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Enzyme immobilization and its applications in food processing: A review

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Abstract

The “immobilized enzymes” are the enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities which can be used repeatedly and continuously. Enzyme immobilization provides an excellent base for increasing availability of enzyme to the substrate with greater turnover over a considerable period of time. Immobilized enzymes are preferred over their free counterpart due to their prolonged availability that reduces redundant downstream and purification processes. The enzymes can be attached to the support by interactions ranging from reversible physical adsorption and ionic linkages to stable covalent bonds. The choice of the most appropriate immobilization technique depends on the nature of the enzyme and the carrier. Such techniques produce immobilized enzymes of varying stability due to changes in the surface microenvironment and degree of multipoint attachment. The industrial applications of immobilised enzymes are progressively increasing. Immobilized enzymes find use in a number of biotechnological products with applications in diagnostics, bio affinity chromatography, and biosensors. Immobilised enzymes find wide applications in the food industry. With these immobilised enzymes, it is possible to obtain different types of sugar syrups, lactose free milk, clarified and debittered juices and wines. Immobilised enzymes can be employed for the production of different active packaging material like oxygen scavenging, anti-microbial films. However, commercialization of immobilized enzymes is still at a slower pace because of their costs and storage problems. Research should be focused to overcome the current limitations related to immobilization techniques, so as to expand the horizon for all-round application. In future, immobilized enzymes are going to play a vital role in various industries including pharmaceuticals, chemicals, food and fuel.

Keywords: Enzymes, immobilisation, good industry, biosensors, active packaging

1. Introduction

Enzymes are biological catalysts with high selectivity's. Enzymes are comprised of a protein component (biopolymer) and cofactors or prosthetic groups most of the times (Datta *et al.*, 2013) ^[9]. These biological catalysts speed up the chemical reaction, at which equilibrium is achieved without altering its position, and undergoing no insignificant chemical change in itself (Mohamad *et al.*, 2015) ^[28]. In recent years the application of enzymes in different industries is continuously increasing. The industrial applications of enzymes include food (baking, dairy products, starch conversion) and beverage processing (beer, wine, fruit and vegetable juices), animal feed, textiles, pulp and paper, detergents, biosensors, cosmetics, health care and nutrition, wastewater treatment, pharmaceuticals and chemical manufacture and bio fuels such as biodiesel and bio-ethanol (Homaei *et al.*, 2013) ^[18].

However, the applications and desirable traits of enzyme are often hampered by their sensitivity to process conditions, low stability, their cumbersome recovery and reuse and tendency to be inhibited by high concentrations of reaction components (Hernandez and Fernandez-Lafuente, 2011) ^[15]. These drawbacks can be overcome by a technique called by immobilization which results in the development of stable, robust and preferably insoluble biocatalysts (Kourkoutas *et al.*, 2004) ^[24]. Enzyme immobilization may be defined as the confinement of an enzyme to a phase (matrix/support) other than the substrates and products. Inert polymers and inorganic materials are usually used as carrier matrices (Datta *et al.*, 2013) ^[9]. The term immobilized enzymes refers to enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously (Katchalski- Katzir, 1993) ^[22]. Moreover, the attachment of an enzyme to a solid support can increase its resistance to various environmental changes such as pH or temperature (Homaei *et al.*, 2013) ^[18].

2. Advantages and disadvantages of Immobilised enzymes: (Katchalski-Katzir, 1993) ^[22]

Potential Advantages:

- Catalyst/enzyme can be reused.
- Easier reactor operation.
- Easier product recovery.
- Wider choice of reactors.

Potential disadvantages

- Loss in activity
- Reduced activity per unit volume
- Diffusion limitation
- Additional cost

3. Properties of support/carrier material used for immobilisation: (Homaei, 2016) ^[17]

The performance of an immobilized enzyme system is dependent on the characteristics of the matrix/support. Some of the desirable properties of support/matrix are:

- Resistance to physical compression.
- Inert towards enzymes.
- Easy to obtain/ derive/ regenerate.
- Resistant to microbial attack.
- Available readily at low cost.
- Large surface area with high permeability
- Presence of sufficient functional groups for enzyme attachment under non-denaturing conditions.
- Good chemical and thermal stability.

4. Techniques used for immobilisation

A number of methods can be used for immobilising an enzyme varying from reversible physical adsorption and ionic linkages to stable covalent bonds. These techniques can be classified in no. of ways. In one approach immobilization methods are classified into two broad categories: irreversible and reversible methods. Apart from this classification, immobilization methods have also been classified on the basis of type of chemical reaction used for binding as support binding and entrapment methods (Homaei *et al.*, 2016) ^[17].

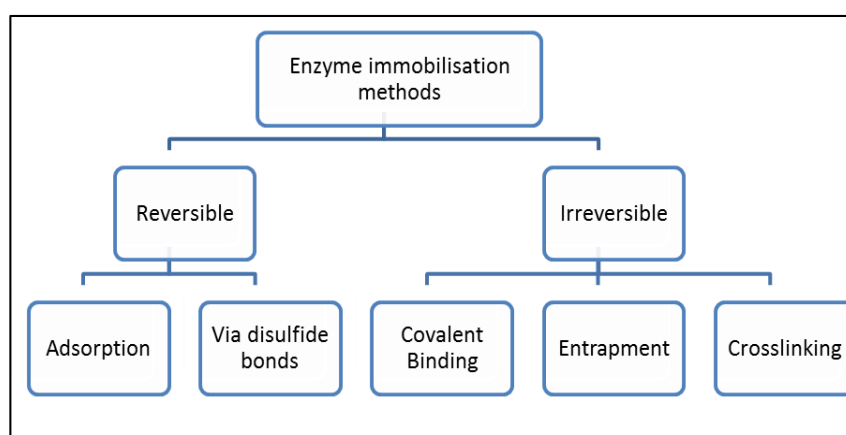


Fig 1: Classification of immobilisation methods into two broad categories: irreversible and reversible methods (Homaei *et al.*, 2016)

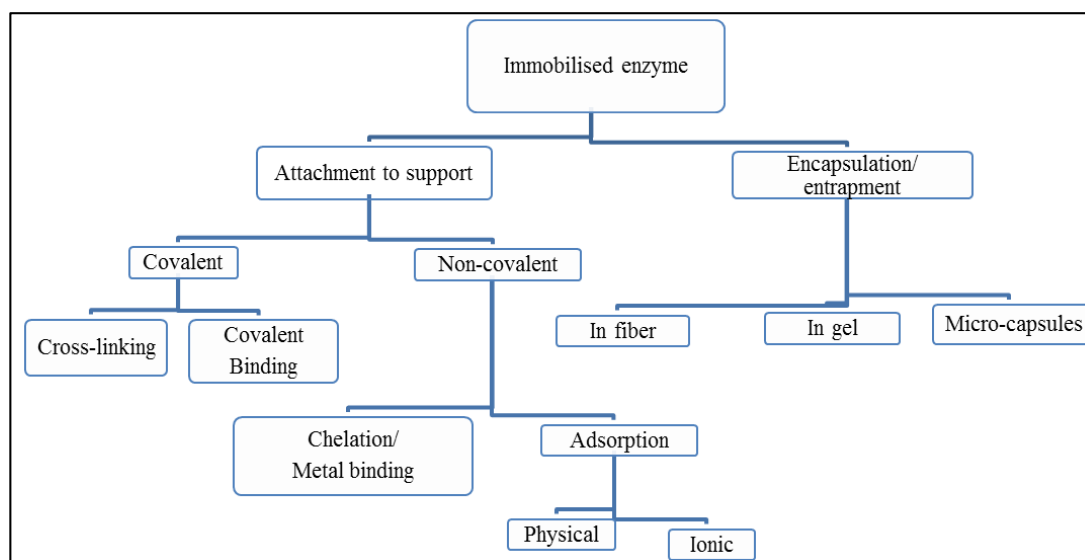


Fig 2: Classification of immobilization methods according to the binding capacity of the carrier material (Homaei *et al.*, 2016) ^[18]

4.1 Methods of Irreversible Enzyme Immobilization

In irreversible enzyme immobilisation the biocatalyst bound to the support cannot be detached without affecting either the biological activity of the enzyme or the support. The most widely used methods of irreversible enzyme immobilization include covalent linking, entrapment or micro-encapsulation, and cross-linking.

a. Immobilisation via formation of Covalent Bonds

It is the most extensively used method of immobilization of enzymes. In this method the covalent bonds are formed between the chemical groups present in enzyme and the chemical groups present on the support. This method results in stable bond formation between enzyme and matrix there by preventing the detachment of enzyme from the support upon

use (Costa *et al.*, 2005) ^[8]. This covalent linking of biocatalysts to matrix emerges from their side chain amino acids like aspartic acid, arginine, histidine and degree of reactivity depending on different functional groups like imidazole, indolyl, phenolic, hydroxyl (Singh, 2009) ^[41]. This technique is used in situations where complete absence of enzyme in the product is desirable. But this method results in the chemical modification of enzyme resulting in loss of enzyme activity (Costa *et al.*, 2005) ^[8].

b. Cross Linking

The cross-linking method is based on the formation of covalent bonds between the enzyme or active molecules by means of bi- or multifunctional reagents (Albayrak and Yang, 2002). This method is also known as copolymerization. Enzymes are joined to one another with the help of bi- or multifunctional reagents (e.g., glutaraldehyde, glutaraldehyde, glyoxal, diisocyanates, hexamethylene diisocyanate, toluene diisocyanate, etc.). This technique is cheap and simple but not used with pure enzymes. The main disadvantage or demerit of this method is that the poly-functional reagents used for cross linking the enzyme may denature or structurally modify the enzyme leading to the loss of catalytic properties (Costa *et al.*, 2005) ^[8].

c. Physical Entrapment

This method consists of the physically trapping the active components into a film, gel, fiber, coating, or microencapsulation (Costa *et al.*, 2005) ^[8]. The polymeric network allows the substrate and products to pass through but retains the enzyme. In this method can be achieved by mixing an enzyme or active molecule with a polymer and then cross linking the polymer to form a lattice structure that traps the enzyme. The advantages of this immobilization method are the extremely large surface area between the substrate and the enzyme, within a relatively small volume. The major disadvantages of this method include the occasional inactivation of enzyme during microencapsulation, leakage of enzyme and the requirement of high enzyme concentration (Nisha *et al.*, 2012) ^[30]. The matrices used for entrapping of enzymes include polyacrylamide gel, collagen, gelatin, starch, cellulose, silicone and rubber.

4.2 Methods of Reversible Enzyme Immobilization

a. Adsorption:

The adsorption of enzymes on materials like activated charcoal, alumina and ion exchange resins is one of the simplest techniques used for restricting the mobility of enzyme (Brady and Jordan, 2009) ^[5]. The enzyme is bound to support by means of non-covalent linkages like ionic, hydrophobic interactions, hydrogen bonding depending on the nature of amino acids available at the surface of enzyme and chemical nature of the support. This technique can be accomplished by mixing an aqueous solution of enzyme with the support material for a period of time followed by washing away of the excess enzyme from the immobilized enzyme on the support. This method of immobilisation is simple causing little effect on enzyme activity and can be reversed by adding fresh enzyme. However the method suffers from the main disadvantage of enzyme desorption from the support in response to changes in the pH, temperature, solvent, and ionic strength or of surrounding medium (Costa *et al.*, 2005) ^[8].

b. Ionic Binding

Immobilization via ionic binding is based, mainly, on ionic binding of enzyme molecules or active molecule to solid supports containing ionic charges. The main difference between ionic binding and physical adsorption is the strength of the interaction, which is much stronger for ionic binding but less strong than covalent binding (Torres *et al.*, 2002) ^[44]. The nature of this non covalent immobilization the process will be reversed by changing the temperature polarity and ionic strength conditions (Nisha *et al.*, 2012) ^[30].

c. Immobilisation via disulfide linkages

This technique involves the formation of disulfide (-S-S-) bonds with the support. The enzymes bearing exposed non-essential thiol (SH) groups can be immobilized onto thiol-reactive supports provided with reactive disulfides or disulfide oxides under mild conditions. The main advantage of this approach is the reversibility of the bonds formed between the activated solid phase and the thiol-enzyme, as the bound protein can be released with an excess of a low-molecular-weight thiol. The possibility of reusing the polymeric support after inactivation of the enzyme may be of interest for the practical use of immobilized enzymes in large-scale processes in industry, where their use has often been hampered by the high cost of the support material (Ovsejevi *et al.*, 2013) ^[31].

d. Affinity Binding

Affinity immobilization exploits specificity of enzyme to its support under different physiological conditions. It can be accomplished by two ways- either the matrix is pre-coupled to an affinity ligand for target enzyme or the enzyme is conjugated to an entity that develops affinity toward the matrix. The advantages of this method are the enzyme is not exposed to any harsh chemicals conditions, conformational changes during immobilization are minimal, and the retention of high activity by immobilized bio molecule (Sardar *et al.*, 2000) ^[37].

5. Application of the Immobilized Enzymes

In recent years the use of immobilized enzymes has increased considerably in various industries like pharmaceuticals, detergents, chemicals and food. The present applications of immobilized enzymes are as under:

a. Biomedical/ Therapeutic Application

Immobilized enzymes are presently used in the diagnosis and treatment of various diseases. Immobilized enzymes are used in biosensors and ELISA for detection of various analytes from complex substrates like stool, blood etc. (Malhotra and Chaubey, 2003) ^[26].

a. Wastewater Treatment/ Bioremediation

The effluents generated by various industries are threat to environment even if these are present in low amounts because of their carcinogenic nature. The enzymes commonly used in the wastewater treatments are preoxidases, laccase, azo reductases lipase. Due to harsh conditions like extreme temperature, low or high pH and high ionic strength these enzymes may lose their activity. This problem can be overcome by immobilization. The immobilized is of high interest for the hydrolysis of oils and fats for treating the waste water from the food industry (Nisha *et al.*, 2012) ^[30].

5.1 Food Industry

Food industry is invariably searching newer and advanced technologies to fulfil the consumer demands. The use of enzymes for transformation of raw materials into useful products has been practised from early times. Enzymes are generally regarded as safe (GRAS) from the legal point of view that has aroused their extensive use in food processing. The addition of few enzymes in purified state to food preparations enhances flavour, aroma, appearance and nutritional value of foods. The main problems associated with the industrial application of enzymes are their cost and unstable nature that can be overcome by immobilisation of enzyme. The applications of immobilised enzymes in food industry are continuously increasing (Khan and Alzohairy, 2010) [23]. The main applications of immobilised enzymes in the food process industry are as follows:

5.1.1. Immobilized enzymes-In the anti-microbial packaging of foods

Microbial contamination of food occurs mainly at their surface during post process handling obligating the use of anti-microbial agents. Antimicrobial active packages are those which are in contact with the food and prevent surface growth of pathogenic micro-organism thereby extending its shelf life (Fucinos *et al.*, 2012) [12]. The immobilization of anti-microbial enzymes on food packages provides a promising form of active packaging systems prolonging the shelf-life of non-sterile, chilled or minimally processed foods (Hanusova *et al.*, 2013) [14]. Immobilization of anti-microbial agents on food package rather than coating it on the surface of the package is more effective in preventing microbial contamination as immobilisation reduces their amount required to achieve the antimicrobial effect as well as prolongs their activity. Enzymes like papain, lysozyme exhibit anti-microbial properties (Manohar *et al.*, 2015) [27].

Hanusova *et al.* 2013 [14] developed an anti-microbial packaging based on immobilized anti-microbial enzymes. The study reported that the immobilisation Glucose oxidase and lysozyme on polyamide and ionomer films enhanced their storage and thermal stability and these immobilised enzymes were less sensitive to pH changes than free enzyme. Furthermore, the polyamide and ionomer films with immobilized glucose oxidase inhibited the growth of bacteria *Escherichia coli* CNCTC 6859, *Pseudomonas fluorescens* CNCTC 5793, *Lactobacillus helveticus* CH-1, *Listeria ivanovii* CCM 5884 and *Listeria innocua* CCM 4030 on agar media.

Manohar *et al.* 2015 [27] studied the anti-microbial activity of papain covalently immobilised to LDPE (low density polyethylene), HDPE (high density polyethylene), LLDPE (linear low density polyethylene) and PCL (polycaprolactam) with curcumin as the photocrosslinker. Anti-microbial activity was determined against *Acinetobacter* sp. isolated from cottage cheese and *Staphylococcus aureus*. It was observed that after 30 days, the free enzyme retained 87 per cent of its original activity, while the immobilized enzyme retained more than 90 per cent of its activity on these polymers. Papain cross linked to LLDPE exhibited the best antibiofilm properties against *Acinetobacter* sp and *Staphylococcus aureus* when compared to the other three polymers. The immobilised enzyme was able to reduce the amount of carbohydrate and protein contents in the biofilms formed by these organisms. Antimicrobial action of Papain crosslinked (PCC) LDPE was tested on beef samples against both the bacterial strains. Meat wrapped with the modified LDPE and stored at 4 °C showed

9 log reductions of these organisms at the end of the seventh day as compared to samples wrapped with the bare polymer.

5.1.2. Immobilized enzymes-In the clarification of juices

Clarification is an important step in juice processing. On industrial level juice clarification is mainly achieved by making use of pectinolytic enzymes (Pinelo *et al.*, 2010) [33]. The pectin a polysaccharide present in middle lamella of cell wall is mainly responsible for turbidity and consistency of fruit juices. The pectinolytic enzymes like polygalactouranase (PG), pectin methyl esterase (PME) etc. hydrolyse pectin and degrades it resulting in juice clarification (Jayani *et al.*, 2005) [19]. The pectinase enzyme possesses excellent catalytic properties but the native enzymes have disadvantages like less stability under operational conditions, difficulty to isolate product and cannot be repeatedly and continuously in an industrial process (Sheldon *et al.*, 2005) [39]. The application of immobilized pectinase in ultrafiltration serves as an alternative to conventional processes (use of chemical finings) for clarification of fruit juice.

Chauhan *et al.* 2015 [7] carried out studies on clarification of pineapple juice using immobilised pectinase enzyme. The immobilisation of pectinase enzyme was carried out via adsorption on celite using glutaraldehyde (2.5%) as cross-linking agent. As compared to free enzyme, immobilised enzyme exhibited maximum activity at higher temperature and lesser incubation time. Furthermore immobilisation was able to retain enzyme activity after repeated use (50 per cent of activity after third cycle of repeated use).

A study conducted by Namika *et al.* 2014 [29] showed similar results using immobilised pectinase enzyme for clarification of plum juice. The surface adsorption onto silica gel was employed for immobilisation of the commercial pectinase. In comparison to free enzyme bound enzyme was more effective in clarifying juice in lesser time and was able to exhibit maximum activity at higher temperature. After 3rd cycle of repeated use about 50 per cent retention in enzyme activity was observed for immobilised enzyme.

5.1.3. Immobilized enzymes-In the production of lactose free milk.

Lactose is a disaccharide composed of glucose and galactose units linked by β -1,4 glycosidic bond. Lactose is main sugar found in milk and milk products. Lactase/ β -galactosidase enzyme produced by walls of small intestine hydrolyzes milk sugar lactose into glucose and galactose (Ghatak *et al.*, 2013) [13]. The lack of lactase enzyme in some people leads to a condition called lactose intolerance characterised by decreased ability to digest lactose. Some of the symptoms of lactose intolerance include abdominal pain, bloating, diarrhoea, gas, and nausea (Heyman, 2006). The high lactose content present in milk products like ice-cream, condensed milk crystallises resulting in inferior quality products (Ghatak *et al.*, 2013) [13]. In addition the presence of high concentration lactose in dairy wastes contributes to environmental pollution as it increases B.O.D. of water (Panesar *et al.*, 2013). The lactose free milk is regular milk, with the addition of the lactase enzyme. Hydrolysis of lactose in milk makes it suitable for lactose intolerant people, improves processing characteristics of milk and reduces environmental pollution (Ghatak *et al.*, 2013) [13].

Ghatak *et al.* 2013 [13] produced lactose free milk using immobilised lactase enzyme. The lactase enzyme was isolated from *Enterobacter cloacae* and immobilised via entrapment using alginate gel (2%). The study revealed that enzyme

exhibited maximum activity at a concentration of 39.33 IU/mg at a temperature of 50 °C and pH 9. The immobilised lactase enzyme was used for the preparation of low lactose milk in a jacketed bed column reactor (height 150mm, internal dia 22mm). The maximum conversion of lactose (46.67%) was observed at a bed height of 89mm using milk (lactose content 4.2%) as substrate at 8 hour operation.

5.1.4 Immobilized enzymes-In cheese ripening

Cheese maturation or cheese ripening is a process carried out for developing unique flavour, aroma and texture in cheese. It is usually accomplished in 6 months to 2 years depending on the cheese variety. But longer maturation periods of cheese increases handling and capital cost (Anjani *et al.*, 2007)^[2]. A variety of methods such as elevation of ripening temperature, use of modified starters or addition of adjunct cultures can be used to enhance cheese ripening, but addition of exogenous enzymes is considered to be the simplest and most specific of all the methods. The addition of free enzymes to milk during cheese making has been found to be undesirable because of loss of enzyme in whey, poor enzyme distribution, reduced yield and poor quality cheese (Kailasapathy and Lam, 2005)^[23]. These drawbacks can be eliminated by making use of encapsulated enzymes. The microcapsule physically separates enzyme from its substrate in the milk and curd mixture during cheese making. The enzyme in whey is only released into the cheese matrix upon capsule breakdown during ripening (Anjani *et al.*, 2007)^[2].

Kailasapathy and Lam, 2005^[23] determined the suitability of gellan, k-carrageenan and a high-melting-fat-fraction of milk fat (HMFF) to encapsulate protease enzymes (Flavourzyme) and their impact in accelerating Cheddar cheese ripening. The rates of enzyme entrapment were 48.2 per cent, 55.6 per cent, and 38.9 per cent for gellan, k-carrageenan and HMFF, respectively. The enzyme capsules were incorporated into milk during cheese manufacture. All the three types of cheese treated with encapsulated enzyme showed higher rates of proteolysis than the control cheese throughout the ripening period. The rate of proteolysis was greater with cheeses made incorporating k-carrageenan capsules containing protease. The k-carrageenan and gellan capsules showed higher retention (90.0% and 91.5%) than milk fat capsules (73.5%). The enzyme losses from gum gel capsules were also lower (5.62% and 8.66% for gellan and k-carrageenan gums, respectively, compared with 17.93% for HMFF capsules).

5.1.5. Immobilized enzymes-In wine processing (haze removal)

Haze or turbidity in a transparent medium is an optical phenomenon resulting from small suspended particles whose presence diverts light from its regular path. Protein haze in wines is an aesthetic problem affecting the quality and consumer acceptance of white wines. At normal temperatures grape proteins in wines are quite stable however during storage these proteins become insoluble/ precipitate out thus leading to haze formation (Vincenzi *et al.*, 2011)^[45]. This quality defect of wines can be overcome by removing grape proteins that have escaped wine making. The most common technique used for removal of insoluble haze forming proteins from wine is adsorption onto bentonite. But this technique has some limitations like its non-specific nature, removing components other than proteins like those responsible for aroma and flavour thus impairing the organoleptic properties of wine. Besides this use of bentonite finings causes variation in mineral composition of wines (Sauvage, *et al.*, 2010)^[38].

The commercial proteolytic enzyme preparations can be used for removal of grape proteins thus serving as an effective alternative to adsorption technique.

A study conducted by Benucci *et al.*, 2016^[8] revealed that immobilised proteolytic enzymes are effective in haze removal in white wines. The unfinned hazy white wines used as samples included Chardonnay, Moscato di Terracina, Malvasia del Lazio, Manzoni bianco, Riesling, Sauvignon blanc, Moscato di Terracina. The stem bromelain (EC 3.4.22.32) and papain (EC 3.4.22.2) were immobilised on commercial chitosan beads by a direct mechanism at pH 3.2. In terms of reducing haze and total protein content immobilised bromelain was found to be more beneficial in all the seven samples than immobilised papain enzyme. When compared to bentonite finings the enzymatic treatment had little or no effect on the mineral content and sensory attributes of wines, suggesting widespread use of immobilized enzymes for haze removal.

5.1.6. Immobilized enzymes- In active packaging/ In package processing (Debitting of fruit juices)

Active packaging may be defined as the packaging system in which active agents are embedded into or on the surface of food packaging materials that can enhance the nutritive value, economics, and stability of food, as well as enable in-package processing (Wong and Goddard, 2014)^[46]. Naringin (4, 5, 7,- Trihydroxy flavanone-7-rhamnoglucoside) is one of the main bitter compounds in citrus juices such as grapefruit and Natsudaikai orange. The presence of naringin (above 50 ppm) and limonin in processed citrus juice causes bitterness due to their synergistic effect thereby reducing the consumer acceptability of citrus juice (Puri and Banerjee, 2000)^[34]. The techniques like adsorption, treatment with ion-exchange resin and use of chemicals can be employed for reducing the naringin levels in juice thereby improving their stability and sensory qualities. However these treatments have several drawbacks. The hydrolysis of naringin by naringinase enzyme can serve as an alternative to these conventional treatments (Del Nobile *et al.*, 2014)^[10]. Naringinase is an enzyme (with α -rhamnosidase and β -glucosidase activity) hydrolyzing naringin to the non-bitter aglycone naringenin (Soares and Hotchkiss, 1998)^[42].

A food grade film developed by Del Nobile *et al.*, 2014^[10] which when in contact with grapefruit juices was able to reduce the naringin concentration of the said juices. The naringin hydrolysing film was based on a crosslinked PVOH matrix containing immobilised naringinase enzyme. The effectiveness of PVOH cross linked film containing immobilised naringinase enzyme in reducing naringin concentration was compared with PVOH cross linked film without enzyme. The film containing immobilised enzyme when in contact with juice resulted in reduction in naringin concentration after 6 hours. The bare film in contact with juice exerted no effect on the concentration of naringin even after 100 hour.

5.1.7. Immobilized enzymes-In the production of flavour esters

The demand for natural flavours is growing day by day with ethyl and hexyl esters being more important and versatile ones (Rodriguez-Nogales *et al.*, 2005)^[35]. The most widely used ester components in food, pharmaceuticals and other industries include ethyl valerate and hexyl acetate having typical flavor of green apple and pear respectively (Chaabouni *et al.*, 2006)^[6]. Mostly these natural flavouring components

have been derived from naturally occurring materials or produced by chemical synthesis. The most beneficial method for synthesis of natural flavours is the lipase enzyme catalyzed synthesis in presence of an organic solvent because of the several advantages like highly specific nature of reaction, the mild operational conditions, higher degree of purity of the products and their acceptability in the food industry (Rosenstein and Gotz, 2000) [36]. But the activity of free lipase is inhibited by short chain fatty acids affecting the synthesis of flavours. The immobilisation of lipase enzyme not only overcomes this limitation but also reduces cost by recycling enzyme.

The flavour esters (hexyl acetate and ethyl valerate) were produced by Chaabouni *et al.*, 2006 [6] immobilised lipases. Lipase isolated from *Staphylococcus simulans* was immobilised on different supports like CaCO₃, Celite 545, Glass beads and Carboxy-Methyl Sephadex. It was reported that the immobilized lipase retained its activity over for five cycles and ten cycles of use in hexyl acetate and ethyl valerate respectively. The most appropriate support for immobilisation of lipase was found to be CaCO₃.

5.1.8. Immobilized enzymes-In the production of syrups/sweeteners

In food industry enzymes are mainly employed for the production of sweeteners. Sucrose is most widely used sweeteners in beverages and other food products. The hydrolysis of sucrose produces invert sugar composed of glucose and levulose in equal amounts (Yadavilli *et al.*, 2013) [47]. Invert sugar is sweeter than sucrose alone and does not crystallise. In addition invert sugar prevents crystallization cane sugar in heavy sugar syrups, exerts more osmotic pressure and is more soluble than glucose and sucrose (Kurup *et al.*, 2005) [25]. Sucrose hydrolysis can be accomplished either by chemical method that involves heating of sucrose in presence of acids like hydrochloric acid, tartaric acid etc at 75–80 °C or by enzymatic treatment with invertase (EC.3.2.1.26) at 30–40 °C. Inversion of sucrose in presence of acid is costly and generates a no. of by-products. Enzymatic hydrolysis occurs at lower temperature, is eco-friendly process generating less toxic and pollutant waste as compared to acid hydrolysis. Furthermore the use of an immobilized enzyme proves to be cost effective as the immobilised enzymes can be recycled thereby acting as an effective alternative to acid hydrolysis for the preparation of invert sugar (Tomotani and Vitolo, 2007) [43].

El-Sayed *et al.* 2015 [11] carried out enzymatic hydrolysis of sucrose producing invert sugar. The free invertase isolated from pea pods was immobilised by two methods *viz.*, covalent binding (cross linking in presence of glutaraldehyde as cross linking agent) and entrapment (entrapment in sodium alginate beads). Because of the weak bonds formed between the carrier and the enzyme covalent binding was found to be inappropriate for enzyme immobilisation. On the other hand entrapment method immobilised free invertase completely (100%) with high activity (1108×10³ µg fructose) and immobilization efficiency 739.2. Degree of sucrose hydrolysis sucrose using immobilized invertase was found to be 33.52 per cent while for the free invertase was 3.9 per cent. The immobilized invertase exhibited higher specific activity (14600 U/mg) about 86.9 per cent times than that of the free enzyme. The optimum conditions for sucrose hydrolysis were immobilised enzyme concentration of 0.02 mg/reaction, temperature of 45 °C and pH 5 was found to be optimum.

5.1.9. Immobilized enzymes-In the analysis of food samples (biosensor)

Biosensors are analytical devices consisting of immobilised biomolecules mainly enzymes that interact with an analyte specifically and produce easily measurable physical, chemical and electrical change. The sensitivity and selectivity of biomolecules along with their stability determine the performance of biosensors. Biosensor has three distinct components - bioreceptor (enzyme, antibody, DNA) that reacts with the analyte and produces a biochemical change, an immobilization surface (conducting polymers, sol-gel films) for the immobilization of biomolecule and transducer unit that converts biochemical reaction into a recognizable signal. In food analysis the use of biosensors is increasing tremendously and is preferred over conventional methods because of their low cost, simplicity and less time consuming features. Some of the common applications of biosensors in food analysis include detection of calcium in milk, evaluation of antioxidant capacity of tea and orange juices, estimation of sulphite in food and beverages, analysing ethanol, glucose and lactate in wine, determination of polyphenolic content of beers (Jugovic *et al.*, 2016) [20].

Basu *et al.* 2007 [3] developed a biosensor based on immobilisation of enzyme for detection of cholesterol in food. The higher concentration of cholesterol in the blood i.e. hypercholesterolemia increases the risk of cardiovascular diseases. Thus, cholesterol is most frequently determined analyte in both clinical as well as food analysis. The biosensor for cholesterol determination was developed by employing immobilised cholesterol esterase and cholesterol oxidase enzymes. These enzymes were immobilised via cross-linking in presence of glutaraldehyde and bovine serum albumin (BSA) on oxygen electrode with gold cathode and Ag/AgCl anode. The immobilized enzymatic membrane was attached to the tip of the electrode by a push cap system. The biosensor exhibited maximum activity at pH of 6 and temperature of 25 °C. The sensor was used for total cholesterol determination in different food samples like meat, eggs. The results obtained were found to be consistent with colorimetric method.

Sharmin *et al.* 2007 [40] developed a phosphate detection biosensor based on immobilised alkaline phosphate enzyme. The alkaline phosphatase was held by covalent linkages on aminated glass fiber disks in presence of glycidoxypropyletrimethoxysilane coupling reagent. The covalent bonding retained its activity and was reused about 4 times without much loss of enzyme activity. The biosensor was used for estimation of phosphate elements in water, raw milk and raw shrimp sample.

5.1.10 Future Prospects

Immobilized enzymes can be of great help in national security, for example, biocatalysts may be incorporated into air filters, masks and clothing to neutralize chemical gases or vapours. In near future immobilised enzymes can be used in treatment of water contaminated with pesticide thus minimizing the effect of pesticides on environment. In food industry immobilised enzymes like glucose oxidase can be employed for removing oxygen from beverages thereby reducing oxidative deterioration by converting it to gluconic acid. Immobilised enzymes can also help in strengthening national security as these can be incorporated in masks, filters neutralising harmful chemicals.

6. Conclusion

Enzyme immobilisation is a highly efficient and cost effective technique used in various fields like food industry, diagnostics, pharmaceuticals and environmental surveillance. This technique is continuously replacing conventional approaches in both lab scale and industrial levels. In food industry these immobilised enzymes can be a great help in producing different products like lactose free milk, sweeteners, clarified wines etc. However immobilised enzymes have some disadvantages like high cost of free enzymes and. The use of immobilised enzymes for production of different active packaging material like oxygen scavenging, anti-microbial films is gradually increasing. However, industrial application of immobilized enzymes is limited because of their costs and storage problems. More research should be focused on overcoming these limitations and expanding the range of applications of these immobilised enzymes.

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