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Review Article

Immobilized enzymes for nutraceuticals production

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Abstract
Due to the various advancements in enzyme development, enzymes have different applications in industries, agriculture, and medical field as well as to make various compounds or molecules. Immobilization refers to the combining the physico-chemical properties of enzyme with the carrier which will enhance the stability of any biocatalyst. The market of nutraceutical products is gaining wide importance. With this view, the present chapter here will focus on various immobilized enzymes with recent advances, and application of immobilized enzymes in production of various nutraceutical compounds. The nutraceutical compounds improve the flavour, structure and digestion of different food products. The immobilized enzymes can be capsulated which can be used effectively for nutraceuticals production. The use of immobilized enzymes for nutraceutical compounds is very eco-friendly, easy with sustainable approach.

Keywords: Carrier, eco-friendly, flavour, proteins, sustainable

Introduction
Enzymes are very important which act as catalysts and speed up the reactions. They have immense importance in pharmaceutical, agricultural, forensic, environmental, medical field, etc. The word 'enzyme' was introduced in 1878 by Wilhelm Kuhne, a German physiologist. The enzymes are proteins and allow the conversion of substrates into products (Robinson, 2015). Each enzyme is specific for a particular substrate. The enzymes can be reused and also are able to function continuously (Bhatia, 2018). The enzymes are produced by bacteria, fungi, actinobacteria viz., *Bacillus*, *Pseudomonas*, *Serratia*, *Herbaspirillum*, *Acinetobacter*, *Burkholderia*, *Streptomyces*, *Aspergillus*, *Trichoderma* sp., etc. The immobilization of enzymes attracted the scientists in 1970s (Khan, 2021) and was applied continuously to the microbial cells. The immobilized enzymes have huge industrial applications. The classification of immobilized enzymes is represented in Fig. 1.

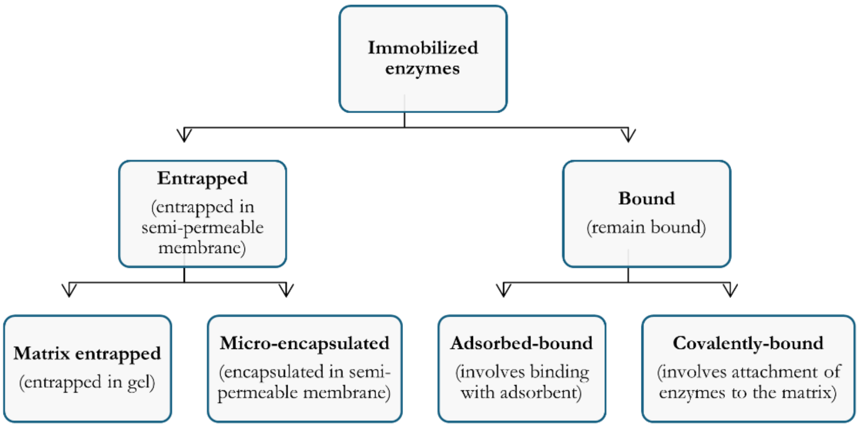

Figure 1 Classification of immobilized enzymes.

Immobilized enzymes can be used for the production of nutraceuticals. Nutraceuticals is the combination of 'Nutrient' and 'Pharmaceuticals' (Shinde *et al.,* 2014). Nutraceuticals refer to the improvement in food or feed, and bioactive compounds qualities which are of beneficial to the humans and also protects against various diseases. Nowadays, nutraceuticals are gaining wide attention and research is being carried due to their immense benefits and applications in industries. The conventional methods for the production of nutraceuticals are costly, time-consuming and not eco-friendly. The use of immobilized enzymes for the production of nutraceuticals is eco-friendly, easy and fast. The examples of nutraceuticals are garlic, omega 3, soybeans, ginger, minerals, vitamins, dietary fibre, hydrolysed proteins, etc.

The review here focuses on market demand for enzymes, immobilized enzymes, and methods of immobilization of enzymes, advantages of immobilization, aspect of nutraceuticals, their types, and use of immobilized enzymes for the production of nutraceuticals.

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List of various enzymes and their importance

The various enzymes and their applications are shown in Fig. 2.

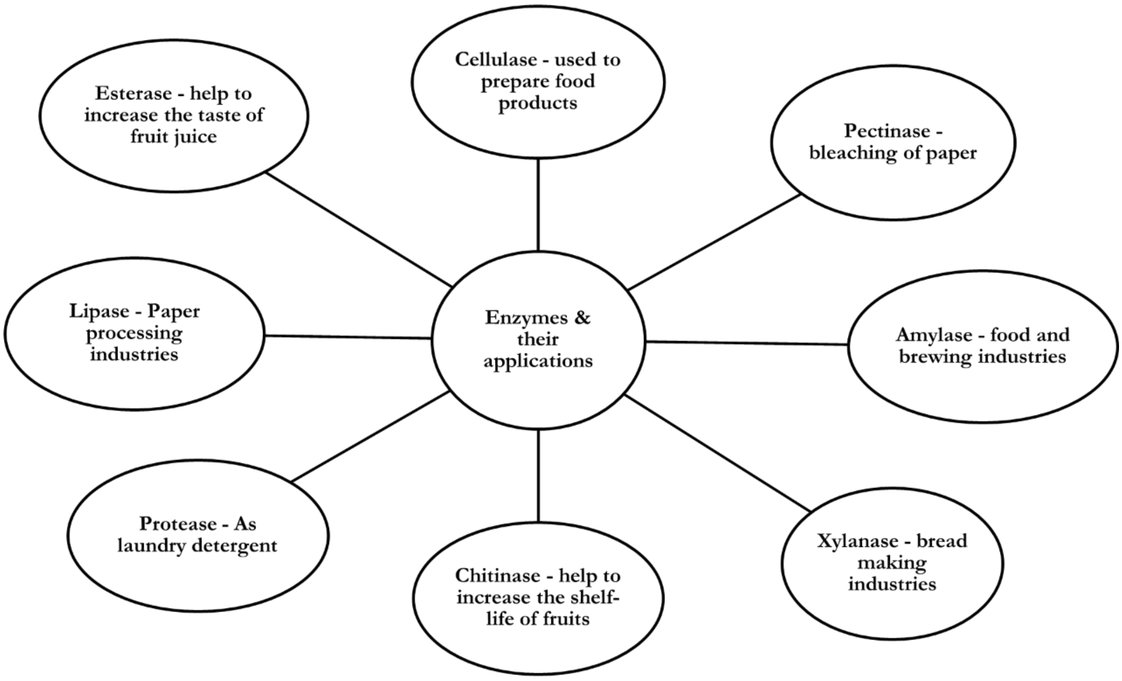

Figure 2 Enzymes and their applications.

Market demand for the enzymes

The global enzymes market size was valued at USD 10.69 billion in 2020 and will expand at a compound annual growth rate (CAGR) of 6.5% from 2021 to 2028 (https://www.grandviewresearch.com/industry-analysis/enzymes-industry). The market demand for enzymes is huge. This huge demand for the enzymes is due their various applications in food, medical, agricultural, and pharmaceutical industries.

Immobilized enzymes

The 'immobilized enzymes' are the enzymes in a particular region with their catalytic activities and can be used again and again. Immobilization separates enzymes from the products and increases the stability of enzymes (Mohamad et al., 2015). This also makes it economical and such immobilized enzymes have various industrial applications. The essential requirements for immobilization are enzymes, types of matrices used and mode of attachment. The enzymes undergo physico-chemical changes after immobilization. It also causes a change in 3-D structure of the proteins (Kalyani et al., 2016). The supports used in immobilization play an important role. These supports can be both organic and inorganic and are viz., clay, charcoal, silica, aluminium oxide, hydroxyapatite, agarose, etc. (Mohamad et al., 2015). The inorganic supports are preferred in immobilization as they are economical and resistant to heat, microbial action, and also have more mechanical strength. But even the organic supports, especially the polymers have attracted their use in immobilization (Pinto et al., 2019). The enzyme will interact with the matrix and form hydrogen or covalent bonds (Xu et al., 2020). The immobilization uses the fact that the functional groups inside chain of various amino acids interact with the support (Mohamad et al., 2015).

Advantages of immobilized enzymes (Basso & Serban, 2019)

The immobilized enzymes have the following advantages:

i) Can be reused and process is economical.
ii) The immobilized enzymes have more effective operational stability.
iii) Reduces environmental pollution in comparison to chemical process.
iv) Sustainable approach.
v) can be used as biocatalysts in various industries

The combination of enzyme with carrier when immobilized is represented in Fig. 3.

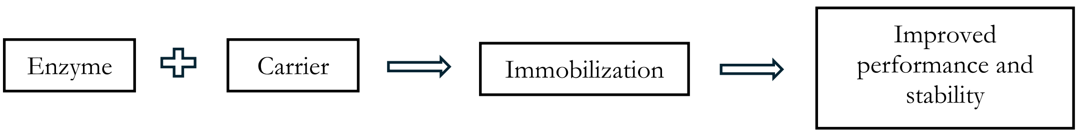

Figure 3 Combination of enzyme with carrier when immobilized.

Methods for immobilization of enzymes

The methods for immobilization of enzymes are adsorption, covalent binding, and entrapment (Fig. 4).

Adsorption

Adsorption is one of the methods for immobilization of enzymes. This involves van der Waals, hydrophobic or electrostatic interactions. In this method, there is no major change in the configuration of enzymes. This method of immobilization of enzymes is economical and fast (Lyu et al., 2021).

Covalent binding

The covalent binding is also of the technique to immobilize the enzymes. The strong covalent bonds are formed between the enzymes and the support material used. This is more appropriate method for immobilization of enzymes which can be used for industrial applications. There is major change in the conformation of the enzymes when immobilized by this method (Lyu et al., 2021).

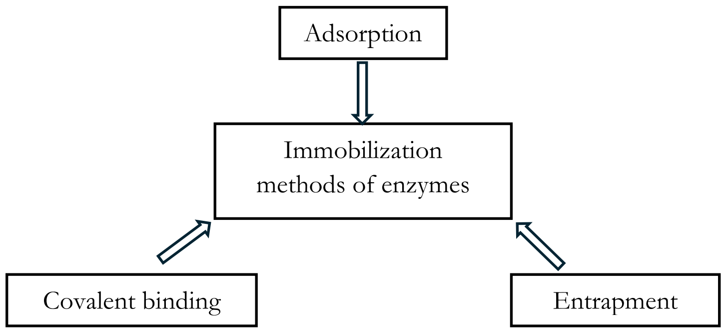

Figure 4 Methods for immobilization of enzymes.

Entrapment

In the entrapment method, the enzyme is trapped in the matrix used and protect from adverse environmental conditions. The enzymes can also be encapsulated in entrapment method (Lyu et al., 2021).

Nutraceuticals

Nutraceuticals are obtained from food which are known give more essential health benefits in addition to the nutritional value in foods. There are basically three categories of nutraceuticals viz., non-traditional, traditional, and commercial nutraceuticals (Chaudhari et al., 2017). The types of nutraceuticals are shown in Fig. 5.

Non-traditional nutraceuticals include fortified and recombinant; traditional include chemical constituents such as nutrients, herbals and phytochemicals, prebiotics, probiotic microorganisms, nutraceutical enzymes, and commercial nutraceuticals include dietary supplements, functional and medicinal food and pharmaceutical compounds (Chaudhari et al., 2017). The nutraceuticals play a very important role in health promotion such as antioxidant activity; prebiotics help in disease prevention and healing processes; digestion system; help in reproduction by influencing the hormones; stem cell growth; and also help in excretory system (Das et al., 2012).

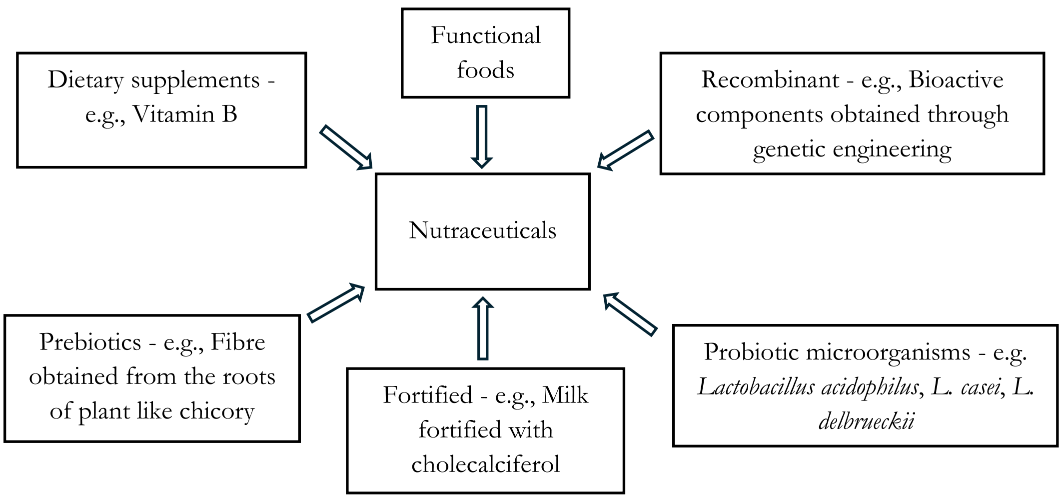

Figure 5 Types of nutraceuticals.

Nutraceuticals based on food availability
The nutraceuticals based on food availability are traditional and non-traditional nutraceuticals (Chanda et al., 2019). The traditional nutraceuticals are available naturally.

Immobilized enzymes for nutraceutical production
The various types of wastes generated viz., food, animal, plants, etc. can be collected and processed into fine powder. The use of enzymes to get nutraceutical products is eco-friendly. The enzymes used undergo hydrolysis and reduced in volume. After this, the enzymes are immobilized and are termed as 'immobilized enzymes. These immobilized enzymes after removal of solids can be processed to get nutraceutical products. The process of use of immobilized enzymes to get nutraceutical products is represented in Fig. 6. The immobilized enzymes have advantages in food industries because their use is eco-friendly, and the reactions can be carried at low temperature.

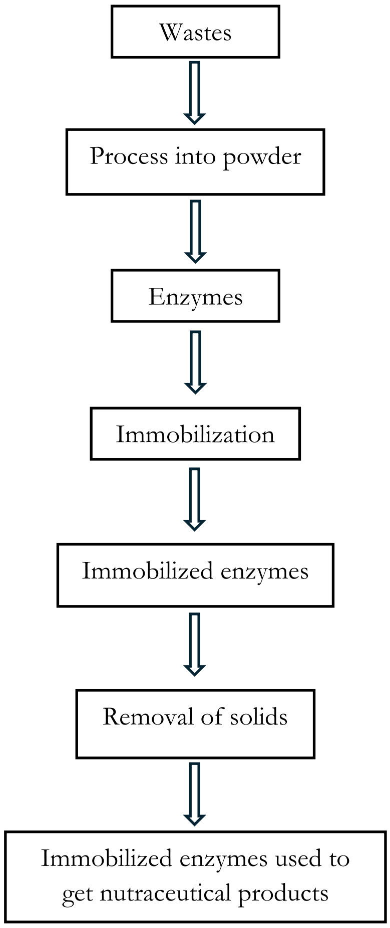

Figure 6 Process of use of immobilized enzymes to get nutraceutical products.

Use of immobilized enzymes for nutraceuticals production

The conventional methods for the production of biodiesel are costly and time-consuming. Biodiesel has great demand worldwide. Lipase is important enzyme for the biodiesel production. The use of immobilized lipase will reduce the cost of downstream process. The production of biodiesel involves pyrolysis and transesterification reactions. The transesterification reaction of oil is carried by immobilized lipase. There is a report on immobilized enzymes in bio-renewables production (Franssen et al., 2013). There is a study on use of immobilized lipase from *Burkholderia* sp. for biodiesel and fatty acids production by transesterification reaction (Rodrigues et al., 2013). The yield of biodiesel obtained was 90%.

Immobilized enzyme such as glycosyl transferases can be used for the production of prebiotic oligosaccharides using sugars such as lactose or sucrose (McNeil et al., 2013). Immobilized enzymes are also used for the production of syrups, confectionaries, amino acids, acrylamide, etc. (Hettiarachchy et al., 2018). They can be used in continuous and batch process.

Immobilized enzymes such as pectinases have applications in food industries. In fruit juices, they increase the production of nutraceuticals i.e., phytochemical compounds such as phenolic compounds (Ravindran et al., 2016). In wine production, these pectinases improve the stability, colour, flavour and anthocyanin content.

Immobilized enzyme lipases impart flavours and fragrances to the food products. Free and immobilized lipases from *Rhizopus* sp. are used in the production of citronellyl butyrate and valerate (Ferreira-Dias et al., 2013). The use of immobilized lipase enzyme to improve the flavour and fragrances of food products is a good alternative to chemical approach. Lipases improve the flavour of soft cheeses and texture of toffees. The enzyme lipase produced by *A. niger, R. oryzae* and *C. cylindracea* when immobilized has important application in bakery to increase the volume and shelf-life of breads (Shamim et al., 2018). There are reports on production of nutraceuticals using immobilized enzyme lipase from *Candida antarctica* and *Lactobacillus ruteri* (Nedovic et al., 2003). The immobilized lipase and proteases enzymes are used in dairy industries for cheese ripening and to enhance or impart the flavour to cheese-like products (Mandake et al., 2020). Natural antioxidants such as vitamins C and E are the best accepted for food applications.

D-tagatose as important nutraceutical

D-tagatose is important nutraceutical because it has anti-diabetic and antioxidant property, and also helps to control obesity (Roy et al., 2018). The immobilized enzymes can be used for production of nutraceutical D-tagatose. L-arabinose isomerase enzyme from *Bacillus coagulans*, *Geobacillus thermodenitrificans*, *Thermotoga neapolitana* produce D-tagatose.

Nutraceuticals and their therapeutic benefits

The nutraceuticals have several therapeutic benefits which are shown in Fig. 7. These include treatment of cervical cancer and arthritis, as antioxidants, in control of diabetes, immunomodulators, etc. (Shinde et al., 2014). There is a report on menaquinone-7 nutraceutical production by solid state fermentation using *Bacillus subtilis* (Ahmad et al., 2013). Menaquinone-7 is important homologue of vitamin-K and is useful in synthesis of blood coagulation factor.

Fermentation is common way for food preservation. The lactic acid bacteria (LAB) are the best group of bacteria which can increase nutraceutical value of food products. This use of LAB to increase nutraceutical value of food is gaining wide interest by the researchers. The LAB proteases yield small peptides which have many useful benefits to the health (Castro et al., 2019).

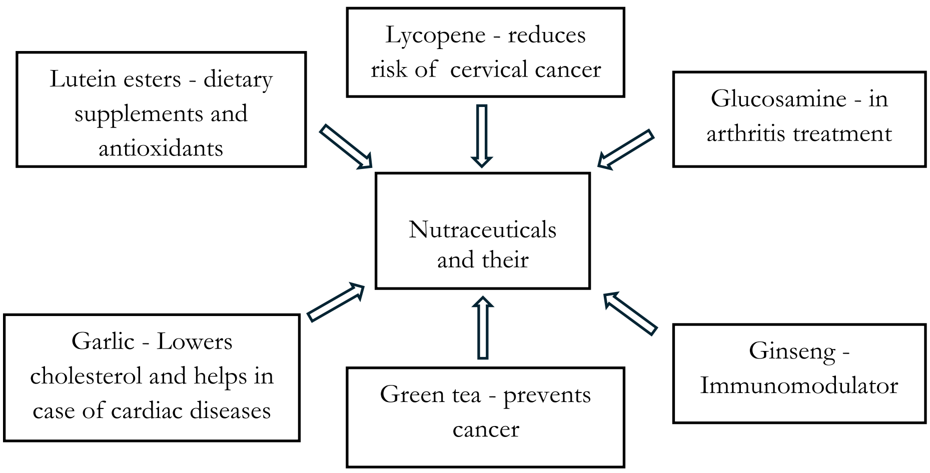

Figure 7 Nutraceuticals and their therapeutic benefits.

Conclusions and Future Perspectives

The use of immobilized enzymes for the nutraceuticals production will be of great use as the nutraceuticals can be used to treat diseases, improve the flavour and quality of food products. The nutraceuticals will also have the application to add aroma to the food products and in bakery industries.

More research is needed in the area of various immobilized enzymes for nutraceuticals production. The new methods of immobilization of enzymes for production of nutraceuticals must be developed. More research on use of nutraceuticals in the medical field must be focused. The commercialization for the production of nutraceuticals using immobilized enzymes on a large is needed.

Conflict of Interests: The authors declare no conflict of interest.

Data Availability Statement: The data that support the finding of this study are available from the corresponding author, upon reasonable request.

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