CHAPTER 1

INTRODUCTION

Lipases are water soluble enzymes which have the ability to hydrolyze triacylglycerols to release free acids and glycerol. Lipases are extracellular enzymes produced by fungi, yeasts, bacteria, plants and animals where bacterial lipases are more economical and stable. Microbial lipases are widely diversified in their enzymatic properties and their substrate specificity which make them very suitable for industrial applications. These are in great demand because of potential applications (Sirisha et al., 2010). Bacterial lipases are used extensively in food and dairy industry for the hydrolysis of milk fat, cheese ripening, flavor enhancement and lipolysis of butter fat and cream. They are used in detergent industry as moisturizing agents. In textile industry they are used to increase fabric absorbency. They are used for synthesis of biodegradable polymers or compounds. Lipase production is affected by type and concentration of carbon and nitrogen sources, pH of the medium, the growth temperature and concentration of dissolved oxygen (Jadhav et al., 2013).

Lipases have a vast potential for application in a number of industries, many of which are totally unrelated. The most well-known use of lipase-catalyzed synthesis lies in the interesterification of fats to produce synthetic triglycerides with desirable characteristics. One such application is in the manufacture of cocoa butter equivalent from palm oil. Lipase-catalyzed hydrolysis can be used to obtain fatty acids and glycerol. The castor bean lipase-catalyzed hydrolysis is already operative on a commercial scale to manufacture fatty acids and glycerol. In cystic fibrosis (CF) patients pancreatic enzyme supplements may fail to control malabsorption and malnutrition, due to the inactivation of lipase by gastric acid. An alternative approach is to use exogenous lipases that are stable and active in an acid environment. Trials of acid stable fungal lipase have been but as yet no plant lipase source has been tested. Lipases can be used as digestive aids in the case of lipase deficiency, with its consequences for fat metabolism. Manufacture of lipases by microbial fermentations constitutes is the process is currently popular. Plant lipases can be used as alternative way than other sources because of their availability, less chance of having microbial contamination and they are natural sources. The isolation of the lipase from a plant source, rice bran is possible which can be used as alternative of microbial lipases

(Prabhu et. al., 1999). This study therefore focuses on the isolation of the lipase from plant sources. Lipase from various sources have been used in flavor promotion in processed cheese and in rapid maturing and introduction of flavor in chocolate crumb, in improvement of egg white whipping properties, as well as in the analysis of triacylglycerol. In recent years the growing demand of lipolytic enzymes has been increased due to its potential use in the various manufacturing processes of industrial goods such as detergent industry, food industry and in pharmaceutical industry. There are many plants seeds, especially of tropical origin, upon which no work have been reported.

Lipases serve important roles in human practices as ancient as yogurt and cheese fermentation. However, lipases are also being exploited as cheap and versatile catalysts to degrade lipids in more modern applications. For instance, a biotechnology company has brought recombinant lipase enzymes to market for use in applications such as baking, laundry detergents and even as biocatalysts (Guo and Xu, 2005) in alternative energy strategies to convert vegetable oil into fuel (Gupta et al., 2004; Ban et al., 2001). There are many plants available in our country which are very good sources of lipase enzyme, like: coconut, soy meat, pumpkin seed, winter melon seed, bean seed, lentil, orange peel, banana peel, mustard seed and so on. These plant sources are agriculturally most available in Bangladesh. In this study these plant origins of lipase enzymes are being selected to be researched, so that lipase activity and isolation of lipase from plant sources can be easily find out. Therefore, the present study is undertaken to develop a simple and systematic procedure for obtaining a high yield of the lipase in a pure and usable form. However, the enzyme is found to be very vulnerable to deactivation even under ambient conditions. For an enzyme to be of commercial value, a degree of stability needs to be conferred on it. It is therefore necessary to ascertain conditions under which the enzyme would be reasonably stable. The aim of the present study, therefore, is to establish the determination of the presence of lipase in plant sources, determination of the lipase activity of the plant foods, and the pH stability and heat stability of lipase collected from different plant origins, so that we can find out more stable lipases which can be collected easily by low cost and can be used in different food industry to minimize the presence high costing of lipase purchasing from foreign country.

Aims of the Study:

- Analysis of the lipase activity of different plant food sources

Objectives of the Study:

1. To identify lipase containing plant sources
2. To identify the optimum pH and optimum temperature stability of lipases
3. To provide lipases at low cost to minimize present high costing of lipase purchasing from foreign country for using in industries

CHAPTER 2

REVIEW OF LITERATURE

Lipase, a subclass of esterases is any of a group of fat-splitting enzymes found in the blood, gastric juices, pancreatic secretions, intestinal juices, and adipose tissues. Lipases have the ability of hydrolyzing fats in the digestive tract but also act as a biocatalyst in carrying out acylation and deacylation of many unnatural substrates. (Alberghina et al., 1990; Ejedegba et al., 2007; Dharmsthiti et al., 1997).

Esterases which can hydrolyze triglycerides at the water/oil boundary are termed lipases or, more systematically, triacylglycerol hydrolases (EC 3.1.1.3). Lipases are stable in the terms of reactivity and surrounding environment and are obtained from plants, animals and different microbial flora in good yields (Macraeand et al., Selva Mohan et al., 2008). From enzyme engineering point of view lipases have a tertiary structure (Sherin Basheerandm and Thenmozhi, 2010). It exposes catalytically active site in the presence of organic solvents and lipid phase. These enzymes are industrially optimized for the greater use. In this study an attempt has been made to detect lipase presence in various plant origins and to determine activity of various varieties of plant origins such as coconut, soya meat, bean seed and pumpkin seed. The enzyme activity was estimated and compared using substrate namely, olive oil.

Interest in isolating new enzymes and new enzyme-producing strains for their use in various fields is increasing due to increased awareness of environment and cost issues for major Research and Development. Among these enzymes, lipases, carboxylesterases, cellulases, xylanases, pectinases, amylases and proteases are some of the most important enzymes (Cherry and Fidantsef, 2003; Pandey et al., 2005; Buchholz et al., 2005). Currently microbial lipases are very diverse in their enzymatic properties and substrate specificities, which make them attractive for industrial applications (Macrae and Hammond, 1985; Vulfson, 1994; Pandey, 1999). Microbial lipase catalyzed the hydrolysis of triacyl glycerol to glycerol and free fatty acids in contrast to esterases (Macrae, 1983; Saxena, 1999). Novel biotechnological applications have been successfully recognized using lipases for the synthesis of biopolymers and biodiesel, the production of new pharmaceuticals, agrochemicals, and flavor compounds (Jaeger and Eggert, 2002; Hasan et al., 2006).

Lipases are present in various plant, animal and microbial sources. Lipases which are collected from microbial sources are very much popular at the recent years. But, lipase extraction from plant origins and other natural sources is very less-explored till now. This study therefore focuses on finding out the lipase containing plant sources, banana peel, mustard seed, pumpkin seed, winter melon seed, lentil, orange peel, bean seed, soya meat, eggplant and to determine their activity level in plant sources, coconut, soya meat, bean seed and pumpkin seed.

Rice bran, which comprises the testa and pericarp, is known to be rich in a number of important components such as oil (edible) (Zachariassen et al. and Giasotta, 1964), vitamins, sugars, and proteins. An important potential protein present in the rice bran is lipase. Like some other lipases, rice bran lipase is known to be regioselective (Gandhi, 1997 and Macrae, 1983) and has a preference for LMW (low molecular weight) substrates (Aizono et al., 1973; Noda and Kobayashi., 1968). These and other biochemical and enzymic properties would enable the employment of this lipase for the manufacture of specified products. No efforts have been reported to explore the possibility of extraction of lipase from rice bran for commercial use, a result of either the poor yield of the enzyme or the lack of information on the enzyme content of the different varieties of rice (Anita et al., 1999).

2.1 Seed lipases

Recently, seed lipases have been the focus of much attention as biocatalysts. In some cases, these enzymes present advantages over animal and microbial lipases due to some quite interesting features such as specificity, low cost, availability and ease of purification, representing a great alternative for potential commercial exploitation as industrial enzymes. The modification of fats and oils by transesterification, for instance, can be performed by both chemical and enzymatic catalysis. The industrial transesterification process is currently performed by chemical means, using high temperatures and alkaline metals as the reaction catalyst. In the enzymatic process, lipases can be used as biocatalysts to promote the exchange of triacylglycerol, showing greater efficiency and leaving no residues. However, several studies have indicated that such processes are very expensive due to the high cost of purifying the microbial enzymes (de Castro et al., 2004; Noor et al., 2003). Seed lipases present

certain advantages since they do not necessarily have to be purified in order to perform this and other processes (Caro et al., 2000).

2.2 Oil seed lipases

Oil seed (*Brassica napus L.*) have been shown to be localized in oil bodies or glyoxysomes. They are known to be played an essential role in the mobilization of seed storage lipids to support germination and post-germinative embryonal growth. Since the last decade, lipases often have been perceived by research scientists as one of the most important class of industrial enzymes (Sana et al., 2004). The following are the most widely studied oilseed grains with respect to lipase extraction and characterization: beans (Enujiugha et al., 2004); sunflower seeds (Sagiroglu and Arabaci, 2005; Sadeghipour and Bhatla, 2003); linseed (Sammour, 2005); peanuts (Huang and Moriau, 1978) and cotton seeds (Rakhimov et al., 1970), although lipases from other oilseed sources are currently being investigated.

2.3 Bean lipases

Bean lipases are well known and characterized. They are generally more active at pH levels close to neutrality, with an optimum temperature of 30ºC and specificity for short and medium chain fatty acids. Enujiugha et al. (2004) studied the lipolytic activity of Africa bean seeds (*Pentaclethra macrophylla Benth*) and the enzyme showed greater activity with oils containing short chain fatty acids, especially coconut oil. The optimum temperature of the lipase was 30ºC, but at 80ºC it still showed lipolytic activity, indicating that it is a heat stable enzyme. The optimum pH was close to neutrality.

2.4 Coconut lipases (*Cocos nucifera linn*)

Ejedegba et al. (2007) studied the physicochemical characteristics of semi-purified coconut lipases extracted with acetone. Four substrates were used to evaluate the enzymatic activity: triolein, tripalmitin, olive oil and coconut oil. The lipase showed a greater affinity for coconut oil, with an optimum pH of 8.5 and optimum temperature range of 35-40ºC in triolein.

2.5 Rice bran lipase

Rice (*Oryza sativa*) is one of the most important food cereals for the majority of the world's population. For the most part, polished rice is the staple food, thus giving rise to an important and abundant byproduct, rice bran, obtained during milling of the rice. Bran, which comprises the testa and pericarp, is known to be rich in a number of important components such as oil (edible) (Zachariassen, 1964), vitamins, sugars, and proteins. An important potential protein present in the rice bran is lipase. Like some other lipases, rice bran lipase is known to be regioselective (Gandhi, 1997; Posorske, 1984) and has a preference for low molecular weight substrates. These and other biochemical and enzymic properties would enable the employment of this lipase for the manufacture of specified products.

Table 2.1: The most studied seed lipases and their main features and biochemical properties

Lipase source	Optimum pH	Optimum temperature	Substrate	Application
Africa bean seed (*Pentaclethra macrophylla Benth*)	7.0	30˚C	Coconut	Hydrolysis
French bean seed (*Phaseolus vulgaris*)	7.0	35˚C	Olive oil, triacetin, french bean oil	Hydrolysis
Castor bean seed (Phaseolus vulgaris)	4.5	30˚C	*p*-nitrophenyl butyrate	Esterification
Rapseed (*Brassica napus L.*)	7.0	37˚C	Olive oil	Esterification Transesterification
Barbados nut (*Jatropha curcas L.*)	7.5	37˚C	Olive oil	Hydrolysis
Lupin seed (*Lupinus luteus L.*)	5.0	45˚C	Lupin oil	Hydrolysis
French peanut (*Panchira aquatic Bomabacaceae*)	8.0	40˚C	*p*-nitrophenyl acetate	Hydrolysis
Almond seed (*Amygdalus communis L.*)	8.5	65˚C	Soybean oil	Hydrolysis
Laurel seed (*Laurus nobilis L.*)	8.0	50˚C	Laurel oil	Hydrolysis
Black-cumin seed (*Nigella sativa*)	6.0	45˚C	Olive oil	Hydrolysis EsterficationTrans esterif-ication
Rice seed (*Oryza sativa*)	11.0	80˚C	Olive oil	Hydrolysis
Wheat seed (*Triticum aestivum L.*)	8.0	37˚C	Triolein	Hydrolysis Esterification
Oat seed (*Avena fatuna*)	9.0	65-75˚C	*p*-nitrophenyl palmitate	Hydrolysis
Coconut seed (*Cocos nucifera linn*)	8.5	30-40˚C	Olive oil	Hydrolysis

(Barros et al., 2010)

2.6 Lipase Applications

The versatility of lipases in catalyzing different kinds of reactions associated with their different specificities endows these enzymes with an important and vast application potential (Gandhi, 1997; Sharma et al., 2001; Pastore et al., 2003; Enujiugha et al., 2004; Hasan et al., 2006; Parques et al., 2006; Freire and Castilho, 2008; Yesiloglu and Baskurt, 2008; Isibilar et al., 2008; Polizelli et al., 2008). Lipases are excellent alternatives for classic chemical syntheses with industrial applications, such as in foods, detergents, oleochemicals, pharmaceuticals, fine chemistry, cosmetics and fragrances, paper pulp, leather, biosensors and lipid riched wastewater treatment (Gandhi, 1997; Paques et al., 2006; Freire and Castilho, 2008).

2.6.1 Food

Lipases are employed in food manufacturing to liberate fatty acids into food products by selective hydrolysis of the fats and oils present in many kinds of food. Depending on the carbon chain length and on the degree of unsaturation, the fatty acid obtained provides the food with flavors, colors and unusual smells, playing an important role in the physicalchemical, organoleptic and nutritional properties of many products (Gandhi, 1997; Sharma et al., 2001; Freire and Castilho, 2008). Wheat, barley, corn and canola seed lipases were used to produce low molecular weight esters in an organic environment (Liaquat and Apent, 2000).

2.6.2 Oils and Fats

Lipases show a wide range of applications in oil chemical industries. Their usage reduces expenses with energy and minimizes the heat degradation of compounds in comparison to traditional chemical processes (Freire and Castilho, 2008).

Pancreatic enzyme supplements may fail to control malabsorption and malnutrition, due to the inactivation of lipase by gastric acid (DiMagno, 1977). The pH in the fourth part of the duodenum of 15 CF (cystic fibrosis) patients remained between 3 and 5.5 for more than 2 h after meals (Robinson, 1990). On average the pH was less than 4.0 for 13% of the 2 h postprandial recording time (normal 1%). They found that the degree of fat malabsorption in CF patients correlated with the level of acidity in the fourth part of the duodenum. Bicarbonate, histamine type 2 receptor antagonists and synthetic prostaglandin E1 have been used to increase the pH in the stomach and

small intestine (Regan et al., 1978; Gow et al., 1981; Robinson et al., 1988) but these strategies still do not result in adequate fat absorption. An alternative approach is to use exogenous lipases that are stable and active in an acid environment. Trials of acid stable fungal lipase have been but as yet no plant lipase source has been tested. Plant lipases were first described in 1871 by Muntz (Muntz, 1871). True lipase activity has been identified in a range of seeds, where the storage materials are converted at germination to soluble sugars that can then be transported to the growing tissues to supply structural carbon and energy.'' Lipase has been isolated from germinating and non-germinating seed sources.

CHAPTER 3
MATERIALS AND METHODS

3.1 Site selection for sample

For experimental purpose samples (coconut, banana, lentil, mustard seed, pumpkin seed, soya meat, winter melon seed, bean seed, orange, and eggplant) were collected from Khulshi Mart (a local market of Khulshi, Chattogram, Bangladesh). These samples were first determined for the residual lipase presence, then coconut, soya meat, bean seed, and pumpkin seed were subjected to determine their lipase activity, pH stability and heat stability.

3.2 Study place

The study was conducted at Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram-4225, Bangladesh.

3.3 Detection of the presence of residual lipase enzyme

Sample was mixed with distilled water for making a solution with calcium acetate and some drops of phenol red indicators, by vigorous shaking of the mixture for few seconds and then neutralizing with sodium carbonate solution dropwise the color of the mixture forms slight pink. The solution was incubated at 44˚C for 30 minutes. The change of color from pink or red to yellow determines the presence of lipase enzyme. Detail of sample preparation is given in 3.3.2.

3.3.1 List of chemical ingredients

a. Calcium acetate, analytical-reagent grade
b. Phenol red indicator- 0.02 per cent, w/v
c. Sodium carbonate, 5 per cent, w/v, analytical- reagent grade
d. Formaldehyde, 40 per cent, w/v
e. Distilled water

3.3.2 Preparation of samples

Banana peel: Banana peels were removed. Peels were then washed with distilled water. Then peels were blended in a blender to make a smooth paste. This paste was then used as banana peel sample.

Figure 3.1: Banana peel sample

Figure 3.2: Lentil sample

Lentil: Lentils were soaked into distilled water for two hours. Then taken from the water and blended in a blender to make a smooth paste of lentil for using as sample.

Mustard seed: Mustard seeds were soaked into distilled water for three hours. Then taken from the water and blended in a blender to make a smooth paste of it for using as sample.

Figure 3.3: Mustard seed sample

Figure 3.4: Pumpkin seed sample

Pumpkin seed: Mature seeds of pumpkins were removed by cutting the fruits. Then the peels of the seeds were removed. The seeds were soaked into distilled water for two hours. Then taken from the water and blended the seeds in a blender to make o smooth paste of it for using as sample.

Soya meat: Soya meats were soaked into distilled water for 1 hour. Then taken from the water and blended to make a smooth paste of it for using as sample.

Figure 3.5: Soya meat sample

Figure3.6: Winter melon seed sample

Winter melon seed: Seeds were removed from the fruits. Peels of the seeds were also removed. Then taken it into a blender with adding small amount of distilled water and blended the seeds to make a smooth paste of it for using as sample.

Orange peel: Peels of the oranges were removed. Peels were then washed with distilled water to remove any unwanted particles. By using small distilled water the peels were blended for making a smooth paste of it. This paste was then used as sample of orange peel.

Figure 3.7: Orange peel sample

Figure 3.8: Eggplant sample

Eggplant: Fresh fruits were cutting into small pieces. Then washed with distilled water and blended to make sample as a smooth paste of it.

Bean seed: Seeds of beans were removed and also removed the outer layer of the seeds. Then washed with distilled water and blended to make sample as a smooth paste of it.

Figure 3.9: Bean seed sample

3.3.3 Working principle of chemical ingredients

Calcium acetate is a chemical compound which is a calcium salt of acetic acid. Sodium carbonate is often added to stop an enzymatic reaction. The reason is double:

1. Due to the shift in pH (above 10), most enzymes are inactivate and the enzyme reaction is stopped at each time interval we take a sample.

2. Chromophore that becomes released due to hydrolysis is almost completely present in its anionic form and becomes detectable in the VIS (visible) spectrum.

Fat digestion by lipase enzyme (Hydrolysis of fat):

Digestion of fat produces fatty acids (and glycerol). That neutralizes the alkali; sodium carbonate. Thus lowering the pH and changing the phenol red indicator from pink or red to yellow color. We could use a pH probe or data logger, or another indicator.

When a substitution of a few drops of formaldehyde solution for the calcium acetate, this has the effect of inhibiting any lipase present in the sample.

3.3.4 Process details

2 grams of banana peel, lentil, mustard seed, pumpkin seed, soya meat, winter melon seed, orange peel, eggplant, and bean seed were separately collected in a 8×1-inch stoppered tube and then added 20 ml distilled water , 0.1 gram calcium acetate and 10 drops phenol red indicator and shaken each tube vigorously for 30 seconds. For neutralizing the all mixtures, 5 per cent sodium carbonate solution was mixed dropwise and shaking the tube for making slight pink color first then added extra 2 drops in excess, then incubated the mixtures at 44˚C for 30 minutes. After this time again shaken the mixtures, this time the production of free fatty acid changes the color of the mixtures from pink to yellow or red indicated the presence of the residual lipase enzyme in the samples.

3.3.5 Flow diagram of the detection of the presence of residual lipase enzyme

The flow diagram of the detection of residual lipase enzyme of the following samples is given below (banana peel, lentil, mustard seed, pumpkin seed, soya meat, winter melon seed, orange peel, eggplant, and bean seed):

Transfer about 2g of the sample (banana peel, lentil, mustard seed, pumpkin seed, soya meat, winter melon seed, orange peel, eggplant, and bean seed) to an 8×1- inch stoppered tube

⇩

Add 20 ml of distilled water, about 0.1g of calcium acetate and 10 drops of phenol red indicator

⇩

Shake the mixture vigorously for about 30 seconds

⇩

Neutralise by adding 5 per cent sodium carbonate solution, dropwise. Shaking the tube between each addition until a slight pink color first develops in the suspension.

⇩

Then add 2 drops in excess

⇩

Incubate the mixture at 44˚C for 30 minutes

⇩

After this time shake the mixture again

⇩

The production of fatty acid indicated by the change in color of the liquor from red to yellow, shows the presence of lipase, which can be confirmed by again neutralizing and incubating for a further 30 minutes.

[For control, proceed as described, but substitute a few drops of formaldehyde solution for the calcium acetate. This has the effect of inhibiting any lipase present in the sample.]

3.4 Determination of the activities of lipase (coconut, soya meat, bean seed, and pumpkin seed)

In this section four samples (coconut, soya meat, bean seed and pumpkin seed) were selected for the determination of the activity of lipase enzyme which are tested as good sources of lipase enzyme (section 3.3 above).

3.4.1 Preparation of lipase acetone powder (coconut, soya meat, bean seed, and pumpkin seed)

3.4.1.1 List of chemical ingredients

a. Distilled water
b. Cold acetone

3.4.1.2 Working principle of chemical ingredients

Acetone extraction is a method for separating certain specific substances from a sample of material using the solvent acetone, which is an organic compound. This compound is an excellent solvent and is more volatile than water that is widely used in industrial processes and in laboratories because of its ability to dissolve many organic compounds, including oils, fats and plastics. Pouring acetone into a rag begins evaporating by taking some of the heat with it as it does so hence why it appears slightly cool. Depending on the solubility of the compounds comprising the materials, acetone extraction process can be used to obtain the desired substance from the raw material or to remove unwanted impurities. In this study the cold acetone was used for extracting unwanted oils and impurities from the coconut, soya meat, bean seed and pumpkin seed samples.

3.4.1.3 Process details of coconut lipase acetone powder preparation

Collection of coconut sample: Fresh coconut was collected from the local market. The coconut was then washed properly for removing dirts and any other unusual particles. The coconut was then split into two parts.

Removal of endosperm: The coconut endosperm was then removed from the coating. The endosperm was then washed by distilled water.

Blending: Acetone was kept into refrigerator for overnight to cool the acetone. The coconut endosperm was then blended with the cold acetone in a blender for about 5 minutes. Coconut oil of endosperm was dissolved into the acetone. The suspension was then kept into freezer at 0˚C and stirred continuously to dissolve more coconut oils into the acetone.

Filtration: The suspension was then filtered and washed with cold acetone to remove excess oil from the coconut.

Drying, sieving and storage: The finely ground acetone powder of coconut was then dried by sun drying. The dried acetone powder then sieved to remove any unwanted particles. The dried coconut acetone powder was the stored in cold for the next use.

3.4.1.3.1 Flow diagram of lipase acetone powder preparation (coconut)

The flow diagram of lipase acetone powder preparation from coconut is given below:

Freshly collected coconut

⇩

Removal of the endosperm of the coconut seed

⇩

Washed with distilled water

⇩

The endosperm was then blended in cold acetone in a blender for 5 minutes

⇩

The suspension was kept at 0˚c and stirred for 5 minutes

⇩

Filtered

⇩

Washed free of oil with cold acetone

⇩

The finely ground acetone was dried (sun drying)

⇩

Sieved to remove any unwanted particles

⇩

Stored in the cold

3.4.1.3.2 Figures of lipase acetone powder preparation (coconut)

Freshly collected coconut

Removing endosperm of the coconut

Blending with cold acetone

Sun drying

Sieving of dried powder

Dried Acetone Powder of coconut sample

3.4.1.4 Process details of soya meat lipase acetone powder preparation

Collecting sample: Fresh soya meat was collected from the local market, Khulshi Marts, Chattogram.

Grinding, blending and filtration: The soya meat was then grinded to make powder in a mortar and pestle. The soya meat powder was then blended with cold acetone in a blender. After blending the powder the suspension was then filtered and washed with cold acetone.

Drying, sieving and storage: The acetone powder was then dried under sun drying to remove moisture. After drying the soya meat acetone powder was sieved to remove any unwanted particles. Finally the soya meat acetone powder was stored in the cold for using until the preparation of enzyme solution from it.

3.4.1.4.1 Flow diagram of lipase acetone powder preparation (soya meat)

The flow diagram of lipase acetone powder preparation from soya meat is given below:

Freshly collected soya meat

⇩

Taken some soya meat in a mortar and pestle

⇩

Ground them to make powder

⇩

The soya meat powder was then blended in cold acetone in a blender for 5 minutes

⇩

The suspension was kept at 0˚c and stirred for 5 minutes

⇩

Filtered

⇩

Washed free of oil with cold acetone

⇩

The finely ground acetone was dried (sun drying)

⇩

Sieved to remove any unwanted particles

⇩

Stored in the cold

3.4.1.4.2 Figures of soya meat lipase acetone powder preparation

Fresh soya meat

Grinded soya meat

Blending with cold acetone

Filtration of soya meat suspension

Sun drying of powder

Sieving of dried powder

Dried acetone powder of soya meat

3.4.1.5 Process details of bean seed lipase acetone powder preparation

Collection of sample: Fresh bean seed was collected and the peels were removed. The seeds were washed with distilled water.

Soaking and washing: The bean seeds were then soaked into distilled water for few hours. After soaking the bean seeds were removed from water. The seeds were then washed with distilled water.

Blending: The bean seeds were then blended with cold acetone to remove bean oil by dissolving the oil into the acetone for about 5 minutes. Then the suspension was kept in freezer at 0˚C and stirred continuously.

Filtration: Then the suspension was filtered to remove the acetone containing bean oil and again washed with cold acetone during filtration to remove free of oil from the seeds.

Drying, sieving and storage: After filtration the suspension was sun dried until made properly dried acetone powder. The dried acetone powder was sieved to remove any unwanted particles. Then the bean seed acetone powder was kept in cold for using until the preparation of enzyme solution from it.

3.4.1.5.1 Flow diagram of lipase acetone powder preparation (bean seed)

The flow diagram of lipase acetone powder preparation from bean seed is given below:

Freshly collected bean seed

⇩

Taken some bean seed in a jar

⇩

Soaked them for few hours into distilled water

⇩

After few hours washed out the water and washed the seeds using distilled water

⇩

The bean seeds were then blended in cold acetone in a blender for 5 minutes

⇩

The suspension was kept at 0˚c and stirred for 5 minutes

⇩

Filtered

⇩

Washed free of oil with cold acetone

⇩

The finely ground acetone was dried (sun drying)

⇩

Sieved to remove any unwanted particles

⇩

Stored in the cold

3.4.1.5.2 Figures of bean seed lipase acetone powder preparation

Soaking of freshly collected bean seed

Blending with cold acetone

Sun drying

Storing dried acetone powder of bean seed

3.4.1.6 Process details pumpkin seed lipase acetone powder preparation

Collecting sample and drying the seeds: Fresh properly ripen pumpkins were collected. Seeds were removed from the pumpkins. Seeds were then washed with distilled water. The pumpkin seeds were then sun dried.

Removing peels and soaking: After drying the peels of the seeds were removed. The seeds then soaked into distilled water for few hours to make them soft. After few hours of soaking the seeds were removed from the water and washed with distilled water.

Blending: Then the soft seeds were blended with cold acetone in a blender for about 5 minutes. Then kept the suspension in freezer and continuously stirred.

Filtration, drying, sieving and storage: Then the suspension was filtered and washed free of oils. Then the suspension was dried after filtration under sun drying. After drying the pumpkin seed acetone powder was sieved to remove any unwanted particles. The acetone powder was kept in cold for further use until the preparation of lipase enzyme solution from it.

3.4.1.6.1 Flow diagram of lipase acetone powder preparation (pumpkin seed)

The flow diagram of lipase acetone powder preparation from pumpkin seed is given below:

Freshly collected well ripen pumpkin seed

⇩

Drying and removal of peels of the seeds

⇩

Taken the seeds in a jar after removing of the peels

⇩

Soaked them into distilled water for few ours

⇩

After few hours washed out the water and the seeds were washed using distilled water

⇩

The pumpkin seeds were then blended in cold acetone in a blender for 5 minutes

⇩

The suspension was kept at 0˚c and stirred for 5 minutes

⇩

Filtered and washed free of oil with cold acetone

⇩

The finely ground acetone was dried (sun drying)

⇩

Sieved and stored in the cold

3.4.1.6.2 Figures of pumpkin seed lipase acetone powder preparation

Freshly collected pumpkin seed

Sun drying of seeds

Peeling of the seeds

Soaking of seeds

Blending with cold acetone

Sun drying

Storing dried acetone powder of pumpkin seed

3.4.2 Preparation of enzyme solution (coconut, soya meat, bean seed, and pumpkin seed)

The enzyme solution was prepared by weighing out 2 g of the acetone powder and homogenized for 2 min with 20 ml cold 0.9% NaCl. The suspension was then centrifuged for 5 min at room temperature and the supernatant which contains the enzyme was stored at 4°C. The enzyme solution was prepared immediately before the assay.

3.4.2.1 List of chemical ingredients

a. Acetone powder
b. Cold 0.9% Nacl

3.4.2.2 Process details of enzyme solution preparation (coconut, soya meat, bean seed, and pumpkin seed)

Weighing: First of all 2g of each of the coconut acetone powder, soya meat acetone powder, bean seed acetone powder and pumpkin seed acetone powder were respectively taken in four separate jars.

Homogenization: Then 0.9% Sodium chloride solution was kept in refrigerator for overnight to make it cold. 20 ml of cold 0.9% sodium chloride was mixed with each of the four acetone powder respectively. The mixtures were then homogenized for about 2 minutes.

Centrifugation: After homogenized, the four mixtures were taken in 4-5 centrifuge tubes and set into the centrifuge machine for about 5 minutes at normal room temperature and 4000 rpm. Centrifuge made two separate parts in each centrifuge tube of the reaction mixtures. The supernatants which were containing the lipase enzyme were separated in four different stoppered tubes.

Storing: The four stoppered tubes of the coconut enzyme solution, soya meat enzyme solution, bean seed enzyme solution and pumpkin seed enzyme solution were stored at 4˚C temperature. The enzyme solutions were prepared immediately before the assay.

3.4.2.3 Flow diagram of the preparation of lipase enzyme solution (coconut, soya meat, bean seed & pumpkin seed)

The flow diagram of the preparation of lipase enzyme solution from acetone powder of coconut, soya meat, bean seed and pumpkin seed is given below:

Weighing out 2g of the coconut acetone powder, 2g of the soya meat acetone powder, 2g of the bean seed acetone powder and 2g of the pumpkin seed acetone powder separately

⇩

Homogenized the each acetone powder separately for 2 min with 20 ml cold 0.9% Nacl

⇩

The suspension was then centrifuged for 5 min at room temperature

⇩

The supernatant which contains the enzyme was stored at 4˚C

⇩

The enzyme solution of coconut, soya meat, bean seed and pumpkin seed were prepared separately immediately before the assay

3.4.3 Assay of enzyme activity

This involves the separation of free fatty acids released in the form of their copper salts using chloroform extraction, which is subsequently determined colorimetrically at 440 nm.

3.4.3.1 List of chemical ingredients

a. Copper reagent (9 volume of aq. 1M triethanolamine, 1 volume of 1N acetic acid, and 10 volume of 5% $Cu(NO_3)_2$-$3H_2O$)
b. Diethyldithiocarbamate reagent (0.1 % w/v) solution of sodium diethyldithiocarbamate in n-butanol
c. Tris buffer (0.05 M, pH 8.0) including 0.5 % sodium taurocholate
d. Olive oil (as substrate, 1 ml of commercial product was diluted with water to 30 ml)
e. Chloroform solution
f. The chloroform solution of stearic acid (1.0 µEq/ml)- standard solution of fatty acid
g. Carbamate reagent

3.4.3.2 Primary preparations of chemical ingredients

a. **Copper reagent:** 9 volume of aqueous 1 M triethanolamine solution, 1 volume of 1 N acetic acid and 10 volume of 5% $Cu(NO_3)_2$-$3H_2O$) were mixed properly for preparing the copper reagent.
b. **Diethyldithiocarbamate reagent:** 0.1g of sodium diethyldithiocarbamate was mixed in few ml of n-butanol first and then up to 100 ml of n-butanol.
c. **Tris buffer:** 0.05 M tris buffer was prepared by adding 6.06 g tris hydrochloride (Tris-HCl) and 1.39g tris base into distilled water up to 1000 ml, combined all ingredients and mixed well and adjusted the pH up to 8.0. Then 0.5 g sodium taurocholate was mixed into 100 ml of 0.05 M tris buffer.
d. **Substrate:** 1 ml of commercial olive oil was diluted with distilled water to 30 ml.
e. **Standard solution:** 1 µEq of stearic acid was mixed into 1 ml of chloroform solution to make the standard solution.

3.4.3.3 Procedure for the assay of enzyme activity

3.4.3.3.1 Enzyme activity of coconut enzyme solution

3.4.3.3.1.1 Preparation of control and incubated solution of coconut enzyme

Incubation system:

The incubated and control mixture of coconut enzyme solution is prepared by the following procedure:

- 0.05 M Tris-buffer (pH 8.0) containing sodium taurocholate (0.5%) - 1.0 ml
- Substrate (olive oil & water mixture) - 1.0 ml
- Sample (Enzyme solution of coconut) - 1.0 ml

1. 1.0 ml of the incubation mixture was taken as the control right after the addition of sample to the system.
2. After the incubation for 1 hour at 37˚C, 1.0 ml of the mixture was also taken for the determination of liberated FFA (free fatty acids).

3.4.3.3.1.2 Flow diagram of the assay of enzyme activity of coconut enzyme (Colorimetric micro-determination of fatty acids):

After the preparation of control and incubated mixture of coconut enzyme solution, the following procedure is required to determine the assay of enzyme activity of coconut lipase enzyme by the colorimetric micro-determination of fatty acids method.

The flow diagram is given below:

The control mixture or the incubated mixture of coconut enzyme solution stated as above [(1) or (2)] was placed in a 10-15 ml stoppered centrifuge tube with 5.0 ml chloroform and 2.0 ml copper reagent

⇩

The tube was stoppered and shaken vigorously for at least 2 minutes

⇩

It was then centrifuged for a few minutes to separate the phases cleanly and the upper phase was aspirated by a fine hypodermic needle

⇩

2 ml of chloroform portion was pipetted into a clean dry tube

⇩

Then 2.0 ml of carbamate reagent was added to this tube

⇩

After the solution was mixed, the extinction was read at 435 nm against a blank solution which was prepared by adding 0.5 ml of water to stoppered centrifuge tube instead of (1) or (2)

⇩

The standard solution was subjected to the same procedure

⇩

The unit of lipase activity of coconut lipase enzyme solution was calculated from the following equation: (2) – (1) FFA µEq/ml/hr

In this procedure the blank solution was prepared in the lab right before the reading of the assay of enzyme activity in the UV-probe machine by colorimetric micro-determination method. For preparing the blank solution, we used water in the replacement of incubated mixture and control mixture solutions. The rest of the chemicals such as chloroform, copper reagent, carbamate reagent etc. were the same. The standard solution was prepared by using stearic acid in chloroform (preparatory procedure is stated as above) instead of the control mixture and the incubated mixture. The rest of the chemicals were the same such as chloroform, copper reagent, carbamate reagent etc. By using the blank solution the control mixture, the incubated mixture and the standard solution was read at 435 nm wavelengths to determine the activity of coconut lipase enzyme.

3.4.3.3.2 Enzyme activity of soya meat enzyme solution

3.4.3.3.2.1 Preparation of control and incubated solution of soya meat enzyme

Incubation system:

The incubated and control mixture of soya meat enzyme solution is prepared by the following procedure:

- 0.05 M Tris-buffer (pH 8.0) containing sodium taurocholate (0.5%) - 1.0 ml
- Substrate (olive oil & water mixture) - 1.0 ml
- Sample (Enzyme solution of soya meat) - 1.0 ml

1. 1.0 ml of the incubation mixture was taken as the control right after the addition of sample to the system.
2. After the incubation for 1 hour at 37˚C, 1.0 ml of the mixture was also taken for the determination of liberated FFA (free fatty acids).

3.4.3.3.2.2 Flow diagram of the assay of enzyme activity of soya meat enzyme (Colorimetric micro-determination of fatty acids):

After the preparation of control and incubated mixture of soya meat enzyme solution, the following procedure is required to determine the assay of enzyme activity of soya meat lipase enzyme by the colorimetric micro-determination of fatty acids method.

The flow diagram is given below:

The control mixture or the incubated mixture of soya meat enzyme solution stated as above [(1) or (2)] was placed in a 10-15 ml stoppered centrifuge tube with 5.0 ml chloroform and 2.0 ml copper reagent

⇩

The tube was stoppered and shaken vigorously for at least 2 minutes

⇩

It was then centrifuged for a few minutes to separate the phases cleanly and the upper phase was aspirated by a fine hypodermic needle

⇩

2 ml of chloroform portion was pipetted into a clean dry tube

⇩

Then 2.0 ml of carbamate reagent was added to this tube

⇩

After the solution was mixed, the extinction was read at 435 nm against a blank solution which was prepared by adding 0.5 ml of water to stoppered centrifuge tube instead of (1) or (2)

⇩

The standard solution was subjected to the same procedure

⇩

The unit of lipase activity of soya meat lipase enzyme solution was calculated from the following equation: (2) – (1) FFA µEq/ml/hr

In this procedure the blank solution was prepared in the lab right before the reading of the assay of enzyme activity in the UV-probe machine by colorimetric micro-determination method. For preparing the blank solution, we used water in the replacement of incubated mixture and control mixture solutions. The rest of the chemicals such as chloroform, copper reagent, carbamate reagent etc. were the same. The standard solution was prepared by using stearic acid in chloroform (preparatory procedure is stated as above) instead of the control mixture and the incubated mixture. The rest of the chemicals were the same such as chloroform, copper reagent, carbamate reagent etc. By using the blank solution the control mixture, the incubated mixture and the standard solution was read at 435 nm wavelengths to determine the activity of soya meat lipase enzyme.

3.4.3.3.3 Enzyme activity of bean seed enzyme solution

3.4.3.3.3.1 Preparation of control and incubated solution of bean seed enzyme

Incubation system:

The incubated and control mixture of bean seed enzyme solution is prepared by the following procedure:

- 0.05 M Tris-buffer (pH 8.0) containing sodium taurocholate (0.5%) - 1.0 ml
- Substrate (olive oil & water mixture) - 1.0 ml
- Sample (Enzyme solution of bean seed) - 1.0 ml

1. 1.0 ml of the incubation mixture was taken as the control right after the addition of sample to the system.
2. After the incubation for 1 hour at 37˚C, 1.0 ml of the mixture was also taken for the determination of liberated FFA (free fatty acids).

3.4.3.3.3.2 Flow diagram of the assay of enzyme activity of bean seed enzyme (Colorimetric micro-determination of fatty acids):

After the preparation of control and incubated mixture of bean seed enzyme solution, the following procedure is required to determine the assay of enzyme activity of bean seed lipase enzyme by the colorimetric micro-determination of fatty acids method.

The flow diagram is given below:

The control mixture or the incubated mixture of bean seed enzyme solution stated as above [(1) or (2)] was placed in a 10-15 ml stoppered centrifuge tube with 5.0 ml chloroform and 2.0 ml copper reagent

⇩

The tube was stoppered and shaken vigorously for at least 2 minutes

⇩

It was then centrifuged for a few minutes to separate the phases cleanly and the upper phase was aspirated by a fine hypodermic needle

⇩

2 ml of chloroform portion was pipetted into a clean dry tube

⇩

Then 2.0 ml of carbamate reagent was added to this tube

⇩

After the solution was mixed, the extinction was read at 435 nm against a blank solution which was prepared by adding 0.5 ml of water to stoppered centrifuge tube instead of (1) or (2)

⇩

The standard solution was subjected to the same procedure

⇩

The unit of lipase activity of bean seed lipase enzyme solution was calculated from the following equation: (2) – (1) FFA µEq/ml/hr

In this procedure the blank solution was prepared in the lab right before the reading of the assay of enzyme activity in the UV-probe machine by colorimetric micro-determination method. For preparing the blank solution, we used water in the replacement of incubated mixture and control mixture solutions. The rest of the chemicals such as chloroform, copper reagent, carbamate reagent etc. were the same. The standard solution was prepared by using stearic acid in chloroform (preparatory procedure is stated as above) instead of the control mixture and the incubated mixture. The rest of the chemicals were the same such as chloroform, copper reagent, carbamate reagent etc. By using the blank solution the control mixture, the incubated mixture and the standard solution was read at 435 nm wavelengths to determine the activity of bean seed lipase enzyme.

3.4.3.3.4 Enzyme activity of pumpkin seed enzyme solution

3.4.3.3.4.1 Preparation of control and incubated solution of pumpkin seed enzyme

Incubation system:

The incubated and control mixture of pumpkin seed enzyme solution is prepared by the following procedure:

- 0.05 M Tris-buffer (pH 8.0) containing sodium taurocholate (0.5%) - 1.0 ml
- Substrate (olive oil & water mixture) - 1.0 ml
- Sample (Enzyme solution of pumpkin seed) - 1.0 ml

1. 1.0 ml of the incubation mixture was taken as the control right after the addition of sample to the system.
2. After the incubation for 1 hour at 37˚C, 1.0 ml of the mixture was also taken for the determination of liberated FFA (free fatty acids).

3.4.3.3.4.2 Flow diagram of the assay of enzyme activity of pumpkin seed enzyme (Colorimetric micro-determination of fatty acids):

After the preparation of control and incubated mixture of pumpkin seed enzyme solution, the following procedure is required to determine the assay of enzyme activity of pumpkin seed lipase enzyme by the colorimetric micro-determination of fatty acids method.

The flow diagram is given below:

The control mixture or the incubated mixture of pumpkin seed enzyme solution stated as above [(1) or (2)] was placed in a 10-15 ml stoppered centrifuge tube with 5.0 ml chloroform and 2.0 ml copper reagent

⇩

The tube was stoppered and shaken vigorously for at least 2 minutes

⇩

It was then centrifuged for a few minutes to separate the phases cleanly and the upper phase was aspirated by a fine hypodermic needle

⇩

2 ml of chloroform portion was pipetted into a clean dry tube

⇩

Then 2.0 ml of carbamate reagent was added to this tube

⇩

After the solution was mixed, the extinction was read at 435 nm against a blank solution which was prepared by adding 0.5 ml of water to stoppered centrifuge tube instead of (1) or (2)

⇩

The standard solution was subjected to the same procedure

⇩

The unit of lipase activity of pumpkin seed lipase enzyme solution was calculated from the following equation: (2) – (1) FFA µEq/ml/hr

In this procedure the blank solution was prepared in the lab right before the reading of the assay of enzyme activity in the UV-probe machine by colorimetric micro-determination method. For preparing the blank solution, we used water in the replacement of incubated mixture and control mixture solutions. The rest of the chemicals such as chloroform, copper reagent, carbamate reagent etc. were the same. The standard solution was prepared by using stearic acid in chloroform (preparatory procedure is stated as above) instead of the control mixture and the incubated mixture. The rest of the chemicals were the same such as chloroform, copper reagent, carbamate reagent etc. By using the blank solution the control mixture, the incubated mixture and the standard solution was read at 435 nm wavelengths to determine the activity of pumpkin seed lipase enzyme.

3.4.4 Determination of the pH stability

For pH stability, enzymes were incubated using different pH buffers. The reaction mixtures were incubated as per standard assay and the residual lipolytic activities were then determined using olive oil as substrate. The assay was carried out using pH 5.0 to pH 8.0 of the reaction mixtures.

3.4.5 Determination of the temperature stability

For temperature stability, different temperatures were experimented on the reaction mixtures to determine the optimum heat stability of coconut lipase, soya meat lipase, bean seed lipase and pumpkin seed lipase. To observe the heat stability of the experimented lipases from different plant origins incubator was used to give different temperature to the reaction mixtures.

In this study the temperature range was from 25˚C to 40˚C. This temperature stability determination procedure was as same as the lipase enzyme activity determination procedure, where different temperature treatments were given to the reaction mixtures of lipase enzymes by incubator to find out the optimum heat stability of the enzymes.

CHAPTER 4

RESULTS

4.1 Detection of the presence of residual lipase enzyme in the samples

Table 4.1: Results of the residual lipase detection test

Item	Result	Remarks	Figure No.
Banana peel	Negative	No color change	4.1
Lentil	Positive	Pink to yellow (strong)	4.2
Mustard seed	Positive	Pink to yellow	4.3
Pumpkin seed	Positive	Pink to yellow	4.4
Soya meat	Positive	Pink to yellow	4.5
Winter melon seed	Positive	Pink to yellow	4.6
Orange peel	Positive	Red to yellow (good)	4.7
Eggplant	Positive	Deep pink to light yellow	4.8
Bean seed	Positive	Pink to yellow	4.9

4.1.1 Banana peel

Banana peel showed negative result. Lipase enzyme was not found in the peel as there was no remarkable color change in the banana peel according to the procedure.

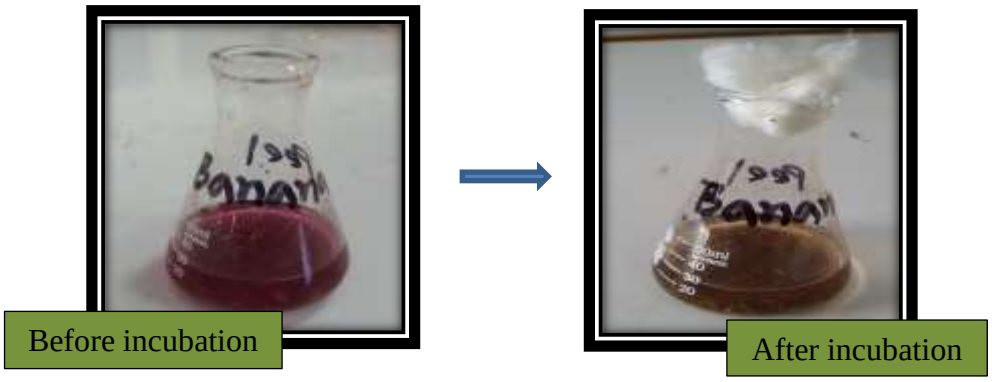

Figure 4.1: Detection of lipase enzyme in banana peel

4.1.2 Lentil

Lentil showed positive result. The color of the lentil sample changed strongly from pink to yellow.

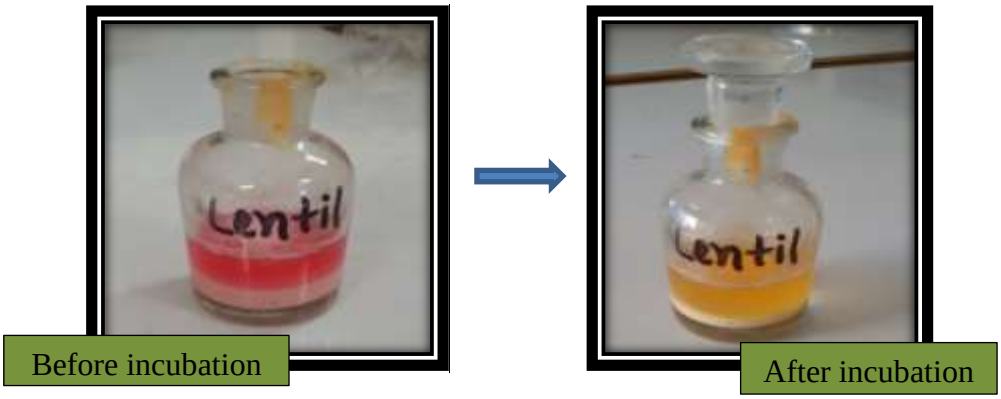

Figure 4.2: Detection of lipase enzyme in lentil

4.1.3 Mustard seed

Mustard seed sample was showed positive result in this study as the color of the liquor was changed from pink to yellow.

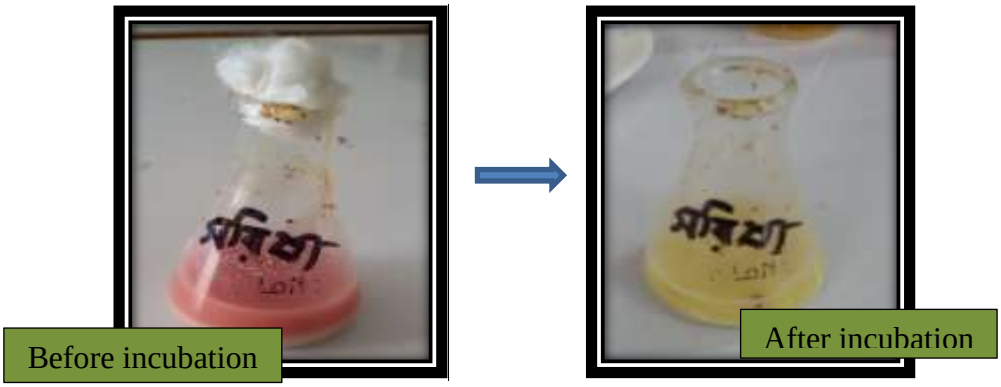

Figure 4.3: Detection of lipase enzyme in Mustard seed

4.1.4 Pumpkin seed

Pumpkin seed enzyme detection test showed positive result.

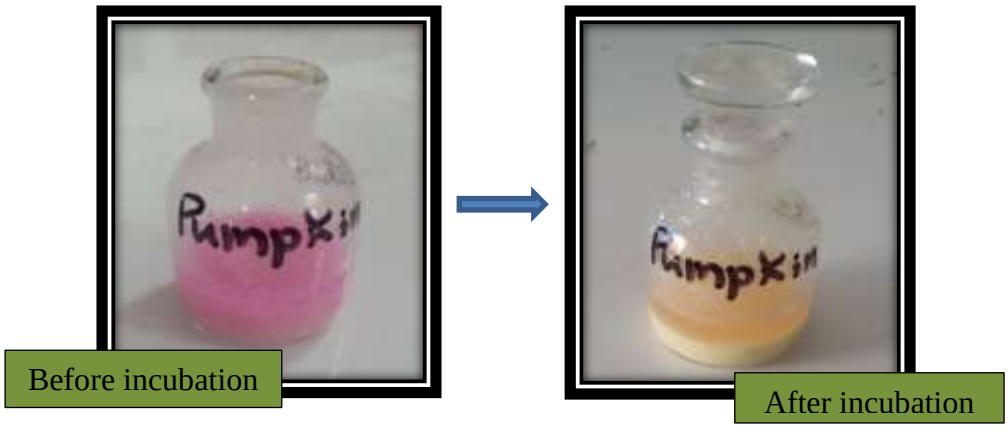

Figure 4.4: Detection of lipase enzyme in Pumpkin seed

4.1.5 Soya meat

Soya meat sample test was positive. As there was a clear color changed from pink to yellow.

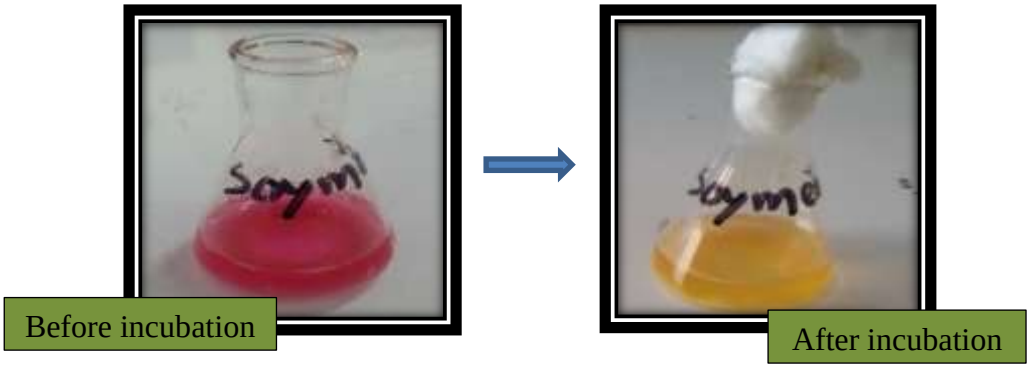

Figure 4.5: Detection of lipase enzyme in Soya meat

4.1.6 Winter melon seed

Lipase detection test was positive in winter melon seed sample.

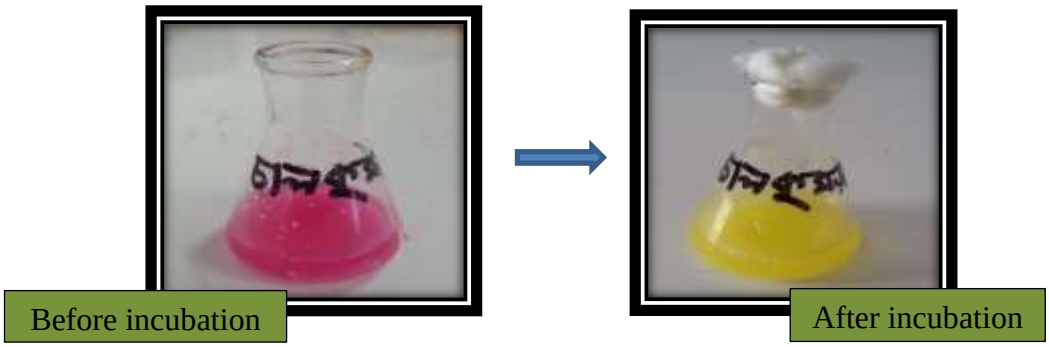

Figure 4.6: Detection of lipase enzyme in Winter melon seed

4.1.7 Orange peel

The lipase detection test in orange peel was positive.

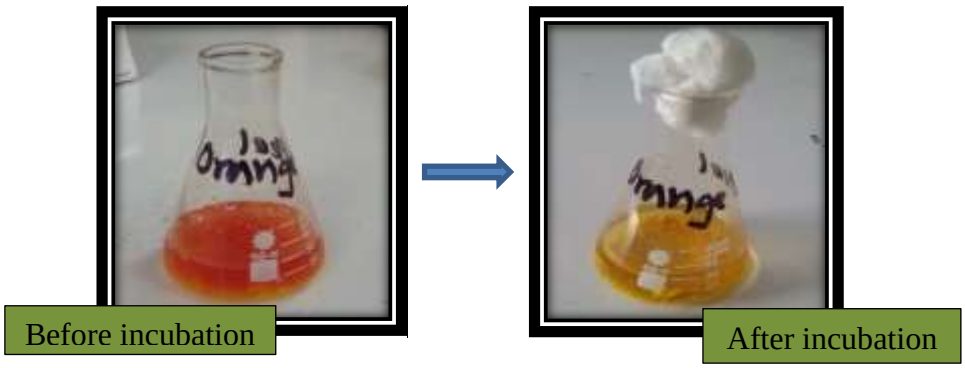

Figure 4.7: Detection of lipase enzyme in Orange peel

4.1.8 Eggplant

The lipase detection test in eggplant was positive. But, the color was changed at very little amount. Due to low level of color changed from deep pink to light yellow, it indicated that the lipase enzyme was present at very little amount in the eggplant sample solution. From this test of this study, it is indicated that eggplant is not a good source of lipase enzyme.

Figure 4.8: Detection of lipase enzyme in Eggplant

4.1.9 Bean seed

The lipase enzyme detection test in bean seed sample was positive. The color of the liquor changed from pink to yellow, which indicated that there was the production of free fatty acids in the solution and this free fatty acid can be produced by the hydrolysis of fat which was present in the sample solution by lipase enzyme. So, the bean seed sample was containing lipase enzyme.

Figure 4.9: Detection of lipase enzyme in Bean seed

4.2 Activities of lipases

The enzyme activity assay was carried out at different pH, ranges from pH 5.0 to pH 9.0, at different temperature treatment and at normal room temperature. The enzymes appear to show a range of different activity depending on the environmental condition.

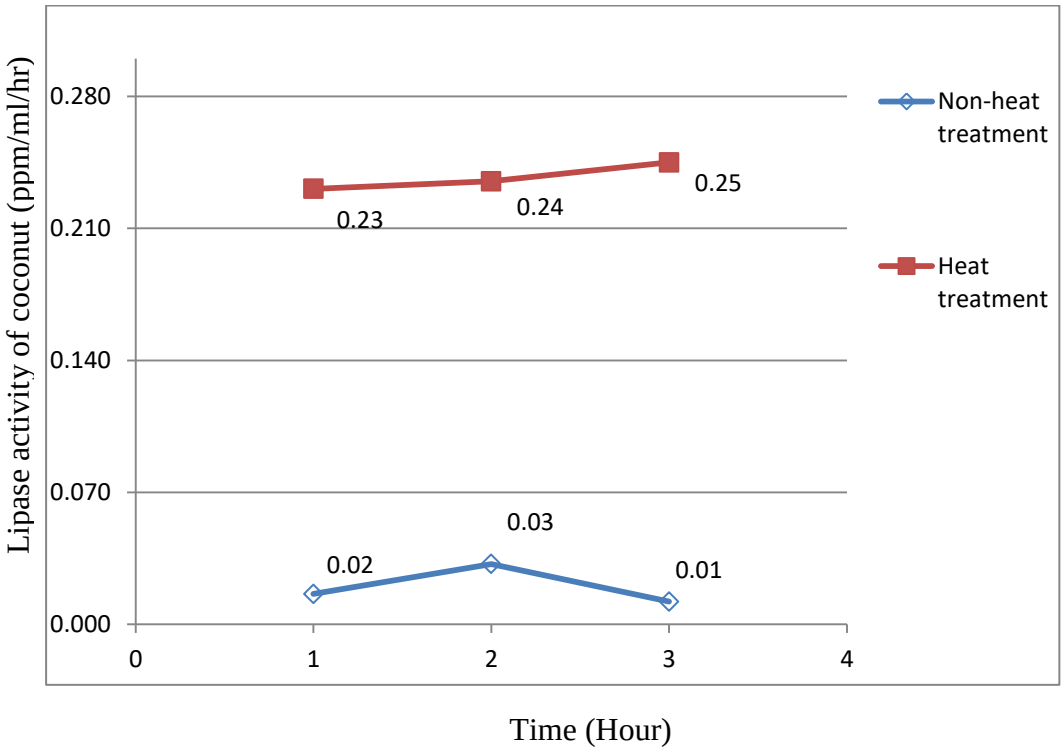

Figure 4.10: Effect of heat treatment on the activities of coconut lipases at pH 9.0 (ppm/ml/hr)

From the above figure, this is observed that, heat treatment increased the enzyme activity of coconut lipase at pH 9.0 and the coconut enzyme was less active in non-heat treatment medium at the same pH. So, the coconut lipase enzyme had the affinity for higher heating medium than normal room temperature, but the coconut enzyme had lower activity at pH 9.0 and this enzyme showed its highest activity at pH 8.0.

Table 4.2: Effect of heat treatment on the activities of coconut lipases at pH 8.0 (ppm/ml/hr)

Time (per hour)	Non-heat treatment(ppm/ml/hr)	Heat treatment(ppm/ml/hr)
1st hour	2.00	2.36
2nd hour	2.14	2.57
3rd hour	2.10	2.60
4th hour	2.16	2.79
5th hour	2.17	2.91

Table 4.2 shows that there is increased in the activities of coconut lipases, when the pH of the medium is decreased from pH 9.0 to pH 8.0. It is important to find out the optimum heat stability of a plant lipase enzyme to use it in the production of foods. This table shows the highest lipase activity of the coconut sample when 37˚C temperature is applied on the reaction medium for 1 hour. On the other hand at normal room temperature, where no further heat treatment is applied on the lipase solution, the activity of the lipase is decreased. So this study shows that coconut lipases are more stable at high temperature and its activity is increased by increasing the time period until reaching a stable activity.

Table 4.3: Effect of heat treatment on the activities of coconut lipases at pH 7.0 (ppm/ml/hr)

Time (per hour)	Non-heat treatment(ppm/ml/hr)	Heat treatment(ppm/ml/hr)
1st hour	0.03	0.10
2nd hour	0.10	0.17
3rd hour	0.18	0.24
4th hour	0.24	0.27
5th hour	0.44	0.50

At pH 7.0 the activity of coconut lipases decrease from 2.17 ppm/ml/hr to 0.44 ppm/ml/hr on non-heat treatment and coconut lipase activity decreases from 2.91 ppm/ml/hr to 0.50 ppm/ml/hr on heat treatment at 5th hour of the assay reading in relation to the pH 8.0 respectively. The effect of heat treatment on coconut lipase at

pH 7.0 investigation also shows that the activity of coconut lipase was higher than non-heat treatment. So heat treatment increases the activity of coconut lipases also at pH 7.0.

Table 4.4: Effect of heat treatment on the activities of Soya meat lipases at pH 7.0 (ppm/ml/hr)

Time (per hour)	Non-heat treatment(ppm/ml/hr)	Heat treatment(ppm/ml/hr)
1st hour	---	---
2nd hour	0.08	---
3rd hour	0.14	---

Table 4.4 shows when heat was applied on soya meat lipases they showed no activity at pH 7.0. On the other hand, when heat was not applied on soya meat lipases they showed no activity at 1st hour of the colorimetric assay reading, but little activity was showed at 2nd hour of the assay reading and the activity of soya meat lipases increased at 3rd hour of the assay reading at the same pH. So there were the findings from the study that, at pH 7.0 heat treatment inactivate the soya meat lipases (heat sensitive) and at normal room temperature the activity of soya meat lipases were so little.

Table 4.5: Effect of heat treatment on the activities of Soya meat lipases at pH 8.0 (ppm/ml/hr)

Time (per hour)	Non-heat treatment(ppm/ml/hr)	Heat treatment(ppm/ml/hr)
1st hour	2.52	1.77
2nd hour	3.07	1.79
3rd hour	3.16	1.76
4th hour	3.18	1.74
5th hour	3.21	1.73

At pH 8.0, soya meat lipases showed highest activities at non-heat treatment, but at high temperature they showed smaller activities than non-heat treatment at the same pH. So, the finding was that soya meat lipases were heat sensitive, but showed higher activity at pH 8.0 than pH 7.0. So these lipases can be used in normal room temperature at pH 8.0 to get highest activity.

Table 4.6: Effect of heat treatment on the activities of Bean seed lipases at pH 8.0 (ppm/ml/hr)

Time (hour)	Non-heat treatment(ppm/ml/hr)	Heat treatment(ppm/ml/hr)
1st hour	4.01	2.41
2nd hour	4.11	2.44
3rd hour	4.12	2.47
4th hour	4.15	2.50
5th hour	4.16	2.53

In the Table 4.6, we can see a great amount of good activity of bean seed lipase at non-heat treatment which is highest at the 5th hour of the assay reading that is 4.16 ppm/ml/hr at pH 8.0. On the other hand, when heat treatment was applied to the reaction medium the activity of the bean seed lipase was decreased from 4.16 ppm/ml/hr to 2.53 ppm/ml/hr at the 5th hour of the assay reading. From these data one thing is found that bean seed lipase is very active at pH 8.0 when the temperature is normal room temperature, but when the heat was increased the activity of the bean seed lipases decreased so much. So this enzyme is suitable for using in the alkaline environment where the room temperature is about to 25-30˚C, but not suitable over 25-30˚C temperature environment.

Table 4.7: Effect of heat treatment on the activities of Pumpkin seed lipases at pH 8.0 (ppm/ml/hr)

Time (hour)	Non-heat treatment(ppm/ml/hr)	Heat treatment(ppm/ml/hr)
1st hour	2.26	2.09
2nd hour	2.33	2.10
3rd hour	2.34	2.15
4th hour	2.39	2.17
5th hour	2.48	2.20

In the Table 4.7, it shows greater activity of pumpkin seed lipases at the pH 8.0 when the temperature was normal room temperature that is no extra heat was applied to this reaction medium of the pumpkin seed enzyme solution. But, when extra high heat

treatment was applied to the medium the activity of the pumpkin seed lipases was decreased. So these seed lipases are suitable for using in the alkaline medium at normal room temperature of our country.

Table 4.8: Activities of lipases at pH 8.0 (ppm/ml/hr)

Time (per hour)	Coconut	Soya meat	Bean seed	Pumpkin seed
1st hour	2.36	1.77	2.41	2.09
2nd hour	2.57	1.79	2.44	2.10
3rd hour	2.60	1.76	2.47	2.10
4th hour	2.79	1.74	2.50	2.11
5th hour	2.91	1.74	2.53	2.20

Table 4.8 shows that the activities of lipases of coconut, soya meat, bean seed and pumpkin seed respectively at pH 8.0 as per hour. Coconut lipases acted maximally at pH 8.0 and its activity was increased at every hour which was increased from 2.36 ppm/ml/hr at the first hour to 2.91 ppm/ml/hour at the 5th hour. So the finding is that coconut lipase can be used for long period applications in any field of lipase activity and it is more stable lipase at pH 8.0. Soya meat lipases showed their maximum activity at the 2nd hour, gradually decreased over every hour of time period at pH 8.0. So this enzyme should be used immediately after preparing the enzyme solution of it for getting good activity at this alkaline pH. Bean seed lipases showed gradually increasing activity over every hour of time period at pH 8.0. So it can be used for many hours after lipase production at this alkaline medium. Pumpkin seed lipases also showed gradual increases of the activity in the alkaline pH of 8.0 over every hour of time period.

Table 4.9: Activities of lipases at pH 7.0 (ppm/ml/hr)

Time (hour)	Coconut	Soya meat	Bean seed	Pumpkin seed
1st hour	0.10	---	2.02	1.01
2nd hour	0.18	---	2.05	1.34
3rd hour	0.24	---	2.10	1.26
4th hour	0.27	---	2.10	1.53
5th hour	0.50	---	2.12	1.79

Table 4.9 shows that at pH 7.0 coconut lipases have very small amount of activity which is gradually increased over every hour of time period. So this enzyme can be used at pH 7.0 but it may give lower activity of it than the activity of it in pH 8.0. At pH 7.0 soya meat lipases showed no activity. So this lipase is not appropriate for using in this pH. Bean seed lipases showed good activity than the other three samples of coconut, soya meat and pumpkin seed lipases at pH 7.0. So, bean seed lipase can be used in pH 7.0. Pumpkin seed lipases acted more than coconut and soya meat lipases at pH 7.0 but lower than bean seed lipases. So, the finding is that at pH 7.0 the mostly active lipase enzyme was the bean seed lipase.

Table 4.10: Activities of lipase at pH 6.0 (ppm/ml/hr)

Time (hour)	Coconut	Soya meat	Bean seed	Pumpkin seed
1st hour	0.00	---	1.07	0.02
2nd hour	0.01	---	1.10	0.04
3rd hour	0.02	---	1.34	0.05
4th hour	0.03	---	1.61	0.06
5th hour	0.02	---	1.73	0.07

Table 4.10 shows that at pH 6.0, coconut lipase has a negligible amount of lipase activities, soya meat lipase has no activity, bean seed lipase has smaller amount of activity and pumpkin seed lipase has also a negligible amount of lipase activity. So, in acidic medium these enzymes are not appropriate for using in any food industry. But, bean seed lipase showed some activity. So, bean seed lipase can be used in acidic medium.

Table 4.11: Activities of lipase at pH 5.0 (ppm/ml/hr)

Time (hour)	Coconut	Soya meat	Bean seed	Pumpkin seed
1st hour	---	---	0.40	---
2nd hour	---	---	0.50	---
3rd hour	---	---	0.67	---
4th hour	---	---	1.12	---
5th hour	---	---	2.01	---

Table 4.11 shows the activities of different lipases at pH 5.0. At this acidic medium having pH 5.0, coconut lipase, soya meat lipase and pumpkin seed lipase showed no activity. So, these lipases were not suitable for using in the acidic medium. Bean seed lipase showed smaller amount of activity in pH 5.0. So, this enzyme is active at acidic medium where the pH is 5.0.

Table 4.12: Activities of lipases at different pH on average (ppm/ml/hr)

Sample	pH 8.0	pH 7.0	pH 6.0	pH 5.0	Remarks
Coconut	2.65	0.26	0.01	---	Suitable for alkaline environment
Soya meat	1.76	---	---	---	Suitable for alkaline environment
Bean seed	2.47	2.08	1.37	0.91	Can be used in both alkaline and acidic environment
Pumpkin seed	2.12	1.39	0.06	---	Suitable for alkaline environment

Table 4.12 shows the activities of coconut, soya meat, bean seed and pumpkin seed lipases at pH 8.0, pH 7.0, pH 6.0, and pH 5.0 respectively. From this study, coconut lipases acted maximally at pH 8.0 because at this pH the coconut lipases showed highest activity of 2.65 ppm/ml/hr than the other pH. The 2nd highest activity of coconut lipases was at pH 7.0 that was 0.26 ppm/ml/hr. The 3rd highest activity of coconut lipases was 0.01 ppm/ml/hr at pH 6.0 which is negligible amount of activity. From these results, this study shows that the coconut lipase is suitable for using in alkaline medium and in acidic medium, its activity is decreased. The optimum pH range of coconut lipase was from pH 6.0 to pH 8.0. Soya meat lipases showed no activity at pH 7.0, pH 6.0 and pH 5.0. These lipases only showed its activity in the alkaline medium having pH 8.0 and that was 1.76 ppm/ml/hr. So, the finding from this study is that the soya meat lipases can be used in alkaline medium and in acidic medium this enzyme is not active. The optimum pH of soya meat was pH 8.0. Bean seed lipases showed good activity in both acidic and basic medium. In alkaline medium it showed highest result of 2.47 ppm/ml/hr. At pH 7.0 the activity was 2.08 ppm/ml/hr, at pH 6.0 the activity was 1.37 ppm/ml/hr and at pH 5.0 the activity was

0.91 ppm/ml/hr. As it showed its activity in both the alkaline and acidic medium, the bean seed lipase can be used in both the higher and lower pH environment. The optimum pH range of the bean seed lipase was from pH 5.0-pH 8.0. Pumpkin seed lipases showed highest activity at pH 8.0 that was 2.12 ppm/ml/hr, the 2nd highest result was 1.39 ppm/ml/hr at pH 7.0 and the 3rd highest activity was 0.06 ppm/ml/hr at pH 6.0 and it showed no activity at pH 5.0. So, the finding is that the pumpkin seed lipase is suitable for alkaline environment and the optimum pH range of this lipase is from pH 6.0-pH 8.0.

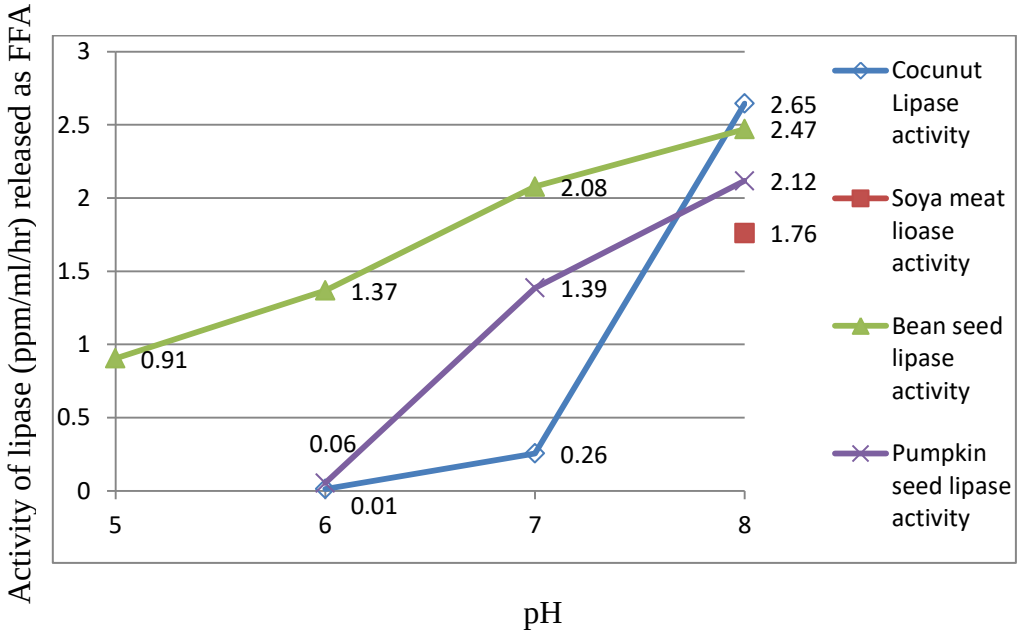

Figure 4.11: Effect of pH on activity of lipases isolated from coconut, soya meat, bean seed and pumpkin seed

The above figure shows that the effect of pH on the activity of lipases isolated from coconut, soya meat, bean seed and pumpkin seed lipases. From the figure, this is observed that at pH 8.0, all the four enzymes showed their highest activities. Coconut lipase had the activity of 2.65 ppm/ml/hr, soya meat lipase had the activity of 1.76 ppm/ml/hr, bean seed lipase had the activity of 2.47 ppm/ml/hr and pumpkin seed lipase had the activity of 2.12 ppm/ml/hr released as FFA at pH 8.0. From these data, this is clear that the coconut lipase had the highest activity at pH 8.0 among the other three enzymes of soya meat, bean seed and pumpkin seed. From the figure, this is also observed that soya meat enzyme had only activity at pH 8.0 and at other pH of 7.0, 6.0 and 5.0, the soya meat enzyme showed no activity. From the above figure, this is also observed that at pH 5.0, pH 6.0, pH 7.0 and pH 8.0, bean seed lipases were more

active than all the other three enzymes of coconut, soya meat and pumpkin seed. At pH 5.0, coconut lipase, soya meat lipase and bean seed lipase were inactive, only bean seed lipase was active at this pH.

Table 4.13: Activities of lipases as heat treatment vs non-heat treatment at different pH (ppm/ml/hr)

Sample	Heat treatment Vs. Non-heat treatment				Remarks
	pH 8.0	pH 7.0	pH 6.0	pH 5.0	
Coconut	2.65/2.11	0.26/0.20	0.01/0.01	---	Heat stable
Soya meat	1.76/3.01	---	---	---	Heat sensitive (High)
Bean seed	2.47/4.14	2.08/4.08	1.37/2.63	0.91/1.34	Heat sensitive (High)
Pumpkin seed	2.12/2.33	1.39/1.63	0.06/0.09	---	Heat sensitive

Table 4.13 shows the activities of lipases when heat treatment was applied to the samples vs when heat treatment was not applied to them at different pH. Coconut lipase showed its highest activity at heat treatment and smaller activity than this when heat was not applied to it at pH 8.0. At pH 7.0, the activity of coconut decreased at both the heat treatment and non-heat treatment level, but it still showed higher affinity for heat treatment and lower activity for non-heat treatment level. When the pH was 6.0, the coconut lipase still had higher affinity for heat treatment and lower for non-heat treatment. And at pH 5.0, the coconut lipase was not active. So, from these data values of this study we can say that coconut lipase is a heat stable lipase and it can be used in higher heating field of food industry applications. And at normal room temperature of our country, coconut lipase showed low activity. From the above table, soya meat lipase shows higher affinity for non-heat treatment and decreases its activity when heat is applied to the enzyme. This was observed that when heat treatment was applied to the soya meat lipase enzyme the activity of the lipase was 1.76 ppm/ml/hr and when heat treatment was not applied to the enzyme the activity of the enzyme was increased to 3.01 ppm/ml/hr released as free fatty acids. At pH 7.0,

6.0 and 5.0 soya meat enzyme showed no activity at the both point of heat treatment and non-heat treatment level. So, from this study this was observed that soya meat lipase is a highly heat sensitive enzyme. And this enzyme is also highly sensitive at acidic medium. When the assay was carried out at pH 8.0 for bean seed lipases, it showed great result of activity and that was 4.14 ppm/ml/hr when heat treatment was not applied to the enzyme medium. But this is remarkable that, when heat treatment was applied to the medium the activity of the same bean seed lipases was greatly decreased to 2.47 ppm/ml/hr released as FFA. It proved that the bean seed lipase is highly heat sensitive. The bean seed lipases also showed heat sensitivity at pH 7.0. The activity was 4.08 ppm/ml/hr at non-heat treatment, but the activity decreased to 2.08 ppm/ml/hr when heat treatment was applied to it. At pH 6.0 and pH 5.0, the bean seed lipase also showed higher affinity for lower heating medium and it was sensitive in higher temperature. So, the bean seed lipase was highly heat sensitive at both the acidic and alkaline medium. This was observed that, pumpkin seed lipases were also heat sensitive. When heat was applied to the pumpkin seed lipases at pH 8.0, the activity of the lipase was 2.12 ppm/ml/hr and when it was carried out in a normal room temperature the activity of the lipase was increased to 2.33 ppm/ml/hr released as FFA. The pumpkin seed lipases also showed higher affinity for lower temperature at pH 7.0, pH 6.0 and pH 5.0 and this enzyme showed heat sensitivity when heat treatment was applied to the enzyme medium by lowering the activity of the enzyme.

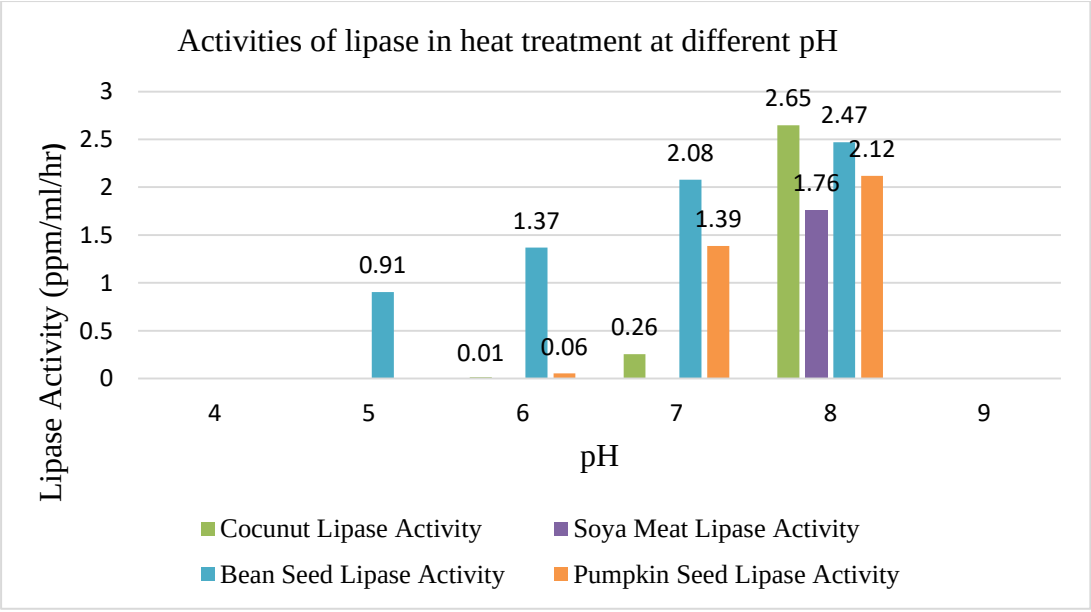

Figure 4.12: Activities of lipases in heat treatment at different pH

In figure 4.12 the activities of coconut lipase, soya meat lipase, bean seed lipase and pumpkin seed lipase are showed in heat treatment at different pH of pH 5.0, pH 6.0, pH 7.0 and pH 8.0. In this figure, the highest activity of lipase in heat treatment was at pH 8.0 and that lipase was coconut lipase and the activity was 2.65 ppm/ml/hr released as FFA. The lowest activity of lipase in heat treatment was at pH 6.0 and that lipase was also coconut lipase and the activity was 0.01 ppm/ml/hr released as FFA.

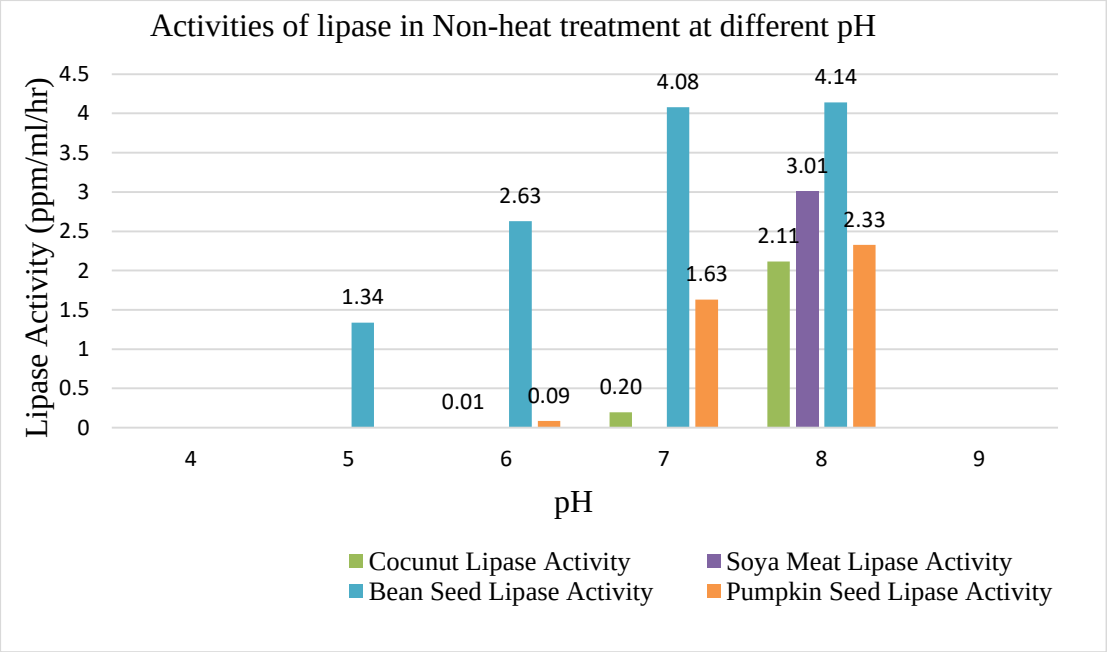

Figure 4.13: Activities of lipases in non-heat treatment at different pH

In the above figure 4.13, the activities of coconut lipase, soya meat lipase, bean seed lipase and pumpkin seed lipase were showed in non-heat treatment at different pH pf pH 5.0, pH 6.0, pH 7.0 and pH 8.0. At non-heat treatment the highest activity was at pH 8.0 and that lipase was bean seed lipase and the activity of bean seed lipase was 4.14 ppm/ml/hr released as FFA. At the same non-heat treatment environment the lowest lipase activity was at pH 6.0 and that lipase was coconut lipase and the activity of coconut lipase was 0.01 ppm/ml/hr released as FFA.

CHAPTER 5

DISCUSSION

In this study during the lipase enzyme detection test, banana peel showed negative result; mustard seed and pumpkin seed showed good presence of lipase enzyme; orange peel showed great amount of presence of lipase enzyme; bean seed, soya meat, lentil and winter melon seed showed positive result of lipase presence; and finally, eggplant showed a negligible amount of lipase enzyme presence in it. The lipase enzyme activity determination test showed different activities for different acidic and alkaline medium and for different temperature range.

The effect of pH on the activity of lipase from many sources has been studied. In this study, all the four samples were acted maximally at pH 8.0. Coconut lipase had the highest activity at pH 8.0 among the other three samples of soya meat, bean seed and pumpkin seed lipases. So, coconut lipase should be used at pH 8.0 which is also showed by another study (Ejedegba et al., 2007). They showed that the coconut lipase acted maximally in mildly alkaline pH, a broad optimum pH of 7.5 with the native substrate to 8.5. The optimum pH of most lipases studied showed that it is either in alkaline pH region or in the acidic pH region, which shows that two types of lipases exist. In this study there were also two types of plant lipases found those were alkaline and acidic lipases. The soya meat lipase, coconut lipase and pumpkin seed lipase were active at alkaline medium and only bean seed lipase was active in both the acidic and alkaline medium. Some of the acid lipase includes castor bean lipase at pH 4.0 – 4.2 (Ory et al., 1960) germinating seedling of Cucumeroropsis edulis with pH 5.0 (Opute, 1975) and Aspergilus lipase with pH 6.5 (Fu et al., 1995). Alkaline lipases occurs in germinating rape seed at pH 8.5 (Wetter, 1957; Hoppe and Theirmer, 1997) rice bran pH 7.5 – 8.0 (Funatsu et al., 1971), F. Oxyspprium lipase at pH 8.6 and 7.0 depending on the substrate (Maria et al., 1991), C. bonducella seeds at pH 7.0 (Vajanti et al., 2001), Hibiscus canabinus seeds pH 7.0 (Kausar and Akhtar, 1979), the coconut lipase studied belongs to this last class of lipase. Soya meat lipase was inactive at pH 5.0-pH 7.0, so it is not appropriate for using at acidic medium and this lipase was only active at pH 8.0. Pumpkin seed lipase and coconut lipase were almost inactive at acidic pH and these lipases were more active at alkaline pH. So, from this study this is showed that bean seed lipase can be used in both the acidic and alkaline medium. This

study also showed that coconut lipase was most active enzyme for using in the alkaline medium.

Lipases have been found to be active over a wide range of temperature. In this study an optimum temperature range of 30– 40°C was observed on average. At higher temperature treatment, coconut lipase showed highest activity and bean seed lipase showed lowest activity. That is at higher heat area of about 35-40˚C, coconut lipase was mostly active and at the same temperature, the bean seed lipase was not active like in lower temperature. The bean seed lipase showed its highest activity on an optimum range of temperature about 30-34˚C. There are some other studies which support the findings of this study where some microbial lipases act at –20°C (Brokenheff and Jensen, 1974), Sesanum indicum lipase act at 37°C (Kumar and Murphy, 1966), melon lipase with optimum temperature of 37°C (Opute, 1975) and Aspergillus sp. lipase with optimum temperature of 30-40°C (Fu et al., 1995). Others include Cajanus cajan L. seed lipase at 30°C (Khan et al., 1991; Dahot et al., 1989), Carissa carandas fruit lipase at 30°C (Mala and Dahot, 1995), C. bonducella L. seed lipase at 30°C (Vajanti et al., 2001). Soya meat lipase and pumpkin seed lipase showed higher activity at an optimum temperature range of 30-34˚C and they showed lower activity at the temperature range of 35-40˚C.So, these two enzymes are suitable for using in the industries where the temperature range are nearly within 30-34˚C. The optimum temperature of coconut lipase activity was 35-38˚C which also supports by a study where showed that the coconut lipase enzyme acted maximally at 35°C (Ejedegba et al., 2007). So, the coconut lipase can be used at this temperature at alkaline pH for getting good result. Coconuts are available in our country. So, if isolation of coconut lipase enzyme is possible, this study showed that coconut lipase enzyme can be used in the food industries where alkaline environment and more than 34˚C is available for long time for the purpose of manufacturing many food products like cheese ripening, flavor enhancing of cheese, emulsification in bakery products, flavor enhancing of bakery products, flavor enhancing in milk, chocolate, and production of volatile materials in black tea during tea fermentation and many other uses. However further studies should be carried out on the isolation of enzyme from these respective plant origins.

CHAPTER 6

CONCLUSIONS

This study determined the optimum pH and temperature of some plant lipases where we found that coconut lipase was suitable for alkaline environment and it was heat stable, soya meat lipase was also suitable for alkaline medium and it was highly heat sensitive, bean seed lipase was only suitable in both the alkaline and acidic environment but it was highly heat sensitive, finally, pumpkin seed sample was suitable for alkaline environment and it was heat sensitive. All the samples except banana peel can be used as lipase source. Further study is needed for identifying yield.

CHAPTER 7

RECOMMENDATIONS AND FUTURE PERSPECTIVES

Due to resource limitation, the isolation of lipase enzymes from various plant foods was not possible. Interestingly, the isolation procedure of lipase enzyme from plant origins requires gel filtration machine, which is not available in our country. If we could manage the gel filtration machine and if we had enough time for isolating lipase enzyme from various plant foods, this study would be more effective for future perspective. In this study coconut, soya meat, bean seed and pumpkin seed showed different activities at different pH and temperature ranges. If fund will available in future, we would expect to continue our research and flourish the real aims of this project by clearly investigating the existing scenario of the plant origins containing lipase enzymes. In future, this study might be an intense research if many other plant sources of lipase enzyme will include determining the activity and the isolating of these plant lipases will possible.

REFERENCES

Aizono Y., Funatsu M., Sugano M., Hayashi K. and Fujiki Y., Enzymatic Properties of Rice Bran Lipase. Agric. Biol. Chem. 1973, 37, 2031-2036.

Alberghina L., Schmid R.D., Verger R., 1991. Contributions to the CEC-GBF International workshop, Braunschweig 1990.

Anita V. Prabhu, Shreehas P. Tambe, Neena N. Gandhi, Sudhir B. Sawant, and Jyeshtharaj B. Joshi, "Rice Bran Lipase: Extraction, Activity, and Stability", Biotechnol. Prog., 15, pp 1083−1089, 1999.

Atul S. Bhangale, Kathryn L. Beers, and Richard A. Gross, (2012). "Enzyme-Catalyzed Polymerization of End-Functionalized Polymers in a Microreactor". Macromolecules. 45 (17): 7000–8.

Ban K., Kaieda M., Matsumoto T., Kondo A. and Fukuda H., (2001). "Whole cell biocatalyst for biodiesel fuel production utilizing Rhizopus oryzae cells immobilized within biomass support particles". Biochem Eng J. 8 (1): 39–43.

Barros M., Fleuri L. F., and Macedo G. A. January - March, 2010. Seed lipases: sources, and properties – A review. Brazilian Journal of Chemical Engineering 27 (01), 15 – 29.

Brocker Loff H. and Jensen R.G., (1974). Lipotytic enzymes. Academic Press New York San Franscisco London pp. 1 – 3.

Buchholz K., Kasche V. and Bornscheuer U.T., "Enzymes in organic chemistry. In: Biocatalysts and enzyme technology", Wiley-VCH Verlag GmbH and Company, KGaA, Weinheim, pp. 127–131, 2005.

Caro Y., Villeneuve P., Pina M., Reynes M. and Graille J., Lipase Activity and Fatty acid Typoselectivities of Plant Extracts in Hydrolysis and Interesterification. Journal of the American Oil Chemists' Society, 77, No. 4, 350 (2000).

Cherry J.R., and Fidantsef A.L., "Directed evolution of industrial enzymes: an update", Current Opinion in Biotechnology, XIV (4), pp.438–443, 2003.

De Castro H.F., Mendes A.A. Santos, J.C. and Lima, C.A., Modificação de Óleos e Gorduras por Biotransformação. Química Nova, 27, No. 1, 146 (2004).

Dharmsthiti S., Ammaranond P., Biotechnol Appl Biochem 1997; 26:111–6.

DiMagno E.P., Malagelada J.R. and Go V.L.. Moertal C. G. Fate of orally ingested enzymes in pancreatic insufficiency. N. Engl. J. Med. 1977; 296: 131 8-22.

Ejedegba B. O., Onyeneke, E. C., and Oviasogie P. O. 2007. Characteristics of lipase isolated from coconut (Cocos nucifera linn) seed under different nutrient treatments. African Journal of Biotechnology, 6(6), 723-727.

Enujiugha V. N., Thani F. A., Sanni T. M. and Abigor R. D., Lipase Activity in Dormant Seeds of the African oil bean (Pentaclethra macrophylla Benth), Food Chemistry, 88, No. 3, 405 (2004).

Freire G.D.M. and Castilho F.L., Lipases em Biocatálise. In: Bon et al. (org). Enzimas em biotecnologia: Produção, Aplicação e Mercado. Rio de Janeiro, Interciência (2008).

Fu X., Hu X., Gao K. and Duan J., (1995). Oil and fat hydrolysis with lipase from Aspergillus sp. JAOCS 72(5): 527 – 531.

Funatsu M., Aizono Y., Hayashi K., Watanable M.and Eto M., (1971). Biochemical studies on rice bran lipase part 1. Purification and physical properties. Agric. Biol. Chem. 35(5): 734 – 742.

Gandhi N. G., Application of Lipase. Journal of the American Oil Chemists' Society, 74, No. 6, 621 (1997).

Ghosh P.K., Saxena R.K. and Rani Gupta, Microbial lipases production and applications. Sciences Progress (1996)119-157.

Gow R., Francis P., Bradbear R. and Sheperd R., Comparative study of varying regimens to improve steatorrhoea and creatorrhoea in CF: Effectiveness of an enteric coated preparation with and without antacids and cimetidine. Lancet 1981; 8255: 1071-4.

Guo Z. and Xu X., (2005). "New opportunity for enzymatic modification of fats and oils with industrial potentials". Org Biomol Chem. 3 (14): 2615–9.

Gupta R., Gupta N. and Rathi P., (2004). "Bacterial lipases: an overview of production, purification and biochemical properties". Appl Microbiol Biotechnol. 64 (6): 763–81.

Harding K.G; Dennis J.S; von Blottnitz, H; Harrison S.T.L (2008). "A life-cycle comparison between inorganic and biological catalysis for the production of biodiesel". Journal of Cleaner Production. 16 (13): 1368–78.

Hasan F., Shah A.A., and Hameed A., "Industrial applications of microbial lipases," Enzyme and Microbial Technology, XXXIX (2), pp. 235–251, 2006.

Hoppe A. and Theimer R.R., (1997). Degradation of oil bodies isolated from cotyledons during germination of rape seed seedlings. J. Plant Physiol 151: 471–478.

Hoppe A. and Theimer R.R., (1997). Rape seed lipase pH dependent specificity for native body autolysis and lipolysis of artificial oil droplets. J. Plant Physiol 151: 390- 398.

Huang A.H.C., Lin Y.H., Wang S.M., Characteristics and Biosynthesis of Seed Lipases in Maize and Other Plant Species. Journal of the American Oil Chemists' Society, 65, No. 6, 897 (1988).

Isbilir S.S., Ozcan M.H. and Yagar H., Some Biochemical Properties of Lipase from Bay Laurel (Laurus nobilis L.) Seeds. Journal of the American Oil Chemists' Society, 85, No. 3, 227 (2008).

Jaeger K.E. and Eggert T., "Lipases for biotechnology," Current Opinion in Biotechnology, XIII (4), pp. 390– 397, 2002.

Jadhav S., Chougule D., Rampure S., 2013. Lipase production from banana peel extract and potato peel extract. Universal Research Publications, 2013.

Kausar N., Akhtar M.W., (1979). Isolation and characterisation of Hibiscus canabinus (Kenof) seed lipase. Pak J. Biochem. 12: 58 – 64.

Khan M.Y., Dahot M.V., Noomrio M.H., (1991). Investigation of lipase activity from Cajanus cajan L seed. Pack J. Sci. Ind. Res, 34: 384 – 386.

Kumark S., Murphy S.K., (1966). Studies on lipolytic enzymes of oil seeds part 1 Sesanum Indicum (Gincelly) Enzymol. 14(7): 243 – 249.

Kundu, Santanu; Bhangale, Atul S; Wallace, William E; Flynn, Kathleen M; Guttman, Charles M; Gross, Richard A; Beers, Kathryn L (2011). "Continuous Flow Enzyme-Catalyzed Polymerization in a Microreactor". Journal of the American Chemical Society. 133 (15): 6006–11.

Macrae, A.R., Lipase-Catalyzed Interesterification of Oils and Fats. J. Am. Oil Chem. Soc. 1983, 60, 291-294.

Macrae A.R., "Lipase-catalyzed interesterification of oils and fats", Journal of American Oil Chemistry Society, LX, pp. 243-246, 1983.

Macrae A.R. and Hammond R.C., "Present and future Applications of Lipases", Biotechnology Genetic Engineering Review, III, pp.193-217, 1985.

Mala V., Dahot M.U., (1995). Lipase activity of Carissa carandas fruit. Sci. Int. (Lahore) 7: 161 – 164.

Maria de mascena Diniz maia, Marcia Mania Camargo de morais, Marcos Antonio de Morais, Edwardo Henrique Msagalhaes Melo, Jose Luiz de Lima Fitro (1999). Revista de Microbiologia 30: 304 – 309.

Muntz H., Sur la germination des graines olegineuses. Am. Chem. PhyS. 1871; 22: 472-85.

Noda M. and Kobayashi K., Lipids of Leaves and Seeds III Enzymic Hydrolysis of Rice Bran Lipids. Nippon Nogei Kagaku Kaishi 1968, 42, 574-580.

Noor I.M., Hasan M. and Ramachandran K.B., Effect of Operating Variables on the Hydrolysis Rate of Palm Oil by Lipase. Process Biochemistry, 39, No. 13 (2003).

Opute F.I., (1975). Lipase activity in germinating seedling of Cucumenpsis edulis J. Exp. Bot. 26 (92): 379-386.

Ory R.L., (1969). Acid Lipase in the Caster bean Lipids 4: 177–185. Savendson A (2000). Lipase protein Engineering. Biochemica et Biophysica Acta 1543: 223 – 238.

Pandey A., "Types of fermentation and factors affecting it. In: Biotechnology", Food fermentation , Joshi, V.K. and Pandey, A.(eds.), Educational Publishers, New Delhi , pp. 383-426, 1999.

Pandey A., Webb C., Soccol C.R. and Larroche C., "Enzyme Technology", 1ª Ed. New Delhi: Asiatech Publishers, Inc, cap. II , pp.760, 2005a.

Paques F.W. and Macedo G.A., Lipases de Látex Vegetais: Propriedades e Aplicações Industriais: A Review. Química Nova, 29, No. 1, 93 (2006).

Pastore M.G., Costa V.S. and Koblitz M.G.B., Purificação Parcial e Caracterização Bioquímica de Lipase Extracelular Produzida por Nova Linhagem de Rhizopus sp. Ciência e Tecnologia de Alimentos, 23, No. 2, 135 (2003).

Polizelli P.P. Tiera M.J. and Bonilla-Rodriguez G.O., Effect of Surfactants and Polyethylene Glycol on the Activity and Stability of a Lipase from Oilseeds of Pachiraaquatica. Journal of the American Oil Chemists' Society, 85, No. 8, 749 (2008).

Posorske L.H., Industrial-Scale Applications of Enzymes to the Fats and Oil Industry. J. Am. Oil Chem. Soc. 1984, 61, 1758-1760.

Prabhu A.V., Tambe S.P., Gandhi N.N., Sawant S.B., and Joshi J.B., 1999. Rice Bran Lipase: Extraction, Activity, and Stability. Biotechnol. Prog. (15), 1083−1089.

Rakhimov M.M., Dzhanbaeva N.R. and Yuldashev P.K., Specificity the Lipase of Cottonseed. Chemical of the Natural Compounds, 6, No. 5, 616 (1970).

Regan P.T., Malagelada J.R., DiMagno E.P., Go V.L. Rationale for the use of cimetidine in pancreatic insufficiency. Mayo C:in. Proc. 1978; 53: 79-83.

Robinson P.J., Sly P. D., Smith A.L., Effect of misoprostal on fat malabsorption in CF. Arch. Dis. Child. 1988; 63: 1081 -2.

Robinson P.J., Smith A.L., Sly P.D., Cystic fibrosis and its relationship to fat malabsorption. Dig. Dis. Sci. 1990; 10: 1299-1304.

Sadeghipour H.R. and Bhatla S.C., Light-enhanced Oil Body Mobilization in Sunflower Seedlings Accompanies Faster Protease Action on Oleosins. Plant Physiology and Biochemistry, 41, No. 4, 309 (2003).

Sagiroglu A. and Arabaci N., Purification and Characterization of Lipase from Sunflower Seed. Preparative Biochemistry and Biotechnology, 35, No. 1, 37 (2005).

Sammaour R.H., Purification and Partial Characterization of an Acid Lipase in Germinating Lipidbody Linseedlings. Turkish Journal of Botany, 29, No. 3, 177(2005).

Sana N.K., Hossain I., Haque E.M. and Ranajit Kumar Shaha. "Identification, purification, and characterization from germinating oil seed (*Brassica napus* L.)". Pakistan journal of biological sciences 7(2): 246-252, 2004.

Saxena R.K., Ghosh P. K., Gupta R., Davdison W.S., Bradoo S., and Gulati S., "Microbial lipases: Potential biocatalysts for the future industry", Current Sciences, LXXVII, pp. 101-115, 1999.

Selva T. Mohan, Palavesamandg. A. Immanvel., African J Biotechnol2008; 7(15): 2728-2735.

Sherin A. Basheerandm, Thenmozhi. Int J Chem Sci 2010; 8(5): S57-S67.

Sirisha E., Rajasekar N. and Narasu M.L., Isolation and Optimization of lipase producing organism from oil contaminated soils. Advance in biological rearch4es (5); (2010).

Vajanti Mala Pahoja, Umar Dajo M., Mumtaz Ali Sethar (2001). Online. J. Biol. Sci. 1(8): 775-778.

Vulfson E.N., "Industrial applications of lipases. In lipases: Their structure, biochemistry and application." Edited by Poul Woodley and Steffen B. Peterson, Cambridge University Press. pp. 271- 286, 1994.

Wetter L.R., (1957). Some properties of Lipase present in Germinating rape seed. J. Am. Oil Chem. Soc. 34: 66–69.

Yesiloglu Y. and Baskurt L., Partial Purification and Characterization of Almond Seed Lipase. Preparative Biochemistry & Biotechnology, 38, No. 4, 397 (2008).

Zachariassen B., Giasotta V.N., Prospects of Rice Bran Industry in India. Chem. Age India 1964, 15, 194-198.

Appendix A: Figures of Sample

Coconut

Soya meat

Bean seed

Pumpkin seed

Orange peel

Banana peel

Eggplant

Lentil

Mustard seed

Winter melon seed

Brief Biography

Sultana Nazia passed the Secondary School Certificate Examination in 2009 from Firoz Shah City Corporation Girls' High School, Chattogram and then Higher Secondary Certificate Examination in 2011 from Omargani M.E.S. College, Chattogram. Sultana Nazia obtained her B.Sc. (Hons.) in Food Science & Technology in 2017 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. She had completed 6 months' industrial placements in various food industries, Hospitals, government and Non-government organizations regarding food quality control, Food Processing and Human nutrition. She had obtained a certificate of completion on "Industrial Training Programme" from School of Food Science and Technology, Universiti Malaysia Terengganu, Malaysia on 1^{st}-30^{th} January 2017. She had also obtained a certificate of participation in "International Linked Programme: Global Arena Leadership Camp" organized by Guidance and Counselling Units, Tengku Bariah Secondary School, Malaysia in January, 21^{st} 2017. She had also completed training course on "Quality Control and Unit Operations in Food Industry" under Training Institute for Chemical Industries, Polash, Narshingdi, Bangladesh for 1 month and on Training Programme at Food and Nutrition Department, Chattogram Diabetic General Hospital, Khulshi, Chattogram for 1 month. Now, she is a candidate for the degree of MS in Department of Applied Human Nutrition & Dietetics under the Faculty of Food Science and Technology; CVASU. She has immense interest to work in determining lipase enzyme activities which are present in many plant origins of our country.