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IMMOBILIZATION OF LIPASE FROM PLANT IN PECTIN

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Lipases from the wheat(Triticum austevium) germ and bran were immobilized in pectin with different degrees of esterification. The immobilized lipases were characterized in term of its thermal and pH stability. The optimum temperature for soluble lipases and immobilized lipases at 40 ^{0}C and 50 ^{0}C respectively. This shows that the resisted of immobilized lipases against denaturation. The pH for soluble and immobilized lipases were at 8.0. This shows that the immobilization did not alter the general character of the lipases.

Lipases (EC 3.1.1.3-Triacylglycerol acyl ester hydrolases) are versatile enzyme that possessing an intrinsic capacity to catalyze cleavage of carboxyl ester bond in tri-,di, and monoacylglycerols (the major constituents of animal, plant, and microbial fats and oil). Lipases catalyze a series of different reactions hydrolysis, ester synthesis, alcoholysis and transesterification. Lipases are unique enzymes in that they require interfacial activation for full catalytic performance. Mammals, bacteria and plant in large amounts produce lipase. Lipases have been purified from several different sources by using several different methodologies[1,4,9,10].

Lipases have been used successfully and extensively in industry. For instant, lipases have been extensively in the dairy industry, oleo chemical industry, in producing structured triacylglycerids, organic chemical processing, detergent formulation, synthesis of biosurfactant, paper manufacture, cosmetics and pharmaceutical processing. The disadvantages of using soluble enzymes in solution, the cost of enzymes is very high, difficulty to remove enzymes from the end product streams and thermal instability of soluble enzymes hampered the wide application of the enzymes. Some of these disadvantages could be avoided or minimized by immobilizing enzymes[1,2,3,7,9].

Many methods for immobilization of lipases are available, each involving a different degree of the complexity and efficiency. The various methods used can be subdivided into two main categories; chemical methods, where covalent bonds are formed with lipase, and the physical methods, where weaker interaction or mechanical containment of lipase is utilized [5,6,7]. Adsorption and entrapment are included in physical immobilization. Covalent bonding, ionic bonding and cross-linking are encountered in chemical immobilization.

Lipases immobilized by entrapment displaced better activities and stabilities when the trapping resin is hydrophobic. Lipases have also been immobilized on polyacrylamide using a diazonium intermediate, on a polyaminopolystyrene resin by azo linkages. Another procedure for attachment of lipase to agarose consists of the first reacting agarose beads N,N'-ethylenediamine to obtain aminoethyl agarose. Immobilization of enzymes through covalent attachment to solid support typically result in a significant activity loss of the linking chemistry modifying other parts of the enzymes, especially near the active sites of the enzymes[3,7,8,9].

Pectin is a polymeric carbohydrate, which has a high molecular weight and is present in all plants, primarily in the form of protopectin. Pectin has an important influence on plant

cells since protopectin and cellulose form the structure of the cell walls. Pectin is derived from the peel of citrus fruits (lemon, lime and orange) or from apple pomace. These raw materials are used because they produce pectin of superior quality, contain a relatively large amount of pectin and are available in sufficient quantities to make them commercially viable. Pectin consists primarily of a chain of galacturonic acid units, which are linked by α-1,4 glucosidic bonds. The galacturonic acid chain is partially esterified as methyl esters. The pectin molecules have a molecular weight of up to 150,000 and a degree of polymerisation of up to 800 units. The functional properties of pectin are largely determined by the degree of esterification (DE) of the pectin molecules. Pectin is traditionally used in a wide range of fruit-based products in which it acts as a gelling agent. Pectin is also used to adjust the mouthfeel of fruit-based beverages and as a protein stabiliser in acidified milk products such as yoghurt. Pectins with a DE of 50% or more are known as high-ester pectins and are capable of forming gels in aqueous systems with high contents of soluble solids and low pH values. Pectins with a DE of less than 50% are known as low-ester pectins. This group of pectins is divided into two sub-groups, i.e. conventional low-ester (LC) pectin and amidated low-ester (LA) pectin. Both sub-groups are characterised by their ability to form gels in systems with low solids content and a wide pH range. Both types form gels in the presence of calcium[12].

Lipase has been isolated and purified from the germ wheat(*Triticum austevium*). Lipase molecular weight was 143±2 kDa and his optimal conditions were 37°C and pH 8.0. The objective of this study is to immobilize the plant (wheat*Triticum austevium*) lipase on pectin with different degree of esterification and to study the behaviour of the prepared immobilized enzyme [13,14,15].

For experimental purpose wheat (*Triticum austevium*) grem and bran were used. *Pectin with different degree of esterification* (75%, 52%, 31.6%, 23%,17.1% and 9.2%) were prepared from stock standard. Solutions and buffers at different concentrations were prepared and storied in dark at 25°C in the dark. All reagents were of analytical grade and used as received. Lipase was precipitated from wheat (grem and bran) water extract.
Lipase activity assayed essentially as described by Tietz N.W. *et al*[11].The lipase activity was calculated based on the following equation LA= V.50/T Where V= the volume of NaoH (0.05 M) titrated in ml, and T= the time of reaction (5-30 min.). One unit of lipase activity is defined as the amount that liberated one mole of fatty acid under the specific condition. Specific activity of lipase was expressed as the enzyme unite per mg of protein.
Lipase was immobilized from water extract by adding each 100 ml exctract 10ml 2% pectin with different degree of esterification (75%, 52%, 31.6%, 23%,17.1% and 9.2%) at room temperature and storied at 2-4°C for 24 hours. The solution was centrifuged at 5000g for 20 min at 4°C, the supernatant and precipitate were collected for determination of lipase activity and protein concentration. The effects of pH on immobilized and free lipase activity were determined by using 0.05M Tris-Hcl (pH 4.5-9.1) at 37°C. The effect of temperature (20-60°C) on immobilized and free lipase activity were determined at pH 8.0.

Lipases were extracted from wheat germ and bran by using distill water without preliminary conducted defattig at ratio 1:100, at temperature 2-40 °C and in current of 24 hours. The activity of lipase was shown in tab.1.

Characteristics of extracted lipases **Table:1**

Extract	Lipase activity (units/cm^3)	Total protein (mg/cm^3)	Specific activity (units mg^{-1})
Wheat germ	0.013	12.0	0.001
Wheat bran	2.50	2.8	0.891

Lipases are water-soluble proteins and their concentration were based on sedimentation by organic solvents or salts of ammonium. Using of these precipitating agents in extraction process need additional and necessity of clearing of extract from salts. So pectinaceous substances with a various degree esterification were chosen as precipitating agents because of their different density of a negative charge. Acetone was used as control in precipitating protein тab.2.

Purification of lipases from wheat germ **Table:2**

Reagents	Precipitate weight (g)	Moisture (%)	Dry precipitate weight (g)	Total protein (mg/cm^3)	Lipase activity ($units/cm^3$)
acetone	13.2	87.5	1.60	66.4	0.833
Pectin (d.e 75%)	6.90	82.6	1.20	9.60	0.333
Pectin (d.e 52%)	8.44	90.5	0.80	9.70	0.500
Pectin (d.e 31%)	9.62	93.7	0.60	14.9	0.500
Pectin (d.e 23%)	45.9	65.1	16.0	66.6	1.500
Pectin (d.e 9.2%)	42.0	76.9	9.70	68.3	1.170

From the results it is visible, that the high activity of lipases in sediments was shown when pectin with a degree of esterification 23 % was used. However specific activity lipase in sediments were the greatest when pectin with a degree esterification 52 % was used (fig.1). Specific activity of lipase in acetone sediment was 0,013 units/ mg.

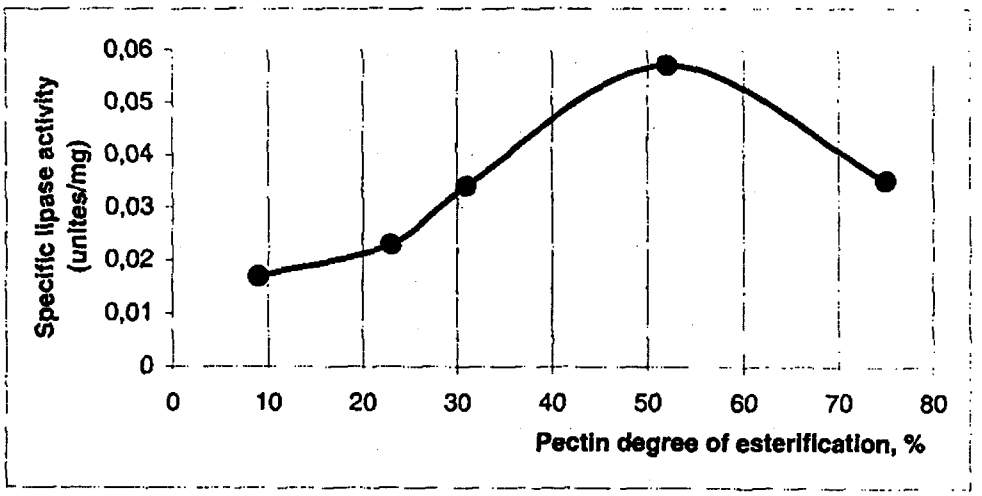

Fig.1: The effect of pectin degree of esterification on the specific lipase activity.

The effect of pH (in the range of 4.5-9.1) and temperature (in the range of 20-60 ^{0}C) on the wheat germ and bran were examined (fig.2 and 3).

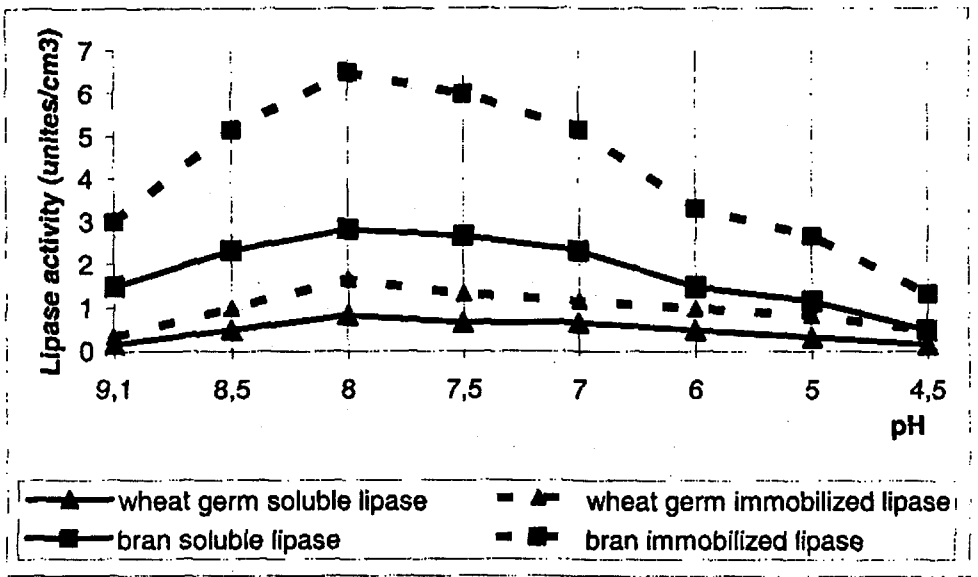

Fig.2: The effect of pH on the activity of wheat germ and bran lipases (soluble and immobilized lipases).

It is shown, that at change pH environments the activity of soluble and immobilized lipases were not distinguish change among themselves. Change in activity of immobilized of bran and a germ. Their activity is maximal was at pH 8,0, change pH leads to decreases in activity . Thus for lipase from bran activity decreases at pH 8.5и 7 for 20 %, whereas for lipase from germ at pH 8,5 on 40 %, at pH 7 on 20 % (fig.2).

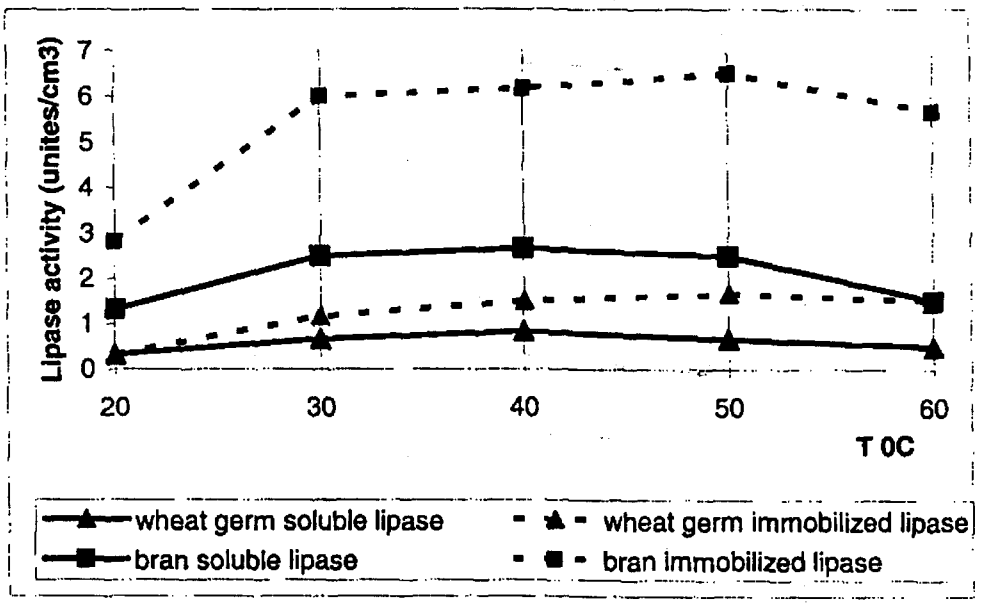

Fig.3: The effect of temperature on the activity of wheat germ and bran lipases (soluble and immobilized lipases)

The optimal temperature for lipase in the general makes 40 ^{0}C for soluble lipase and 50^{0}C for immobilized lipase. The immobilized lipases were more stable than soluble lipases when temperatures were increased (fig.3) which indicated the resistance of immobilized lipases against denaturation.

In conclusion, pectin with different degrees of esterification showed a promising future of applying polymer as support for biocatalyst as it allows easy immobilization technique.

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