

Comparison of polyphenol oxidase activity in different parts of deep water pink shrimp (*Parapenaeus longirostris*) by using L-DOPA substrate in Marmara Sea during the fishing season

Marmara Denizinde av sezonu boyunca derin su pembe karidesi (*Parapenaeus longirostris*) farklı dokularındaki polyfenol oksidaz enzim aktivitesinin L-Dopa substratı kullanılarak karşılaştırılması

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Özet: Melanosis derin su pembe karidesinin pazar kaybı için önemli bir problemdir. Bunun sorumlusu olan polifenol oksidaz (PPO) enzim aktivitesidir. PPO'nun özelliğini daha iyi anlamak ve oluşan aktiviteyi düşürme amacı için, ölüm sonrası derin su pembe karidesi türünün (*Parapenaeus longirostris*) farklı dokularında polifenol oksidaz (PPO) enzim aktivitesinin tespiti çalışılmıştır. Aktivite analizleri yedi ayrı dokuda çalışılmıştır: kafa (sefalalotoraks+karapas), karapas, dış iskeletin abdomen bölgesi, yüzme bacakları, yürüme bacakları, uropodlar ve telson bölgelerinde ayrı ayrı analizleri yapılmıştır. Sonuçlar doğrultusunda derin su pembe karidesinde PPO aktivite dağılımının kafadan telsona doğru azaldığı tüm çalışma boyunca tespit edilmiştir. Ayrı dokularda yapılan dağılım incelemesinde %70 amonium sulfat çökeltmesi sonucunda en yüksek aktivite kafa ve abdomen arasında kalan bölgede görülmüştür. Fakat cinsiyetler arasında istatistiksel anlamda önemli bir fark görülmemiştir. Ayrıca PPO aktivite düzeylerinin kıyaslanması tüm av sezonu boyunca yapılmıştır. Türün önemi bu türün ekonomik değerinden gelmektedir ve literatürde Marmara Denizinden avlanan türle ilgili PPO aktivitesi ile ilgili kaynağa rastlanmamıştır. Elde edilen veriler Marmara Ddenizinde bulunan erkek ve dişi karideslerin mevsimsel aktivite düzeylerinde göstermektedir.

Anahtar kelimeler: Karides, PPO aktivitesi, kara benek, melanosis.

Abstract: Melanosis is a major problem associated with the marketing loss of deep water pink shrimp. This is mediated by polyphenoloxidase (PPO), activity. To better understanding of PPO characteristics should pave a way for lowering melanosis so tissue distribution of polyphenol oxidase (PPO) activity was studied in deep water pink shrimp (*Parapenaeus longirostris*) post mortem. Activity analyzed in 7 different tissues; the head (cephalothorax+carapace), carapace, exoskeleton of the abdomen, pereopods, pleopods, uropods and telson individually. Due to the results, PPO activity of deep water pink shrimp was determined to be lower from head to telson during the research period. Partial tissue distribution showed that the highest activity was determined in the parts between head and the abdomen by using 70% ammonium sulfate fractions. But statistical significant correlation cannot be detected between the genders. And also comparison between the PPO levels of deep water pink shrimp during the fishing season was performed. The importance of these species came from their economic values, and no available data about the PPO activity in the literature could be observed in Marmara Sea. The determined data showed us the seasonal activity levels of male and female shrimps in Marmara Sea.

Keywords: Shrimp, PPO activity, black spot, melanosis.

INTRODUCTION

Discoloration in crustaceans, called melanosis or black spot, is a natural post-mortem process caused by the polymerization of phenols into insoluble black pigments which were the melanins (Zamarano et al., 2009). Phenol polymerization is catalyzed by polyphenol oxidase (also called phenol oxidize), an enzymatic complex found in almost all organisms. The term polyphenol oxidase (PPO) is generally used to refer to two similar enzymes involved in phenol oxidation: tyrosinases (EC 1.14.18.1), which catalyze the o-hydroxylation of monohydroxyphenols (i.e. monophenolase or cresolase activity) and the oxidation of o-dihydroxyphenols to

o-quinones (i.e. catechol oxidase or diphenolase activity), and catecholoxidases (EC 1.10.3.1) which only catalyze the oxidation of o-dihydroxyphenols. The o-quinones may react nonenzymatically with a variety of compounds in the presence of O₂ and form melanins. Monophenol oxidases generally also act as o-diphenoloxidases, as reported in Kim et al. (2000) and Ramirez et al. (2003), often at a faster rate

Deep water pink shrimp (*Panapeanus longirostris*) is one of the important products with an increasing exportation value in the Turkish economy. And discoloration in this shrimp specie is an important loss in market value. The presence of

melanins strongly reduces the marketability of the products (Kim et al., 2000). During post-mortem storage of crustaceans, proPPO can be also activated into PPO by the action of proteolytic enzymes leaching from the digestive tract (Ali et al., 1994). And then protein hydrolysis by these proteases generates substrates for active PPO (Ali et al., 1994). This was the reason why PPO has been studied in different tissues in shrimp species.

Sulphites are the most common and effective additives used to prevent melanosis in crustaceans. However, a search for alternative compounds was initiated, after the use of sulphites was found to be related to allergic and asthmatic reactions in some consumers (McEvily et al., 1991). The effectiveness of traditional sulphites and 4-hexylresorcinol as a melanosis-inhibiting chemical has been demonstrated both in laboratory and on board experiments (McEvily et al., 1991; Montero et al., 2001; Martínez-Alvarez et al., 2005). Recently, Montero et al. (2004) reported that deepwater pink shrimp (*Parapenaeus longirostris*) were highly sensitive to melanosis, and that there was an increase in the inhibition of melanosis after the shrimp were treated with increasing concentrations of 4-hexylresorcinol. But there is no published study in the literature that compared the partial tissue distribution of PPO activity of this specie in Marmara Sea during the fishing season. Since in this and other shrimps, as well as in prawns, melanosis is first detected in the head and then down spreads to the other tissues during chilled storage, the aim of the current study was determining PPO activity levels in different tissues. Moreover, determining the activity of PPO could be useful to explain, design, and prepare an appropriate formula of preventers to control melanosis in seasons.

MATERIAL AND METHODS

Sampling of shrimp tissues

Deep water pink shrimp (*Parapenaeus longirostris*) caught by using a commercial shrimp fishing vessel in Bandırma (in the South coast of Marmara Sea) during the fishing season (except May, June, July and August in 2010). Every month 20 kg of male and 20 kg of female specimens were selected for the research. Samples were placed in polystyrene boxes without using any chemical preventer. And flake ice was used in the polystyrene boxes to keep them cool in transfers. Packages were stored at +4° C immediately after arrival of the vessel to the port (about 5 h after catch), later transported to the processing factory which was located in Izmir by using cool truck. The samples were differentiated from the whole immediately due to their gender after arrival. And head (cephalothorax + carapace), carapace, exoskeleton of the abdomen, pereopods, pleopods, uropods and telson were taken separately (Fig 1) and kept in -80° C deep freezer for a night. The selected tissues were mixed with liquid nitrogen, grounded to a fine powder using a coffee mill and stored at -80° C for enzyme purification.

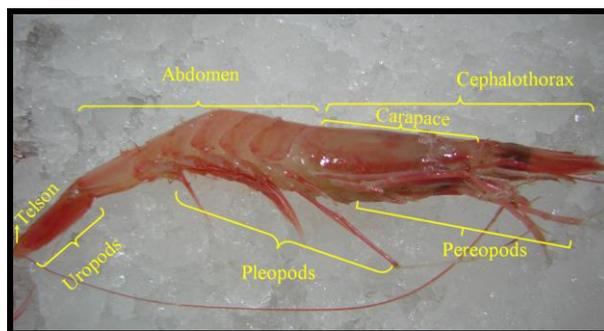


Figure 1. Sampling parts of deep water pink shrimp (*Parapenaeus longirostris*)

Partial purification of PPO

PPO was extracted according to Simpson et al. (1988) with slight modifications. One part of ground powder was added to three parts (w/v) of 0.1 M sodium phosphate buffer (pH 7.2) containing 1 M NaCl, 0.2% Brij 35. The suspension was stirred for 30 min at 4° C and then centrifuged at 8 000g for 30 min at the same temperature (4° C). The supernatant was filtered through 4 layers of muslin and the remaining extract was fractionated with ammonium sulfate ((NH₄)₂SO₄). The supernatant was fractionated with ((NH₄)₂SO₄) 70% saturation and re-suspended in 0.1 M sodium phosphate buffer (pH 7.2) and protein precipitation was collected by centrifugation at 23 500 g for 30 min (4° C). And the pellet obtained was dissolved in a minimum volume of 0.05 M sodium phosphate buffer, pH 7.2 and dialyzed against 50 volumes of the same buffer at 4° C for 1 day with 3 changes.

PPO Enzyme activity

PPO activity was determined with using Perkin Elmer Lambda 25 UV Visible spectrometer (USA) equipped with a Nüve circulating water bath (Nüve Co., Turkey) equipped with thermostatic controller. To determine the PPO activity L-DOPA (3,4-Dihydroxy-L-phenylalanin) was used as a substrate. And the enzyme activity was measured continuously at 475nm, 35°C, for 10 min in a UV-spectrophotometer. One unit of enzymatic activity was defined as an increase in absorbance of 0.001 per minute for 1 ml of enzyme at 35 °C (Bartolo and Birk, 1998; Cong et al., 2005). And the amount of enzyme which caused an increase in absorbance of 0.001/min. were defined as eu/mL (enzyme unit per mL).

The temperature for PPO activity was used by measuring the enzyme activity at 35°C temperature using a circulating water bath. The effect of temperature on the activity of PPO was tested in the standard reaction solutions (buffer and substrate) at the appropriate temperature (35°C) for the enzyme. Three milliliters of reaction mixture contained 0.6 ml of substrate, 2.3 ml of 0.1 M buffer solution, and 0.1 ml of enzyme solution. All assays were performed in triplicate.

Free L-tyrosine determination

The free L-tyrosine content was measured according to the colorimetric method of Lowry et al. (1951). Using bovine

serum albumin as the standard. The extraction was done by using 6% perchloric acid. 0.4 ml of extraction, 0.2 ml Folin-Ciocalteus-fenol and 0.4 ml of NaOH (1M) were mixed. Then after stabilized in 10 minutes the mixture was read by using UV - spectrophotometer in 578 nm.

Statistical analysis

Statistical analysis was carried out using SPSS (SPSS, 1999, Version 9.0. Chicago, IL, USA) One way anova by Duncan's multiple range test. This test was used to compare the differences among means between the months on tissues. The results are presented as means \pm SD with the significance level set at $p < 0.05$ under varying storage periods. And also data obtained from the samples were subjected to an independent sample - T - test (SPSS 16.0), at a confidence level of 95 %. The test was selected to compare 2 independent shrimp sample groups (male and female shrimps) to estimate the difference in recorded data.

RESULTS AND DISCUSSION

Study was design to determine the tissue distribution of PPO during the fishing season; therefore differentiation between the genders was also determined. For this aim comparison inside of the gender was investigated with monthly sampling by the researchers. High PPO activities were monitored in the extracts of carapace, cephalothorax, abdomen exoskeleton and preopods. And lower activity was shown by the extract from the uropods to telson. In every month different data were determined in male and female samples extracts (Table 1, Table 2). But it can be said that lower activities were seen in pleopods, uropods and telson extracts.

Data were taken from 7 different tissue in monthly comparison between the male samples tissue and the highest

values were taken due to the tissues in months as follows; February in a sequence carapace, uropods and pereopods, in March pereopods, abdomen and head, in April abdomen, pereopods, carapace, in September the sequence was determined as pereopods, abdomen and head, in October pereopods, abdomen and head, in November pereopods, abdomen and head and in December the sequence was abdomen, carapace and head. As mentioned before the first 3 sequences were changed almost in carapace, abdomen, pereopods and head. As expected the spread of the melanosis (browning) was seen between the parts head and pereopods in male samples. In statistically comparison in head tissue activity values only February and December data were significantly different from the other months sampling. The lowest value in head was taken in February and the highest activity value can be seen in December in Table 1. Statistical comparison was performed between the months in the same tissue parts due to the taken data in parts head, carapace and abdomen the highest PPO activity data were taken in December with significant differences ($p < 0.05$) On pereopods between March, April, September and October no statistical differences determined ($p > 0.05$). The lowest data were taken in February on pereopods in male samples. On tissues pleopods and uropods the highest activity data were taken in October with a significant different ($p < 0.05$) then other months. And on telson the highest data was taken in December. PPO from the cephalothoraxes of various crustaceans such as pink shrimp (*Parapenaeus longirostris*; *Penaeus duorarum*) (Zamorano et al. 2009), white shrimp (*Penaeus striiferus*) (Chen et al. 1997), prawn (*Penaeus japonicus*) (Montero, 2001b) and lobster (*Nephrops norvegicus*; *Homarus Americanus*) (Yan et al., 1991) have been purified and characterized.

Table 1. PPO enzyme activity of male samples (eu min⁻¹ mL⁻¹)

Months	Head (cephalothorax + carapace)	Carapace	Exoskeleton of the abdomen	Pereopods	Pleopods	Uropods	Telson
February	393.67 \pm 3.51 ^a	595.87 \pm 1.63 ^{ac}	241.27 \pm 2.97 ^a	446.67 \pm 6.66 ^{ac}	220.00 \pm 3.00 ^a	467.33 \pm 14.47 ^a	279.33 \pm 4.16 ^{ab}
March	465.60 \pm 6.92 ^{ab}	357.93 \pm 6.33 ^b	468.40 \pm 9.61 ^b	678.13 \pm 11.20 ^{bc}	281.47 \pm 6.22 ^b	217.87 \pm 23.89 ^b	178.00 \pm 7.00 ^a
April	482.00 \pm 15.72 ^b	574.00 \pm 7.21 ^a	611.00 \pm 11.53 ^{bcd}	577.67 \pm 9.50 ^{abc}	344.07 \pm 12.43 ^{bd}	266.00 \pm 14.00 ^b	272.67 \pm 14.74 ^{ab}
September	500.93 \pm 55.62 ^b	537.33 \pm 75.08 ^a	563.73 \pm 157.06 ^{bc}	784.13 \pm 174.05 ^{bc}	315.80 \pm 60.25 ^{ab}	231.00 \pm 46.94 ^b	211.33 \pm 63.89 ^a
October	479.67 \pm 21.22 ^b	582.80 \pm 18.05 ^a	731.00 \pm 22.11 ^{cd}	867.67 \pm 38.08 ^b	708.33 \pm 12.74 ^c	570.33 \pm 30.62 ^c	358.00 \pm 16.09 ^{bc}
November	465.60 \pm 6.92 ^{ab}	372.60 \pm 24.50 ^b	535.07 \pm 58.87 ^b	678.13 \pm 11.20 ^{bc}	433.67 \pm 38.68 ^d	217.87 \pm 23.89 ^b	178.00 \pm 7.00 ^a
December	645.67 \pm 48.88 ^c	677.00 \pm 19.92 ^c	774.33 \pm 12.86 ^d	616.67 \pm 17.01 ^c	636.33 \pm 54.24 ^c	535.67 \pm 32.62 ^{ac}	389.67 \pm 73.05 ^c

^x Data are expressed as mean \pm SD (n=3)

^y Different superscript letters in the same column indicate significant differences ($p < 0.05$)

Table 2. PPO enzyme activity of female samples.

Months	Head (cephalothorax + carapace)	Carapace	Exoskeleton of the abdomen	Pereopods	Pleopods	Uropods	Telson
February	464.00 \pm 2.0 ^a	371.93 \pm 9.10 ^a	535.40 \pm 2.25 ^a	483.67 \pm 1.53 ^a	225.20 \pm 2.31 ^a	285.67 \pm 4.04 ^a	429.67 \pm 4.51 ^a
March	455.93 \pm 1.90 ^a	351.27 \pm 5.66 ^a	417.73 \pm 7.51 ^b	575.20 \pm 9.13 ^{bc}	226.87 \pm 7.58 ^a	226.20 \pm 10.30 ^b	272.47 \pm 4.50 ^b
April	526.00 \pm 11.14 ^a	479.93 \pm 13.01 ^{ac}	883.67 \pm 23.63 ^c	383.33 \pm 29.37 ^c	355.27 \pm 10.01 ^b	223.33 \pm 10.41 ^b	255.57 \pm 17.83 ^b
September	653.33 \pm 7.23 ^b	802.33 \pm 175.52 ^b	761.00 \pm 4.58 ^d	621.67 \pm 37.86 ^b	521.67 \pm 37.86 ^c	499.67 \pm 51.19 ^c	348.33 \pm 46.46 ^c
October	655.67 \pm 17.90 ^b	463.00 \pm 7.55 ^a	726.33 \pm 28.15 ^d	836.67 \pm 2.89 ^d	544.33 \pm 29.26 ^c	550.67 \pm 33.61 ^c	326.20 \pm 26.82 ^{cd}
November	534.27 \pm 30.36 ^a	428.00 \pm 63.50 ^a	528.67 \pm 51.39 ^a	520.00 \pm 40.36 ^{ae}	520.00 \pm 26.91 ^c	226.20 \pm 10.30 ^b	272.47 \pm 4.50 ^{bd}
December	513.00 \pm 66.46 ^a	682.80 \pm 91.03 ^{bc}	797.67 \pm 36.36 ^d	779.67 \pm 12.86 ^d	625.00 \pm 26.06 ^d	570.33 \pm 30.62 ^c	372.67 \pm 28.57 ^{bc}

^x Data are expressed as mean \pm SD (n=3)

^y Different superscript letters in the same column indicate significant differences ($p < 0.05$)

In female samples due to the monthly comparison sequence was as follows; on February the highest PPO activity was determined in abdomen, then pereopods and head were the first 3 high activity data. Normally as found in previous studies, the spread of melanosis began from the cephalothorax (Zamarona et al. 2009; Nirmal and Benjakul, 2012), but in our findings the data show the different spread beginning from the abdomen. Between the months March and December higher activity data were taken from the part between head and pereopods. Highest activity of March was determined in pereopods and followed with head and abdomen. When the April data compared with others the highest PPO data was taken from abdomen in female samples. This value was the highest determined PPO activity in female samples. In September the sequence was as follows; carapace, abdomen and head. All the other months the determined activity values were all higher than the previous months but no correlation was determined between the tissue parts. A recent study by Zamarano et al. (2009) evaluated PPO activity in partially purified extracts from different parts of deep water pink shrimp (*Parapenaeus longistris*). They found higher enzyme activity in carapace extracts, but marked melanosis developed on the

cephalothorax and head after 1 day at 4°C. Even after 7 days at 4°C there was no melanosis in the carapace, confirming that the development of melanosis in different tissues depend on another factor in addition to PPO levels. When the head activity compared statistically significant differences were determined (p<0.05) in February, April, September and December. The highest activity value was determined in December in the head. The reasons for determining different PPO activities depend on the weather conditions and the temperature. Nirmal and Benjakul (2012) found 95.7 (u/mg) specific PPO activity using ammonium sulfate fraction from the cephalothorax of pacific white shrimp (*Litopenaeus vannamei*). When the carapace tissue compared due to the months the highest value determined in September. This value was significantly (p<0.05) different than the other months except December activity value. In the abdomen part the highest activity value in the study was determined in April with a significant difference (p<0.05). In the months October and December no significant difference was determined in pereopods PPO activities. The highest activity was seen in December on pleopods and in February on uropods. The highest enzyme activity of telson was determined in February in female samples (Table 2).

Table 3. PPO enzyme activity comparison between the genders (eu min⁻¹ mL⁻¹)

Months		Head		Exoskeleton of the abdomen				
		Carapace	Pereopods	Pleopods	Uropods	Telson		
February	M	393.67±3.51 ¹	595.87±1.63 ¹	241.27±2.97 ¹	446.67±6.66 ¹	220.00±3.00 ¹	467.33±14.47 ¹	279.33±4.16 ¹
	F	464.00±2.00 ²	371.93±9.10 ²	535.40±2.25 ²	483.67±1.53 ²	225.20±2.31 ¹	285.67±4.04 ²	429.67±4.51 ²
March	M	465.60±6.92 ¹	357.93±6.33 ¹	468.40±9.61 ¹	678.13±11.20 ¹	281.47±6.22 ¹	217.87±23.89 ¹	178.00±7.00 ¹
	F	455.93±1.90 ¹	351.27±5.66 ¹	417.73±7.51 ²	575.20±9.13 ²	226.87±7.58 ²	226.20±10.30 ¹	272.47±4.50 ²
April	M	482.00±15.72 ¹	574.00±7.21 ¹	611.00±11.53 ¹	577.67±9.50 ¹	344.07±12.43 ¹	266.00±14.00 ¹	272.67±14.74 ¹
	F	526.00±11.14 ²	479.93±13.01 ²	883.67±23.63 ²	383.33±29.37 ²	355.27±10.01 ¹	223.33±10.41 ²	255.57±17.83 ¹
September	M	500.93±55.62 ¹	537.33±75.08 ¹	563.73±157.06 ¹	784.13±174.05 ¹	315.80±60.25 ¹	231.00±46.94 ¹	211.33±63.89 ¹
	F	653.33±7.23 ²	802.33±175.52 ²	761.00±4.58 ¹	621.67±37.86 ¹	521.67±37.86 ²	499.67±51.19 ²	348.33±46.46 ¹
October	M	479.67±21.22 ¹	582.80±18.05 ¹	731.00±22.11 ¹	867.67±38.08 ¹	708.33±12.74 ¹	570.33±30.62 ¹	358.00±16.09 ¹
	F	655.67±17.90 ²	463.00±7.55 ²	726.33±28.15 ¹	836.67±2.89 ¹	544.33±29.26 ²	550.67±33.61 ¹	326.20±26.82 ¹
November	M	465.60±6.92 ¹	372.60±24.50 ¹	535.07±58.87 ¹	678.13±11.20 ¹	433.67±38.68 ¹	217.87±23.89 ¹	178.00±7.00 ¹
	F	534.27±30.36 ²	428.00±63.50 ²	528.67±51.39 ¹	520.00±40.36 ²	520.00±26.91 ²	226.20±10.30 ¹	272.47±4.50 ²
December	M	645.67±48.88 ¹	677.00±19.92 ¹	774.33±12.86 ¹	616.67±17.01 ¹	636.33±54.24 ¹	535.67±32.62 ¹	389.67±73.05 ¹
	F	513.00±66.46 ²	682.80±91.03 ¹	797.67±36.36 ¹	779.67±12.86 ²	625.00±26.06 ¹	570.33±30.62 ¹	372.67±28.57 ¹

*Data are expressed as mean ± SD (n=3)

†Different superscript numbers in the same column indicate significant differences between the genders (p < 0.05)

Table 4. L-Tyrosine content of samples (eu min⁻¹ mL⁻¹)

Months		Head		Exoskeleton of the abdomen				
		Carapace	Pereopods	Pleopods	Uropods	Telson		
February	M	0.14±0.01 ^{a1}	0.16±0.02 ^{a1}	0.15±0.02 ^{a1}	0.13±0.02 ^{a1}	0.15±0.01 ^{a1}	0.15±0.03 ^{a1}	0.16±0.01 ^{a1}
	F	0.15±0.03 ^{a1}	0.15±0.00 ^{a1}	0.14±0.00 ^{a1}	0.12±0.01 ^{a1}	0.16±0.01 ^{a1}	0.15±0.00 ^{a1}	0.15±0.00 ^{a1}
March	M	0.15±0.01 ^{a1}	0.16±0.00 ^{a1}	0.15±0.01 ^{a1}	0.13±0.00 ^{a1}	0.16±0.01 ^{a1}	0.15±0.00 ^{a1}	0.15±0.00 ^{a1}
	F	0.15±0.01 ^{a1}	0.16±0.01 ^{a1}	0.16±0.01 ^{a1}	0.15±0.01 ^{a1}	0.16±0.00 ^{a1}	0.16±0.01 ^{a1}	0.15±0.00 ^{a1}
April	M	0.14±0.00 ^{a1}	0.15±0.01 ^{a1}	0.14±0.00 ^{a1}	0.1a1±0.00 ^{a1}	0.14±0.00 ^{a1}	0.14±0.00 ^{a1}	0.14±0.01 ^{a1}
	F	0.14±0.01 ^{a1}	0.15±0.01 ^{a1}	0.13±0.01 ^{a1}	0.10±0.00 ^{a1}	0.14±0.00 ^{a1}	0.14±0.01 ^{a1}	0.13±0.01 ^{a1}
September	M	0.14±0.02 ^{a1}	0.15±0.00 ^{a1}	0.14±0.01 ^{a1}	0.13±0.01 ^{a1}	0.15±0.01 ^{a1}	0.13±0.01 ^{a1}	0.15±0.03 ^{a1}
	F	0.13±0.01 ^{a1}	0.15±0.01 ^{a1}	0.15±0.02 ^{a1}	0.13±0.01 ^{a1}	0.14±0.03 ^{a1}	0.14±0.03 ^{a1}	0.14±0.02 ^{a1}
October	M	0.15±0.01 ^{a1}	0.14±0.02 ^{a1}	0.13±0.01 ^{a1}	0.14±0.03 ^{a1}	0.14±0.01 ^{a1}	0.13±0.02 ^{a1}	0.14±0.02 ^{a1}
	F	0.13±0.02 ^{a1}	0.14±0.01 ^{a1}	0.14±0.01 ^{a1}	0.12±0.01 ^{a1}	0.14±0.02 ^{a1}	0.13±0.01 ^{a1}	0.14±0.01 ^{a1}
November	M	0.14±0.01 ^{a1}	0.14±0.01 ^{a1}	0.14±0.01 ^{a1}	0.13±0.01 ^{a1}	0.14±0.02 ^{a1}	0.14±0.01 ^{a1}	0.13±0.01 ^{a1}
	F	0.14±0.02 ^{a1}	0.14±0.02 ^{a1}	0.14±0.01 ^{a1}	0.14±0.01 ^{a1}	0.14±0.02 ^{a1}	0.16±0.01 ^{a1}	0.12±0.02 ^{a1}
December	M	0.13±0.01 ^{a1}	0.15±0.02 ^{a1}	0.14±0.01 ^{a1}	0.13±0.01 ^{a1}	0.14±0.01 ^{a1}	0.14±0.02 ^{a1}	0.14±0.02 ^{a1}
	F	0.14±0.01 ^{a1}	0.14±0.02 ^{a1}	0.14±0.01 ^{a1}	0.15±0.03 ^{a1}	0.14±0.01 ^{a1}	0.14±0.02 ^{a1}	0.14±0.01 ^{a1}

*Data are expressed as mean ± SD (n=3) †Different superscript letters in the same column indicate significant differences between the months and different superscript numbers in the same column indicate significant differences between the genders (p < 0.05).

Table 3 was performed to compare the activity differences of genders in samples. On the month February except the uropod values, all the data from the tissues were determined significantly different ($p < 0.05$) in samples. Table 3 shows that on head, abdomen, pereopods, and telson female samples PPO activities were determined higher than male samples. Pleopods activity value were statistically same ($p > 0.05$). In March sampling period on the parts head, carapace and uropods no significant difference was determined ($p > 0.05$) but on the other hand on abdomen, pereopods and pleopods higher PPO activity and lower PPO activity can be seen on male shrimps ($p < 0.05$). In April sampling male shrimps activity values were found significantly higher on carapace, pereopods and on uropods. And in female samples activities were determined higher on head, abdomen tissues. Previously in the study of Bono et al. (2010) which were monitored the PPO activity of deep water pink shrimp due to the gender and seasons, in all groups (male and female) PPO activity was higher in the late warm season (August and September) in Italy. And the activity was lowest between February and March. Also similar pattern was observed by Zamarano et al. (2009) for samples of the same specie caught in December and April along the coast of Spain. These data strongly support the hypothesis that PPO activity is influenced by seasonal changes. In the current study due to the September results on head, abdomen, pleopods and uropods tissues results showed that female samples were all gave the higher values ($p < 0.05$). And abdomen, pereopods, uropods and telson activities were all determined statistically the same not only in September but also in October ($p > 0.05$) between the genders. In November sampling period except the pereopods tissue female activity values were found almost higher or equal on the rest tissues of the study ($p \leq 0.05$). On the last month of the study in December when head PPO activity was found higher on male shrimps, pereopods PPO activity were higher on female shrimps ($p < 0.05$) and the rest tissues were similar ($p > 0.05$). In the study of Bono et al. (2010) weak PPO activity was observed in the females but those results were unexpected for the researchers of that study. Ogawa et al. (1984) found a well established link between PPO activity, greater body growth and high mean discoloration grade in females. Also in the current study higher PPO activity was found in female samples like Ogawa et al. (1984) and as a inverse of Bono et al. (2010).

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Table 4 shows the result of L-tyrosine contents in both genders. No statistical differences determined not only between the genders but also between the monthly periods.

As mentioned in the study of Zamarano et al., (2009) generally reaction of PPO with phenolic substrates during enzyme extraction and the subsequent polymerization of the intermediate products can lead to enzyme precipitation and loss of enzymatic activity (Chen et al., 1997). Most researchers, including Chen et al. (1997) and Rolle et al. (1991) did not include any protective agents so in the current study no protective agent were used like polyvinylpyrrolidone (PVPP). But no significant correlation was detected between the PPO activities of the tissues. A comment can be suggested about the data in both genders the tissues which had similar L-tyrosine contents in all months but different PPO activities were determined. This results explain that there were different reasons effected the PPO activities like; temperature, nutrition, catching area. But as Zamarano et al. (2009) mentioned mostly the high activity values were determined in the tissues of head, carapace, abdomen and the pereopods. But no linear correlation was found between the male and female samples based on the tissues and the activity values. Overall, increasing trend in PPO with an increasing weather temperature in both samples generally showed the spread of melanosis th parts between the cephalothorax and the abdomen but also pereopods and pleopods have high PPO activity in some months.

CONCLUSION

This study provides evidence of gender and tissue variability of PPO activity in the fishing season of deep water pink shrimp. Partial tissue distribution showed that the highest activity was determined in the parts between head and the abdomen by using 70% ammonium sulfate fractions. But statistical significant correlation cannot be detected between the genders. In future projects, the aim may be to determine the exact amount of sulfites to prevent the evaluation of browning in this specie.

ACKNOWLEDGEMENTS

This study was supported by TUBITAK and the data were taken from the report of Project No: 109O210.

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