition, variables including temperature, water activity, and pH can influence enzymes. Unfortunate changes occur as a result of these variables, so alternatives have been researched to stabilise them. As an example of a stable alternative, enzyme immobilisation enables the reusability of catalysts and their incorporation into packaging. These methods allow for the regulated release of enzymes into food, which enhances specific food products' desirable qualities [38–40]. Despite the fact that enzymes have been the subject of several research, there has been a dearth of evaluations that concentrate on the various types of food enzymes and how they could be employed in packaging.

Consequently, the purpose of this review is to provide a concise overview of the primary food-related enzymes, including those that are endogenous and those that are exogenous, together with their respective classes and subclasses, and the immobilisation of these enzymes in packaging.

**Use of enzymes in the culinary arts:
Enzymes that take part in oxidation:**

The class of enzymes known as food-related oxidoreductases includes polyphenol oxidase (PPO), glucose oxidase, catalase, peroxidase (POD), lipoxygenase, and glucose oxidase (Table 1). Fruits and vegetables have endogenous enzymes called POD and PPO, which are thought to be the primary culprits of browning. Only when things are exposed to oxygen or cut, sliced, or mechanically damaged during transportation or thawing will they brown. Both monophenol oxidase activity, in which the PPO enzyme hydroxylates phenolic substrates in the orthoposition, and diphenol oxidase activity, in which the enzyme oxidises diphenol to ortho-benzoquinone, are necessary for the PPO enzyme to exert its function. In both of these processes, oxygen serves as a co-substratum. Catalysing oxidative processes utilising oxygen as the final hydrogen acceptor or peroxide as the substrate is

the goal of POD. The degradation of vegetables is just one of many things associated with the POD enzyme. Because these substances are heat sensitive, their deactivation signifies proper bleaching. There is an indication role of a type of peroxidase enzyme, lactoperoxidase, in dairy products generally as well [41, 42]. To ensure that the pasteurisation process has not gone over the recommended temperature, it is preferable for the enzyme to remain active in the milk during the pasteurisation process (inactivation temperature: 70–80 °C). In addition, when refrigeration is not an option, you can employ lactoperoxidase, an enzyme that is triggered by the hydrogen peroxide (H₂O₂) found in raw milk and has antibacterial properties, to prevent the growth of microbes. Catalase, found in aerobic creatures, is a tetrameric protein [43]. Hydrogen peroxide (H₂O₂) is a substrate that causes oxidative stress; this enzyme's mechanism of action involves breaking it down into oxygen and water. In the food business, catalase has a purpose in making cheese. Unwanted H₂O₂ residues in cheese are removed by it. It is possible to employ hydrogen peroxide (H₂O₂) in place of heat treatment procedures like pasteurisation in order to preserve the enzymes that are naturally present in cheese and milk. Microbes and bovine liver both contain catalase.

Table 1. Examples of exogenous and endogenous oxidoreductases in food.

Enzyme	Source	Type of application	Main effects of application/use	Reference
Endogenous oxidoreductases				
Peroxidase	Fruits and vegetables	-	Browning of fruits and vegetables/ Adequate bleaching process indicator	Singh et al. (2018)
Polyphenol oxidase	Fruits and vegetables	-	Browning of fruits and vegetables	Singh et al. (2018)
Lactoperoxidase	Milk	-	Pasteurization process indicator	Lara-Aguilar & Alcaine (2019)
Exogenous oxidoreductases				
Catalase	Animals and microorganisms	Cheese production	Conversion of hydrogen peroxide to water and oxygen, removing excess hydrogen peroxide	Kaushal et al. (2018)
Glucose oxidase	Microorganisms: <i>Aspergillus</i> and <i>Penicillium</i>	Food preservation	Removal of free oxygen, inhibition of microorganisms, and maintenance of flavor and color	Li et al. (2019); Kiesenhofer et al. (2017)
Lipoxygenase	Soy plants	Food preservation	Protection of flour in bakery products, ensuring colorless products	Gava et al. (2009); Tu et al. (2018)

Transferase:

Transglutaminase is an example of an enzyme transferase that has found application in the food industry. Its main function is to facilitate the transfer of acyl groups from glutamine residues to various acceptors, specifically the γ -carboxamide group. The protein molecules are subjected to polymerization and cross-linking processes when this enzyme acts on them via ϵ -(γ -glutamyl) lysyl peptide bonds. When the necessary primary amines are not present or when chemical reagents obstruct the lysine ϵ -amine, water can be used as an acceptor, and the transglutaminase process can convert the glutamyl residue to a glutamyl residue through deamidation [44, 45]. While transglutaminase can be found in animals or made from microbes, the latter option can be more cost-effective and offer a wider range of potential uses. Since its discovery in 1989, it has mostly been found in *Streptococcus* spp. bacteria. The gelling qualities of a wide range of food products can be enhanced with the help of transglutaminase. Proteins can be modified post-translationally, and substrates can be deamidated or cross-linked. Modifying functional qualities including viscosity, gelation, solubility, and water holding capacity is possible by the crosslinking of high molecular weight proteins. The reason this enzyme is called "meat glue" is because of its capabilities. This is how transglutaminase can be utilised to enhance the quality and texture of milk and dairy products, as well as the appearance, texture, preservation, and toughness of meat [45, 46]. It boosts the product's hardness, makes the protein film look better and stay put, and even lowers the calorie count in fish-based items.

Enzyme hydrolase:

Each of the six classes of enzymes has an essential function in food production (Schmidt and Salas-Mellado, 2009; Patel et al., 2016), but hydrolases—including amylase, invertase, lactase, lysozyme, lipase, pectinase, and protease—are arguably the most important and consequential (Table 2).

Amylase:

The bacterial and fungal groups *Bacillus*, *Pseudomonas*, and *Clostridium* are the primary sources of amylase. Historically recognised as the first enzyme capable of digesting starch, it plays a crucial role in the starch, bread, sugar, and beverage (beer) industries. Kirchloff found it and separated it in 1811. The starch and glycogen α -1,4 and α -1,6 glycosidic linkages are hydrolyzed by the amylase. Depending on the kind of anomeric sugar produced by the reaction, it is typically separated into α - and β -amylase and classed as either endo or exoamylase. α -amylase, which is derived from fungi or malted cereals, is utilised in bakery goods due to its versatility; it can be added to flour and used to make dough. This enzyme enhances the rate of fermentation and decreases the dough's viscosity, leading to better product volume and texture [47, 48]. Also, it helps make more fermentable sugars, which enhance the bread's flavour, crust, colour, and overall quality. While brewing, α -amylase can be used to hydrolyze starch as it gelatinizes, resulting in a lower must viscosity and making retrograding starch more difficult. This is done by mixing resting hot water with ground malt to degrade proteins and starch, and to produce soluble malt extract, the wort. In contrast, β amylase is

employed in the production of maltose syrups that find application in the food, beer, and

pharmaceutical sectors.

Table 2. Examples of exogenous and endogenous hydrolases in food.

Enzyme	Source	Type of application	Main effects of application/use	Reference
Endogenous hydrolases				
Pectinase	Fruits and vegetables	-	Alteration of the texture of fruits and vegetables during the stages of ripening, storage, and processing	Ordoñez et al. (2005)
Exogenous hydrolases				
α -Amylase	Microorganisms and malted cereals	Bakery and brewing products	Improvement in volume and texture, color, flavor, quality of bakery products; beer brewing process	Mishra et al. (2017); Zhang et al. (2018)
β - Amylase	Microorganisms and malted cereals	Syrup manufacturing	Releases carbohydrates from smaller chains needed for fermentation process by brewer's yeast, providing sugars that will serve to form alcohol in beer	Duan et al. (2019)
Cellulase	Microorganims	Fruit juices and winemaking	Improve the texture, quality, yield of various food products	
Invertase	Microorganisms, plants and animals	Sweets in general, artificial honey, jams, confectionery, drinks, and so on	Prevents crystallization of sugary products	Manoochchri et al. (2020); Trujillo Toledo et al. (2019)
Lactase (β -galactosidase)	Microorganisms	Dairy products	Improvement in the taste and color of milk and dairy products, incorporation of greater creaminess in the products, reduction of the maturation time of cheeses	Gava et al. (2009)
Lipase	Stomach and pancreas of man and animals and filamentous fungi, yeasts, and bacteria	Cheese	Improvement of texturing and flavor and development of cheese flavor and cheddar cheese production	Aravindan et al. (2007); Guerrand (2017); Raveendran et al. (2018); Trbojević Ivić et al. (2016)
Pectinase	Microorganisms	Beverage	Extraction of juice and aroma, removal of mist caused by pectin, whitening, and reduction of turbidity, extraction of natural pigments in wines and manufacture of sparkling wines, extraction of oil, and fermentation of coffee and tea	Dal Magro et al. (2018); Khan et al. (2013)
Lysozyme	Eggs, plants, bacteria, and animal secretion	Foods prone to microbial contamination	Food preservative against gram-positive, gram-negative bacteria and fungi	Liu et al. (2013)

The Enzyme Lactase:

When it comes to the lactose disaccharide, β -galactosidase (lactase) converts it into glucose and galactose, just like invertase can break down a disaccharide into two monosaccharides [49]. Lactose, a sugar that is plentiful in milk and other dairy products, is a carbohydrate that is consumed in large quantities. Nevertheless, a lack of the enzyme lactase in certain organisms can cause lactose intolerance in certain consumers. Those who consume dairy products may have uncomfortable side effects such flatulence, severe stomach discomfort, and intestinal breakdown; these consumers are primarily non-Caucasians, indigenous, and Asian. The sensory changes, particularly the amplified sweetness flavour, cause many people with this disorder to reject lactose-

free dairy products. In this case, it's a viable substitute for lactase that may be added to dairy products to appeal to those customers.

Digestive Enzyme:

Enzymes known as lipases can hydrolyze carboxylic esters of long-chain acylglycerols, which have 10 carbon atoms. These digestive enzymes break down lipids and fats; they are ubiquitous in the pancreas and the stomachs of monogastric animals like humans. The most common microorganisms that produce these substances include *Candida* sp., *Aspergillus* sp., *Rhizomucor* sp., *Rhizopus* sp., *Humicola* sp., *Yarrowia lipolytica*, and *Pseudomonas* sp., although they can also be sourced from filamentous fungi, yeasts, and bacteria for various industrial uses. The food industry prefers lipase

enzymes derived from microorganisms for a number of reasons, including higher stability, the potential for innovation through various strategies [50], and eminent catalytic multifunctionality. They use these enzymes to enhance texturing and flavour, for example, in the creation of cheddar cheese and other cheeses.

The Enzyme Pectinase:

The process of hydrolyzing pectin components, which are often found in plant cell walls and are composed of α -1,4-linked d-galacturonic acid, is carried out by pectinase, an enzyme that can originate from either plants or microbes. Fruit juices have been known to use this enzyme for a variety of purposes in the food industry, including but not limited to: removing pectin-induced mist, whitening and reducing turbidity, extracting natural pigments in wines (resulting in sparkling wines), fermenting coffee and tea, and extracting oil.

What is Lysozyme?

Once the food industry has used various additives to inhibit the growth of spoilage microorganisms and pathogenic microorganisms, one of the most sought-after properties is its antimicrobial capacity. These microorganisms cause food spoilage and harm to consumers. Enzymes like lysozyme have been studied in this context because of this crucial characteristic. Lysozyme, muramidase, or N-acetylmuramic hydrolase is a tiny monomeric protein that can be found in nature in a variety of forms, including eggs, plants, microbes, and animal secretions. It is stabilised by four disulfide bonds among its eight cysteine residues. This enzyme is regarded as a safe food additive in various parts of the world because of its strong bacteriostatic activity against both gram-positive and gram-negative bacteria. As a result, it has significant potential in the food preservation [51-53] business. You have the ability to break down the peptidoglycan chains that are present in the cell walls of both gram-positive and gram-negative bacteria, as stated by Anastas et al. (2021). The enzyme degrades the β -(1,4)-glycosidic linkages between N-acetylglucosamine (NAG) and N-acetylmuramic

acid (NAM) residues in this manner. Involving the carboxylic acid moieties of glutamate-35 (Glu-35) and aspartate-52, hydrolysis takes place at the active site of lysozyme. Glu-35 adds a proton to the glycosidic ether bond between NAG and NAM, forming an oxonium ion. Then, the hydroxy NAG is nucleophilically displaced, and NAM forms an ester bond with Asp-52 at the same time. The final step in scission of the glucosidic link is the hydrolysis of the ester, which yields a terminal hydroxy NAM.

Conclusion:

Enzymes perform a wide variety of important roles in food items, making them indispensable to the food business. The most essential of the six enzyme classes are hydrolases; nevertheless, ligase has received very little attention from the food research community. The other three classes are oxidoreductase, transferase, and lyase. Subclasses can either be endogenous, occurring naturally in a diet, or derived from plants, animals, or microbes. Because of differences in enzyme and food properties, each subclass serves a particular purpose. Immobilisation in packaging is one method of incorporating food enzymes; this protects the enzymes from intrinsic and extrinsic influences that can be harmful. In comparison to directly incorporating enzymes, enzymatic packaging has the advantage of allowing for controlled release of the enzymes, which in turn increases the food's shelf life. Furthermore, the packaging protects the enzyme and makes its stability more efficient. Furthermore, research has shown that this theory holds water; the enzyme is effectively contained within the packaging, which also makes it possible to reuse it. This presents an option for the enzyme sector, as the cost of administering the enzyme is still quite high. But there hasn't been much research on activating packages with enzymes, so this method needs more investigation. There needs to be more of an effort from the industry to keep an eye on the development of active packaging since, despite its effectiveness, it is not widely available (at least in Brazil).

References:

1. Henniger G (2004) Nonindustrial enzyme usage: Enzymes in food analysis. In: Aehle W (ed) *Enzymes in Industry, Production and Applications*, 2nd edn, Wiley-VCH Verlag GmbH & Co. Weinheim, Germany, pp 322–334
2. Whitaker JR (1985) Analytical uses of enzymes. In Gruenwedel D, Whitaker JR (eds) *Food Analysis. Principles and Techniques*, vol 3. Biological Techniques, Marcel Dekker, New York, pp 297–377
3. Bernfeld P (1955) Amylases, α and β . *Methods Enzymol* 1:149
3. Bergmeyer HU (1983) *Methods of Enzymatic Analysis*. Academic Press, New York
4. Beutler H (1984) A new enzymatic method for determination of sulphite in food. *Food Chem* 15:157
5. Raabo E, Terkildsen TC (1960) On the enzyme determination of blood glucose. *Scand J Clin Lab Invest.* 12:402
6. Beutler H, Wurst B (1990) A new method for the enzymatic determination of D - malic acid in foodstuffs. Part I: Principles of the Enzymatic Reaction. *Deutsche Lebensmittel-Rundschau* 86:341
7. Williams DC, Lim MH, Chen AO, Pangborn, RM, Whitaker JR (1986) Blanching of vegetables for freezing – Which indicator enzyme to use. *Food Technol* 40(6): 130.
8. Surrey K (1964) Spectrophotometric method for determination of lipoxidase activity. *Plant Physiology* 39:6
9. Zhang Q, Cavalieri, RP, Powers JR, Wu J (1991) Measurement of lipoxygenase activity in homogenized green bean tissue. *J Food Sci* 56:719
10. Murthy GK, Kleyn DH, Richardson T, Rocco RM (1992) Phosphatase methods. In: Richardson GH (ed) *Standard methods for the examination of dairy products*, 16thedn. American Public Health Association, Washington, DC, p. 413
11. Rocco R (1990) Fluorometric determination of alkaline phosphatase in fluid dairy products: Collaborative study. *J Assoc Off Anal Chem* 73:842
12. Davis CE (1998) Fluorometric determination of acid phosphatase in cooked, boneless, nonbreaded broiler breast and thigh meat. *J AOAC Int* 81:887
13. Christen GL, Marshall RT (1984) Selected properties of lipase and protease of *Pseudomonas fluorescens* 27 produced in 4 media. *J Dairy Sci* 67:1680
14. Kim SM, Zayas JF (1991) Comparative quality characteristics of chymosin extracts obtained by ultrasound treatment. *J Food Sci* 56:406
15. Reyes J, Cavalieri RP (2003) Biosensors. In: Heldman DR(ed) *Encyclopedia of agricultural, food, and biological engineering*, Marcel Dekker, New York, pp 119–123
16. Guilbault GG, Lubrano GJ (1972) Enzyme electrode for glucose. *Anal Chim Acta* 60:254
17. O. Ziouzenkova, G. Orasanu, M. Sharlach, T. E. Akiyama, J. P. Berger, J. Viereck, J. A. Hamilton, G. W. Tang, G. G. Dolnikowski, S. Vogel, G. Duester and J. Plutzky, *Nat. Med. (N.Y.)*, 2007, 13, 695–702.
18. Smiddy, M.A. , T. Huppertz , and S.M. van Ruth , Triacylglycerol and melting profiles of milk fat from several species. *International Dairy Journal*, 2012. 24(2): pp. 64–69.
19. Liu, Z. , et al., Heat stress in dairy cattle alters lipid composition of milk. *Scientific Reports*, 2017. 7(1): p. 961.
20. Benbrook, C.M. , et al., Organic production enhances milk nutritional quality by shifting fatty acid composition: A United States-wide, 18-month study.

- PLoS One, 2013. 8(12): pp. e82429–e82429.
21. Stefanov, I. , B. Vlaeminck , and V. Fievez , A novel procedure for routine milk fat extraction based on dichloromethane. *Journal of Food Composition and Analysis*, 2010. 23(8): pp. 852–855.
 22. Lindmark Månsson, H. , Fatty acids in bovine milk fat. *Food & Nutrition Research*, 2008. 52(1): p. 1821.
 23. Markiewicz-Kęszycka, M. , et al., Fatty acid profile of milk - A review. *Bulletin of the Veterinary Institute in Pulawy*, 2013. 57(2): pp. 135–139.
 24. Liu, Z. , et al., Comprehensive characterization of bovine milk lipids: Triglycerides. *ACS Omega*, 2020. 5(21): pp. 12573–12582.
 25. Moate, P.J. , et al., Grape marc reduces methane emissions when fed to dairy cows. *Journal of Dairy Science*, 2014. 97(8): pp. 5073–5087.
 26. O'Donnell-Megaró, A.M. , D.M. Barbano , and D.E. Bauman , Survey of the fatty acid composition of retail milk in the United States including regional and seasonal variations. *Journal of Dairy Science*, 2011. 94(1): pp. 59–65.
 27. Verma, A. and K. Ambatipudi , Challenges and opportunities of bovine milk analysis by mass spectrometry. *Clinical Proteomics*, 2016. 13(1): p. 8.
 28. Bernardi, N. , et al., A rapid high-performance liquid chromatography-tandem mass spectrometry assay for unambiguous detection of different milk species employed in cheese manufacturing. *Journal of Dairy Science*, 2015. 98(12): pp. 8405–8413.
 29. Yoshimura, Y. and N. Zaima , Application of mass spectrometry imaging for visualizing food components. *Foods*, 2020. 9(5): p. 575.
 30. Rau, J. , et al., Rapid animal species identification of feta and mozzarella cheese using MALDI-TOF mass-spectrometry. *Food Control*, 2020. 117: p. 107349.
 31. Renes, E. , et al., Effect of forage type in the ovine diet on the nutritional profile of sheep milk cheese fat. *Journal of Dairy Science*, 2020. 103(1): pp. 63–71.
 32. Simeoni, M.C. , et al., Determination of free fatty acids in cheese by means of matrix solid-phase dispersion followed by ultra-high performance liquid chromatography and tandem mass spectrometry analysis. *Food Analytical Methods*, 2018. 11(10): pp. 2961–2968.
 33. Kokotou, M.G. , C. Mantzourani , and G. Kokotos , Development of a liquid chromatography-high resolution mass spectrometry method for the determination of free fatty acids in milk. *Molecules (Basel, Switzerland)*, 2020. 25(7): p. 1548.
 34. Devle, H. , et al., A GC – magnetic sector MS method for identification and quantification of fatty acids in ewe milk by different acquisition modes. *Journal of Separation Science*, 2009. 32(21): pp. 3738–3745.
 35. Jenske, R. and W. Vetter , Gas chromatography/electron-capture negative ion mass spectrometry for the quantitative determination of 2- and 3-Hydroxy fatty acids in bovine milk fat. *Journal of Agricultural and Food Chemistry*, 2008. 56(14): pp. 5500–5505.
 36. Sumarmono, J. , M. Sulistyowati , and Soenarto , Fatty acids profiles of fresh milk, yogurt and concentrated yogurt from Peranakan etawah goat milk. *Procedia Food Science*, 2015. 3: pp. 216–222.
 37. Larrouy-Maumus, G. , et al., Discrimination of bovine milk from non-dairy milk by lipids fingerprinting using routine matrix-assisted laser desorption ionization mass spectrometry. 2020.

38. van Ruth, S.M. , et al., Butter and butter oil classification by PTR-MS. *European Food Research and Technology*, 2008. 227(1): pp. 307–317.
39. Brena, B. M., & Batista-Viera, F. (2006). Immobilization of enzymes. In J. M. Guisan (Ed.), *Immobilization of enzymes and cells* (pp. 15-30). Singapore: Springer.
40. Brovetto, M., Gamenara, D., Saenz Méndez, P., & Seoane, G. A. (2011). C–C bond-forming lyases in organic synthesis. *Chemical Reviews*, 111(7), 4346-4403.
41. Casas-Godoy, L., Gasteazoro, F., Duquesne, S., Bordes, F., Marty, A., & Sandoval, G. (2018). Lipases: an overview. In G. Sansoval (Ed.), *Lipases and phospholipases* (pp. 3-38). Singapore: Springer. [http:// dx.doi.org/10.1007/978-1-4939-8672-9_1](http://dx.doi.org/10.1007/978-1-4939-8672-9_1).
42. Cunha, L. R., Soares, N. D. F. F., Assis, F. C. C., Melo, N. R., Pereira, A. F., & Silva, C. B. (2007). Desenvolvimento e avaliação de embalagem ativa com incorporação de lactase. *Food Science and Technology*, 27(1), 23-26.
43. Dal Magro, L., Kornecki, J. F., Klein, M. P., Rodrigues, R. C., & Fernandez-Lafuente, R. (2020). Pectin lyase immobilization using the glutaraldehyde chemistry increases the enzyme operation range. *Enzyme and Microbial Technology*, 132, e109397
44. Anastas, P. T., Rodriguez, A., Winter, T. M., Coish, P., & Zimmerman, J. B. (2021). A review of immobilizations techniques to improve the stability and bioactivity of lysozyme. *Green Chemistry Letters and Reviews*, 14(2), 302-338.
45. Gurumallesh, P., Alagu, K., Ramakrishnan, B., & Muthusamy, S. (2019). A systematic reconsideration on proteases. *International Journal of Biological Macromolecules*, 128, 254-267.
46. Hanušová, K., Vápenka, L., Dobiáš, J., & Mišková, L. (2013). Development of antimicrobial packaging materials with immobilized glucose oxidase and lysozyme. *Open Chemistry*, 11(7), 1066-1078.
47. John, J., Kaimal, K. K. S., Smith, M. L., Rahman, P. K. S. M., & Chellam, P. V. (2020). Advances in upstream and downstream strategies of pectinase bioprocessing: a review. *International Journal of Biological Macromolecules*, 162, 1086-1099.
48. Kamijo, J., Sakai, K., Suzuki, H., Suzuki, K., Kunitake, E., Shimizu, M., & Kato, M. (2019). Identification and characterization of a thermostable pectate lyase from *Aspergillus luchuensis* var. *saitoi*. *Food Chemistry*, 276, 503-510.
49. Alvarado-Ramírez, L., Rostro-Alanis, M., Rodríguez-Rodríguez, J., Sosa-Hernández, J. E., Melchor-Martínez, E. M., Iqbal, H. M. N., & Parra-Saldívar, R. (2021). Enzyme (single and multiple) and nanozyme biosensors: recent developments and their novel applications in the water-food-health nexus. *Biosensors*, 11(11), 410.
50. Kuraishi, C., Yamazaki, K., & Susa, Y. (2001). Transglutaminase: its utilization in the food industry. *Food Reviews International*, 17(2), 221-246.
51. Lara-Aguilar, S., & Alcaine, S. D. (2019). Lactose oxidase: a novel activator of the lactoperoxidase system in milk for improved shelf life. *Journal of Dairy Science*, 102(3), 1933-1942.
52. Almasi, H., Jahanbakhsh Oskouie, M., & Saleh, A. (2021). A review on techniques utilized for design of controlled release food active packaging. *Critical Reviews in Food Science and Nutrition*, 61(15), 2601-2621.
53. Moreno, H. M., Tovar, C. A., Domínguez-Timón, F., Cano-Báez, J., Díaz, M. T.,

- Pedrosa, M. M., & Borderías, A. J. (2020). Gelation of commercial pea protein isolate: effect of microbial transglutaminase and thermal processing. *Food Science and Technology*, 40(4), 800-809.
54. Neifar, S., Cervantes, F. V., Bouanane-Darenfed, A., BenHlima, H., Ballesteros, A. O., Plou, F. J., & Bejar, S. (2020). Immobilization of the glucose isomerase from *Caldicoprobacter algeriensis* on Sepabeads EC-HA and its efficient application in continuous High Fructose Syrup production using packed bed reactor. *Food Chemistry*, 309, e125710.