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The Influence of the Extract Obtained from Giant Red Shrimp (*Aristaeomorpha foliacea*) Shells on Chemical Properties of Cold-Stored Anchovy (*Engraulis encrasicolus*)

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ABSTRACT

This study aims to determine the effects of extract obtained from giant red shrimp (*Aristaeomorpha foliacea*) shells on the changes in chemical parameters of anchovy (*Engraulis encrasicolus*) during 18 days of refrigerated storage. Butylated hydroxytoluene (BHT) was used for the comparison of antioxidant effects. The investigation of changes in fish during refrigerated storage indicated that lipid oxidation significantly increased ($P<0.05$). Compared to control group, BHT and different rates of shell extract were determined to have significant effects on prevention of oxidation. Comparison of total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), peroxide, free fatty acids and pH values indicated that the most positive result was found in the BHT added group, which was followed by the groups containing 0.5% and 0.1% of shell extracts, and control group. In this study, it was concluded appropriate to use natural shell extracts besides synthetic antioxidants during the storage of fish.

Keywords: Giant red shrimp; *Aristaeomorpha foliacea*; Shrimp shell extract; Anchovy; Shelf life; Cold storage

Kırmızı Dev Karides (*Aristaeomorpha foliacea*) Kabuklarından Elde Edilen Ekstraktın Soğukta Depolanan Hamsinin (*Engraulis encrasicolus*) Kimyasal Özellikleri Üzerine Etkisi

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ÖZET

Bu çalışmada, kırmızı dev karides (*Aristaeomorpha foliacea*) kabuklarından elde edilen ekstraktın, hamsi (*Engraulis encrasicolus*)'nin buzdolabında 18 gün depolanması süresince kimyasal parametrelerinde meydana gelen değişimler üzerine etkisinin belirlenmesi amaçlanmıştır. Antioksidan etkiyi kıyaslamak amacıyla butillendirilmiş hidroksi toluen (BHT) kullanılmıştır. Depolama süresince balıklarda meydana gelen değişimler incelendiğinde, süreyle birlikte lipid

oksidasyonunun önemli ($P<0.05$) ölçüde arttığı tespit edilmiştir. Kontrol grubuyla kıyaslandığında, uygulanan BHT ve farklı oranlardaki kabuk ekstraktlarının oksidasyonun önlenmesi üzerine olumlu etkileri gözlenmiştir. Toplam uçucu bazik azot (TVB-N), tiyobarbiturik asit (TBA), peroksit, serbest yağ asitleri ve pH değerleri kıyaslandığında; uygulama grupları içerisinde en olumlu sonuç BHT eklenen grupta bulunurken bunu sırasıyla % 0.5 ve % 0.1 oranında kabuk ekstraktı içeren gruplar ve kontrol grubu izlemiştir. Bu çalışmada balıkların depolanması esnasında sentetik antioksidanların yanı sıra kabuktan elde edilen doğal ekstraktın da kullanılmasının uygun olacağı belirlenmiştir.

Anahtar Kelimeler: Kırmızı dev karides; *Aristaeomorpha foliacea*; Karides kabuğu ekstraktı; Hamsi; Raf ömrü; Soğuk depolama

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1. Introduction

Shrimp catching is important not only in Turkey but also in international fishery. In Turkey, significant amounts of shrimp are hunted in Mediterranean, Aegean and Marmara coasts. Male, jumbo, Karabiga, red and pink shrimp take place among the whole hunted shrimps. In recent years, the economic importance of giant red shrimp (*Aristaeomorpha foliacea*) increased with the raise in the interest towards hunting in deep water species. While they are hunted with bottom trawl and served for fresh consumption, most of them are processed in seafood processing plants and exported.

With the advanced technology in shrimp processing, the assessment of shrimp wastes has been a crucial matter. The wastes of shrimps which are removed from the meat parts in processing factories comprise approximately 40-56% of the whole product (İbrahim et al 1999; Gildberg & Stenberg 2001; Sachindra et al 2006). This rate varies by the species of shrimp and approximately 34% of the total weight consists of head and 14% consists of shell (Binsan et al 2008). These wastes include very valuable bioactive compounds (antioxidants, carotenoids, chitin, peptone, amino acids, peptides, proteins, minerals, enzymes, lipid, flavor compounds and other useful nutrients). They are used as addition agent in foods and also utilized as a protein source in aquaculture and stockbreeding (Gagne 1993; İbrahim et al 1999; Binsan et al 2008).

Chitin and its derivate chitosan rank at the top of the list of bioactive compounds. Approximately

20-30% chitin can be obtained from Crustacea shells. One of the most important compounds that make shrimp shells such valuable is the natural antioxidants. The isolation and identification of natural antioxidants in shell wastes is an important stage for the assessment of shell wastes. A few studies conducted on antioxidant features of shrimp shells so far have researched the characterization and effects of these features on some fish species. In studies conducted so far, it is reported that waste extracts of *Penaeus monodon* species assessed as a potential bioactive substance in shrimp processing factories in Thailand have antioxidant activity (Dajsipun et al 2000); in the extract obtained from *Pandulus jordani* shells, the substance with antioxidant feature is phenol compounds (Seymour et al 1996); once again, in extract obtained from *Pandulus jordani* shells, polar compounds are responsible for antioxidant activity (Li et al 1994) and this extract has positive effects on quality during the storage of some fish (Li et al 1998). However, the number of research conducted on antioxidant feature of shrimp shells is limited with the above mentioned studies and further detailed studies are needed on this subject. For this reason, the present study aims to determine the effects of different concentrations of the shell extract obtained from giant red shrimp, discarded as waste products in sea product processing factories, on chemical properties of anchovy, highly important for Turkish fishery sector, stored in refrigerator.

2. Material and Methods

2.1. Materials

In present study, shell materials were obtained from the wastes of giant red shrimp (*Aristaeomorpha foliacea*). Fresh samples of shrimp processing discards including intact cephalothorax and abdominal exoskeleton were collected from a local shrimp factory. Representative shrimp wastes were selected, put in polystyrene boxes and stored at -20 °C before and during transportation to the laboratory. Shells were completely separated from the shrimp wastes in laboratory and washed in pure water. BHT was used as a commercial antioxidant.

2.2. Preparation of crude extract

Shrimp shells were extracted with ethanol according to the method of Li et al (1998). The mixture was blended until homogeneous with a blender. The slurry was filtered through a funnel using Whatman filter paper (GF/C, Schleicher & Schuell) to remove debris. The filtrate was evaporated to dryness. The dried crude shell extract was resolved by water as a solution dip anchovy treatment. BHT was used as a commercial antioxidant.

Antioxidant activity, total phenol compound and total carotenoid content of the giant red shrimp shells were found as 45.84%, 17.87 mg 100 g⁻¹ and 20.31 mg 100 g⁻¹, respectively (Küçükgülmez & Çelik 2013).

2.3. Fish sample preparation

The anchovy (*Engraulis encrasicolus*) (average weight and length: 12.28±1.71 g and 11.74±0.62 cm, respectively) were purchased from a local fish market (total 17 kg anchovy). They were stored in ice an insulated box and transferred to the laboratory. After the viscera, gills and bone were removed, the anchovy samples were divided into four groups (4 kg each).

Fish fillet samples were dipped into the following solutions as different treatments for 5 min; control containing only distilled water, 0.1% and 0.5% (w v⁻¹) crude antioxidant solutions and 0.005% BHT solution. These concentrations of crude antioxidant solution and BHT were determined based on the

results of a preliminary study made in accordance with the studies of Li et al (1998) and Soyer (1995). Subsequently, fish samples were put into styropor dishes, covered with stretch films, and then stored in the refrigerator (2.7 °C). Experiment was performed with two-parallels. Analyses were made every other three day for 18 days.

2.4. Chemical analysis

Total volatile basic nitrogen (TVB-N) was determined on steam distillation using the Kjeldahl distillation apparatus and titration (Antonocopoulos 1973). Thiobarbituric acid (TBA) number was determined using the method of Tarladgis et al (1960), expressed as mg malondialdehyde kg sample⁻¹ using a conversion factor of 7.8. The peroxide value (PV) was determined by the acetic acid-chloroform method (AOCS 1990) and expressed as meq O₂ kg lipid⁻¹. The free fatty acids (FFA) content of the lipid was determined volumetrically using aqueous sodium hydroxide (0.25 N) and phenolphthalein indicator (1% ethanol) according to IAFMM (1987) method. The pH was determined from homogenates of minced fish and distilled water in a ratio of 1:10 (w v⁻¹) by using a digital pH meter (Lima Dos Santos et al 1981).

2.5. Statistical analysis

The SPSS (SPSS Inc., Chicago, IL, USA) software was used for the statistical analysis. Comparisons among groups were made using one-way analysis of variance (ANOVA), and significant differences were determined by Duncan's multiple range tests at 5% confidence level.

3. Results and Discussion

3.1. Total volatile basic nitrogen (TVB-N)

TVB-N values of various anchovy groups are presented in Table 1. TVB-N values of all groups increased significantly during storage period and TVB-N values of the group including 0.5% shell extract and of the group with added BHT were found much lower compared to other groups (P<0.05). As TVB-N amount in fish is associated with bacterial deterioration and activity of endogenic enzymes,

TVB-N analysis is one of the most commonly used methods for the detection of fish freshness (Vareltzis et al 1997). At the end of the microbial activity, some volatile bases emerge as a result of degradation of protein or non-protein nitrogenous compounds (Yerlikaya et al 2005). While TVB-N amount is low in fresh fish, it increases by the deterioration of fish during the storage period. Generally, samples including 25 mg 100 g⁻¹ TVB-N are considered as “very good”, 30 mg 100 g⁻¹ as “good”, 35 mg 100 g⁻¹ as “marketable” and higher than 35 mg 100 g⁻¹ as “spoiled” (Varlık et al 1993). Accordingly, in the present study, the control group and the other group including 0.1% shell extract exceeded the consumability limit on the 15th day of refrigerated storage and those groups with 0.5% shell extract and added BHT exceeded this limit on 18th day in terms of TVB-N value. These results indicate the positive

effects of the extract of the used shrimp shells and BHT on TVB-N value of fish. Similar to the present study, other studies which used different plant materials as natural antioxidant source reported that TVB-N value of fish is affected in positive direction (Nam & Ahn 2003; Pazos et al 2005).

3.2. Thiobarbituric acid (TBA)

TBA changes in anchovy groups are presented in Table 2. TBA values of all groups statistically significantly increased during refrigerated storage ($P<0.05$). In general assessment of TBA results, it is found that the TBA values of the group with added BHT during the storage period were significantly lower than other groups ($P<0.05$). This positive effect was followed by the group with the added 0.5% shell extract.

Table 1- TVB-N changes of anchovy (*Engraulis encrasicolus*) treated with shrimp shell extract during refrigerated storage (mg 100 g⁻¹)

Çizelge 1- Karides kabuk ekstraktı ile muamale edilen hamsinin (*Engraulis encrasicolus*) buzdolabında depolama süresince TVB-N değişimleri (mg 100 g⁻¹)

Days/Groups	Control	0.1% shell extract	0.5% shell extract	BHT
0	16.93±2.02 ^{a,1*}	17.01±0.81 ^{a,1}	17.14±0.87 ^{a,1}	17.11±0.89 ^{ab,1}
3	18.89±0.68 ^{b,2}	18.01±1.43 ^{ab,12}	16.10±2.07 ^{a,1}	16.79±0.04 ^{a,12}
6	20.40±0.81 ^{bc,3}	19.43±0.15 ^{b,2}	16.64±0.06 ^{a,1}	16.91±0.40 ^{a,1}
9	21.37±0.54 ^{c,2}	21.86±1.66 ^{c,2}	20.40±0.49 ^{b,12}	18.68±0.09 ^{b,1}
12	25.62±0.69 ^{d,3}	22.78±0.63 ^{c,2}	23.40±0.43 ^{c,2}	20.79±0.75 ^{c,1}
15	35.42±0.59 ^{e,3}	35.61±1.18 ^{d,3}	27.20±0.62 ^{d,2}	24.77±0.55 ^{d,1}
18	43.48±1.03 ^{f,3}	40.06±1.17 ^{e,3}	37.24±0.86 ^{e,2}	35.75±1.51 ^{e,1}

*, data are expressed as means±standard deviation; different letters within the column denote significant differences ($P<0.05$); different number within the row denote significant differences ($P<0.05$)

Table 2- TBA changes of anchovy (*Engraulis encrasicolus*) treated with shrimp shell extract during refrigerated storage (mg MDA kg⁻¹)

Çizelge 2- Karides kabuk ekstraktı ile muamale edilen hamsinin (*Engraulis encrasicolus*) buzdolabında depolama süresince TBA değişimleri (mg MDA kg⁻¹)

Days/Groups	Control	0.1% shell extract	0.5% shell extract	BHT
0	1.67±0.38 ^{a,1*}	2.10±0.14 ^{a,1}	1.95±0.17 ^{a,1}	1.68±0.38 ^{a,1}
3	2.77±0.30 ^{b,2}	2.67±0.40 ^{b,2}	2.36±0.05 ^{a,12}	2.03±0.02 ^{a,1}
6	4.70±0.19 ^{c,3}	4.87±0.04 ^{c,3}	4.12±0.30 ^{b,2}	3.70±0.02 ^{b,1}
9	6.45±0.23 ^{d,3}	6.59±0.01 ^{d,3}	5.93±0.66 ^{c,2}	4.15±0.19 ^{c,1}
12	7.01±0.03 ^{e,3}	7.67±0.09 ^{e,3}	6.15±0.25 ^{d,2}	4.94±0.35 ^{d,1}
15	8.62±0.31 ^{f,4}	8.10±0.36 ^{f,3}	6.53±0.34 ^{e,2}	5.70±0.24 ^{e,1}
18	9.08±0.35 ^{g,3}	8.67±0.16 ^{g,23}	7.12±0.31 ^{f,2}	6.20±0.14 ^{f,1}

*, data are expressed as means±standard deviation; different letters within the column denote significant differences ($P<0.05$); different number within the row denote significant differences ($P<0.05$)

TBA value is a commonly used indicator for the determination of lipid oxidation of fish (Sallam 2007; Turhan et al 2009). The detection of TBA amount is based on the malondialdehyde measurement which determines the secondary oxidation products in association with the fish deterioration (Al-Bandak et al 2009). It is reported that consumability limit of the TBA value which is used to determine the rancidity level of oils is between 7 and 8 mg MDA kg⁻¹ (Varlık et al 1993). According to this assessment, the group with added BHT and the group including 0.5% shell extract did not exceed this value during the storage period while control group and the group including 0.1% shell extract exceeded this value on 15th day of the storage. In this study which aims to assess shell extracts as natural antioxidant source, TBA values being an indicator of lipid oxidation support this view. In their study which is very similar to the present study, Li et al (1998) researched the natural antioxidant obtained from shrimp shells of the *Sebastolobus alascanus* fish stored in refrigerator on TBA amount and they obtained similar results.

The effects of natural antioxidants of fish on lipid oxidant are a recently noticeable issue. TBA analyses are widely conducted in order to detect these effects. TBA analysis was used to determine the effect of natural extracts obtained from various plants on lipid oxidation during the storage of various fish species and it is highlighted that these extracts decrease lipid oxidant which is a similar

finding to the present study (Yasin & Abou-Taleb 2007; Selmi & Sadok 2008; Al-Bandak et al 2009).

3.3. Peroxide value

The changes in peroxide values of anchovy groups during the storage period are presented in Table 3. Peroxide value statistically significantly increased in all groups by storage period ($P<0.05$). This increase in peroxide values is an indicator of oxidative rancidity in fish lipids. As fish meat involves high amounts of polyunsaturated fatty acids which can be easily oxidized and lead to high level of peroxide, peroxide levels increase by the storage level (Yasin & Abou-Taleb 2007).

It is observed that in all groups, peroxide level reached the highest level on 15th day of storage and started to decrease on 18th day. It is assumed that the decrease in peroxide level at last stages of the storage may result from the degradation of hydroperoxides being a secondary oxidation product. Because, peroxide analysis is measured with hydroperoxide formation and it gives good results at initial stages of oxidation.

Many studies conducted on the natural and commercial antioxidant usage so as to delay lipid oxidation, investigated the changes of peroxide levels and it is reported that peroxide levels of groups with the added natural and commercial antioxidant were lower than the control group during the storage

Table 3- Peroxide changes of anchovy (*Engraulis encrasicolus*) treated with shrimp shell extract during refrigerated storage (meq O₂ kg⁻¹)

Çizelge 3- Karides kabuk ekstraktı ile muamale edilen hamsinin (*Engraulis encrasicolus*) buzdolabında depolama süresince peroksit değişimleri (meq O₂ kg⁻¹)

Days/Groups	Control	0.1% shell extract	0.5% shell extract	BHT
0	0.61±0.10 ^{a,1*}	0.85±0.44 ^{a,1}	0.87±0.51 ^{a,1}	0.66±0.00 ^{a,1}
3	2.13±0.43 ^{b,1}	1.75±0.37 ^{a,1}	1.50±0.33 ^{b,1}	1.68±0.42 ^{b,1}
6	3.74±0.45 ^{c,2}	3.13±0.33 ^{b,2}	3.57±0.69 ^{c,2}	2.19±0.07 ^{bc,1}
9	6.82±1.44 ^{d,3}	6.62±0.18 ^{d,3}	4.14±0.30 ^{c,2}	2.44±0.23 ^{c,1}
12	7.14±0.38 ^{d,3}	6.28±0.71 ^{d,2}	6.70±0.15 ^{d,23}	3.37±0.07 ^{d,1}
15	10.59±1.44 ^{e,3}	9.14±1.13 ^{e,3}	7.70±0.87 ^{e,2}	5.38±0.64 ^{e,1}
18	5.94±0.62 ^{d,2}	5.21±0.50 ^{c,2}	3.53±0.45 ^{c,1}	5.30±0.33 ^{e,2}

*, data are expressed as means±standard deviation; different letters within the column denote significant differences ($P<0.05$); different number within the row denote significant differences ($P<0.05$)

period which is a similar finding to the present study (Sallam 2007; Yasin & Abou-Taleb 2007; Al-Bandak et al 2009). To conclude, peroxide level of fish meat significantly differs by fish species, initial peroxide level, processing and storage conditions, storage period and the type of the added antioxidant.

3.4. Free fatty acids

Free fatty acid levels of anchovy groups are presented in Table 4. Free fatty acid levels statistically significantly increased in all groups during the 18-day storage period ($P<0.05$).

Although no statistically significant difference was observed between groups at initial periods of storage, free fatty acid values of the anchovy group with added BHT were found statistically lower

than other groups ($P<0.05$) which was followed by the group including 0.5% shell extract, the group including 0.1% shell extract and lastly the control group. Free fatty acids which emerge as a result of the hydrolysis of the lipids in fish stored or frozen in refrigerator are important for the development of rancidity (Chaouky et al 2008). In the present study, the positive effects of shell extract and BHT on this value are remarkable as well. In a similar study conducted on the effects of commercial antioxidants and vacuum packaging on free fatty acids, similar results were obtained (Soyer 1995).

3.5. pH

pH changes in anchovies during the storage period are presented in Table 5. pH values of all groups

Table 4- Free fatty acid changes of anchovy (*Engraulis encrasicolus*) treated with shrimp shell extract during refrigerated storage (oleic acid %)

Çizelge 4- Karides kabuk ekstraktı ile muamale edilen hamsinin (*Engraulis encrasicolus*) buzdolabında depolama süresince serbest yağ asitleri değişimleri (% oleik asit)

Days/Groups	Control	0.1% shell extract	0.5% shell extract	BHT
0	3.21±0.05 ^{a,2*}	3.17±0.35 ^{a,2}	1.93±0.52 ^{a,1}	2.51±0.46 ^{a,12}
3	3.44±1.08 ^{a,1}	3.12±0.06 ^{a,1}	3.12±0.47 ^{b,1}	2.55±0.29 ^{a,1}
6	4.68±0.85 ^{b,2}	3.95±0.10 ^{a,1}	3.91±0.01 ^{bc,1}	3.56±0.10 ^{ab,1}
9	5.30±0.17 ^{b,2}	4.43±0.32 ^{b,1}	4.17±0.04 ^{c,1}	4.16±0.16 ^{b,1}
12	6.69±0.17 ^{c,4}	5.66±0.08 ^{c,3}	5.08±0.00 ^{d,2}	4.32±0.25 ^{bc,1}
15	7.22±0.09 ^{cd,4}	6.03±0.02 ^{c,3}	5.49±0.38 ^{c,2}	4.53±0.32 ^{bc,1}
18	8.09±1.12 ^{d,3}	6.84±0.50 ^{d,2}	6.00±0.33 ^{c,2}	4.74±0.07 ^{c,1}

*, data are expressed as means±standard deviation; different letters within the column denote significant differences ($P<0.05$); different number within the row denote significant differences ($P<0.05$)

Table 5- pH changes of anchovy (*Engraulis encrasicolus*) treated with shrimp shell extract during refrigerated storage

Çizelge 5- Karides kabuk ekstraktı ile muamale edilen hamsinin (*Engraulis encrasicolus*) buzdolabında depolama süresince pH değişimleri

Days/Groups	Control	0.1% shell extract	0.5% shell extract	BHT
0	6.08±0.02 ^{a,12*}	6.04±0.02 ^{a,1}	6.06±0.01 ^{ab,1}	6.11±0.01 ^{b,2}
3	5.99±0.01 ^{a,1}	6.00±0.04 ^{a,1}	6.01±0.05 ^{a,1}	6.02±0.05 ^{a,1}
6	6.16±0.07 ^{b,1}	6.20±0.01 ^{b,1}	6.13±0.07 ^{b,1}	6.12±0.05 ^{b,1}
9	6.34±0.07 ^{c,2}	6.30±0.05 ^{c,12}	6.29±0.04 ^{c,12}	6.20±0.05 ^{c,1}
12	6.63±0.09 ^{d,2}	6.67±0.03 ^{de,2}	6.47±0.01 ^{d,1}	6.38±0.00 ^{d,1}
15	6.97±0.06 ^{e,4}	6.80±0.01 ^{e,3}	6.56±0.01 ^{e,2}	6.41±0.01 ^{d,1}
18	7.18±0.03 ^{f,4}	7.00±0.06 ^{f,3}	6.77±0.02 ^{f,2}	6.50±0.01 ^{e,1}

*, data are expressed as means±standard deviation; different letters within the column denote significant differences ($P<0.05$); different number within the row denote significant differences ($P<0.05$)

decreased on 3rd day of the storage and then started to increase again by storage period. pH value is at low levels initially due to the fact that glycogen turns into lactic acid at post-mortem glucoses stage of fish (Şengör et al 2000). At following stages of the storage, oxido-reduction balance is disturbed due to enzymes and bacteria; changes occur in concentration of free hydrogen and hydroxide ions and thus pH value increases. pH value of fresh fish is 6.0-6.5; consumability value is 6.8-7.0 (Varlık et al 1993; Turhan et al 2001). According to this assessment and considering the pH results of the present study, it is found that pH values of control group and the groups including 0.1% shell extract exceeded the critical limit 6.8-7.0 on 15th day of storage while the group including 0.5% shell extract and the groups with added remained behind the critical limit during the storage. Different studies reported that pH value of fish meat differs by fish species, hunting type, fish processing technology, storage conditions and contamination of microorganism and this is not an absolute criterion for the freshness or quality yet should be considered as a supportive factor to other parameters (Selmi & Sadok 2008).

4. Conclusions

The shell wastes which are not utilized in seafood processing factories in Turkey have a great potential. The assessment of these wastes is very important not only for seafood factories but for other industries. Although there are many studies on the assessment and economic use of wastes of shrimp and other shellfish in the world, the number of studies on their antioxidant feature is very limited. In this scope, to obtain natural antioxidant from wastes of giant red shrimp shells and thus to contribute to economy with wastes through obtaining food protecting materials which are safe for human health and to prevent the environmental damage caused by the unused wastes show the importance of the present study. Consequently, it was concluded that the extract isolated from shrimp shells could be used during the cold storage of fish fillets instead of synthetic antioxidants.

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