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Novel enzymatic processes applied to the food industry

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There is a deep relation between enzymes and their application in food and feed industries. These ‘green’ biological catalysts have altered the way we process our food. The enzymes applied in the food industry have evolved in several ways during several past decades. The desired traits as thermostability, ability to act at wide pH range, non-metal ion dependency, fast reaction rate, wide substrates utilization, etc., have been developed by using single, or integrated approaches as screening, rDNA technology, protein engineering, etc. Immobilization of enzymes has also enabled them to be employed more economically as being able to reuse them with minor loss, or without any loss in the activity. These ‘green’ molecules have exerted a great impact on human life that expresses perspectives of enzymes in the area of food industry.

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Introduction

All living organisms are built-up and maintained by the biological catalysts, called as ‘enzymes’, which catalyze biochemical reactions that are necessary to support life [1•]. Some enzymes have been designed by the nature to form complex molecules from simpler ones, while others have been designed for breaking up the complex molecules into simpler ones; also few are present for modifying the molecules. These reactions involve making and breaking of the chemical bonds existing in the components. Owing to their ‘specificity’, a property of enzyme that allows it to recognize a particular chemical compound, or substrate that they are designed to target; they are useful for industrial processes and are capable of catalyzing the reaction between particular chemicals even though present in mixtures with many chemicals. The application of enzymes in various processes for the production of industrial chemicals, often referred as ‘White biotechnology’ remains a challenge since new biocatalytic processes have to compete economically with the well-established chemical processes that have been optimized since long years [2–4].

Humans recognized the importance of enzymes thousands of years ago, where clarification and filtration of wines and beer are among the earliest examples of application of industrial enzymes. Microbial enzymes find great importance for the development of efficient bioprocesses in several industries, for example, chemical, pulp and paper, textiles, pharmaceuticals, leather, detergent, food and beverages, biofuels, animal feed, personal care, etc. These are exploited in several process based on their unique properties such as high specificity, high rate of catalysis, ability to perform with improved yield and reduced waste generation [5]. Enzymes are usually user-friendly for these processes as they catalyze reactions under mild conditions (e.g., temperature, pH and pressure), do not require protecting the functional groups of the substrate, exhibit longer half-life, higher stereo-selectivity, which yields stereo-selective and regio-selective reaction products at the rate of 10^5–10^8. Moreover, they work on unnatural substrates also [6]. Today, more than 500 products are made via enzymatic processes in several industries [7]. About 150 industrial processes exploit enzymes, or whole microbial cell as catalysts. Microbial enzymes find applications in numerous segments of industries as mentioned above [8]. However, the segment of food and beverage enzymes comprises the largest segment of industrial enzymes [9]. It created revenues of nearly $1.2 billion in 2011, which was expected to grow up to $1.8 billion by 2016, with average annual growth rate of 10.4% [10]. Within food and beverage enzymes segment, the milk and dairy market had the highest sales, with $401.8 million in 2009 [3]. Novozymes is the largest supplier of the industrial enzymes, followed by DSM and DuPont (based on holding stake in Danisco and Genencor Division). North America and Europe are the largest consumers of industrial enzymes; however, the Asia-Pacific region is predicted for a rapid increase in enzyme demand in China, Japan and India.

Current industrial market is very competitive in terms of product cost; therefore, there is a demand for novel, durable and more versatile enzymes in order to develop sustainable and economically viable and competitive production processes. The companies generally compete due to their product quality, performance, use of intellectual property rights, and the ability to innovate among them. Most enzymes available in the market are in recombinant form, which is produced from bacteria, fungi and yeasts. In order to discover new potential enzymes, several strategies and evolution practices have been applied. Apart from the microbial diversity, modern molecular techniques such as metagenomics, protein engineering and genome shuffling are being used to discover novel microbial enzymes with improved catalytic properties [11].

Rational of enzyme's application in food industry

Enzymes have been used for food processing as long as man has processed food. Food industry represents one of the economic sectors where enzymes have found a wide variety of applications. The use of enzymes in food industry is based on three basic aspects, (i) to control the quality of foods (presence, or absence of some enzymatic activities has a great impact in the quality control of the final product), (ii) to modify the properties of some food additives and the food itself (to modify the physicochemical and rheological properties of the foods, for example, the use of enzymes, such as amylases, lipases pectinases, etc.), and (iii) to be used as food additives (enzymes with direct applications in the food industry) [12].

Most of the commercial chemical processes are driven by high temperatures and pressures, which lead to high energy cost and needs high volume of cooling water. These ultimately increase the capital investment. Harsh processes requiring high pressure, temperature, acidity, or alkalinity necessitate heavy investment for designing the specific equipment being able to handle extreme conditions. Most of these processes generate undesired byproducts, which are difficult to separate and exert harmful impact on the environment. To combat with the above drawbacks, enzymes have emerged as a powerful tool [11]. Microbial production of enzymes has gained much importance compared to the plant and animal sources because of being economical as well as versatile. The enzymes are produced by the microorganisms in shorter times without the production of toxic compound.

Enzymes are natural products, which is the most important reason of their widespread application in food industry [13]. Furthermore, their unsurpassed specificity, ability to perform under mild conditions of temperature, pressure and pH along with high rate of reaction and turnover number make them suitable candidates for food industry. Life-cycle assessment (LCA) has confirmed that the implementation of enzyme-based technology has a positive impact on the environment [14]. Microbial food enzymes may be utilized to increase the productivity, efficiency and quality of food products without a costly investment and have the advantage of being produced with simple technology and economically viable downstream processing. A survey on world sales of enzymes ascribed 31% for food enzymes, 6% for feed enzymes and the remaining for technical enzymes [15].

Food processing via biological agent is possibly having the deepest route among all other processes and is historically well-established. Enzymes are environmentally safe, natural and are applied very safely in food and even pharmaceutical industries; still, these are proteins, which, like any protein can cause and have caused in the past allergic reactions, hence, protective measures are necessary in their production and applications.

Applications in various sectors of food industry

Food enzymes comprise enzymes used in baking, brewing, beverage, fruit juice and wine industries, as well as dairy industry and the oils & fats industry. Food enzymes are added to the food during various stages of manufacture, processing, preparation, treatment, packaging, transport or storage of the foods [16,17]. Methods of food processing such as fining (e.g., addition of adsorptive compounds, followed by settling, or precipitation) and clarification (e.g., sedimentation, racking, centrifugation, filtration, etc.) include the removal of excess amounts of certain components to achieve clarity and to ensure the physicochemical stability of the end product [18]. The fining and clarification of fermented beverages often include expensive and laborious work that generates large volumes of disposal, thereby causing a loss of product and the removal of important aroma and flavor compounds from the remaining product. In order to minimize the disadvantages of these harsh processes, an increasing use of enzyme preparations (e.g., proteases, pectinases, glucanases, xylanases, arabinofuranosidases, etc.) are often added to the fermentation media (e.g., grape, must and wine). Emphasis is also given on microbial production of aroma liberating enzymes (e.g., pectinases, glycosidases, glucanases, arabinofuranosidases, etc.) [19]. Improved yeast can be used in the production of better flavored alcoholic beverages [20].

Almost all classes of enzymes have a role in food and feed applications; however, hydrolases are the most prevalent ones. Representative examples of enzyme from each classes and their application in food industry is given in Table 1.

Enzymes used in food processing have historically been considered non-toxic. Still as every coin has two faces, enzymes too have their limitations. Some characteristics arising from their chemical nature and source, such as allergenicity, activity-related toxicity, residual microbiological activity and chemical toxicity are of high concern. These attributes of concern must, however, be addressed in light of the growing complexity and sophistication of the methodologies used in the production of food-grade enzymes. Safety evaluation of all food grade enzymes,