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Original Article

Properties of protease and lipase from whole and individual organ of viscera from three tuna species

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Abstract

Properties of visceral enzymes from yellowfin tuna (*Thunnus albacares*), skipjack tuna (*Katsuwonus pelamis*) and tonggol tuna (*Thunnus albacares*) were studied. The crude enzymes from viscera of yellowfin tuna and skipjack tuna exhibited the highest activities at pH 10.0 whereas it was at pH 9.0 for those from viscera of tonggol tuna. The enzymes were the most stable at their optima pH after 120 min incubation. The optimum temperatures for protease and lipase activities were at 50°C and 60°C, respectively but the extracted enzymes were more stable in the temperature range of 37-40°C. The protease and lipase from spleen were the most thermostable with the half-life of 120 and 90 min at 60°C incubation, respectively. Protease activity from spleen accounted for 45.6% of the total protease activity of the whole tuna viscera.

Keywords: tuna viscera, property, protease, lipase

1. Introduction

Enzymes especially protease from marine sources have received world wide attention with the potential applications due to the diversity of marine sources with different species, habitats and environment. Different types and properties of enzymes from these sources could be achieved and applied to many industries. Examples of these applications were use of chymotrypsin from Atlantic cod (*Gadus morhua*) and pepsin from cod (*Breogadus saida*) in cheese production as a rennet substitute (Brewer *et al*., 1984) and use of trypsin from cod (*Gadus ogac*) for production of different amino acids (Simpson and Haard, 1984).

In our previous studies (Prasertsan and Prachumratana, 2008), tuna viscera from yellowfin tuna (*Thunnus albacares*), skipjack (*Katsuwonus pelamis*), and tonggol tuna (*Thunnus tonggol*) generated during canned tuna processing were accounted for 7.05, 5.44 and 5.18% of the body weight, respectively. In addition, they possessed high enzyme activities. This continued investigation aimed to study the properties of protease and lipase of the crude enzymes from the three tuna species in order to obtain basic data for further exploitation of these enzymes.

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2. Materials and Methods

2.1 Source of enzymes

Whole viscera and individual viscera organ (stomach, liver, pancreas, spleen) of three tuna species : yellowfin tuna (*Thunnus albacares*), skipjack (*Katsuwonus pelamis*), and tonggol tuna (*Thunnus tonggol*) were used as sources of enzymes in this study. They were kindly provided by two seafood processing factories in Songkhla region, Thailand. The viscera were kept frozen at -20°C in sealed plastic bags until needed for enzyme extraction.

2.2 Determination of enzyme activities

Protease activity was measured according to the procedure described by Hagihara *et al.* (1958) in which either casein or hemoglobin were used as substrate and the optical density was measured at the wavelength of 275 nm after incubation for 15 min at 37°C.

Lipase activity was measured by modified method of Winker and Stuckman (1979) using *p*-nitrophenyl palmitate as substrate and the optical density was measured at the wavelength of 410 nm after incubation at 37°C for 15 min.

2.3 Properties of the crude enzymes from viscera of three tuna species

The crude enzymes extracted from the whole viscera and the individual viscera organ of the three tuna species were studied for the effect of pH (pH 2.0-11.0, prepared as above) and incubation temperature (10-70°C) on enzyme activities. For stability test on the enzymes from all sources, effect of the buffer pH (pH 2.0-11.0) and temperature (37-70°C) were studied and samples were taken for determination of residual protease and lipase activities after 0, 30, 60, 90 and 120 min. The activities were reported as relative activities compared with the initial enzyme activities.

3. Results and Discussion

3.1 Properties of the crude enzymes from viscera of three tuna species

The effect of pH in the pH range of 2.0-11.0 on the activity of crude enzymes extracted from viscera of the three tuna species was investigated. Results revealed that the optimum pH for protease (Figure 1) and lipase (Figure 2) activities from all sources of yellowfin tuna and skipjack tuna occurred at pH 10.0 while those of tonggol tuna showed the highest activity at pH 9.0. Enzymes from whole viscera of yellowfin tuna possessed the highest protease and lipase activity of 71.54 and 1.19 U/ml with the specific activities of 3.06 and 0.05 U/mg, respectively. The enzymes activities of skipjack tuna viscera were 60.53 and 0.90 U/ml (2.59 and 0.03 U/mg) whereas those of tonggol tuna viscera were 53.36 and 0.60 U/ml (2.28 and 0.02 U/mg), respectively. The specific protease activities from the whole viscera of these three tuna species (2.28-3.06) were higher than that of the chymotrypsin extracted from caeca or rainbow trout (*Oncorhynchus mykiss*) (0.64 U/mg) (Kristjansson and Nielson, 1991) but lower than that of trypsin from pancreas of crayfish (*Procambarus clarkia*) (Kim *et al.*, 1994). The optimum pH values found in this study were the same as those achieved with the optimum pH of the buffer used for enzyme extraction (Prasertsan *et al.*, 2008). The latter case (optimum at pH 9.0) was the same as that of chymotrypsin from caeca of rainbow trout (*Oncorhynchus mykiss*) (Kristjansson and Nielson, 1991). Both pH optima of this work were higher than that of purified trypsin from pancreas of crayfish (*Procambarus clarkii*) (pH 7.5-8.0) (Kim *et al.*, 1994). The optimum pH was also found to be different depending on the substrate used as in the case of trypsin from caeca and intestine of anchovy (*Engraulis encrasicholus*) which illustrated the optimum pH at 9.5 and 9.0 when casein and BAPNA (benzamidine N-benzoyl-DL-arginine *p*-nitroanilide) were used as the substrates, respectively (Kim *et al.*, 1994).

Unlike the effect of pH on the enzyme activity, the pH stability of the crude enzymes from all sources was determined within a narrow range of pH 9.0-11.0 (pH 9.0, 9.5, 10.0, 10.5 and 11.0). Results demonstrated that the crude enzymes from all sources of both yellowfin tuna (Figure 3-4) and skipjack tuna (Figure 5-6) viscera were most stable at the optimum pH 10.0 and pH 9.0 for enzyme activity from viscera of tonggol tuna (Figure 7-8). Comparison among all sources, the crude enzymes from whole viscera of yellowfin tuna were most stable with the protease residual activities of 96 and 91% and lipase activities of 91 and 89% after incubation for 60 and 120 min, respectively. The pH stability of tuna viscera at pH 9.0-10.0 was similar to that of the serine protease from cod caeca at pH 8.8-9.6 with the optimum pH at 9.6 (Shin and Zall, 1986).

The influence of temperature on enzyme activity was determined at temperature ranging between 10 and 70°C. The optimum temperature for all sources of protease was found to be at 50°C with the highest activity of 90.61 U ml^{-1} (Figure 9) obtained from the viscera of yellowfin tuna. The protease activities decreased to 54 U ml^{-1} at 60°C and sharply declined at 70°C (13 U ml^{-1}). This optimum temperature was lower than those of alkaline protease from four species of freshwater fish and 21 species of marine fish (60-65°C) (Iwata *et al.*, 1974). The dependence of the optimal temperature on pH was illustrated in the case of pancreas of crayfish (*Procambarus clarkii*) whereby the optimum temperature was 60°C at pH 6.8 and 50-60°C at pH 8.1 (Kim *et al.*, 1994). On the other hand, the optimum temperature for all sources of lipase was found to be at 60°C with the highest activity of 1.44 U ml^{-1} (Figure 10) obtained from the viscera of yellowfin tuna. The lipase activity decreased sharply to 0.48 U ml^{-1} at 70°C.

Thermal stability studies at 37-70°C for 120 min indicated that the crude enzymes of yellowfin tuna was most stable at 37°C for both protease (Figure 11-13) and lipase (Figure 14-16). For protease, the residual protease activities were 86.1% and 78.4% after incubation for 60 and 120 min, respectively. At 60°C the corresponding values were 65.0% and 38.5%, respectively. The half-life of protease from crude enzyme was found to be 75 min at 60°C. For lipase, the residual lipase activities were 81.5% and 74.2% after incubation for 60 and 120 min, respectively. At 60°C the corresponding values were 56.2% and 38.0%, respectively. The half-life of lipase of the crude enzyme was found to be 73 min at 60°C. The stability of the crude enzyme at 37°C was similar to those of enzyme from Atlantic cod (*Gadus morhua*) (30-35°C) (Asgeirsson and Bjarnason, 1991) or chymotrypsin from caeca and intestine of rainbow trout (*Oncorhynchus mykiss*) (<40°C) (Kristjansson and Nielson, 1991).

4. Conclusion

Enzymes extracted from viscera of the three tuna

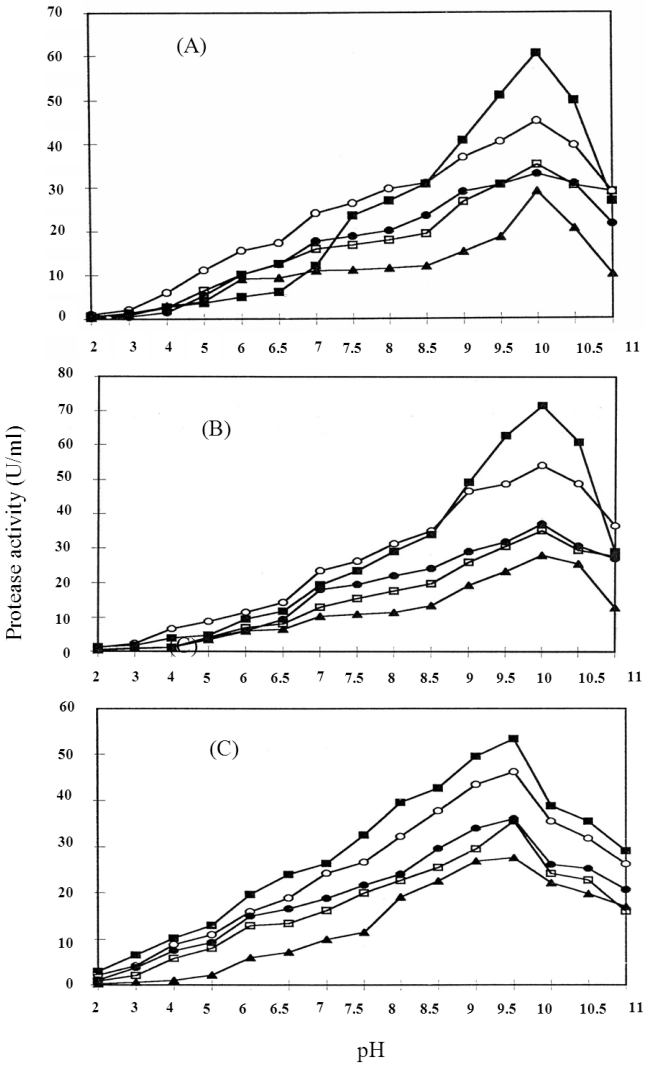

Figure 1. Effect of buffer pH on protease activity of crude enzymes extracted from stomach (▲), spleen (O), liver (●), pancrease (□) and whole viscera (■) of (A) skipjack tuna (*Katsuwonus pelamis*), (B) yellowfin tuna (*Thunnus albacares*) and (C) tonggol tuna (*Thunnus tonggol*).

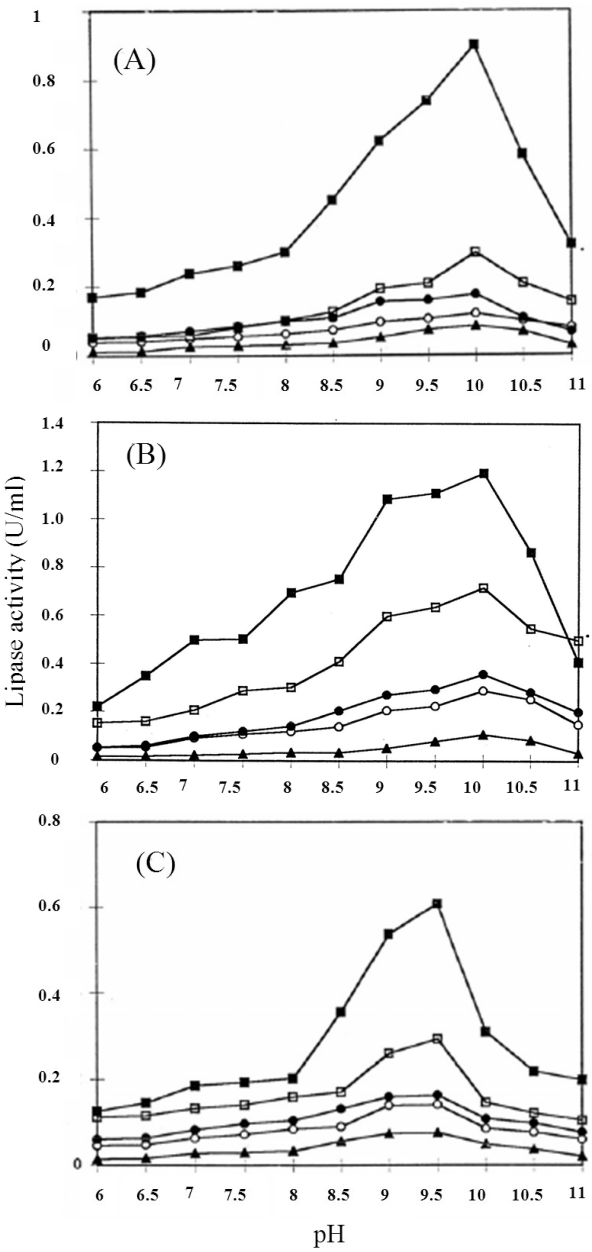

Figure 2. Effect of buffer pH on lipase activity of crude enzymes extracted from stomach (▲), spleen (O), liver (•), pancreas (□) and whole viscera (■) of skipjack tuna (*Katsuwonus pelamis*) (A), yellowfin tuna (*Thunnus albacares*) (B) and tonggol tuna (*Thunnus tonggol*) (C).

species; yellowfin, skipjack and tonggol tuna, had the highest protease and lipase activities at alkaline pH (10, 10 and 9) and most stable at their optima pH after 120 min incubation. Although their optimum temperatures for protease and lipase activities were 50°C and 60°C, respectively, they were more stable at lower temperatures (37-40°C). Spleen possessed the most thermostable enzymes compared to other sources.

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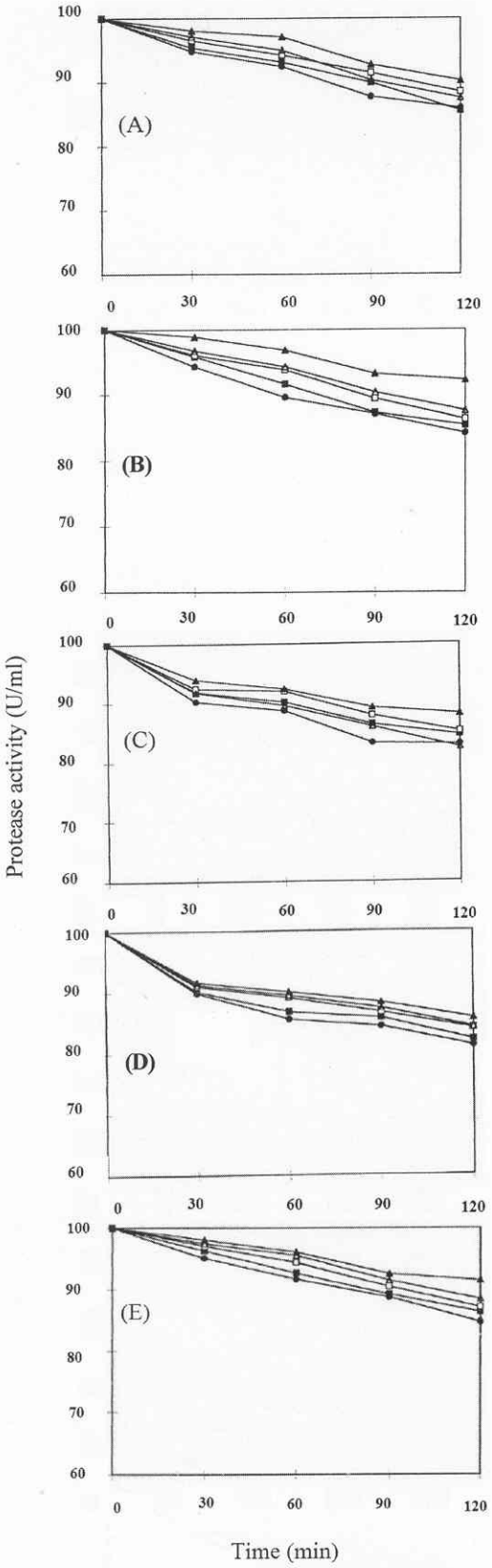


Figure 3. Effect of pH on the stability of protease extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of yellowfin tuna (*Thunnus albacares*)

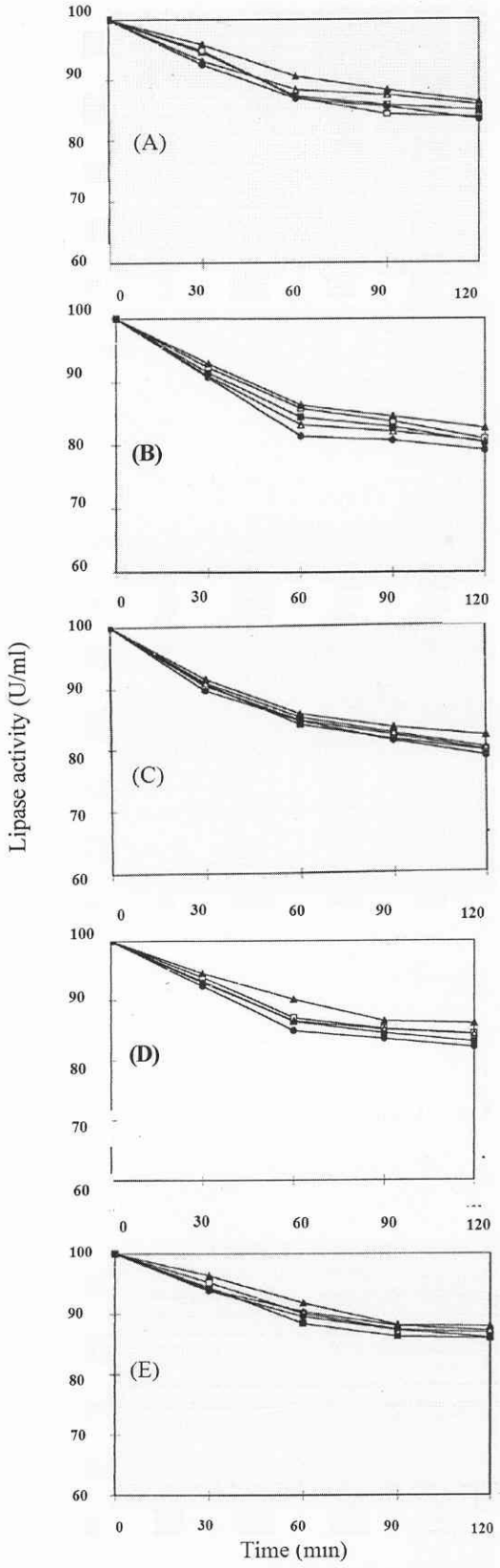


Figure 4. Effect of pH on the stability of lipase extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of yellowfin tuna (*Thunnus albacares*)

pH 9.0 (■), 9.5 (□), 10.0 (▲), 10.5 (△), and 11 (•)

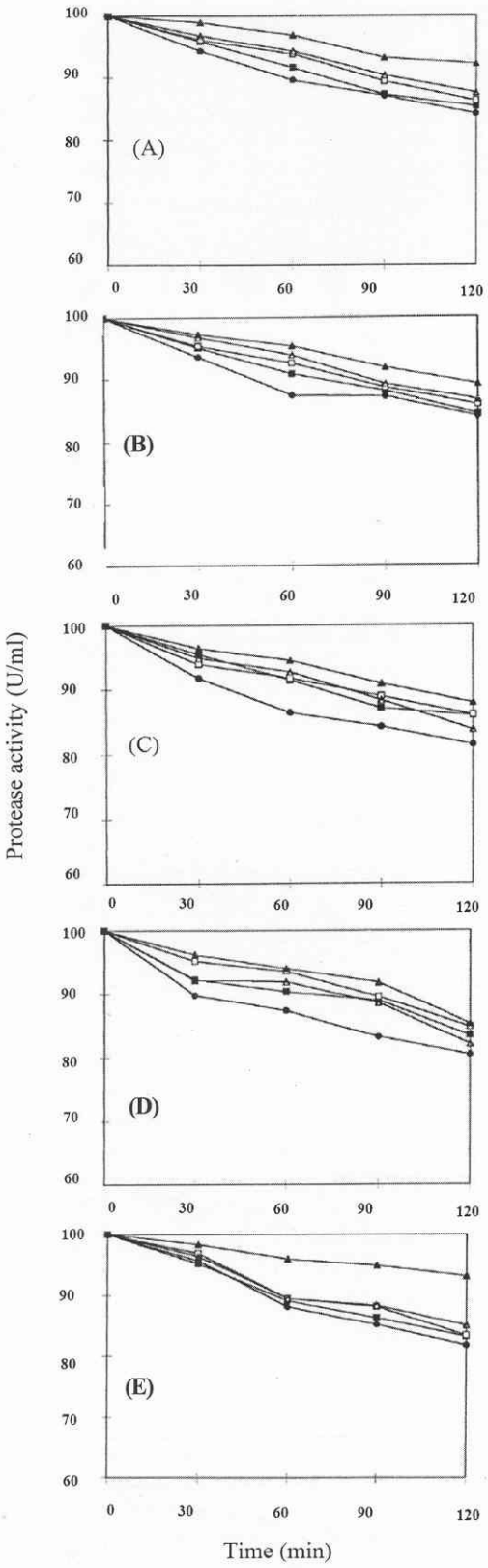


Figure 5. Effect of pH on the stability of protease extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of skipjack tuna (*Katsuwonus pelamis*)

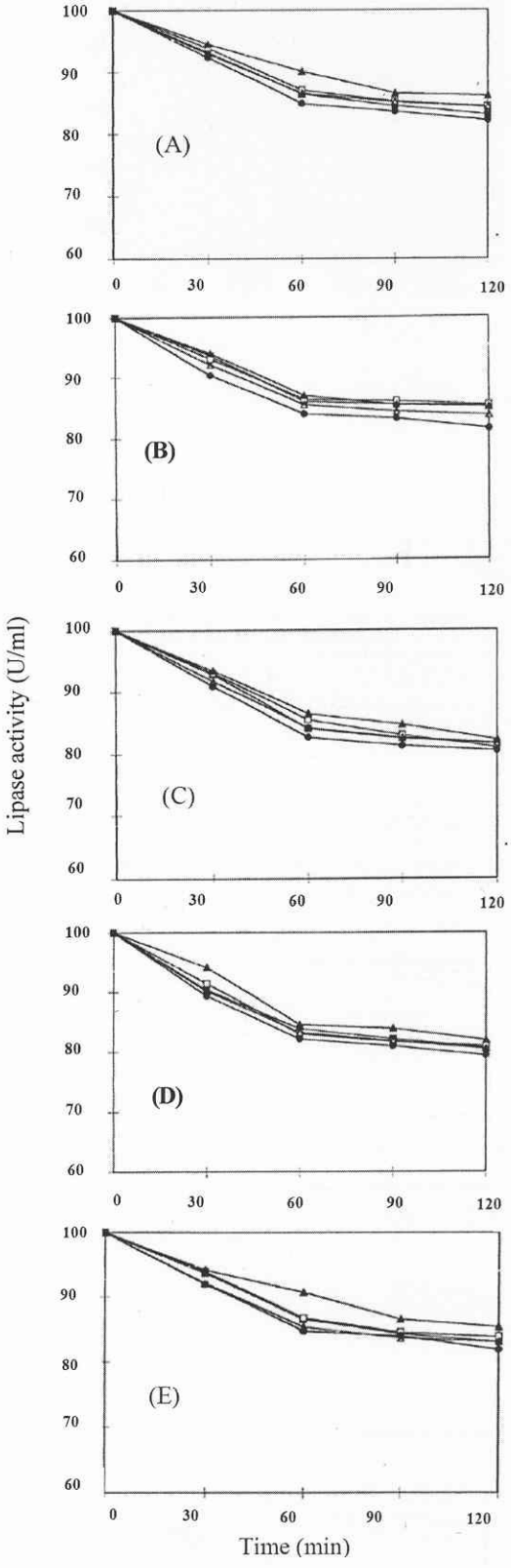


Figure 6. Effect of pH on the stability of lipase extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of skipjack tuna (*Katsuwonus pelamis*)

pH 9.0 (■), 9.5 (□), 10.0 (▲), 10.5 (△), and 11 (•)

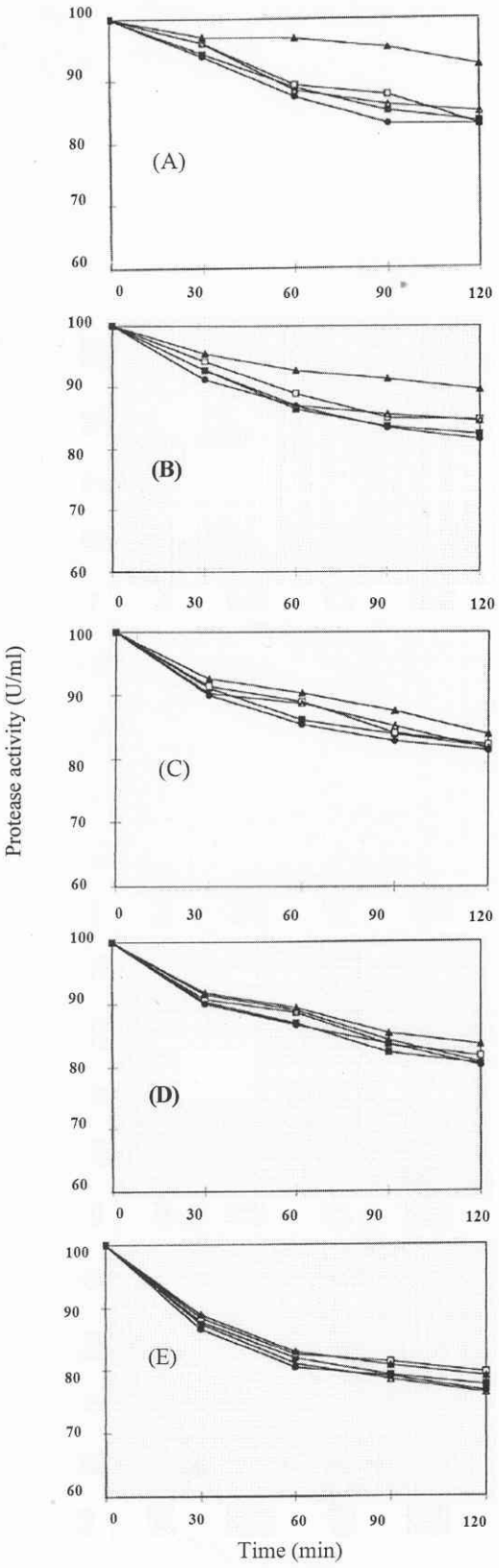
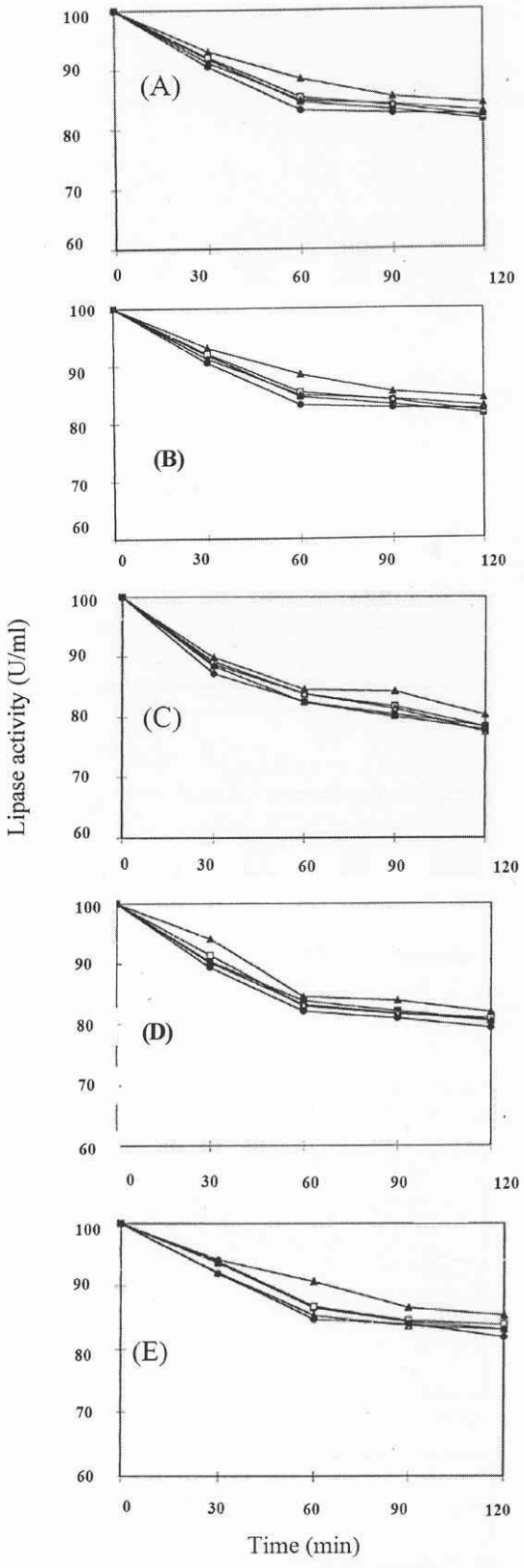


Figure 7. Effect of pH on the stability of protease extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of tonggol tuna (*Thunnus tonggol*)



pH 9.0 (■), 9.5 (□), 10.0 (▲), 10.5 (△), and 11 (•)

Figure 8. Effect of pH on the stability of lipase extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of tonggol tuna (*Thunnus tonggol*)

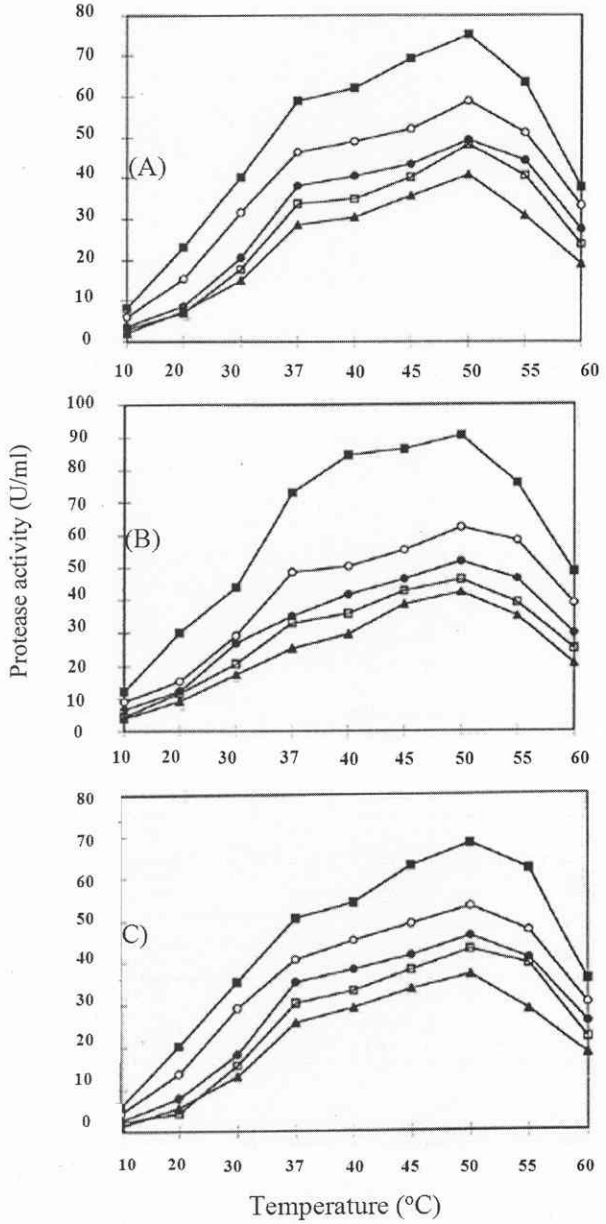


Figure 9. Effect of temperature on protease activity of enzymes extracted from stomach (▲), spleen (O), liver (•), pancreas (□), and whole viscera (■) from skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*) and tonggol tuna (*Thunnus tonggol*)

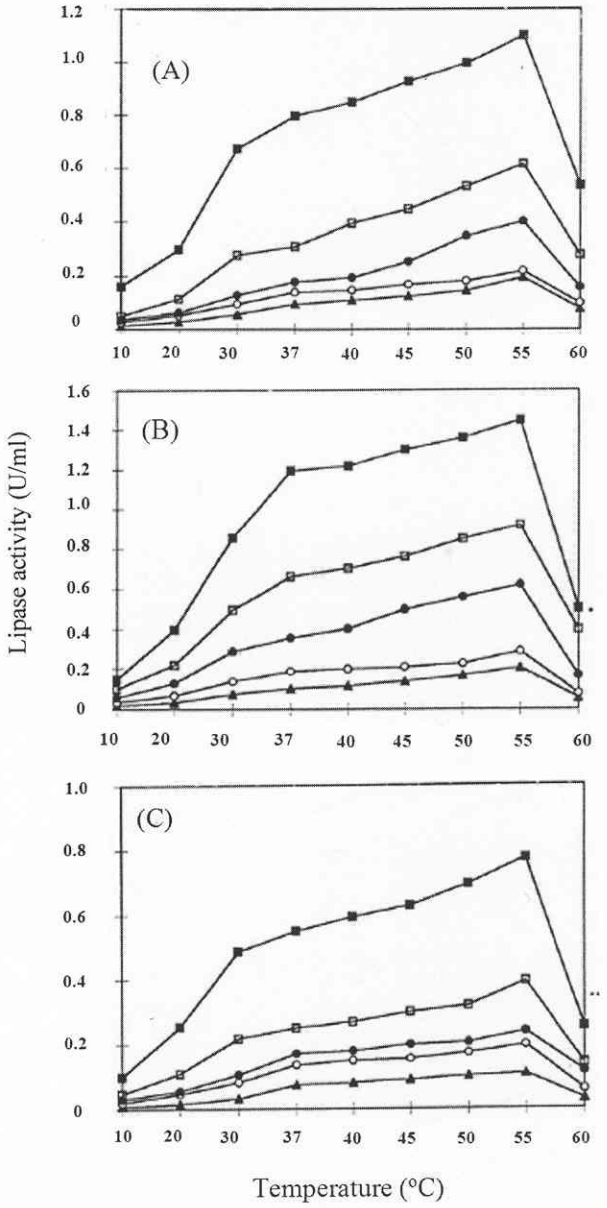


Figure 10. Effect of temperature on lipase activity of enzymes extracted from stomach (▲), spleen (O), liver (•), pancreas (□), and whole viscera (■) from skipjack tuna (*Katsuwonus pelamis*), yellowfin tuna (*Thunnus albacares*) and tonggol tuna (*Thunnus tonggol*)

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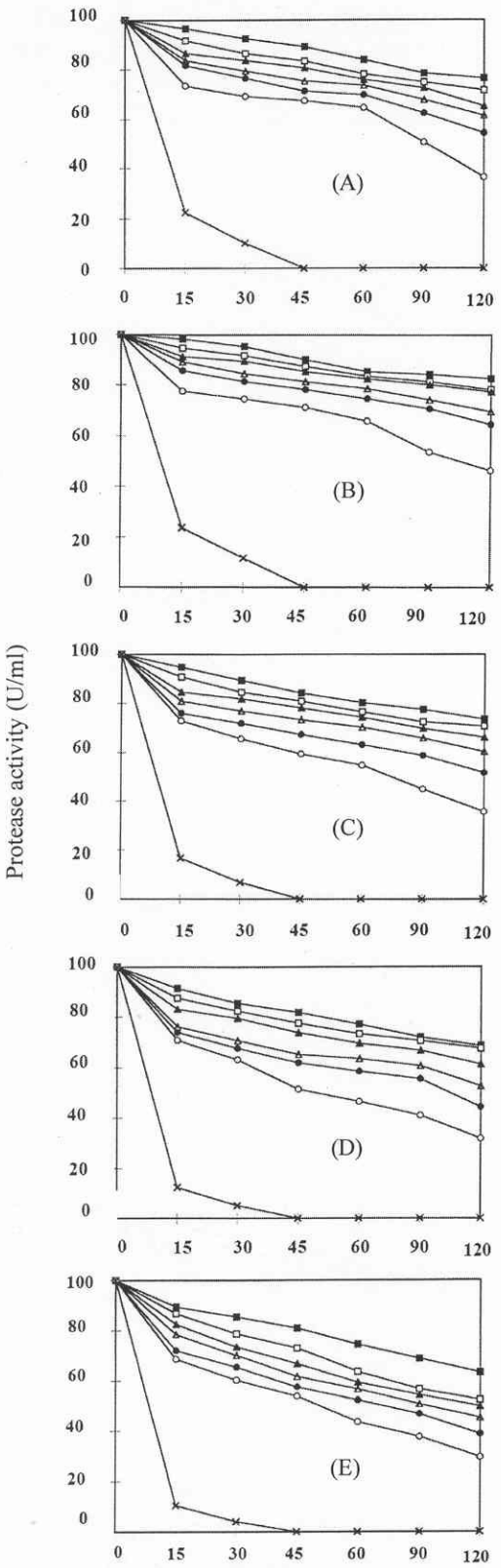


Figure 11. Effect of temperature on the stability of protease extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of skipjack tuna (*Katsuwonus pelamis*)

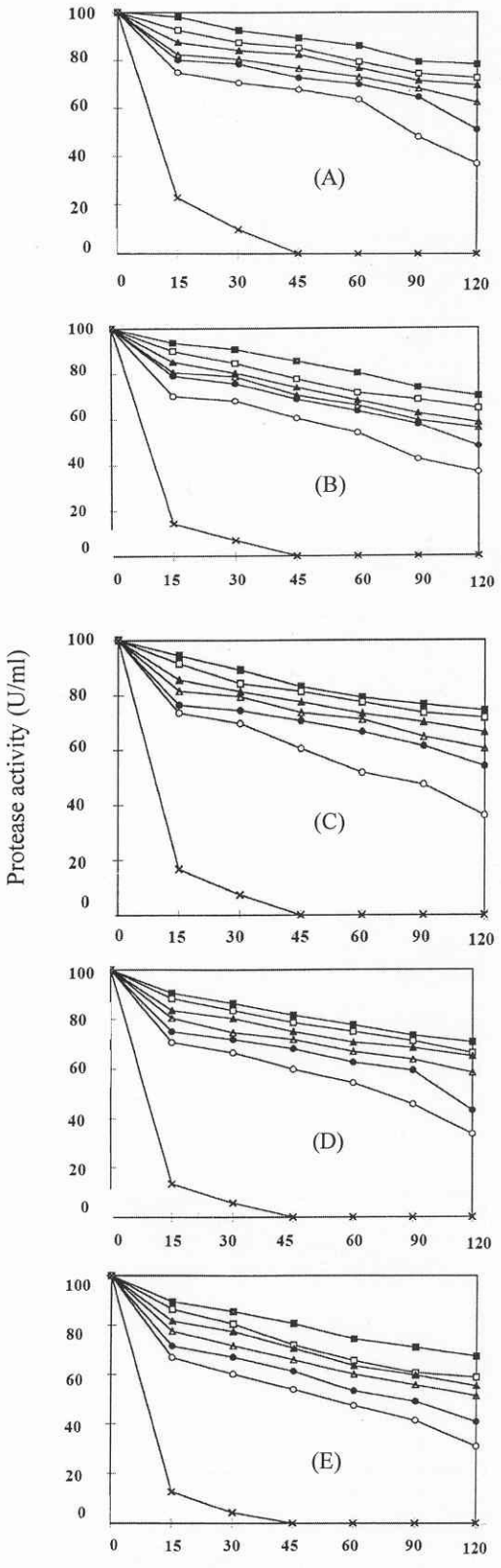


Figure 12. Effect of temperature on the stability of protease extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of yellowfin tuna (*Thunnus albacares*)

37°C (■), 40°C (□),45°C (▲),50°C (△), 55°C (•), 60°C (o) and 70°C (×)

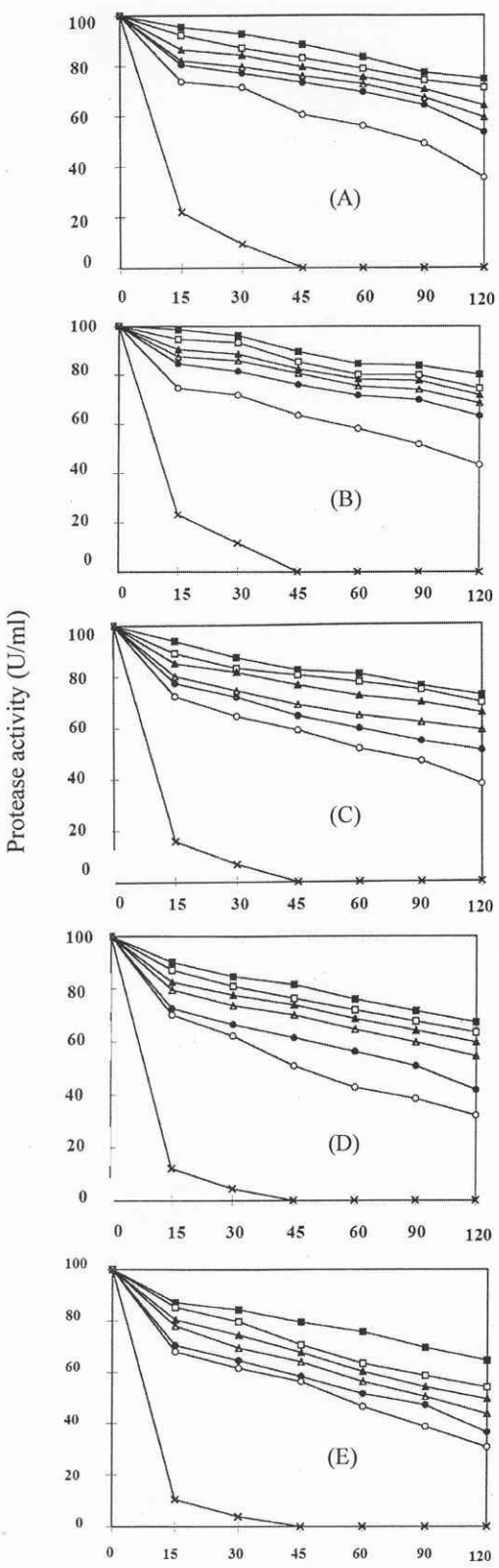


Figure 13. Effect of temperature on the stability of protease extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of tonggol tuna (*Thunnus tonggol*)

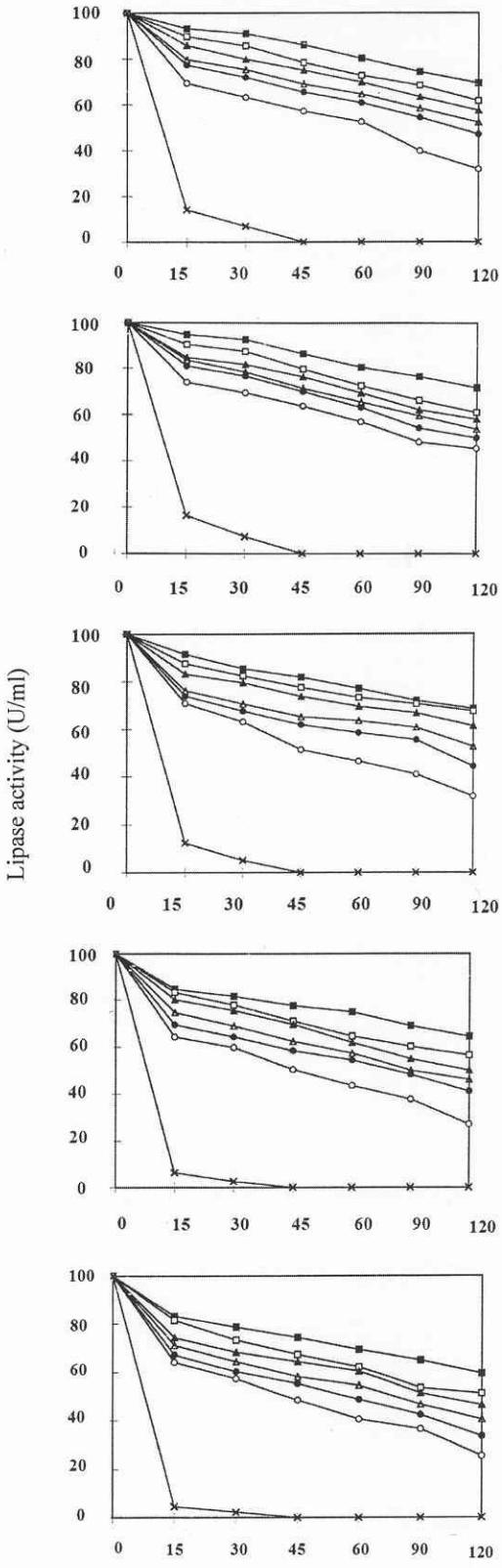


Figure 14. Effect of temperature on the stability of lipase extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of skipjack tuna (*Katsuwonus pelamis*)

37°C (■), 40°C (□), 45°C (▲),50°C (△), 55°C (•), 60°C (o) and 70°C (×)

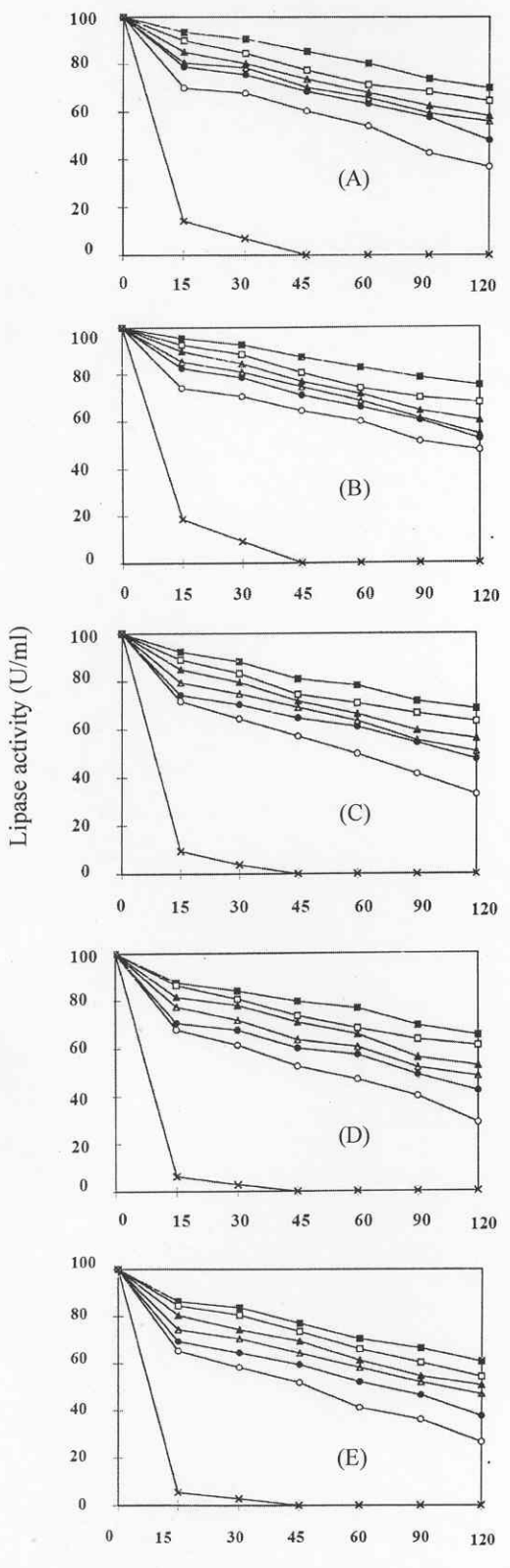
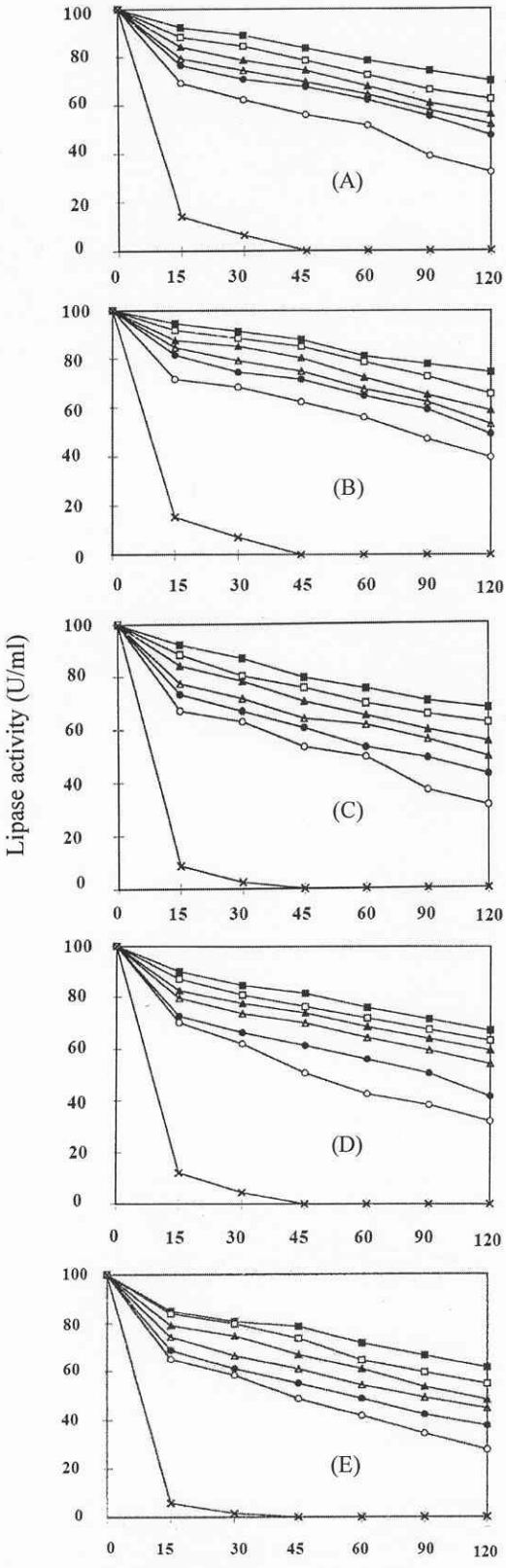


Figure 15. Effect of temperature on the stability of lipase extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of yellowfin tuna (*Thunnus albacares*)



37°C (■), 40°C (□), 45°C (▲), 50°C (△), 55°C (•), 60°C (o) and 70°C (×)

Figure 16. Effect of temperature on the stability of protease extracted from whole viscera (A), spleen (B), liver (C), pancreas (D) and stomach (E) of tonggol tuna (*Thunnus tonggol*)