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Macro and Micro Element Composition of Smoked and Marinated Shrimps (*Parapenaeus longirostris*): Its Potential Impact on Consumer Health

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ABSTRACT: The aim of this study is to determine the effect of smoking and marination process on mineral concentration of shrimp meat. The samples obtained from the Tuzla Region of the Marmara Sea were first smoked and then marinated after the pre-treatment. Mineral analysis was carried out in ICP-MS after microwave wet digestion process on both the shrimps immersed in boiling water after caught (group A), smoked (group B), and marinated shrimps after smoking (group C). According to the results of the study, the macro element concentration after smoking process (group B) was found to be higher than the other groups. Changes were observed in the microelement and heavy metal content of the shrimps because of smoking and marination process. Heavy metal concentrations of the groups were below the limits recommended by international organizations. The Hazard Quotient (HQ) of the groups was below the limit value of 1 and therefore all groups can be said to be safe for human consumption.

Keywords- *Parapenaeus longirostris, heavy metals, health risk assessment, shrimp, smoking, marinating*

Dumanlanmış ve Marine Edilmiş Karideslerin (*Parapenaeus longirostris*) Makro ve Mikro Element İçeriği: Tüketici Sağlığı Üzerindeki Potansiyel Etkisi

ÖZET: Bu çalışmanın amacı, karides etinin mineral konsantrasyon düzeyleri üzerine dumanlama ve marinasyon işleminin etkisini belirlemektir. Marmara Denizi Tuzla Bölgesinden temin edilen örnekler, uygulanan ön işlemlerin ardından önce dumanlanmış ve ardından marine edilmiştir. Hem avlanma sonrası kaynar suya daldırılıp çıkartılan karideslerde (A grubu), hem de dumanlanan (B grubu) ve dumanlama sonrası marine edilen karideslerde (C grubu) mikrodalgada yaş yakma işleminden sonra ICP-MS de mineral analizi gerçekleştirilmiştir. Çalışma sonuçlarına göre dumanlama sonrası (B grubu) makro element konsantrasyonu diğer gruplara kıyasla daha yüksek bulunmuştur. Karideslerin mikro element ile ağır metal içeriğinde dumanlama ve marinasyon sonucunda değişimler gözlemlenmiştir. Grupların ağır metal konsantrasyonları uluslararası organizasyonların tavsiye ettiği limitlerin altındadır. Grupların tehlike katsayısı 1 sınır değerinin altındadır ve bu nedenle tüm grupların insan tüketim için güvenli olduğu söylenebilir.

Anahtar Kelimeler- *Parapenaeus longirostris, ağır metal, sağlık risk değerlendirmesi, karides, dumanlama, marinasyon*

1. Introduction

The most important criterion in a product offered for human consumption is its reliability in terms of public health. For this reason, many chemical, microbiological and physical contaminations should be prevented in the process of preparing seafood for consumption. In the first place, fishing from safe waters is an aspect that should be considered, as the quality of the water affects the nutritional content of the final product. The heavy metal content of seafood is one of the most discussed issues in seafood consumption. Fish, shrimp, crab, and bivalves are used in metal accumulation tests organisms and are commonly consumed by human (Primavera 1997; Aytekin et al. 2019). The pH, temperature, alkalinity of pollutants, sampling style (Barajas et al. 2006; Amin et al. 2011), size, feeding habits, and ecological zone (Kwok et al., 2014) influence the bioaccumulation of heavy metals. However, heavy metal pollution in shrimp is not only important to the shrimp itself, but also because of the non-carcinogenic health risks associated with shrimp consumption (Sarkar et al., 2016).

Shrimp is a very tasty, highly connective tissue aquatic product fished from the Turkish seas. The shrimp species common in Turkey are green tiger shrimp, caromate shrimp, giant gamba shrimp, and deep-water pink shrimp. In total, 5.177 tons of shrimp were caught in 2020, of which 3.515 tons were deep-water pink shrimp. All the deep-water pink shrimps caught are offered for human consumption (TUIK, 2021). The edible part of the shrimp is the abdominal region, and its meat is rich in protein (18-20%). It is an easily digestible food because it is poor in connective tissue. It is a good source of vitamin D and vitamin B12 and an excellent source of selenium (Varlık et al., 2007).

Smoking is a method that has been used in food processing technology for many years. Besides giving flavour to the product, it provides a reduction in the microbiological load because of the heat treatment used. Marination gives flavour to the food without heat treatment. However, it also has a protective effect due to additives such as salt, acid, and spices. Numerous studies (Hoq et al., 2003; Kpoclou et al., 2013) have been undertaken on smoking or marination, to give flavour and to determine the changes in the nutrient content of the product as a result of the processing methods. However limited studies (Şengör et al., 2004; Özoğul et al., 2010; Akintola et al., 2013; Çağlak et al. 2015) have been carried out on the combined processing of seafood, especially smoked and marinated seafood.

There are studies in the literature on the mineral content of different shrimp tissue (Madany et al., 1996; Guhathakurta & Kaviraj, 2000; Pourang & Amini, 2001; Pourang et al., 2005; Yanar & Çelik, 2006; Sriket et al., 2007; Hasan et al., 2021), but limited. The study, which includes nutritional composition, the shelf life, chemical, sensory and microbiological properties of smoked and marinated shrimps, was previously published by Eyüboglu and Kocatepe (2021). In this study, the mineral content of deep-water pink shrimps, which shelf life and nutritional composition were determined by Eyüboğlu and Kocatepe (2021), were investigated. The aim of this study is to determine the metal content of deep-water pink shrimp marinated after smoking and to determine the consumer healthy assessments.

2. Materials And Methods

Sampling: The sampling and processing were carried out as determined in Eyüboglu and Kocatepe (2021). In the present study, deep-water pink shrimps (*Parapenaeus longirostris*) from Tuzla Istanbul, Sea of Marmara, Turkey were used. The shrimps were immersed for 1

minute in boiling water at the port, then transported to the laboratory in ice, their shell and internal organs were removed. Shrimp meats firstly smoked and secondly marinated.

The smoking process: shrimp salted for 10 min in salt brine (brine ratio was 1:2), filtered (15 min) and pre-dried at 30°C for 20 min, then smoked at 60°C for 10 min. The shrimps were smoked on wire mesh trays in a convective smoking kiln.

Then the shrimps were marinated (brine: 1% alcohol vinegar, 2.2% salt, and 0.4% citric acid); (shrimp:brine ratio was 1:9) 4°C for 2 days.

Groups were as follow;

Group A: Shrimp immersed for 1 minute in boiling water, Group B: smoked shrimp meat, and Group C: smoked+marinated shrimp meat. Samples were made ready for metal analysis. 500 grams of raw and processed product was used for each replication. The study was carried out in triplicate with two replications.

Analytical procedure: The metal analyses of raw and processed shrimp samples were measured by ICP/MS (Agilent Technologies / 7700X ICP-MS Systems, Santa Clara, U.S.). Analytical quality control was chieved using Agilent reference (Std. 1: Agilent 8500-6940 2A (10 mg kg -1 in %5 HNO3): Li, Be, Na, Mg, K, Ca, Rb, Sr, Cs, Ba, V, Cr, U, Mn, Fe, Co, Ni, Cu, Zn, Ag, Cd, Al, Ga, As, Se, Tl, Pb; Std. 2: Agilent 8500-6940 Hg (10 mg kg -1 in %5 HNO3): Hg) materials. The results of the study were given as mg kg-1 wet weight. The measurement limit values of metals were; Li: 0.1132, Ga: 0.02863, Be: 0.002037, As: 0.6655, Na: 0.3834, Se: 0.2816, Mg: 0.07421, Rb: 0.002855, K: 0.7839, Ag: 0.04828, Ca: 8.166, Cd: 0.004838, V: 0.02506, Sb: 0.01398, Cr: 0.02936, Cs: 0.002211, Fe: 0.1959, Hg: 0.03357, Co: 0.006183, Pb: 0.003045, Ni: 0.05721, U: 0.002152, Cu: 0.06849, Al: 0.426, Mn: 0.03901, Zn:2.676, Sr: 0.01669, Ba: 0.03248 (Milestone, 2018).

Metal pollution index (MPI)

MPI was calculated by taking the geometric mean of metals such as Zn, Fe, As, Sr, Al, Cu, Mn, Rb, Se, Ba, Ni, Cr, B, Ti, Hg, Cd, Pb in the samples (Usero et al. 1996; Abdel-Khalek et al. 2016). If the MPI value is less than 2, it is considered that degree of pollution is not impacted (Jamil et al. 2014).

Estimated daily intake (EDI)

Estimated daily intake (EDI) is proportional to the amount of seafood consumption and therefore the daily intake of metals taken with seafood. The Turkish Statistical Institute reported that the average amount of fish consumed per Captiva in 2019 was 6.3 kg per year. EDI values of metals were calculated with the following formula:

EDI=(Cm*W)/m

Cm is the concentration of metals in shrimp meat; W represents daily average consumption of fish given as: 0.017 kg day^{-1} for estimated daily intake (EDI) (Human body weight 70 kg).

Hazard Quotient (HQ)

One of the indexes used to determine the negative effects of metal intake on human health is the hazard quotient (HQ). The HQ is calculated by adjusting the EDI and the reference dose (Rf D mg kg^{-1} year) determined by different organizations. The RfD values for Hg and Cd had been reported by the U.S. EPA as 0.0003 and 0.001 mg kg-1 day-1, respectively. For Pb, the RfD value of 0.0035 mg kg^{-1} day^{-1} stated in previous studies was used (Harmanescu et al. 2011, Bat et al. 2020, Bat et al. 2021). The HQ was calculated by using the equation below:

HQ=EDI/Rf D

The fact that the HQ is less than 1 indicates that there is a health risk in the food in terms of the specified metal (Yee 2010).

Statistical Analysis

Samples were analysed in triplicate and data were given as mean and standard error. Descriptive statistics (mean, standard deviation and range), normality test (Shapiro Wilk's and Levene's), analysis of variance (ANOVA), followed by post hoc Tukey tests, and t-test were conducted using Minitab Statistics 17. $p<0.05$ values were considered as statistically significant difference.

3. Result And Discussion

Macro elements

The concentration of the macro elements (Na, Mg, K, Ca, P) identified in groups are shown in Table 1. The macro element content of A group was K>P>Na>Ca>Mg, respectively. Öksüz et al. (2009) stated that the macro elements contained in the same shrimp species were Ca>Na>K>P>Mg. Sriket et al. (2007) reported the Mg contents of black tiger shrimp and white tiger shrimp as 431 and 361 mg kg^{-1}, respectively. In addition, Yanar and Çelik (2006) indicated that the Na and K contents of green tiger shrimp and speckled shrimp were 1608, 2261 and 1511, 2372 mg kg^{-1} respectively. The Na, K and Mg contents of the deep-water pink shrimps used in this study were higher than was founded in this literature. El-Said et al. (2021) reported the Na and K contents of *P. longrostris* as 9.8 and 4.4 mg g^{-1}. These values were high compared to the samples in present study. The highest Na values were obtained in smoked shrimp (Group B). The Na, Mg, Ca, and P contents of the shrimp meat increased with the smoking process, while these values in the final product decreased with the marination process (Group C). The main reason for this was the loss of water resulting from the heat treatment applied to the shrimp meat, and the proportional increase in the macro element level. The water content of the product increased and the macro-mineral content decreased with the subsequent soaking in the marinating brine. Bello (2013) stated that the K, Ca, Mg, and Na content of raw shrimp meat decreases with the smoking process. Shrimp meat has a high phosphorus (P) content. This content increased even more with the smoking process. Similarly, Hoq et al. (2003) found that the phosphorus content of smoked *M. brevicornis* was a high phosphorus (P) content. There were very good ratios between Ca and P in both groups A and B. However, both Ca and P loss were observed in the final product (Group C) with the marination process. The maximum loss of K was observed during the marination process. The Na/K ratio of shrimp was below 1 but increased with the processing. This ratio was as high as 47.47 mg kg^{-1} in the smoked and marinated shrimp (Group C). It has been reported that low Na:K ratio in diets with higher K intake and lower Na intake may help prevent some risk factors related to cardiovascular diseases, hypertension, and low HDL cholesterol (Okada et al., 2020). The Na content of the final product increased due to both the pre-brine process for flavouring before the smoking process and the marination made in the Na-containing brine after smoking.

Table 1. The macro elements concentration detected in the groups (mean concentration ± sd mg kg $^{-1}$ ww)

	Na	Mg	K	Ca	P	Na/K ratio
Group A	2597.4±31.1c	595.9±8.8b	2708.2±36.3a	1270.6±23.4b	2611.5±33.3b	0.96
Group B	9569.6±114.7a	622.1±2.4a	1959.2±4.2b	1616.5±12.7a	2896.5±9.9a	4.88
Group C	7301.0±4.5b	125.5±0.6c	153.8±0.5c	658.9±1.9c	1406.9±3.0c	47.47

n=6, Different superscripts in the same column mean significantly different at the 5% probability level (*P<0.05*).

Micro elements

Shrimp is a good source of minerals. Micro elements are especially important for humans and other organisms because they are included in the structure of enzymes. The mineral content is very important for good health, although, at times, they could pose a threat if their proportions are too high (Ravichandran et al., 2009). The micro element content of A, B, and C Groups are shown in Table 2. The Zn content of shrimp is higher than that of other micro elements. Similar to our study, it has been determined that the Zn content of shrimps (Heu et al., 2003; Aytekin et al., 2019) and other crustaceans (Hossain & Khan, 2001; Miramand et al., 2001; Mitra et al., 2010) is higher than other micro elements. Zinc is a mineral that is given to children as a supplement because it strengthens the immune system and increases appetite. The amount of iron, which is of great importance in the transport of oxygen in the blood and the prevention of anaemia (DeMaeyer et al. 1989), is an element that is provided from infancy in terms of public health. The Fe content of the deep-water pink shrimp used in the present study was higher than those detected in different shrimp species in previous studies (Öksüz et al., 2009; Sriket et al., 2007; Yanar & Çelik, 2006). The effect of the processing methods applied in all micro element concentrations, except for selenium and titanium, was statistically different ($p<0.05$). Selenium was not lost after the smoking process ($p>0.05$), it decreased in the final product due to the effect of salt and acid in the marination process ($p<0.05$). Chromium is a mineral that is necessary for the body in that it contributes to the functionality of the hormone insulin, and is a component of the glucose tolerance factor (GTF) (Mertz 1993). In the last few decades, the contamination of hexavalent chromium, in both terrestrial and aquatic ecosystems, has increased as a result of various anthropogenic activities. Overexposure to hexavalent chromium can result in higher accumulation in human and animal tissues, resulting in toxic and harmful health effects (Prasad et al., 2021). According to the U.S. Department of Health and Human Services (USHHS), 60 µg of Cr is taken daily from food in the normal diet (ATSDR, 2000). The Cr levels observed in this study were below the permissible limit value of 2.0 mg kg $^{-1}$ set by the WHO (2008). Selenium is an element of great importance in human health and nutrition. In recent years, it has been reported that selenium is especially effective in preventing methyl mercury toxicity (García-Sevillano et al., 2015; Ralston et al., 2008). Also, some studies have shown that moderate doses of Se can reduce the negative effects of Cr (VI) in terms of oxidative damage (Hao et al., 2017). The daily intake of selenium also differs depending on age. A daily Se intake of 70 µg day $^{-1}$ is recommended for adults. 100 g of smoked marinated shrimp meets the daily Se intake of an adult individual.

Table 2. The micro elements concentration detected in the groups (mean concentration ± sd mg kg $^{-1}$ ww)

	Group A	Group B	Group C
Zn	20.28±0.06b	81.87±0.45a	16.84±0.02c
Fe	7.39±0.02c	12.34±0.06b	22.45±0.08a
As	10.97±0.12a	10.22±0.05b	1.11±0.01c
Sr	12.49±0.19b	14.31±0.09a	5.16±0.02c
Al	7.94±0.03c	8.74±0.12b	15.61±0.15a
Cu	8.01±0.03b	10.38±0.07a	6.01±0.02c
Mn	0.51±0.01b	0.67±0.01a	0.17±0.01c
Rb	0.53±0.01a	0.38±0.00b	0.06±0.00c
Se	1.03±0.03a	1.06±0.02a	0.79±0.01b
Ba	0.57±0.01b	0.84±0.01a	0.49±0.00c
Ni	0.06±0.00c	0.16±0.00a	0.08±0.00b
Cr	0.02±0.00c	0.04±0.00b	0.05±0.00a
B	3.51±0.02b	4.90±0.02a	2.60±0.02c
Ti	0.40±0.01b	0.48±0.03b	0.65±0.05a

n=6, Different superscripts in the same column mean significantly different at the 5% probability level (*P<0.05*).

Heavy metals

The concentrations of heavy metals in Group A, B, and C were below safety limits as shown in Table 3. There were significant differences in Hg and Pb levels between groups. The heavy metal content of the shrimps varied depending on the processing step. The mean heavy metal contents detected in Group A and B were Hg> Pb>Cd, while it is Pb>Hg>Cd in smoked and marinated shrimp. Heavy metals are not essential for human life and have properties that can threaten human health. The limit values with regard to Pb, Cd, and Hg were detailed by the Turkish Food Codex (2011) and The European Commission Regulation (EU) (2014) as shown in Table 3. In the present study, heavy metal content not exceeded the limit values in the groups. Abdennour et al. (2000) reported that the Pb and Cd contents of *P. longirostis* in northeast Algeria as between 0.55-2.3 and between 0.46-0.89 mg kg $^{-1}$, respectively. The Pb and Cd contents of all groups in this study were lower compared to previous studies. The Cu and Cd content of the deep-water pink shrimp were reported as 1.33 and 0.23 mg kg -1 by Gökoğlu et al. (2008) and 2.2 and 0.78 mg kg -1 by Öksüz et al. (2009), respectively. While the processing methods did not affect the Cd content of the shrimp ($p>0.05$), it caused an increase in Pb content. The Pb concentration in shrimp meat ranged from 0.54 to 1.16 mg kg -1 (Sarkar et al., 2016). The heavy metal contents of all groups in the present study were lower than those found in these studies. Smoke components were thought to increase the Pb content of the product. It was reported by Bello (2013) that different processing methods such as smoking, boiling and sun-drying cause increases and decreases in the mineral content of shrimp. Depending on the processing methods, some nutritive elements in foods may decrease or increase during the process, while changes in antinutrients may occur (Bradbury & Holloway, 1998).

Table 3. The mean heavy metal concentration detected in groups (mean concentration ± sd mg kg $^{-1}$ ww)

	Cd	Hg	Pb
Group A	0.01±0.00a	0.06±0.00c	0.05±0.00c
Group B	0.01±0.00a	0.09±0.00a	0.08±0.00b
Group C	0.01±0.00a	0.08±0.00b	0.12±0.00a
Limits value	0.05[a] / 0.25[b]	0.5[a] / -[b]	0.3[a] / -[b]

[a] Turkish Food Codex (2011) WHO (2008)
[b] EU (2014)

n=6, Different superscripts in the same column mean significantly different at the 5% probability level (*P<0.05*).

Health Risk through the consumption of shrimp

Metal Pollution Index (MPI)

The Metal pollution index values are shown in Fig. 1. If the MPI value is less than 2, contamination could not be detected; if it is between 2 and 5, it is very low; if it is between 5 and 10, there is low contamination (Jamil et al., 2014). In the present study, the MPI values were less than 2 in the shrimp and the processed shrimps. The highest MPI value (0.93) was found in the smoked shrimp.

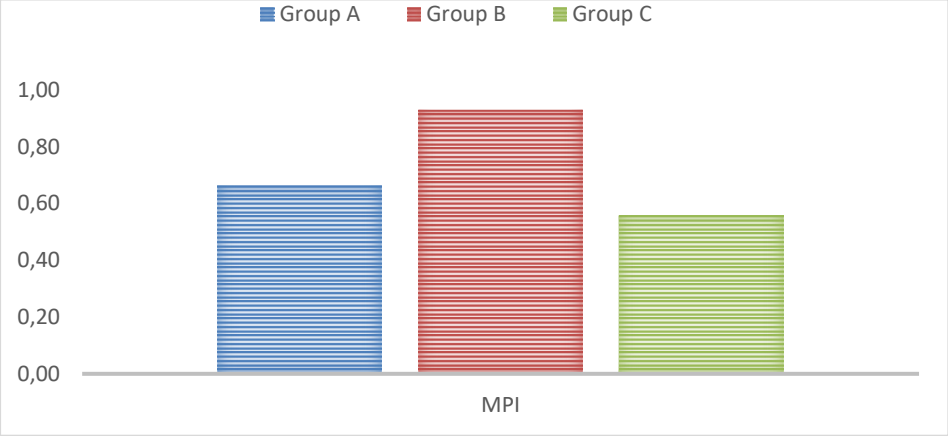

Fig. 1. Metal Pollution index (MPI) values of groups

Estimated Daily Intake (EDI) and Hazard Quotient (HQ)

The EDI values of trace metals resulting from the average shrimp consumption of adults in Turkey are presented in Table 4. The results revealed that the EDI values for the examined and processed shrimps were below the recommended values, indicating that health risks associated with the intake of these heavy metals (Hg, Cd, Pb) through the consumption of ready-to-eat food (smoked marinated shrimp (Group C) were non-existent. The fact that the HQ was above 1 indicates that there is a health risk in the food with regard to the specified metal (Yee 2010). The HQ values of shrimp and processed shrimp did not exceed the recommended limit.

Table 4. The EDI and HQ for Hg, Cd and Pb through the consumption of groups

		Group A		**Group B**		**Group C**	
	RfDo	**EDI**	**HQ**	**EDI**	**HQ**	**EDI**	**HQ**
Hg	0.0003[c]	1.46E-05	0.048571	2.186E-05	0.072857	1.94E-05	0.064762
Cd	0.001[a]	2.43E-07	0.000243	2.429E-07	0.000243	2.43E-07	0.000243
Pb	0.0035[b]	1.21E-05	0.003469	1.943E-05	0.005551	2.91E-05	0.008327

[a] US EPA (2017)
[b] Bat et al. (2021)
[c] US EPA (2018)

n=6, Different superscripts in the same column mean significantly different at the 5% probability level (*P<0.05*).

4. Conclusion

In conclusion, this study examined changes in mineral concentration in smoked and marinated products, produced from deep-water pink shrimp caught from the Marmara Sea, to determine whether they it poses a risk to public health. It found that the mineral content of the shrimp changed as a result of the smoking and marination processes. In general, it was determined that the macro and micro element content increased proportionally with the smoking process, and then decreased as a result of the marination process. It was determined that while the Cd content of deep-water pink shrimp did not change depending on the processing methods., the Pb content increased. In addition, it was found that the consumption of both shrimp and the final products considered in the study does not pose a risk to human health. However, considering the Na/K ratio of the final product, the Na content was quite high. Reduced sodium salts can be used in the marination process to reduce the Na/K ratio of the final product. For this reason, it can be recommended that individuals with heart and high blood pressure problems may be recommended to consume this product in limited quantities.

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