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Purification and Characterization of Lipase from the Liver of Carp, *Cyprinus carpio* L. (1758), Living in Lake Tödürge (Sivas, Türkiye)

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Abstract

In this study, a lipase from the liver of *Cyprinus carpio* Linnaeus (1758) living in Tödürge Lake (Sivas) was purified with purification parameters of 90.38 µmol/dk.mg protein specific activity and 75.50 fold purity. The purification procedure consisted of preparation of homogenate, precipitation with polyethylene glycol-6000 (PEG-6000) and chromatographic techniques including in Q sepharose, sephacryl S 200 HR and phenyl sepharose CL-4B, respectively. The purified lipase showed a single band with approximately a molecular weight of 74 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). pH and temperature optimums for the purified enzyme were determined as 8.0 and 37°C using p-nitrophenyl butirate (p-NPB) as substrate. K_m and V_{max} values were found as 0.17 mM p-NPB and 2.6 µmol/ml.dk, respectively. It was observed that surface active agents, such as sodium dodecyl sulfate (SDS), Triton X-100 and Na-taurocholate act as inhibitor on lipase activity.

Keywords: *Cyprinus carpio*, lipase activity, Q sepharose, sephacryl S 200 HR, phenyl sepharose CL-4B, Tödürge Lake.

Tödürge Gölü (Sivas, Türkiye)'nde yaşayan *Cyprinus carpio* Linnaeus (1758)'nun Karaciğerindeki Lipaz'ın Saflaştırılması ve Karakterizasyonu

Özet

Bu çalışmada, Tödürge Gölü'nde yaşayan *Cyprinus carpio* Linnaeus (1758)'nun karaciğerinden 90.38 µmol/dk.mg protein spesifik aktivite ve 75,50 katlık saflaştırma parametreleri ile bir lipaz saflaştırılmıştır. Saflaştırma prosedürü homojenatın hazırlanması, poletilen glikol-6.000 (PEG-6000) ile çöktürme ve Q sefaroz, sefakril S 200 HR, fenil sefaroz CL-4B'yi kapsayan kromatografik tekniklerden oluşmuştur. Saflaştırılmış lipaz, sodyum dodesil sülfat-poliakrilamit jel elektroforezi (SDS-PAGE) ile yaklaşık 74 kDa'luk bir molekül ağırlığı ile tek bant göstermiştir. Optimum pH ve sıcaklık, substrat olarak p-nitrofenil butirat (p-NPB) kullanarak sırasıyla 8,0 ve 37°C olarak belirlenmiştir. K_m ve V_{max} değerleri, sırasıyla, 0,17 mM p-NPB ve 2,6 µmol/ml.dk olarak bulunmuştur. Sodyum dodesil sülfat (SDS), Triton X-100 ve Na-taurokolat gibi yüzey aktif maddelerin lipaz aktivitesi üzerinde inhibitor olarak etki ettikleri gözlenmiştir.

Anahtar Kelimeler: *Cyprinus carpio*, lipaz aktivitesi, Q sefaroz, sefakril S200 HR, fenil sefaroz CL-4B, Tödürge Gölü

Introduction

Lipids are essential for living systems and constitute a major part of the earth's biomass. They have structural roles in membranes, are the most potent energy storage molecules and involved in cell signaling. Lipases and esterases, which are lipolytic enzymes, are required for the lipids to participate in these functions during their metabolism. This means that these enzymes are involved in the breakdown and the turnover of these water-insoluble compounds, mobilizing them both within the cells of an individual organism and the transfer of these molecules between organisms (Beisson *et al.*, 2000; Gilham and Lehner, 2005).

Lipases (E. C. 3.1.1.3) can be broadly defined as enzymes that catalyze the hydrolysis of ester bonds in substrates such as vitamin esters, phospholipids, triglycerides (TGs) and cholesteryl esters (Kurtovic *et al.*, 2009). They catalyze their substrates at lipid-water interface, called interfacial activation, a phenomenon that can be traced to the unique structural characteristic of this class of enzymes (Beisson *et al.*, 2000; Reetz, 2002). Lipases have been widely used in the food and other industrial applications and thus there is an increasing demand in discovering new enzyme sources having unusual characteristics to suit particular applications.

Compared with proteases and other hydrolytic enzymes in carbohydrate metabolism, lipases are relatively less well studied and in this regard, lipases from aquatic animals are even less well known than those found microbial, plant and mammalian sources (L'opez-Amaya *et al*., 2001; Aryee *et al*., 2007).

Fish tissue by-products such as the head, frame, viscera, the skin, scales and specially the digestive organs together with glands are potentially rich source of enzymes such as lipases and numerous biochemicals. Pyloric cecum, stomach, pancreas, and intestines as digestive organs found in fish remain unexplored lipolytic enzyme sources that could have unique characteristics. Hovewer, the studies focusing on the purification of fish tissue lipases appear to be much more limited (Kurtovic *et al.*, 2009). For these reasons, it appears that recent studies have been focused on lipases from aquatic organisms and major part of these studies has been dealt with the digestive lipases. Recently, the presence of lipase activity has been showed in *Scophthalmus maximus* (Izquierdo and Henderson, 1998), *Pagrus major* (Iijima *et al*., 1998), *Mugil cephalus* (Aryee *et al*., 2007), *Cirrhinus reba* (Islam *et al*., 2009), *Sardinops sagax caerulea* (Noriega-Rodriguez *et al.*, 2009), *Sardinella aurita* (Smichi *et al*., 2010), *Oncorhynchus tshawytscha* and *Macruronus novaezelandiae* (Kurtovic *et al.*, 2010) and *Penaeus vannamei* (Rivera-Perez *et al.*, 2010). Gjellesvik (1991) purified and characterized a bile salt dependent lipase (BSDL) for the first time from the pancreatic tissue of Atlantic cod *Gadus morhua* L. Recent a few studies conducted on larval turbot *S. maximus* has been explained that this species also has the BSDL activity and BSDL activity did not affected in turbot larvae fed rotifers with a high or low lipid content (Hoehne-Reitan *et al.*, 2001a; 2001b; 2003).

The Lake Tödürge is 56 km from Sivas and a typical cyprinid lake populated by eight fish species and commercial fishing is carried out in this lake by co-operatives (Unver and Erk'akan, 2005). There is no study assessing the biochemical features of fish species from the Lake Tödürge. We thought that fish species living in this lake would be important resources of protein and fatty acids in terms of feeding of local folk. For this reason, we investigated some fish species from the lake regarding with both fatty acid composition and lipase enzyme. In this part of our study, we are presenting the purification and some basic kinetic properties of the liver lipase enzyme of *Cyprinus carpio* from the Lake Tödürge, which is located in the centre of Anatolia, as a first biochemical study.

Materials and Methods

Biological Material

Cyprinus carpio individuals used in this study were hunted from the Lake Tödürge in October. The liver samples were removed under icy conditions and stored at -20°C until used.

Sample Preparation for Lipase Extraction

The frozen livers were thawed at 4°C and room temperature, respectively and cleaned by flushing with distilled water followed by rinsing with cold 0.9% NaCl solution. The liver samples were chopped small pieces in order to homogenize.

Preparation of Enzyme Extract and Purification Procedure

Chopped liver sample was homogenized using Electromag M 11 homogenizer in 60 mM Tris-HCl buffer, pH 7.40, containing 0.25 mM D-Mannit and 1 mM Na_4EDTA (buffer A). Homogenate were filtered using a glass wool and then centrifuged (Beckman Coulter) at 60.000 x g for 1 hour at 4°C. The precipitate was discharged and the supernatant was filtered using glass wool and this fraction was exposed to 30% (w/v) PEG-6000 precipitation at 8000 x g for 20 minutes at 4°C. The resulting precipitate was redissolved in buffer A and used in next steps.

The Q sepharose anion exchange material was filled into the column (1 x 20 cm) and washed with distilled water of 100 ml in order to refine from ethanol. After this step, the column was equilibrated with 20 mM Tris-HCl, pH 7.80, containing 1 mM Na_4EDTA and 0.2 mM DTT (buffer B). An aliquot of 5 ml of PEG-6000 precipitate was applied to the column. The unbound proteins was eluted washing equilibration buffer and proteins bound the column were eluted using 100, 200, 300, 400, 600, 800 and 1000 mM NaCl in buffer B. The fraction showing lipase activity was pooled and concentrated and desalted using a Millipore Amicon Ultra centrifugal filter device (10 kDa MWCO, SIGMA-ALDRICH Inc. St. Louis, USA). The concentrated material was applied into a sephacryl S 200 HR gel filtration column (1 x 30 cm) as a second chromatographic step, which was previously equilibrated with 20 mM Tris-HCl, pH 7.80, containing 0.2 mM DTT (buffer C). Elution of proteins from column was carried out using buffer C until the absorbance at 280 nm of eluate reaches to a minimum. Fraction having lipase activity pooled and consantrated and applied into a phenyl sepharose CL-4B hydrophobic interaction column (1x20 cm), equilibrated with 20 mM Tris-HCl, pH 7.80, containing 150 mM ammonium sulphate ($(NH_4)_2SO_4$) (buffer D). This chromatographic step involved firstly washing the column with buffer D to remove loosely or unbound proteins and then washing with 10, 20, 50% isopropanol series in the buffer D.

Estimation of Protein Amount

Protein concentration (mg/ml) was estimated using a micro-scale Bradford protein quantization

method (Bollag *et al.*, 1996) using Bovine Serum Albumin (BSA) as standard.

Enzyme Assay

Lipase activity was assayed as reported by Bülow and Mosbach (1987) with slight modifications. The stock substrate solution was 50 mM of p-NPB in ethanol and activity buffer was 50 mM Tris-HCl, pH 8.0, containing 4% ethanol. Lipase activity was assayed spectrophotometrically by measuring the rate of hydrolysis of p-NPB at 405 nm and 30°C in a Cecil 5000 series UV double beam spectrophotometer. The change in the absorbance at 405 nm and 30°C was read at 30 s intervals for a period of 5 min against blank. The reaction mixture composed of 2930 µl activity buffer, 10 µl of enzyme solution and 60 µl of p-NPB. One unit of activity was defined as the amount of enzyme that catalyze the release of 1 µmol of p-nitrophenol (p-NP) per min under assay condition. The extinction coefficient of p-NP was taken as 11.500 $M^{-1}cm^{-1}$ (Metin and Akpınar, 2000).

Effect of pH on the Activity of Liver Lipase

The activity of liver lipase from *C. carpio* was examined within the pH range of 5.0 and 10.0 using the following buffer solutions of 50 mM phosphate buffer, pH 5.0-7.5 and Tris-HCl buffer, pH 8.0 and 10.0. Liver lipase was exposure a pre-incubation for 20 min in interested pH values and standard activity assay using p-NPB as substrate in 1 mM final concentration in reaction tube were evaluated for every pH solution.

Effect of Temperature on the Activity of Liver Lipase

The temperature optimum of lipase purified from *C. carpio* liver was tried to determine by equilibrating the enzyme solution of 10 µl at 5, 10, 20, 30, 33, 35, 37, 38, 40, 45, 50 and 60°C for 20 min. At the end of incubation period, the enzyme solution was assayed using p-NPB (1 mM final concentration and pH 8.0).

Kinetic Study

The effect of substrate concentration (p-NPB) ranging from 0.16 mM to 2 mM on the reaction rate was assayed at 30°C and pH 8.0. The kinetic parameters containing apparent K_m and V_{max} were calculated by nonlinear regression using "GraphPad Prism version 5.00 for windows, Graphpad software".

Effect of Surface Active Compounds on Liver Lipase Activity

In this section, the effect of sodium dodecyl sulphate (SDS), Na-taurocholic acid and Triton X-100 was evaluated under standard activity assay conditions containing interested chemical agent in the ratio of 0.5 % by incubating them in given times.

Electrophoretic Studies

SDS-PAGE under reducing conditions was carried out based on the method of Laemmli (1970) using 8 and 10% gels. The gels stained with silver staining.

Results

Liver lipase from *C. carpio* living in the Lake Tödürge was purified by three-step chromatographic approaches as described in material method section. All purification steps and the results obtained have been exhibited in Table 1 and Figures 1-7. 30% (w/v) PEG-6000 application was important at the gaining and the concentrating of the lipase activity in a smaller volume but did not provide any purification parameters and had more less the similar purification parameters when compared with the supernatant obtained from the centrifugation of crude homogenate at 60.000x g for 1 hour. As a first chromatic step, Q sepharose anion exchange chromatography provided only one fraction corresponding one protein peak having lipase activity in a concentrated manner by eluting 300 mM NaCl (Figure 1). We also repeated the same data in the purification of liver lipase from other fish species (unpublished data). Purification parameters of this step were calculated as 29.72 µmol/dk.mg specific activity and 24.83-fold purity. SDS-PAGE study carried out by silver staining showed that the fraction having the lipase activity collected from Q sepharose had many contaminant protein bands. However, it appears that this chromatographic step discharged a huge amount of

Table 1. Purification steps of the liver lipase from *Cyprinus carpio*

Step	Activity (µmol/ml.dk) Mean±S.E	Protein (mg/ml) Mean±S.E	Σ Volume (ml)	ΣActivity (µmol/dk) Mean±S.E	Σ Protein (mg) Mean±S.E	Specific activity (µmol/dk.mg)	Yield (%)	Fold (Times)
Homogenate	32.15±0.28	26.85±1.37	40	1286±4.39	1074±11.07	1.197	100	1
Supernatant (60.000xg)	26.57±0.61	20.61±2.08	38	1010±7.01	783.18±9.93	1.289	72.92	1.07
30 % (w/v) PEG-6000	52.60±2.05	43.44±1.83	15	789±4.73	651.6±3.28	1.210	61.36	1.01
Q sepharose	15.98±1.16	0.537±0.33	12	191.757±3.19	6.45±0.97	29.729	14.911	24.836
Sephacryl S200-HR	11.755±0.88	0.2218±0.10	10	117.55±2.48	2.218±0.21	52.99	9.1407	44.269
Phenyl sepharose CL-4B	2.35±0.24	0.026±0.03	3	7.05±0.04	0.078±0.03	90.384	0.548	75.508

S.H.: Standard Error. Mean: Each data is average of three repeated experiment.

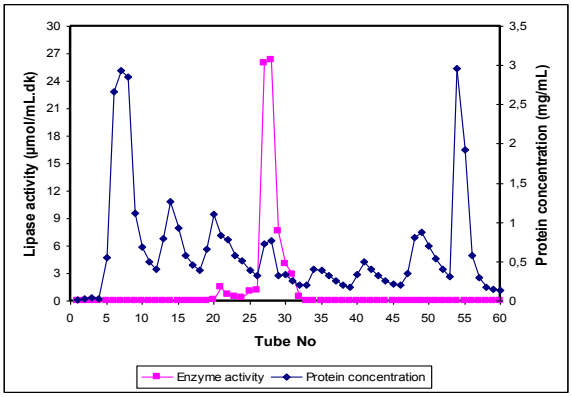

Figure 1. Q sepharose anion exchange chromatography of liver lipase. Elutions were collected as fractions of 3 ml and enzyme activity and protein concentration were expressed as "µmol/mL.dk" and "mg/ml", respectively.

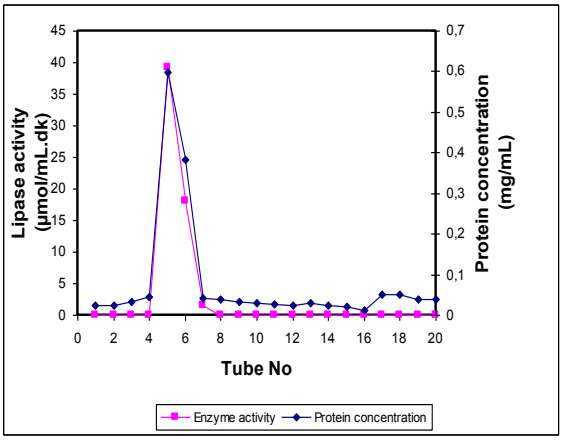

Figure 2. Sephacryl S 200-HR gel filtration chromatography of liver lipase. Elutions were collected as fractions of 2 ml and enzyme activity and protein concentration were expressed as "µmol/ml.dk" and "mg/ml", respectively.

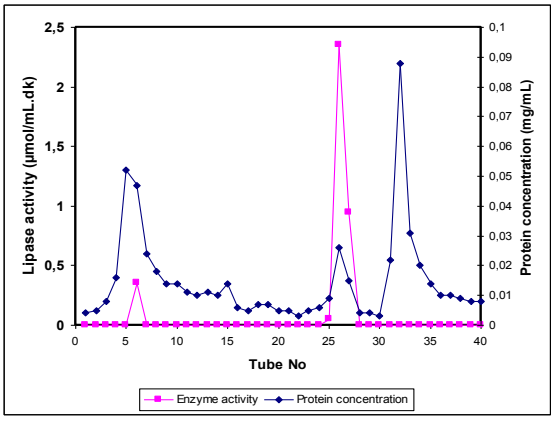

Figure 3. Phenyl sepharose CL-4B hydrophobic interaction chromatography of liver lipase. Elutions were collected as fractions of 3 ml and enzyme activity and protein concentration were expressed as "µmol/ml.dk" and "mg/ml", respectively.

contaminant proteins (Figure 4). After concentrating the fraction having lipase activity, this fraction was applied onto the sephacryl S 200 HR gel filtration chromatography. With this step, only one protein fraction having the lipase activity was defined, corresponding only one protein peak (Figure 2). In the result of this chromatographic approach, a 52.99 µmol/dk.mg of specific activity and 44.26-fold purity was obtained in terms of purification parameters. In the last chromatographic step in which phenyl sepharose CL-4B was used, only a small lipase activity was observed by the eluting with buffer D

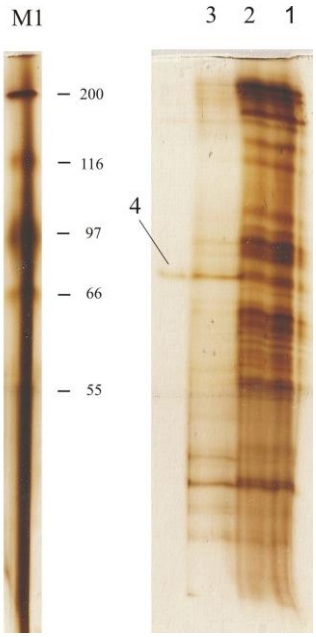

Figure 4: Analysis of purified liver lipase (SDS-PAGE). M1, molecular mass marker (Sigma); 1: Liver homogenate of *C. carpio*, 2: PEG-6000 precipitate of the liver homogenate of *C. carpio*; 3: solution obtained after Q sepharose chromatography; 4: purified liver lipase of *C. carpio* after the last step Phenyl sepharose CL-4B chromatography.

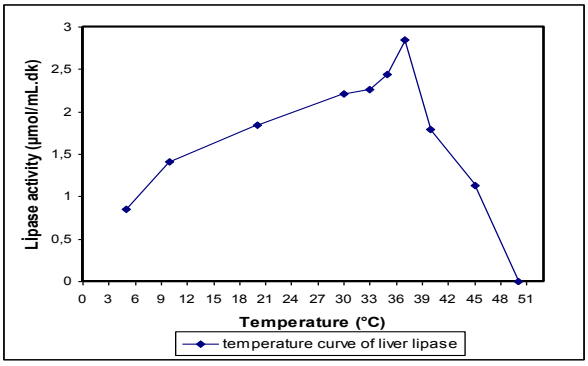

Figure 5. Effect of temperature on liver lipase activity of C. carpio.

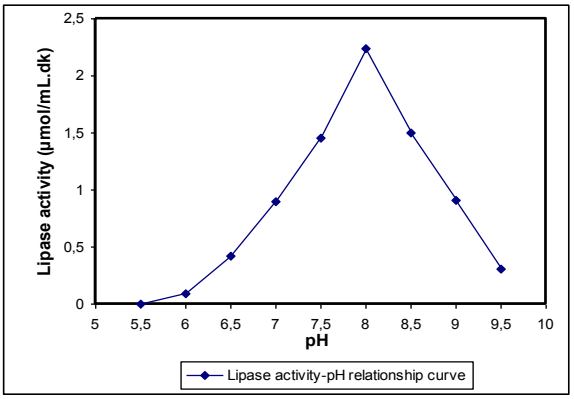

Figure 6. Effect of pH on liver lipase activity of C. carpio.

used in this chromatographic step (Figure 3). We could not detect any lipase activity in the eluting with 10 and 20% (v/v) of isopropanol. However, in eluting with 50% (v/v) of isopropanol, a major lipase fraction was obtained and when checked with SDS-PAGE using silver staining, there was only one activity band, corresponding approximately 74 kDa molecular weight. Purification parameters of this step were calculated as 90.38 µmol/dk.mg of specific activity and 75.50-fold purity.

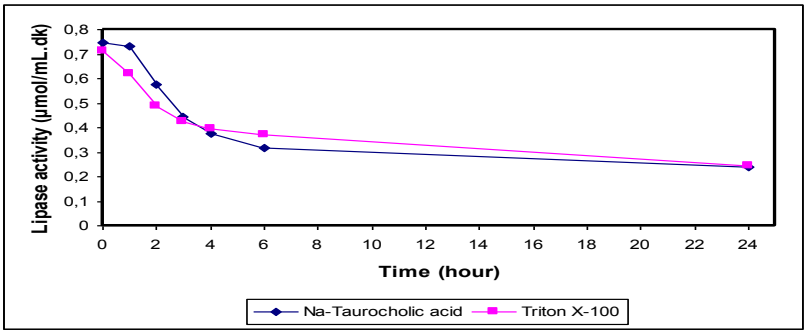

Figure 7. Effect of Na-taurocholicacid and Triton X-100 on liver lipase activity of *C. carpio.*

The effect of temperature on liver lipase activity was measured over a range from 5 to 50°C. Together with the increase in temperature, lipase activity also showed increases. The highest lipase activities were determined between 30 and 37°C and the temperature optimum was defined as 37°C. it was observed that there was decrease when increased the temperature to 40°C and in our study it could not be detected any lipase activity at 50°C (Figure 5).

When assessed the effect of pH on liver lipase activity, liver lipase showed a high activity at the pH values between 7.0 and 8.5. In general, the lowest lipase activities were obtained at acidic pH values, specifically at pH 6.0. The pH optimum for liver lipase was to be found 8.0 (Figure 6).

The effect of substrate amount was assessed using p-NPB. In the p-NPB applications ranging from 0.16 to 2.00 mM, the lipase activity showed linear increases until 1.33 mM p-NPB application. However, between 1.33 to 2.00 mM p-NPB applications, the enzyme exhibited a steady-state kinetic. From all these data, kinetic values for liver lipase were obtained using the Michaelis-Menten model. Catalytic properties of liver lipase were found as K_m=0.17 mM p-NPB and V_{max}= 2.6 µmol/ml.dk

The effects of 0.5 % SDS, Triton X-100 and Na-taurocholic acid were determined in terms of liver lipase. All these chemical agents were the potential inhibitor of liver lipase in the dosage investigated. Na-taurocholic acid and Triton X-100 exhibited similar pattern, decreasing the lipase activity at the end of two hours. However, inhibitor effect of SDS was much more obvious and SDS-treated liver lipase completely lost its activity within 1 hour.

Discussion

In this study, we purified an enzyme showing lipase activity from *C. carpio* liver and this is the first biochemical report from the lake Tödürge in Türkiye.

Lipases obtained from different sources are usually subjected to certain pre-purification steps before they are purified further (Palekar *et al.*, 2000). In our study, 30% (w/v) PEG-6000 was used as a pre-purification step and this step did not provide any purification parameter but it was important in terms of the obtaining nearly all the lipase activity in precipitate. Only two studies used PEG in the pre-purification step of the purification of lipase from fish (Kurtovic *et al.*, 2010) and insect fat body (Arrese and Wells, 1994). These studies also could not record any purification with PEG precipitation of proteins in homogenate.

It is indicated that in general, lipases from higher plant and animal sources require a large number of chromatographic steps in order to obtain the same level of purification as those obtained from microbial sources (Palekar *et al.*, 2000). In our study we used three-step purification in order to purify the liver lipase from fish. In the first chromatography, Q sepharose showed that proteins bound to column were eluted as eight major protein peaks and only the elution of 300 mM NaCl showed lipase activity. From the data presented Table 1, this chromatographic step resulted in approximately 25-fold purification together with specific activity of 29.72 µmol/dk.mg. Iijima *et al.* (1998) also used Q sepharose as a first chromatographic step in order to purify from the hepatopancreas of red sea bream and they obtained only 3.8-fold purity together with a specific activity of 0.85 µmol/dk.mg. Amara *et al.* (2010) purified a lipase from snail hepatopancreas, using DEAE cellulose anion exchange chromatography in the first step and in this study, 2.4-fold purity with 3.5 U/mg specific activity was obtained.

A few study using gel filtration column reported very high purification parameters. One of them used Bio-gel A 0.5 M in order to purify hepatic triacylglycerol lipase from rainbow trout, *Oncorhynchus mykiss* and this study reported 27.000-fold purity (Harmon *et al.*, 1991). Rivera-Perez *et al.* (2010) used superdex 200 column as a first chromatographic step to purify a digestive lipase from *P. vannamei* and obtained 17.46-fold purity and 1252 U/mg specific activity. Smichi *et al.* (2010) used sephacryl S 200 column in order to purify a digestive lipase from *S. aurita* and 10.5-fold purity together with 20 U/mg specific activity was obtained. Sephacryl S 200 gel filtration chromatography used in our study resulted in different purification parameters when compared these studies (Table 1).

It seems that purification of lipase using

hydrophobic matrix works well in microbial sources. Diogo *et al.* (1999) studied comprehensively hydrophobic interaction chromatography of *Chromobacterium viscosum* lipase on polypropylene glycol immobilized on sepharose and they found that 20 % (w/v) ammonium sulphate in phosphate buffer had the total retention of lipase on column. In the preliminary studies carried out in our laboratory, we could not obtain the lipase from the column by using ammonium sulphate fractionation (decreasing salt series from starting 1 M of ammonium sulphate) and ethylene glycol elution (increasing series of ethylene glycol until 100% (v/v) in the buffer, the data have not seen in here). A recent study has been reported that isopropanol also can be used safely in eluting lipase activity from column (Ferrer *et al.*, 2000) and we modified this technique for fish lipase as described in material method section and in our study, lipase was eluted with 50% (v/v) isopraponal after fractionation 10 and 20 (v/v) % of isopropanol. With this step, liver lipase from *C. carpio* was purified 75-fold purity having 90.38 µmol/dk.mg specific activity. From all these results, we may suggest that purification parameters obtained may show differences depending on which chromatographic techniques are conducted.

In our study, liver lipase showed its optimum activity at 37°C. Optimum temperatures were found to be 15°C for *O. mykiss* liver lipase (Harmon *et al.*, 1991), 25-35°C for *Gadus morhua* digestive lipase (Gjellesvik *et al.*, 1992), 35°C for *M. novaezelandiae* (Kurtovic *et al.*, 2010), 35°C for *C. reba* dorsal part lipase (Islam *et al.*, 2009), 37°C for *S. aurita* digestive lipase (Smichi *et al.*, 2010), 60°C for crab digestive lipase (Cherif *et al.*, 2007). During optimum pH studies we could not obtain lipase activities in acidic pH values until pH 6.0. The optimum pH value was determined as 8.0 for *C. carpio* liver lipase in present study. Many studies found to be the optimum pH values were between the pH values of 7.0-9.0 in fish species (Harmon *et al.*, 1991; Iijima *et al.*, 1998; Aryee *et al.*, 2007; Noriega-Rodriguez *et al.*, 2009; Smichi *et al.*, 2010). Islam *et al.* (2010) found that the optimum pH value of dorsal part lipase purified from *C. reba* was 5.5.

Using different substrates, it appears that lipases purified different tissues of fish have been exhibited different K_m and V_{max} values. We defined the K_m and V_{max} values for *C. carpio* liver lipase as 0.17 mM and 2.6 µmol/ml.dk using p-NPB, respectively. Harmon *et al.* (1991) found the K_m and V_{max} values as 0.28 mM and 0.016 nmol/h/mg, using tributyrin for *O. mykiss* liver lipase. Another study focused on liver lipase of *O. mykiss* determined K_m and V_{max} values as 0.12 mM and 0.40 U/mg, respectively, using p-nitrophenyl acetate (p-NPA) as substrate (Metin and Akpınar, 2000). Aryee *et al.* (2007) indicated that medium and long chain p-nitrophenyl substrates was useful substrates for the lipase purified from the viscera of *M. cephalus* and using p-nitrophenyl palmitate (p-NPP) as a substrate they found to be 0.22 mM and 20 $\mu min^{-1}mg^{-1}$ of K_m and V_{max}, respectively.

In limited number of the studies assessing the effects of the surface active materials, it has been indicated that these agents may increase the activity of lipases as well. For example, the effect of 0.01 % of SDS on the lipase of *Penicillium aurantiogriserum* at 28°C and 1 hour incubation was indifferent. In the same study, in Triton X-100 application under these conditions, it was found that the lipase lost the half of its activity (Lima *et al.*, 2004). However, Mogensen *et al.* (2005) found that low concentrations of ionic, non-ionic and zwitter ionic detergents increased the activity of lipase purified from *Thermomyces lanuginosus*, while decreased higher concentrations of detergents. In our study, we found that SDS was a strong and potent inhibitor of the liver lipase. Triton X-100 and Na-taurocholate showed similar effects and in general, these two chemical decreased the activity at the end of two hours incubation periods. From this point of view, the effects of the surface active materials appear to be dosage dependent and show differences in the lipases purified from different sources.

Mukundan *et al.* (1985) indicated that the lipase they purified from the hepatopancreas of *Sardinella longiceps* was a glycosylated enzyme having a molecular weight of 54-57 kDa. In this study, we determined the molecular weight of the liver lipase of *C. carpio* as 74 kDa. Other studies found the molecular weight of lipase as 40-43 kDa for *O. mykiss* liver lipase (Harmon *et al.*, 1991), 64 kDa for *P. major* hepatopancreas lipase (Iijima *et al.*, 1998), 84 kDa for *C. reba* dorsal part lipase (Islam *et al.*, 2009), 43 kDa for *S.aurita* digestive lipase (Smichi *et al.*, 2010). As can be seen from these results, we may conclude that lipases have different molecular weight depending on the tissue and the fish species under investigation.

With this study, we purified and characterized a lipolytic enzyme having lipase activity from the liver of *C. carpio* living in the Lake Tödürge. As indicated in the text, the research has been focused on the lipases of digestive systems of aquatic organisms. In here, we have reported the purification of an intracellular lipolytic enzyme with its basic kinetic features.

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