EFFECTS OF DIFFERENT PARAMETERS ON THE LIPOLYTIC ACTIVITIES OF LIPASE AND PHOSPHOLIPASE EXTRACTED FROM *PRUNUS ARMINICA* MEAL OF HUNZA CULTIVAR

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ABSTRACT: *Prunus Arminica.L* seeds of Hunza cultivar were defatted with chloroform, methanol mixture (2:1v/v) by ultrasonic assisted extraction technique. The lipase and phospholipase extracted, incubated at 40^0C and pH 6 in citrate buffer for 1 hour. The lipolytic activities of enzymes were measured at different temperatures i,e 30-70^0C , pH i,e 3-9 and solvents i,e n-heptane , Di-isopropyl ether and Cyclo hexane. 10 % emulsion of lecithin and olive oil used as the substrate for phospholipase and lipase substrates respectively. The enzymes showed stability at lower temperatures i,e 35-50^0C and slightly acidic to neutral i.e pH 5 to pH 7. Concentration of liberated fatty acids was the indicator of the enzymes activity which were measured by taking absorbance at 440nm. Stearic acid with different concentrations in hexane and methanol (1:1v/v) were used for the standard curve. Cu-TEA reagent with small quantity of $CaCl_2$ was used for maximum extraction of Cu-FFA and for stable value of blanks in the present study. The enzymes showed maximum activities at 45^0 C temperature and 6 pH.

Keywords: Ultrasonic extraction, *Prunus Arminica*, Olive oil, Lecithin, Lipase, Stearic acid Phospholipase.

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INTRODUCTION

Lipase and phospholipase are the enzymes which are responsible for hydrolization. In seeds theses enzymes hydrolyze triacylglycerols at the oil water interphase and form the free fatty acids along with the glycerols - a byproduct (Gadge, P.P *et al*., 2011 and Abolemonaem *et al.*, 2011). Lipase and phospholipase are abundantly found in animals, microorganisms and vegetables (Ejedegba *et al.,* 2007). The vegetable source of enzymes are most important because of their unique properties of substrate specificity, enantiomeric and regio selectivity (Krishna *et al.,* 2002). Furthermore, the enzyme specially lipase of vegetable origin are of low cost, easily available and easy to purify (Polizelli 2000 and Paques 2006).The importance of lipase and phospholipase are gaining hype in recent years because of their utilization in food, pharmaceutical, Oils/ fats, fine chemicals and cosmetic industries (Freire *et al.*, 2008 and Athawale *et al.*, 1999).

Along with the industrial importance of enzymes there are some serious disadvantages of enzymes as well. The lipolytic activity of lipase is responsible for the development of rancidity along with the deterioration of the oil (Cancino *et al*., 2008) which effects the nutritional values, shelf life and sensory aroma properties of food products, cosmetic and personal care products. Present study was focused on the impact of different parameters to observe the activities of lipase and phospholipase in oil bearing seeds of apricots. Apricots Scientifically known as *Prunus Arminica* are valuable source of protein (21-26%) and oil (40-45%), (Gupta, 2012) and the fruit is used mainly in the preparation of jams and nectars for export and locally consumption. Breakthrough technology made the consumers increasingly aware about the quality, health promoting components and nutritional composition in food and food products. With the factor of high yield, Apricot oil is an excellent source of monounsaturated fatty acids (94.56%) with essential fatty acids while Saturated fatty acids present in lower quantity (4.45%) (Manzoor *et al.,* 2012). The ratio of saturated, monounsaturated , polyunsaturated unsaturated fatty acids makes the health promoting composition of the oil. Unsaturated essential fatty acids are ideal for edible consumption and helpful in lowering the chances of Cardio vascular diseases (CVD) risks (hifza *et al.,* 2007). Further apricot Plants are well known for their great medicinal, commercial and economical importance. Plants different parts are used in traditional medicine in crude form, furthermore it is a home remedy of cough, Asthma, Bronchitis, anemia and fever. Kernel oil of apricot seeds is used in the preparation of many cosmetic products, moisturizing creams, baby oils, massaging oils, face scrubs and lip balms. Apricot is preserved in the dried form for the utilization as an off season main food of northern areas of Pakistan (Hussain *et al.,* 2010).

Due to such utilization of apricots and the deteriorating characteristic of enzymes in oils, the present study was conducted to observe the enzymes activities in

vast range of pH, temperatures and organic solvents to establish an optimum condition at which lipase and phospholipase shows their maximum activities. As the consumption of fruits and oils of apricot was noticed mainly in the Gilgit Balitistan areas especially Hunza valley, so Hunza cultivar was selected for the present study.

MATERIAL AND METHODS

Collection of *P. arminica* seeds: The fruit of *P. arminica* was collected from northern Karimabad area of Hunza-Pakistan in mid of June (2016). The fruit and trees were identified by the local residents. Seeds were obtained by breaking the stones manually into two parts with hammer. Seeds samples were kept in refrigerator prior to analysis.

Extraction of *P. arminica* seeds oils: The seeds of apricot were ground into the fine powder by grinder and defatted by ultrasonic assisted extraction technique. The extraction completed in 30minutes by keeping 15ml/g solvent and seed ratio at 300W sonic power. Mixture of 2:1v/v chloroform, methanol was used as a solvent to extract the oil from the seeds.

Extraction of enzymes from *P. arminica* seed meal: 20 gm of defatted seeds were suspended in 0.1M citrate buffer i,e citric acid 0.1M and disodium hydrogen phosphate 0.2M at pH 6 and was shaked for one hour at 45^0C. The supernatant containing the enzyme was obtained by centrifugation for 15 minutes at 4000 rpm (Zhong and Glatz, 2006).The extract containing enzyme was then diluted further with 200ml distilled water and 200ml of 0.1M citrate buffer while maintaining the pH at 6. The extracted enzymes from both lipase and phospholipase were stored in refrigerator for observing the enzymes activities under different conditions of temperature and pH (Hifza *et al*., 2007).

Preparation of substrates: Lipase substrate was prepared by blending 10% emulsion of pure triacylglycerol of olive oil with10% acacia gum emulsion to determine the lipase activity .10% lecithin emulsion was used as a substrate for phospholipase enzyme (Michael *et al*., 2001).

Preparation of Standard curve of stearic acid: 0.1M stearic acid prepared in chloroform, hexane (1:1 v/v) and used as a stock solution.50 -500µL of this solution took in ten different tubes along with 5.0ml chloroform, hexane(1:1v/v) and 2.5ml Cu-TEA reagent. Tubes were closed and centrifuged for 30 minutes. The upper organic layer was separated and took absorbance at 440.0nm after adding 0.5ml of 0.1% sodium diethyldithiocarbamate solution (Maliks *et al*., 2000).

RESULT AND DISCUSSION

Enzymes play a vital role in vivo synthesis and in the breakdown of a number of organic compounds in animals and plants. The present study was conducted for lipase and phospholipase enzymes of Hunza apricot cultivar seeds, which were involved in the degradation of lipids. The enzymes were hydrolyzed for triacylglycerols and phosphoacylglycerols respectively and the liberated fatty acids served as the indicator of their activities.

Table-1. Absorbance of different concentrations of stearic acid.

Stearic acid concentration (µL)	Absorbance of stearic acid at 440.0nm
50	0.09
100	0.15
150	0.289
200	0.399
250	0.537
300	0.759
350	0.899
400	0.96
450	1.289
500	1.119

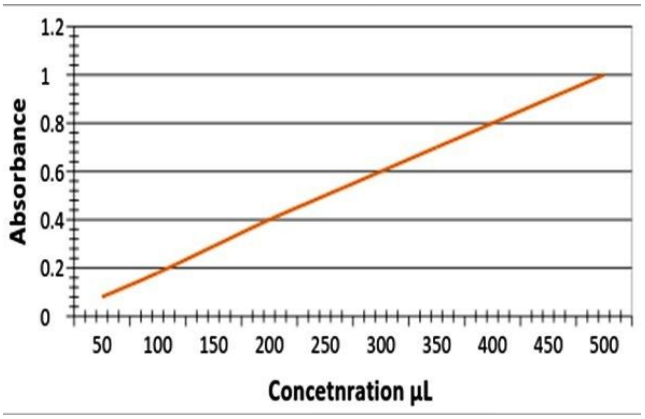

Figure-1. Stearic acid standard curve

Effect of pH: Four milliliter fraction each of lipase and phospholipase extracts were mixed with 5ml substrate and was shaked on orbital shaker for 1 hour at 45 ˚C. 0.1 ml of 0.1M $CaCl_2$ and phosphate buffer of pH 6 was added after incubation in each flask (Eze, S.O.O. and Ezema, B.O. 2012). Liberated fatty acids were extracted with the mixture of hexane and chloroform (1:1v/v) in 2.5ml of Cu-TEA reagent were added in the flasks of lipase and phospholipase and centrifuged for 15.0 minutes at 4000 rpm. Took the absorbance of upper layer at 440.0nm after the addition of 0.5ml of 0.1% diethyldithiocarbamate. Different experiments were conducted at the pH ranging between 3 to 9 by using

citric acid and sodium hydrogen phosphate. The Fig - 2 showed the absorbances of liberated fatty acids due to the lipolytic activity of lipase as a result of hydrolysis of substrates at different pH.

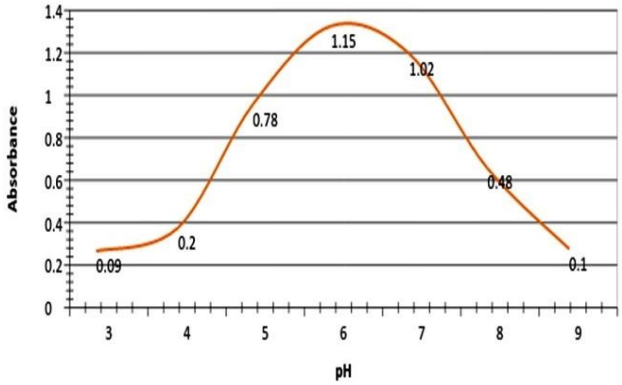

Figure-2. Absorbance of liberated fatty acids at different pH due to lipase

Table-2. Activity of lipase in *P. arminica* seed at various pH.

pH	Concentration of liberated fatty acids µL	Lipolytic activity µU
3	44.9	0.361
4	99.88	0.80
5	349	2.8
6	509.99	4.08
7	460.1	3.6808
8	230.5	1.844
9	60	0.48

a. Derived from standard stearic acid curve
b. Derived from Guven's equation

Table - 2 depicted the activity of the enzyme lipase at different pH from 3-9. The results showed maximum activity at pH 6.

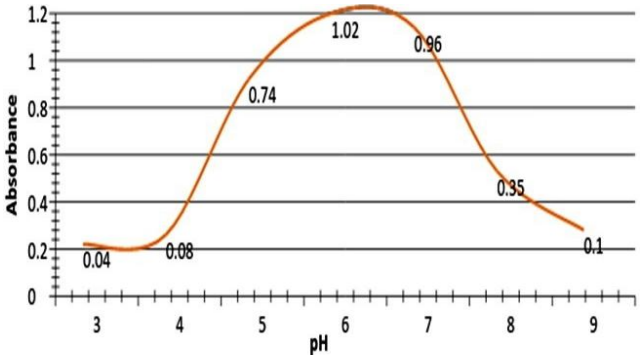

Figure-3. Absorbance of liberated fatty Acids at different pH due to enzyme phospholipase

The results exhibited that both the activities lipolytic due to lipase and phospholipase were maximum at pH 6.

Effect of Temperature: Temperature play a vital role in the activity of enzymes. Lipolytic activities of lipase and phospholipase with the variation of temperature from 30 to 70 ˚C with an increase of 5 ˚C at pH 6, were monitored to observe the impact of temperatures.

Fig-4 showed the absorbance of liberated fatty acid due to hydrolysis by the lipolytic activity of lipase at various temperatures.

Table-3. Lipolytic activity of phospholipase at different pH of *prunus arminca* seed.

pH	Concentration of liberated fatty acids µL (a)	Phospholipase activity µU (b)
3	30.1	0.2408
4	52	0.416
5	339	2.712
6	458	3.664
7	430	3.44
8	168	1.344
9	60	0.48

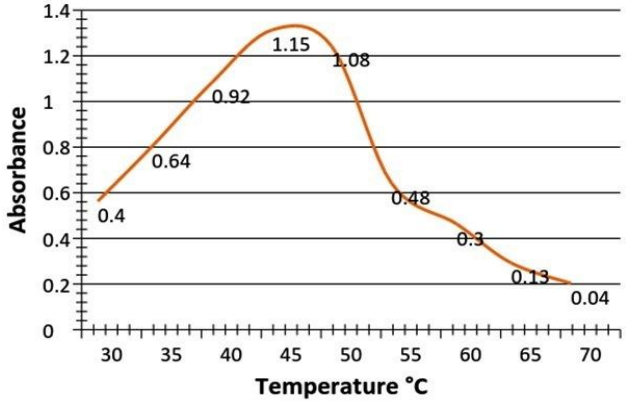

Figure-4. Absorbance of liberated Fatty Acids with variation of Temperatures with lipase enzyme

Table-4 showed the concentration of fatty acids and activities of enzymes at different temperature.

Table-4. Lipase activity at different temperatures and constant pH 6.

Temperature °C	Concentration of liberated fatty acids µL	Lipolytic activity of lipase µU
30	189	1.512
35	295.5	2.364
40	414	3.312
45	510.1	4.08
50	479.5	3.836
55	210.1	1.6808
60	149.9	1.199
65	74	0.592
70	31	0.248

The maximum lipolytic activity of lipase was observed at 45°C. Fig- 5 showed the frequencies of absorbance of liberated fatty acids at different temperatures due to the lipolytic activity of phospholipase.

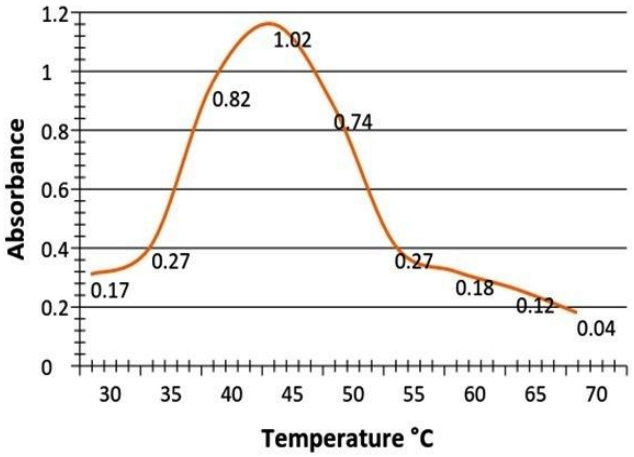

Figure-5. Absorbance of liberated fatty Acids at different temperature due to phospholipase

Table-5. Phospholipase activity at different temperature and constant pH 6.

Temperature (°C)	Concentration of liberated fatty acid µL	Lipolytic activity of phospholipase µU
30	94.8	0.7584
35	136	1.088
40	371	2.968
45	460.6	3.6848
50	340.4	2.7232
55	135.6	1.0848
60	95.2	0.7616
65	70.2	0.5616
70	30	0.24

Maximum lipolytic activity of lipase and phospholipase was observed at 45°C. However as the temperature started increasing, the activity resulted decreasing trend with the downfall of the liberation of fatty acids. Which justified that mostly enzymes were protein in nature which started denaturing at higher temperature.

Effect of solvents: Organic nonpolar solvents were used to determine the activities of lipase and phospholipase. n-Heptane, diisopropyl ether and cyclohexane were used to observe the activities of enzymes.

Fig - 6 showed the absorbance relevant to fatty acid liberated by the action of lipase.

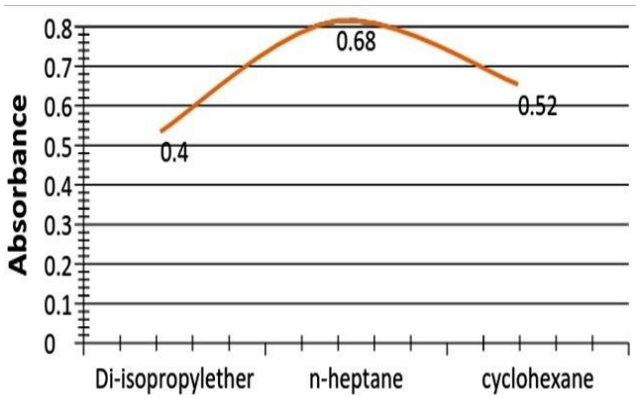

Figure-6. Absorbance of fatty acids due to lipase

Table-6. Lipolytic activity of lipase of *P. arminca* seed in different solvent.

Solvent	Concentration of liberated Fatty Acids. µL	Lipolytic Activity of lipase µU
Di-isopropyl ether	190.2	1.5216
n-heptane	312	2.496
Cyclohexane	240.4	1.9232

Table -6 shows the activities of lipase in different organic solvents.

Fig- 7 depicted the liberated fatty acids frequencies of absorbance at 440.0nm in different organic solvents due to lipolytic activity of phospholipase.

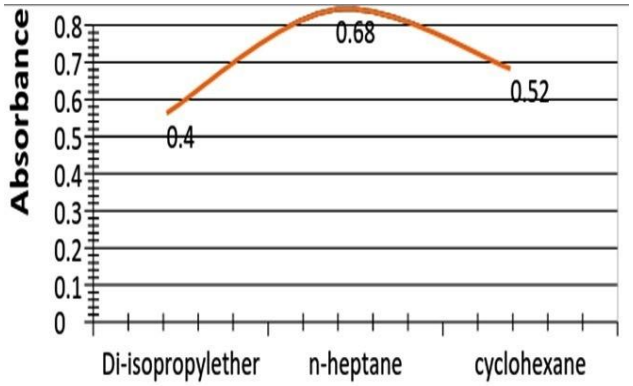

Figure-7. Absorbance of fatty acids due to phospholipase

Table-7. Phospholipase activity of *P. arminca* seed in different organic solvent.

Solvent	Concentration of fatty acids. µL	Lipolytic activity of phospholipase µU
Di-isopropyl ether	150.1	1.2008
n-heptane	269.9	2.1592
Cyclohexane	230.2	1.8416

Table 6 and 7 showed the activities of lipase and phospholipase enzymes in different solvents and results indicated that in n-heptane, both the enzymes showed their maximum activities. Concentration of fatty acids was the indicator for the exhibition of the enzymes activities.

Apricot seeds are the major source of oil and due to storage these oil seeds also produced lipase and phospholipase. Present study showed lipase and phospholipase characterization at different pH, temperature and solvents. Likewise different vegetable seeds were studied for lipase characterization by various researchers and found the maximum activity of lipase at same pH (Eze and Chilaka; 2010, Hifza *et al*., 2007 and waheed *et al*., 2001). Lipase was produced from a germinating seeds which showed variations depending on the time of germination reported by previous researcher (Abigor *et al* .,2002, Miled *et al*., 2000). The extracted apricot lipase and phospholipase enzyme activity increased with an initial increase in pH and optimum activity was noted at pH 6 suggesting slightly acidic nature of the enzyme. Using caster lipase Altaf, (1997) observed an optimum pH of 6 with phosphate buffer. Optimum PH from 6 – 7.5 was observed for lipase from some seeds of Lupin, Pinus and hazelnut (Grogorevea *et al*., 1977; Hammer and Murphy, 1993; Metwalli, 1983; and Bonvehi, 1996)

The lipase had an optimum temperature of 45^0 C which is similar to the work reported in Jatropha Curcas. L (Abigor *et al*., 2002). Enujiugha *et al*., (2004) further observed a gradual decline in the activity of lipase from Conphor nut with successive increase in temperature from 30 – 80^0 C . Likewise, organic solvents have effect on lipase and phospholipase activity. Our results were in agreement with the Hifza *et al*., 2007; Waheed *et al*., 2001, which reported n-heptane as an activator of both enzymes i.e lipase and phospholipase while diisopropyl ether and cyclohexane were found to be moderator solvents with respect to activation of enzymes.

The concentration and type of substrate played vital role in the activation and extraction of enzymes.10% emulsion of lecithin and olive oil gave the ideal results of hydrolysis of fatty acids. Same substrates and their concentrations were reported to be ideal by the previous workers while observing the hydrolysis results by changing the concentration of emulsion and the substrates on the Candida rogasa (ammar *et al*., 2017; Ismail and Sagiroglu , 2005).

Conclusion: On the basis of above observations, it could be inferred that *P. arminica* seeds can be a cheap source of lipase and phospholipase in slightly acidic range with optimum temperature of 45˚C. From the product point of view, the activity of lipase and phospholipase can be used as quality indicator for optimizing storage and processing conditions. To obtain high quality product it is necessary to inactive lipase and phospholipase that catalyzes the hydrolytic breakdown of lipids in apricot seeds. As a product, it can be used in as a detergent in oil industry as a value addition products.

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