Lipolytic activity of viscera extract from three freshwater fish species in Phatthalung, Thailand: Comparative studies and potential use as dishwashing detergent additive

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ABSTRACT

Lipolytic activities of viscera extract from three freshwater fish species including hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*), snakehead (*Channa stiata*) and Nile tilapia (*Oreochromis niloticus*) were studied. Optimal activity from all species was at pH 7.0–8.5 and 40–45 °C. Among all species tested, Nile tilapia showed the highest activity, followed by snakehead and hybrid catfish. The enzymes were stable to heat treatment up to 40 °C and over a pH range of 7.0–10.0 for 30 min. Lipases from the viscera of three fish species were enhanced in the presence of NaN_3. However, the lipase activities from all species were inhibited by $MgCl_2$, $MnCl_2$, $HgCl_2$, $AlCl_3$, $CuCl_2$, EDTA, EDAC, DEPC, PMSF, CTAB, sodium percarbonate and sodium polyacrylate. The Nile tilapia lipase was more stable against surfactants than both snakehead and hybrid catfish lipases. The effect of extractants on lipase recovery from Nile tilapia viscera was studied and its stability in the presence of different commercial dishwashing detergents was also investigated. Nile tilapia viscera powder isolation with 50 mM Tris-HCl, pH 7.0 containing 1.0 M NaCl and 0.2% (v/v) Brij 35 gave a higher lipase recovery than other extractants tested ($p < 0.05$). The Nile tilapia lipase exhibited substantial stability and compatibility with tested commercial dishwashing detergents for 15–30 min. Considering its characteristics, Nile tilapia crude enzyme containing lipase activity may be considered a potential candidate for future application in detergent processing industries.

1. Introduction

The growing consumer demand for healthy fish products has led to a thriving fish processing industry worldwide. Processing of fish includes: scaling, cutting, filleting, cooking, salting and canning. When fish fillets or cans are produced, a large fraction (30–80%) of fish (flesh, heads, bones, fins, skin, tails and viscera) are left to waste. Fish waste is usually disposed of in landfills or in the sea resulting in environmental problems which underscore the need for a proper utilization of fish wastes for the recovery of valuable products. Fish waste can be utilized as ingredients in animal feed and fertilizer (Zhao et al., 2011) or used for the recovery of valuable biomolecules, especially enzymes such as proteinase (Klomklao et al., 2004) and lipase (Kuepethkaew et al., 2017).

In Thale Noi, Phatthalung province, Thailand, the freshwater processing industries, especially fermentation and drying, are becoming increasingly important because they are one of the main income

generators. Three freshwater fish species commercially used in the processing industries include hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*), snakehead (*Channa stiata*) and Nile tilapia (*Oreochromis niloticus*). Fish viscera, byproducts from processing industries, are generated. However, they are also a potential source of lipases. Fish lipases exhibit some characteristics and properties that complement those of lipases from mammalian and microbial sources due to the evolutionary pathways, diets and habitats of fish (Kurtovic et al., 2009). In general, fish lipases have cold-adapted properties, which are known to show more catalytic activity and stability than mammalian lipases, thereby making them suitable for applications in food processing at low temperatures (Sae-leaw and Benjakul, 2018).

Lipases (EC 3.1.1.3) belong to a class of hydrolyzes and water soluble enzyme(s) which catalyzes the hydrolysis of insoluble triacylglycerols to generate free fatty acids, diacylglycerols, monoacylglycerols and glycerol. Lipases catalyze a variety of reactions including hydrolysis, transesterification and interesterification of other esters and the

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ester synthesis, and exhibit a range of regio-, enantio- and stereo-selective transformation properties (Geoffry and Achur, 2018). Lipases constitute the most important group of biocatalysts for biotechnological applications (Benjamin and Pandey, 1998). Based on their versatility of being activated only when adsorbed on to an oil–water interface, lipases have emerged as a stupendous enzyme to catalyze dynamic reactions with promising uses in organic chemical processing, synthesis of biosurfactants, the oleochemical industry, dairy industry, agrochemical industry, paper manufacturing, nutrition, cosmetics and pharmaceutical processing (Geoffry and Achur, 2018) as well as synthesis of biodiesel (Kimtun et al., 2017). Lipases are also added to household and industrial laundry detergents and household dishwashers, where their function is in the removal of fatty residues and cleaning clogged drains. The cleaning power of lipase detergents had increased markedly (Hasan et al., 2010). Recently, lipase from the hepatopancreas of Pacific white shrimp were used as detergent and its efficacy was comparable to commercial laundry detergents (Sae-leaw and Benjakul, 2018).

Compared with other hydrolytic enzymes (e.g. proteases and carbohydrases), lipases from fish are relatively less studied. Lipases from aquatic animals, especially freshwater fish, are even less well known versus their counterparts from mammalian, plant and microbial sources (Kuepethkaew et al., 2017). This study aimed to characterize the biochemical properties of lipase from the viscera of three freshwater fish species, including hybrid catfish, snakehead and Nile tilapia commonly consumed and used for processing in Thale Noi, Phatthalung province, Thailand. Their compatibility with various commercial dishwashing detergents was also investigated.

2. Materials and methods

2.1. Chemicals

Acetone, tris (hydroxymethyl) aminomethane, Brij35, Triton X-100 and gum arabic were acquired from Merck (Darmstadt, Germany). Bovine serum albumin (BSA) and p-nitrophenylpalmitate were purchased from Sigma Chemical Co. (St. Loius, MO, USA). Sodium dodecyl sulfate (SDS) was obtained from Fluka (Buchs, Switzerland). The surfactants and other chemicals used were of analytical grade and were procured from Merck (Darmstadt, Germany).

2.2. Preparation and isolation of viscera extract from three freshwater fish

Whole viscera from three species of freshwater fish including hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*), snakehead (*Channa stiata*) and Nile tilapia (*Oreochromis niloticus*) were collected from a local market in Thale Noi, Phatthalung, Thailand. Samples were placed in polyethylene bags and imbedded in a polystyrene box containing ice with an ice/sample ratio of 2:1 (w/w) and transported to our laboratory at Thaksin University, Phatthalung campus within 30 min. Thereafter, the samples were cut and homogenized into powder in three volumes of acetone at −20 °C for 30 min according to the method of Kuepethkaew et al. (2017). The homogenate was filtered in vacuo on Whatman No.4 filter paper. The residue obtained was then homogenized in two volumes of acetone at −20 °C for 30 min, and then the residue was left at room temperature until dried and free of acetone odor.

To prepare the viscera extract, the viscera powder was suspended in distilled water at a ratio of 1:9 (w/v) and stirred continuously at 4 °C for 30 min. The supernatant was recovered by centrifuging the slurry at $5000 \times g$ at 4 °C for 30 min (Biofuge Stratos Bestell-Nr. 75005289, Germany). The supernatants were collected and referred to as "viscera extract". The viscera extracts from three species of freshwater fish were used for further study (Fig. 1).

2.3. Enzyme assay and protein determination

Lipase activity of viscera extract from each fish was determined spectrophotometrically using p-NPP as substrate according to the method of Kuepethkaew et al. (2017). One volume of 8.0 mM substrate solution in isopropanol was mixed just before use with nine volumes of 50 mM Tris-HCl buffer pH 7.5 containing 0.4% (w/v) Triton X-100 and 0.1% gum arabic. This solution (0.9 mL) was equilibrated at 37 °C and the reaction was started by the addition of 0.1 mL of the enzymatic solution. The variation of optical density at 410 nm was monitored against a blank without enzyme using a Shimadzu ultraviolet (UV)-1700 spectrophotometer (Kyoto, Japan). The amount of liberated p-nitrophenol was determined at 410 nm during the first 5 min of reaction. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 μmol of p-nitrophenol per minute under the assay conditions. Protein concentration was determined by the Biuret method (Robinson and Hodgen, 1940) using BSA as a standard.

2.4. pH and temperature profiles

Lipolytic activity was measured within the pH range of 6.0–10.0. Different buffers were used for different pH conditions: 0.2 M phosphate buffer for pH 6.0–7.5, 50 mM Tris-HCl for pH 7.5–9.0 and 0.1 M glycine-NaOH for pH 9.0–10.0. The activity was assayed at 37 °C for 5 min. For the temperature profile study, the activity was assayed at different temperatures (20, 30, 40, 45, 50, 55, 60, 70 and 80 °C) for 5 min at the optimal pH found for the respective segment.

2.5. Thermal and pH stability

Viscera extract were incubated at various temperatures (0, 10, 20, 30, 40, 50, 60, 70 and 80 °C) for 30 min, followed by cooling in iced water. The residual activities were assayed using p-NPP as a substrate and the activities were reported as the relative activities (%) compared with the initial enzyme activities. The effect of pH on enzyme stability was evaluated by measuring the residual enzyme activities after incubation at various pHs (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) for 30 min at room temperature. The residual activities were assay with p-NPP as previously described.

2.6. Effect of some chemicals

Different chemicals (CaCl₂, MgCl₂, MnCl₂, HgCl₂, AlCl₃, CuCl₂, NaN₃, ethyldiaminetetraacetic acid (EDTA), phenylmethanesulfonyl fluoride (PMSF), 1-(3-Dimethylaminopropyl)-3-ethlcarbodiimide) (EDAC), diethyl pyrocarbonate (DEPC), sodium percarbonate and sodium polyacrylate) were mixed with the enzyme solution to obtain the final concentration of 1 mM (Kuepethkaew et al., 2017). The control contained the enzyme without the tested chemical. The mixtures were kept at room temperature for 30 min and the remaining activity was determined using p-NPP as a substrate.

2.7. Effect of some surfactants

Different surfactants (Tween 20, Tween 80, Triton X-100, gum arabic, sodium dodecyl sulfate (SDS), and cetyltrimethylammonium bromide (CTAP)) were added to the viscera extract to obtain a final concentration of 1 mM. The lipase activity was assayed after incubation for 30 min at room temperature. The residual activity was determined and reported as the relative activity compared with the original activity.

Fish viscera yielding the highest lipase activity were selected for further study.

2.8. Effect of extraction media on the recovery of lipase from the viscera of selected freshwater fish

2.8.1. Effect of extractants

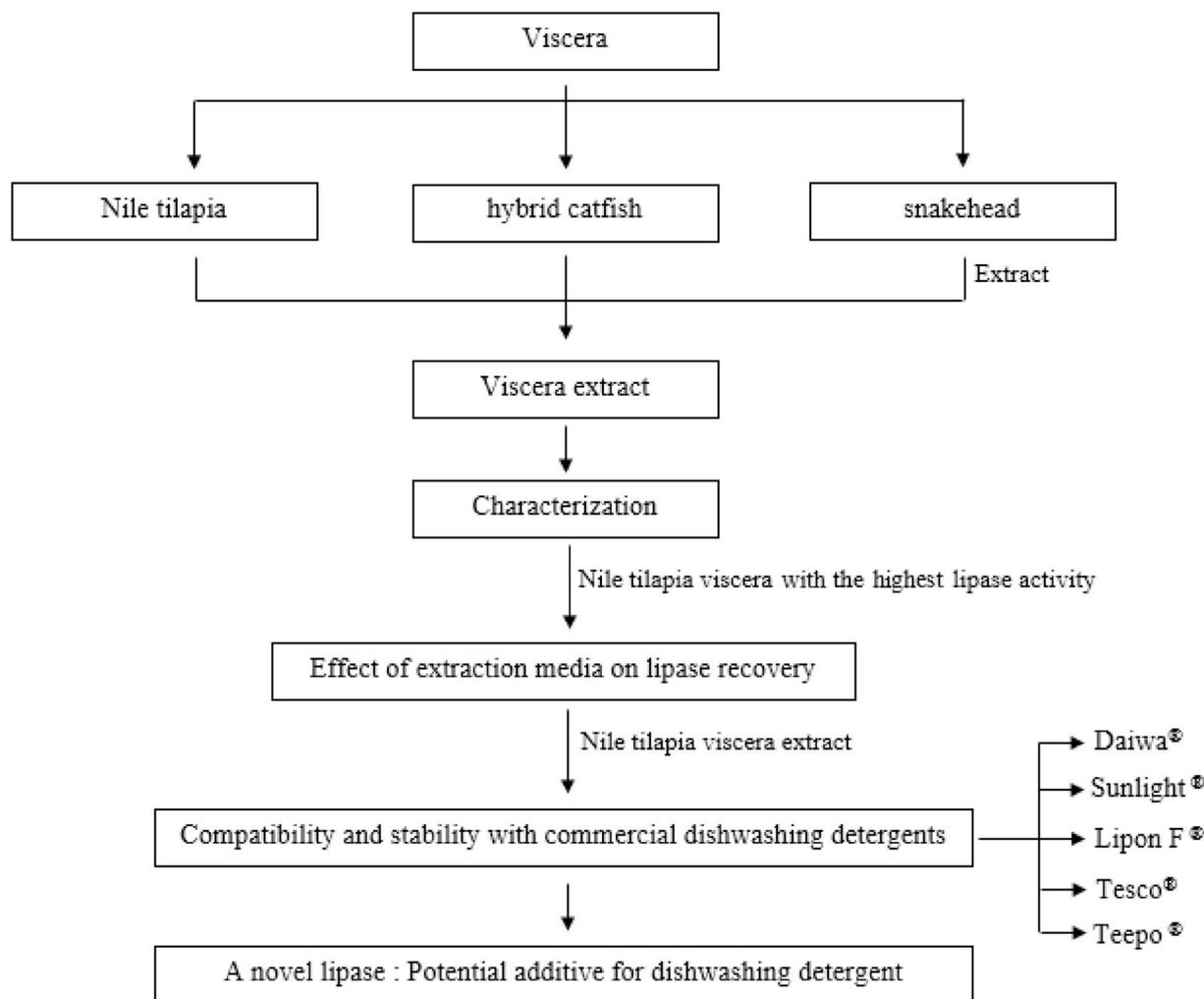Different extraction media, (distilled water, 50 mM sodium

Fig. 1. Outline of the process for fish lipase extraction and characterization.

phosphate buffer, pH 7.0, and 50 mM Tris-HCl, pH 7.0) were used to extract lipase from the viscera of selected freshwater fish. The medium was added into the defatted viscera powder at a ratio of 1:9 (w/v) and stirred continuously at 4 °C for 30 min. The supernatant was recovered by centrifuging the slurry at $5000 \times g$ at 4 °C for 30 min. The lipase activity and protein content in the extracts were determined, and the yield and specific activity of the extracts obtained using different media were compared. The extraction media used for solubilizing the lipase, which was able to extract lipase with the highest yield, was selected for further steps.

2.8.2. Effect of NaCl concentration

Defatted viscera powder was suspended in 50 mM Tris-HCl, pH 7.0 containing different NaCl concentration (0, 0.25, 0.5, 0.75, 1, 1.25 and 1.50 M) at a ratio of 1:9 (w/v) and stirred continuously at 4 °C for 30 min. The supernatant was recovered by centrifuging the slurry at $5000 \times g$ at 4 °C for 30 min. The lipase activity and protein content in the extracts were measured. The extraction yield and specific activity of the extracts were calculated. The extractant showing the highest yield was chosen for further steps.

2.8.3. Effect of surfactant

Defatted viscera powder was suspended in 50 mM Tris-HCl, pH 7.0 containing 1 M NaCl and 0.2% (v/v) different surfactants (Brij35, Tween20, Tween80, Triton X-100, and SDS) at a ratio of 1:9 (w/v) and stirred continuously at 4 °C for 30 min. The supernatant was recovered by centrifuging the slurry at $5000 \times g$ at 4 °C for 30 min. The lipase

activity and protein content in the viscera extracts were measured. The extraction yield and specific activity of the extracts were calculated. The extractant rendering the highest yield was chosen for preparation of crude lipase extract.

2.9. Compatibility and stability with commercial dishwashing detergents

The compatibility and stability with commercial dishwashing detergents of the samples were performed according to the method of Kuepethkaew et al. (2017) with a slight modification. The detergents tested were Daiwa® (Standard Manufacturing, Thailand), Sunlight® (Uniliver, Thailand), Lipon F® (Lion, Thailand), Tesco® (Mit Mongkol Industry, Thailand) and Teepo® (Sherwood Corporation, Thailand) (Fig. 1). The lipases contained in these detergents were inactivated by heating the diluted detergents for 1 h at 65 °C prior to addition of the enzyme preparation. The viscera extract was incubated with different detergents for 15 and 30 min at room temperature, and then the remaining activities were determined under the standard assay conditions. The enzyme activity of the control without dishwashing detergent, incubated under the similar conditions, was taken as 100%.

2.10. Statistical analysis

A completely randomized design was used throughout this study. Data were subjected to analysis of variance and mean comparison was carried out using Duncan's multiple range test (Steel and Torrie, 1980). For pair comparison, T-test was used. Statistical analysis was performed
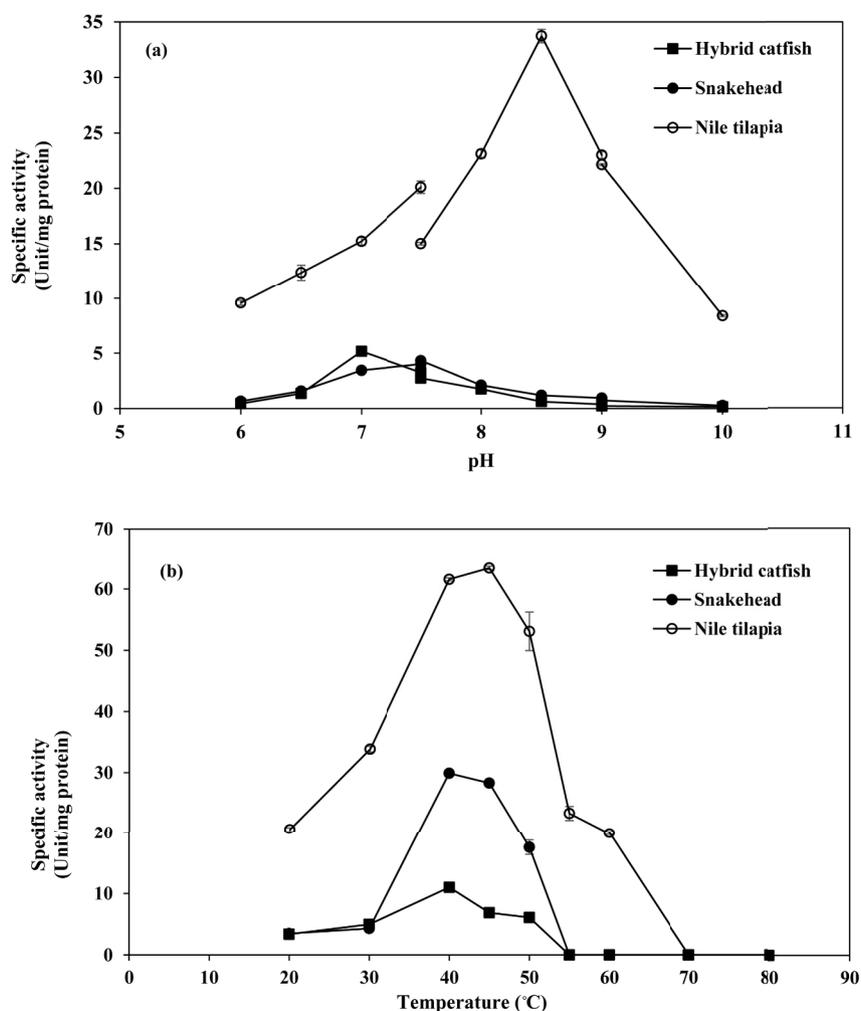
Fig. 2. pH (a) and temperature (b) profiles of lipases from the viscera of different freshwater fish species. Bars represent the standard deviation from triplicate determinations.

using a SPSS package (SPSS 11.5 for windows, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. pH and temperature profiles

Lipase activities of viscera extract from three freshwater fish species were investigated at different pH values (6.0–10.0). Results revealed that the lipase activity had an optimal pH of 7, 7.5 and 8.5 for hybrid catfish, snakehead and Nile tilapia, respectively (Fig. 2a). These activities from all fish were reduced drastically above their optimal pH. A change in pH affects both the substrate and enzyme by changing the charge distribution and conformation of the molecules (Klomklao et al., 2006). The optimum pH value for the three fish species lipase activity was close to those described for other fish species such as grey mullet viscera (*Mugil cephralus*; pH 8.0) (Aryee et al., 2007), Pacific white shrimp hepatopancreas (*Litopenaeus vannamei*; pH 8.5) (Kuepethkaew et al., 2017) and neon flying squid hepatopancreas (*Ommastrephes bartramii*; pH 7.0) (Takahashi et al., 1996).

The temperature profiles of viscera extract from all fish are displayed in Fig. 2b. The lipase activities were measured at temperatures ranging from 20 to 80 °C using *p*-NPP as substrate at their optimum pHs. Results showed that the maximum activities of lipase for the hybrid catfish, snakehead and Nile tilapia were found at temperatures of 40, 40 and 45 °C, respectively. These results were similar to those reported for

crayfish (40 °C) (López-López et al., 2003), and whiteleg shrimp (30–40 °C) (Rivera-Pérez et al., 2011), which were slightly lower compared to marine snail (50 °C) (Zarai et al., 2012) and grey mullet (50 °C) (Aryee et al., 2007). However, these results were different from those reported for lipase from neon flying squid and cod (25 °C), in which *p*-nitrophenyl laurate and *p*-nitrophenyl myristate were used as substrate, respectively (Aryee et al., 2007; Takahashi et al., 1996). The difference in temperature optima might be due to several factors including the varying mechanical properties of the homologous lipases and the different substrate used for measurements, since different substrates do exhibit temperature activity differences with enzymes (Kuepethkaew et al., 2017).

3.2. Lipase activity in viscera from different fish species

Viscera lipase activities of all fish species tested are displayed in Table 1. Under their optimal condition, viscera extract of Nile tilapia showed the highest specific activity when *p*-NPP was used as a substrate, followed by those of snakehead and hybrid catfish, respectively. It is speculated that differences in the level of viscera lipase activity among fish species might be related to growth and physiological change in fish such as metabolism, season, habitat temperature and depth, spawning, age, size and diet (Klomklao et al., 2004).

Table 1
Activity of viscera lipases from different freshwater fish species*.

Fish species	Total activity (x10³ Unit)	Total protein (mg)	Specific activity (Unit/mg protein)	Lipase activity (x10³ Unit/g tissue)***
Hybrid catfish	2.72 ± 0.14[a]	246.99 ± 3.50[a]	11.01 ± 0.58[a]	5.43 ± 0.29[a**]
Snakehead	12.87 ± 0.11[b]	430.24 ± 3.43[b]	29.91 ± 0.25[b]	25.81 ± 0.12[b]
Nile tilapia	32.21 ± 0.15[c]	507.54 ± 1.61[c]	63.46 ± 0.28[c]	64.42 ± 0.29[c]

* Activity was analyzed using *p*-NPP as a substrate under their optimal condition.

** The different letters in the same column denote significant differences ($p < 0.05$).

*** Mean ± SD from triplicate determinations.
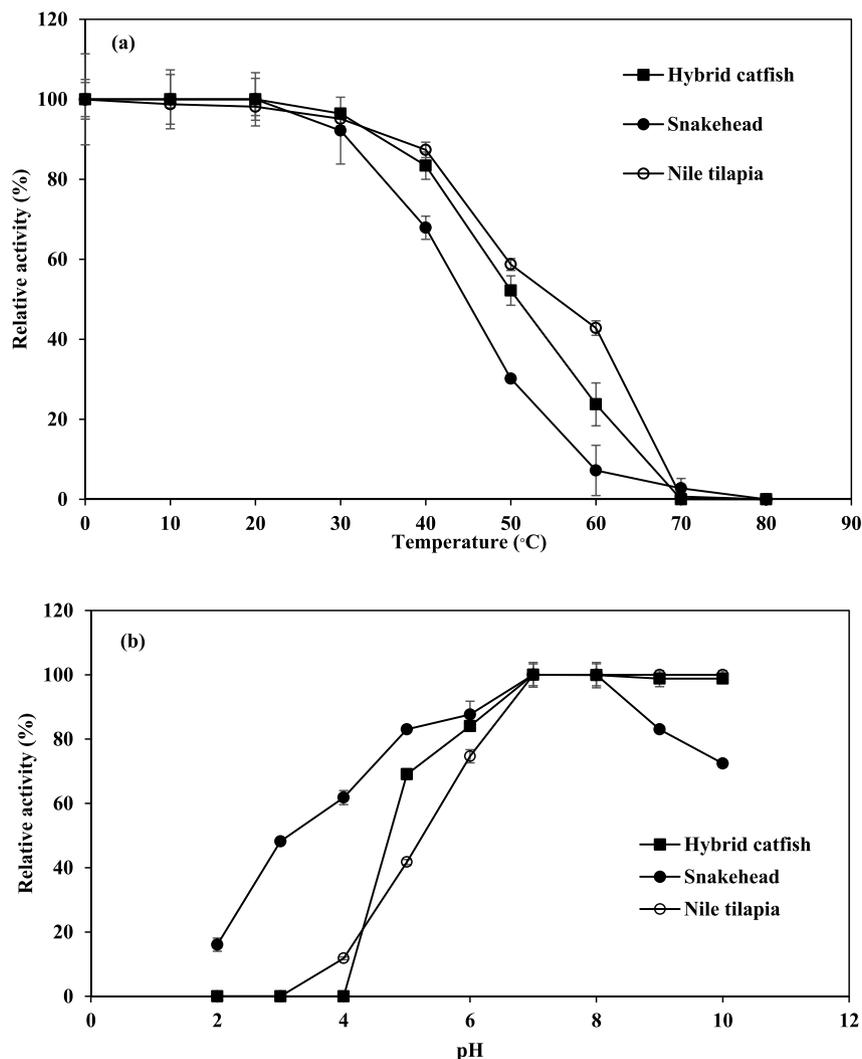
Fig. 3. Thermal (a) and pH (b) stability of lipases from the viscera of different freshwater fish species. Bars represent the standard deviation from triplicate determinations.

3.3. Thermal and pH stability

The thermal stability of lipase from all fish viscera extracts are shown in Fig. 3a. The enzymes were stable when incubated at temperatures up to 40 °C for 30 min. Nevertheless, a sharp decrease in activities was noticeable at temperatures above 40 °C. No activity remained at 80 °C, suggesting complete loss in activity caused by a thermal denaturation of the lipase. Kuepethkaew et al. (2017) reported that lipase from the hepatopancreas of Pacific white shrimp was stable when incubated at temperatures up to 40 °C for 30–120 min. The lipase from grey mullet viscera was active within the temperature range of 20–60 °C (Aryee et al., 2007). For pH stability, both hybrid catfish and Nile tilapia viscera extracts were found to be mostly stable in alkaline pH values ranging from 7.0 to 10.0 for 30 min. However, viscera extract snakehead was stable in the pH range of 7.0–8.0 with an exposure time of 30 min and the lipase stability slightly decreased at a pH value above pH 8.0 (Fig. 3b). At a pH value below 7.0, the stability of the enzyme from all fish species sharply decreased. Aryee et al. (2007) reported that lipase was stable between pH 7.0 and 10.0 during incubation at 25 °C for 30 min. The lipase from the hepatopancreas of Pacific white shrimp was stable in the pH range of 7.0–10.0 with an exposure time of 30–120 min (Kuepethkaew et al., 2017). Both lipase and phospholipase from grey mullet viscera were found to be mostly stable in an alkaline pH value ranging from 7.5 to 9.0 (Smichi et al., 2013). The stability of the enzyme at a particular pH might be related to the net charge of the enzyme at that pH (Klomklao et al., 2006). At extreme pH levels, strong

Table 2

Effect of some chemicals on the activity of lipases from the viscera of different freshwater fish species*.

Substance	Relative activity (%)***		
	Nile tilapia	Snakehead	Hybrid catfish
Control	$100.00 \pm 1.82^{h**}$	100.00 ± 0.98^h	100.00 ± 0.91^j
CaCl$_2$	110.50 ± 2.25^i	60.93 ± 2.03^f	79.24 ± 2.19^i
MgCl$_2$	91.21 ± 2.78^g	77.00 ± 0.78^g	55.78 ± 2.85^f
MnCl$_2$	89.21 ± 1.56^g	62.73 ± 1.04^f	39.62 ± 0.25^e
HgCl$_2$	88.71 ± 0.61^g	30.52 ± 1.35^d	25.84 ± 2.02^c
AlCl$_3$	77.61 ± 2.48^f	60.82 ± 3.90^f	73.99 ± 1.67^h
CuCl$_2$	64.40 ± 1.68^e	9.21 ± 0.69^{ab}	18.95 ± 1.07^b
NaN$_3$	107.34 ± 1.92^i	110.41 ± 2.46^i	109.02 ± 3.47^k
EDTA	87.42 ± 1.76^g	$80.46 + 2.88^g$	69.89 ± 3.08^g
EDAC	29.31 ± 1.94^c	49.88 ± 2.39^e	9.27 ± 1.14^a
DEPC	33.79 ± 0.61^c	11.22 ± 0.95^{ab}	30.19 ± 2.94^d
PMSF	14.63 ± 1.31^a	12.23 ± 0.32^b	15.50 ± 1.13^b
Sodium percarbonate	21.44 ± 1.91^b	7.41 ± 0.90^a	29.53 ± 0.25^d
Sodium polyacrylate	53.88 ± 2.74^d	16.97 ± 1.46^c	28.47 ± 2.33^{cd}

* Activity was analyzed using p-NPP as a substrate under their optimal condition.

** The different letters in the same column denote significant differences ($p < 0.05$).

*** Mean $\pm$ SD from triplicate determinations.

intramolecular electrostatic repulsion caused by high net charge, results in swelling and unfolding of the protein molecules (Klomklao et al., 2010). Since tolerance in the alkaline range of viscera extract from three freshwater fish was observed, this can be considered as a potential candidate for application in processes that are conducted in an alkaline range such as detergent applications (Balaji and Jayaraman, 2014).

3.4. Effect of some chemicals on the lipase activity

The viscera lipases from Nile tilapia, snakehead and hybrid catfish were incubated with various compounds and their relative activities were determined after incubation for 30 min at room temperature (Table 2). Generally, similar results were observed in all fish species. The lipase activities from the three fish species were found to increase slightly in the presence of NaN$_3$, while the lipase retained approximately 107–110% relative activity. Kuepethkaew et al. (2017) reported that NaN$_3$ at concentrations of 1 and 10 mM increased lipase from Pacific white shrimp hepatopancreas by 2 and 17%, respectively. One and 10 mM NaN$_3$ activated grey mullet by 23 and 30%, respectively (Aryee et al., 2007).

Various metal ions were also studied for their effects on the lipase activities from the viscera of three freshwater fish species. Treatment with Ca^{2+} at 1 mM inhibited the activity of viscera lipases from both snakehead and hybrid catfish. Aryee et al. (2007) found that 1 and 10 mM Ca^{2+} decreased grey mullet digestive lipase by 4% and 7%, respectively. However, the viscera lipase from Nile tilapia was slightly enhanced in the presence of Ca^{2+} (110% relative activity), which was in agreement with results obtained from the lipase from halotolerant *Bacillus* sp. VITL8 (Balaji and Jayaraman, 2014). Calcium ion has been reported to form complexes with ionized fatty acids, changing their solubility and behaviors at interfaces (Gulomova et al., 1996). Also, the calcium-induced increase on lipase activity could be attributed to the complex action of calcium ion on the released fatty acids. Moreover, the enzyme structure was stabilized because of the binding of calcium ions to the lipase, bridging the active region to a second subdomain of the protein and, hence, stabilizing the enzyme tertiary structure (Kim et al., 2000).

In general, lipases are strongly inhibited by Hg^{2+} (a thiol group inhibitor). This is likely due to the proximity of -SH group to the catalytic and interfacial binding site, but spatially remote from the catalytic site. This may have induced the marked loss of activity (Aryee et al., 2007). The catalytic effect of lipases might be considered to be of

Ser, His, and Glu or Asp residues. Thus, the bulky Hg^{2+} group might cause steric interference to the approach of the substrate to the active site (Islam et al., 2009). From the results, the viscera lipase of all fish species retained approximately 88%, 30% and 25% relative activity for Nile tilapia, snakehead and hybrid catfish, respectively. In the case of Mg^{2+}, Mn^{2+}, Al^{3+} and Cu^{2+}, there was a decrease in lipase activities. Other metal ions slightly inhibited lipase activity, which may be due to the fact that transition metal ions change the conformation of the protein to a less stable form (Joseph et al., 2012). The inhibitory nature of metals has been thought to be due to the interaction of ions with charged side chain groups of surface amino acids, thus influencing the conformation and stability of the enzyme (Rahman et al., 2006).

EDTA at 1 mM had a strong inhibitory effect on the activity of hybrid catfish lipase with only 69% residual activity after incubation at room temperature for 30 min although 1 mM EDTA caused a slight decrease in the activities of viscera lipases from Nile tilapia and snakehead (13–20%). The effect of group specific reagents (DEPC, PMSF, EDAC) on lipases from the viscera of three freshwater fish was also investigated. Strong inhibition of viscera lipases from three fish species by DEPC, PMSF and EDAC was observed. The results indicated the respective involvement of histidine, serine and carboxylate for their catalytic activity (Ramakrishman et al., 2016). Ramakrishman et al. (2016) reported that lipase activity from *Enterococcus faecium* MTCC5695 was inhibited by DEPC, PMSF, EDAC and NBS.

Lipase activities of viscera extracts from all species decreased with the incubation of a bleaching agent (sodium percarbonate) (Table 2). The bleaching agent released hydrogen peroxide, an oxidizing agent, when dissolved into water. This could easily lead to the oxidization of some amino acids and cause the inactivation of enzymes. For sodium polyacrylate, the only dispersing agent tested in this study, the activity of lipases from both snakehead and hybrid catfish were strongly affected by sodium polyacrylate, whereas Nile tilapia lipase showed more than 53% stability compared to the control, similar to the result of Naganthran et al. (2017). From the results, the differences in lipase activities inhibited/activated by some chemicals were found, The results probably implied the differences in binding properties of those chemicals of the lipases in viscera extract of the three freshwater fish species.

3.5. Effect of some surfactants on lipase activity

Surfactants possess the common characteristic of lowering the surface tension when added in small amounts to water, and this could influence enzyme catalysis. (Aryee et al., 2007; Ogino et al., 2000). The effect of surfactants (1.0 mM) on the lipase activity from the viscera of three freshwater fish was investigated after a 30 min duration at room temperature (Table 3). Different surfactants had different influences on lipase activities. For non-ionic surfactants, the viscera lipase from Nile tilapia showed a high stability toward non-ionic surfactants such as Triton X-100, Tween 20 and Tween 80 with the residual activity higher than 83%. However, the viscera lipase from both hybrid catfish and snakehead were strongly inhibited by these non-ionic surfactants. The lipase from *Penicillium aurantiogriseum* was also inactivated in the presence of Triton X-100 (Lima et al., 2004). The lipase from the hepatopancreas of Pacific white shrimp was evaluated as highly stable against surfactants (Tween 20, Tween 80 and Triton X-100) (Kuepethkaew et al., 2017). In this study, gum arabic had no appreciable effect on lipase activity from the viscera of either Nile tilapia or snakehead at the 1% level, although it caused a slight (26%) decrease in activity from hybrid catfish viscera. Peng et al. (2010) reported that 1.2% gum arabic increased the lipase activity of *Pseudomonas aeruginosa* CS-2 with a 130% relative activity. Gum arabic is most commonly used for hydrolysis of triacrylglycerol, giving emulsion which can be stored for several weeks. It has generally been assumed that gum arabic was simply an emulsifying agent stabilizing emulsions without interfering with the lipase assay itself (Kuepethkaew et al., 2017). In addition, after 30 min of incubation of the viscera lipases of Nile tilapia, snakehead and hybrid

Table 3
Effect of surfactants on the activity of lipases from the viscera of different freshwater fish species*.

Surfactants	Relative activity (%)***		
	Nile tilapia	Snakehead	Hybrid catfish
Control	100.00 ± 3.29[d]**	100.00 ± 7.48[c]	100.00 ± 3.91[e]
Tween 20	86.61 ± 2.50[c]	53.55 ± 1.42[b]	40.41 ± 2.36[c]
Tween 80	83.15 ± 1.08[c]	47.32 ± 1.70[b]	29.25 ± 2.04[b]
Triton X-100	86.96 ± 1.28[c]	35.53 ± 2.94[a]	39.06 ± 2.89[c]
Gum arabic	100.29 ± 1.88[d]	100.10 ± 3.11[c]	74.16 ± 2.20[d]
SDS	71.01 ± 2.55[b]	38.82 ± 2.04[a]	29.79 ± 2.03[b]
CTAB	66.57 ± 0.75[a]	39.29 ± 0.97[a]	9.54 ± 1.56[a]

* Activity was analyzed using *p*-NPP as a substrate under their optimal condition.
** The different letters in the same column denote significant differences ($p < 0.05$).
*** Mean ± SD from triplicate determinations.

catfish with 1% SDS as an anionic surfactant, the lipases exhibited 71, 38 and 29% of the initial activity, respectively. Hence, SDS destabilized the viscera lipases of all fish species tested in this study. Ungcharoenwiwat and H-Kittikun (2015) found that 1% SDS inactivated the lipase *Burkholderia* sp. EQ3 activity. The ionic interactions between enzyme and the SDS head group may cause the inactivation of globular protein (Naganthran et al., 2017). The cationic CTAB strongly destabilized lipases of all fish species. Consequently, the viscera lipase of all fish species might have precipitated and lost its functionality. The stability and improvement of enzymes by surfactants therefore vary depending on the enzymes and their characteristics (Rahman et al., 2013). Wang et al. (2009) reported that 1% CTAB slightly inhibited the enzyme activity of *B. cepacia* ATCC 2541.

The goal of the present investigation was to produce lipase, which could be suitable for using in dishwashing detergents. Therefore, it is relevant to test lipase stability in the presence of this additive. Overall, Nile tilapia viscera yielded the highest lipase activity (Table 1) and it was evaluated as highly stable against surfactants, delineating itself as a desirable additive for better detergent formulation. Hence, Nile tilapia viscera was selected for further investigation in lipase recovery and its application in dishwashing detergents.

3.6. Effect of extraction media on the lipase recovery from the viscera of Nile tilapia

3.6.1. Effect of extractants

The effect of extraction media on viscera lipase extraction from Nile tilapia is shown in Table 4. Viscera extract using 50 mM Tris-HCl, pH 7.0 showed a higher lipase activity than those extracted with distilled water and 50 mM sodium phosphate buffer, pH 7.0 when *p*-NPP was used as substrate ($p < 0.05$). The results suggested that Tris-HCl buffer had a greater efficiency to isolate lipase than sodium phosphate and distilled water. Tris-HCl buffer might favor solubilization of lipase associated with the cell membrane by increasing the charge of enzyme and proteins (Klomklao et al., 2010). The repulsion between the

Table 5
Effect of NaCl concentrations on the recovery of lipase from the viscera of Nile tilapia*.

NaCl concentration (M)	Total activity (x10³ Unit)	Total protein (mg)	Specific activity (Unit/mg protein)***
0	35.23 ± 1.44[b]**	531.23 ± 4.23[c]	66.32 ± 2.72[a]
0.25	35.95 ± 1.01[b]	572.01 ± 2.01[d]	62.85 ± 1.76[a]
0.5	36.23 ± 1.27[bc]	574.18 ± 0.77[d]	63.10 ± 2.21[a]
0.75	38.33 ± 1.07[c]	593.78 ± 1.36[e]	64.55 ± 1.81[a]
1	41.89 ± 0.87[d]	591.00 ± 4.20[e]	70.88 ± 1.48[b]
1.25	36.93 ± 1.12[bc]	499.22 ± 2.81[b]	73.98 ± 2.24[b]
1.5	32.67 ± 1.04[a]	448.82 ± 6.21[a]	72.79 ± 2.32[b]

*The defatted Nile tilapia viscera powder was extracted in 50 mM Tris-HCl, pH 7.0 containing different NaCl concentrations at 4 °C for 30 min and hydrolytic activity was analyzed using *p*-NPP as substrate at pH 8.5 and 37 °C.
**The different letters in the same column denote the significant differences ($p < 0.05$).
***Mean ± SD from triplicate determinations.

Table 6
Effect of some surfactants on the recovery of lipase from the viscera of Nile tilapia*.

Surfactants	Total activity (x10³ Unit)	Total protein (mg)	Specific activity (Unit/mg protein)***
Control	41.59 ± 0.60[d]**	593.34 ± 1.56[e]	70.10 ± 1.21[b]
SDS	32.57 ± 0.50[a]	457.02 ± 4.84[a]	71.27 ± 1.11[b]
Tween 80	35.47 ± 0.64[b]	499.92 ± 1.27[b]	70.95 ± 1.28[b]
Triton X-100	37.58 ± 0.86[c]	568.54 ± 11.51[d]	66.10 ± 1.52[a]
Tween 20	38.20 ± 0.79[c]	514.52 ± 5.65[c]	74.24 ± 1.54[c]
Brij 35	42.31 ± 0.54[d]	504.29 ± 4.35[bc]	83.90 ± 1.06[d]

*The defatted Nile tilapia viscera powder was extracted in 50 mM Tris-HCl, pH 7.0 containing 1.0 M NaCl in different surfactants at 4 °C for 30 min and hydrolytic activity was analyzed using *p*-NPP as substrate at pH 8.5 and 37 °C.
**The different letters in the same column denote the significant differences ($p < 0.05$).
***Mean ± SD from triplicate determinations.

enzyme and tissues might lead to the ease of the extraction of lipase from the viscera. Kuepethkaew et al. (2017) reported that 50 mM Tris-HCl buffer, pH 7.0 was the optimum medium to dissolve Pacific white shrimp hepatopancreas lipase. From this result, 50 mM Tris-HCl, pH 7.0 was selected as the extractants for the recovery of Nile tilapia viscera lipase since the viscera extract had the maximum lipase activity.

3.6.2. Effect of NaCl concentration

Tris-HCl buffer containing different NaCl concentrations were used to extract the lipase from Nile tilapia viscera (Table 5). The lipolytic activity apparently increased ($p < 0.05$) when the NaCl concentration was increased from 0 to 1 M ($p < 0.05$). Addition of salt at low ionic strength can enhance solubility of a protein by neutralizing charges on the surface of the protein, reducing the ordered water around the protein and increasing entropy of the system (Poonsin et al., 2016). However, there was no further increase in the lipolytic activity with

Table 4
Effect of extraction media on the recovery of lipase from the viscera of Nile tilapia*.

Extraction media	Total activity (x10³ Unit)	Total protein (mg)	Specific activity (Unit/mg protein)***
Distilled water	30.38 ± 1.09[a]**	488.36 ± 5.07[a]	62.21 ± 2.22[ab]
50 mM Na-Phosohate, pH 7.0	32.60 ± 1.80[a]	565.35 ± 2.92[c]	57.66 ± 3.19[a]
50 mM Tris-HCl, pH 7.0	35.92 ± 1.22[b]	536.79 ± 2.03[b]	66.92 ± 2.28[b]

*The defatted Nile tilapia viscera powder was extracted in different media at 4 °C for 30 min and hydrolytic activity was analyzed using *p*-NPP as substrate at pH 8.5 and 37 °C.
**The different letters in the same column denote the significant differences ($p < 0.05$).
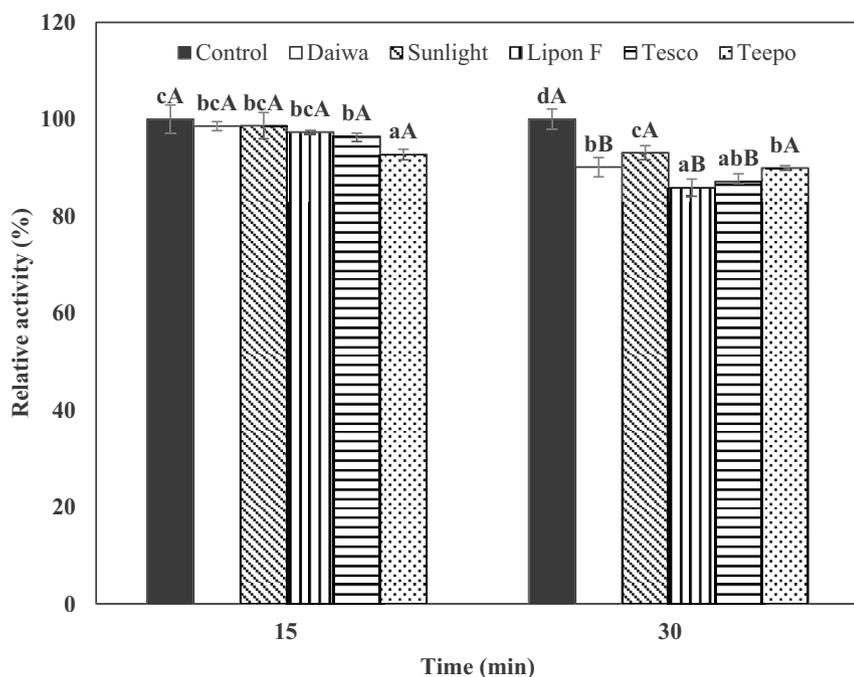***Mean ± SD from triplicate determinations.

Fig. 4. Stability of lipase from the viscera of Nile tilapia in the presence of various commercial dishwashing detergents. Bars represent the standard deviation from triplicate determinations. Different lowercase letters on the bars within the same incubation time indicate significant differences ($p < 0.05$). Different uppercase letters on the bars within the same commercial dishwashing detergents indicate significant differences ($p < 0.05$).

NaCl above 1 M. The decrease in lipase activity might be because of the enzyme denaturation caused by the salting out effect with increasing concentration of NaCl (Klomklao et al., 2004).

3.6.3. Effect of surfactant

Table 6 shows the influence of some surfactants on the recovery of lipase from Nile tilapia viscera. Addition of SDS, Tween 20, Tween 80 and Triton X-100 in 50 mM Tris-HCl, pH 7.0 containing 1 M NaCl mainly decreased the yield. On the other hand, the highest yield or lipase activity and specific activity were obtained when the Nile tilapia viscera powder was extracted with 50 mM Tris-HCl, pH 7.0 containing 1.0 M NaCl and 0.2% (v/v) Brij 35 ($p < 0.05$). The yield of lipase extracted with the aid of Brij 35 was approximately 1.08-fold higher than that of lipase extracted without Brij 35. Poonsin et al. (2016) reported that Brij 35 addition to crude extract from albacore tuna spleen led to a small increase in trypsin activity. The lipase recovery from the hepatopancreas of Pacific white shrimp was strongly enhanced in the presence of Brij 35 (Kuepethkaew et al., 2017). The Brij 35 was added to facilitate improved extraction of soluble cell material and to emulsify the small amount of lipid present in viscera extract to prevent lipid interference with the lipase activity (Klomklao et al., 2010). Therefore, 50 mM Tris-HCl, pH 7.0 containing 1.0 M NaCl and 0.2% (v/v) Brij 35 was selected as the extraction medium for lipase in Nile tilapia viscera.

3.7. Stability of the lipase with commercial dishwashing detergents

Due to the high activity and stability of the viscera lipases from Nile tilapia in an alkaline pH range and their relative activity toward different surfactants, viscera lipases may eventually be used as a detergent additive. Hence, the compatibility of viscera lipase from Nile tilapia with some commercial dishwashing detergents (Daiwa®, Sunlight®, Lipon F®, Tesco® and Teepo®) was studied. The viscera lipase was preincubated in the presence of various commercial dishwashing detergents for 15 and 30 min at room temperature, and the results are summarized in Fig. 4. High lipase resistance for 15 and 30 min was observed because retained activity was above 85%. The viscera lipase retained 98% of its activity in the presence of Daiwa®, Sunlight® and Lipon F® after 15 min incubation at room temperature. At 30 min incubation, the lipase was found more stable in Daiwa® and Sunlight® than those (Lipon F®, Tesco® and Teepo®) of all dishwashing detergents.

However, more than 85% of its initial activity was observed in the presence of all dishwashing detergents after 30 min incubation at room temperature. The lipolytic activity varied with each dishwashing detergent, indicating that the enzyme performance in detergents depends on a number of factors, including the compounds in dishwashing detergents. Hence, the lipase from viscera of Nile tilapia, which is stable and works at alkaline pH with suitable wash conditions for enzymated-detergent powders and liquids, has good potential for use in the dishwashing detergent industry (Hasan et al., 2010), especially for most formulations available in Thailand.

4. Conclusion

Viscera extract from three freshwater fish species contained lipases with optimum pH and temperatures of 7.0–8.5 and 40-45 °C, respectively. Nile tilapia exhibited the highest lipase activity followed by snakehead and hybrid catfish. Lipase from Nile tilapia viscera can be successfully extracted using 50 mM Tris-HCl buffer, pH 7.0 containing 1.0 M NaCl and 0.2% (v/v) Brij 35. The high stability of the lipase from the viscera of Nile tilapia towards surfactants and commercial dishwashing detergents makes it a novel lipase for further commercial utilization as a potential additive in dishwashing detergent formulations.

Conflicts of interest

No conflict of interest is declared.

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