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THE EFFECT OF DEPURATION ON BACTERIAS, MINERAL MATTERS, FATTY ACIDS AND AMINO ACIDS IN CARPET SHELL (*RUDITAPES DECUSSATUS* LINNAEUS, 1758) AND THE EVALUATION OF QUALITY INDEXES IN TERMS OF HUMAN HEALTH

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ABSTRACT

In this study, we have evaluated the effect of depuration on the bacterias, the mineral matters, the fatty acids and the amino acids in the carpet shells and the quality indexes in terms of human health. The carpet shells that underwent the depuration process for a total of 72 hours in a closed loop system were analyzed every 12 hours. There was a significant decrease in the total number of bacteria depending on the duration of depuration. *E. coli*, *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Vibrio parahaemolyticus* and *Vibrio cholera* could not be determined in the carpet shells during depuration. 48-hour processing time was determined to be significant for a decline in Co, Fe, Al, Cu, and Mn levels due to depuration in the carpet shells. Zn, Cu, Ni and Cr levels were found to remain in the national and international limit values during the study. Al and Mn levels were envisaged to be appropriate for all the thresholds specified in terms of human health. The values of total essential amino acids (∑EAA) and non-essential amino acids (∑NEAA) at the end of depuration process were higher than their initial values and the EAA/NEAA ratio was determined between 0.82-1.09. It was found in the carpet shells during depuration that polyunsaturated fatty acids (PUFA) were between 57.64-58.88%, saturated fatty acids (SFA) were between 23.72-25.46% and monounsaturated fatty acids (MUFA) were between 8.51-9.75%. EPA, DHA and omega-3 levels which play an important role in human nutrition were found to be high. Moreover, the quality indexes of the fatty acid (AI, TI, FLQ, w6/w3, h/H) were determined to be at appropriate values for human health.

KEYWORDS:
Carpet shell, Depuration, mineral matter, Fatty acid, Amino acid, microbiology

INTRODUCTION

Environmental pollution which occurs due to population growth causes the contamination of life sources and the deterioration of the ecosystem. When water environment which constitutes a large part of the ecosystem is used as a receiver and remover for used waters and other wastes, it suffers from more pollution compared to air and soil in the ecosystem [1]. Bivalve molluscs which live in the sedimentary structure in the marine environment have a high nutritional value. They contain the large amounts of the protein, the vitamin A, B, C, D, the various minerals and the fatty acids. However, there can be harmful and toxic substances in their bodies because they have water filtration feature [2].

Today, nutrition is very important for human health and so we should use water resources effectively. However, all adverse effects that can harm human health should be removed and minimized. Bivalve molluscs can concentrate contaminants on their own tissues in higher concentrations compared to the sea water in their environment [3]. Bivalves that are grown in contaminated waters accumulate the microorganisms including bacteria and viruses in their body. Then the consumption of these products as raw or undercooked may pose a significant risk to human health [4, 5]. At this point, the depuration process should be implemented in order to remove the accumulations which occur due to exposure to the contamination factors with a negative impact on human health [6].

The Regulation (EC) No. 854/2004 [7] and the Regulation (EC) No. 629/2008 [8] were adopted by the European Union and Turkey in terms of the depuration process and product safety in bivalve molluscs. According to the Regulation (EC) No. 854/2004 [7], bivalve mollusc production areas are divided into three different classes (A, B and C) in terms of microbiological quality values, and also the conditions are specified for the depuration process [6]. In addition, according to the Regulation (EC)

No. 629/2008 [8], the threshold limit values of the mineral substances are specified for human consumption of bivalve molluscs.

Bivalves are highly valued because they are used in human nutrition in many countries with a coastline all over the world [9]. According to FAO records, the production of carpet shell was reported to be 4002 tons across the globe in 2014. The total production of carpet shell was 83.4 tons in Turkey in 2013. It is collected from the Western Black Sea and the Aegean coasts in Turkey and marketed especially to the European countries [10, 11].

In the literature, the effects of the depuration process on bacteria and heavy metal content of shellfish were studied in general. However, the changes in the amino acids and the fatty acids of the products due to the depuration process were not demonstrated. The purpose of this study was to determine the effects of the depuration process on the microbiological, chemical and nutritional aspects of carpet shells and safe intake levels for human. It was also aimed to determine the effects of the depuration process on the mineral matters, the fatty acids and the amino acids and to identify safe intake levels for human health and to evaluate important indexes (AI, TI, FLQ, ω-6/ω-3, h/H, ΣEAA/ΣNEAA).

MATERIALS AND METHODS

Carpet shell (*Ruditapes decussatus*) used in the study were collected by hand from İzmir Bay, the Aegean Sea (Turkey). The dead or damaged products were removed. The live carpet shell, with an average height of 41.09±2.58 mm, width of 27.17±1.63 mm and weight of 12.02±2.18 g, were placed in purification pools of 450 dm^3 of water by 2 repetitions in amounts of 50 kg. After the sea water supplied with deep water pump passed through a carbon filter and UV lamp (40000 $\mu Ws/cm^2$) in a closed circuit purification system, it was transferred to the pools where the products were placed. The sea water temperature and flow rate of the closed-circuit purification system was set as 16 °C and 2.64 L/s, respectively. A purification process was carried out for the samples taken at the beginning and every 12 hours for a total of 72 hours.

The preparation process for the mineral matter analysis of all samples was done according to FAO Technical Report 158 [12, 13, 14]. The concentration of the metal analysis was calculated by the dry weight, and it was expressed in wet weight units.

Total bacterial analysis was performed using Plate Count Agar (PCA), and total coliform bacteria and *E. coli* counts were performed using Violet Red Bile Agar (VRB) [15, 16]. *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Vibrio cholera* analyses were carried out according to the FDA [17], Pal and Marshall [18], Halkman [16] and THSK [19]. *E. coli*, *S. aureus*, *Salmonella* spp, *L. monocytogenes*, *V. parahaemolyticus* and *V. cholera* colonies, which were identified as suspected, were selected and purified. Biochemical tests for bacteria identification were carried out in accordance with Arda [20]. In addition, DNA isolation, DNA sequence analyses following the increase of the target gene sequence were performed by MACROGEN INC. (Seoul-KOREA) in exchange for the purchase of services.

Crude fat content was determined using a solvent extractor Velp SER 148/6 (Velp Scientifica, Milano, Italy) with petroleum ether (130 °C). Crude protein, crude ash and dry matter content was determined according to the AOAC [21] Method 2.507, AOAC [21] Method 7.009 and AOAC [22] Method 985.14, respectively.

Amino acid samples were sent to TUBITAK-MAM Food Institute for analysis. In amino acid analysis, an in-house method was created by modifying those of Dimova [23] and Gheshlaghi et al. [24], and the sample analysis was carried out. The analysis process was performed using a UFLC (Ultra Fast Liquid Chromatography) device and a UV detector.

Analysis of fatty acid methyl esters (FAME %) was done according to Tufan et al. [25]. The determination of fatty acids was conducted on gas chromatography-mass spectrometry (GC-MS) equipment (QP2010 Ultra with AOC-20i+s model auto sampler) using a mass selective detector (GC-MS QP 2010 PLUS) equipped with GC/MS solutions software (Shimadzu, Kyoto, Japan). FAME mix standards were separated on a Restek RT-2560 column (USA Cat no: 13199 Serial no: 47623-07; 100 m × 0.25 mm internal diameter and the fatty acid content (%) in the carpet shell was calculated as mg/100g according to Weihrauch et al. [26].

Lipid quality indices as atherogenicity index (AI), thrombogenicity index (TI), fish lipid quality (FLQ) and hypocholesterolemic/hypercholesterolemic ratio (h/H) ratio were calculated as follows [27, 28, 29].

Statistical analysis was done by one-way analysis of variance (ANOVA) using the JMP 5.0.1 (SAS) package program. For statistical testing of differences according to depuration time, Tukey's test was used [30].

RESULTS AND DISCUSSION

In this study, the amounts of cobalt (Co), aluminium (Al), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni), cadmium (Cd), chromium (Cr), lead (Pb) obtained from the carpet shells were shown in Table 1. The values of Co was decreased at 24 (1.14 mg/kg) and 48 (1.11 mg/kg) hours, and also it was determined to be different from other groups ($P<0.05$). Although the value of Al

which was initially measured as 20.02 mg/kg showed instability during the depuration process, it was significantly lower especially after 48 hours and was measured as 11.43 mg/kg at 72 hours ($P<0.05$). The value of Fe was initially measured as 87.29 mg/kg and showed an irregular distribution during the depuration process. However, it was measured as 75.98 mg/kg at 60 hours and this value was significant as the lowest value ($P<0.05$). When the distribution of Cu and Mn values was analyzed during the depuration process, they showed an increase during the first 24 hours and then a steady decrease. Their values were respectively 10.76 mg/kg and 1.05 mg/kg at the end of depuration and they showed significant differences from other depuration durations ($P<0.05$). The value of Cr was initially measured as 0.36 mg/kg and showed an irregular distribution during the depuration process. However, it showed a significant decrease especially at 60 hours and the difference was detected with the value of 0.32 mg/kg ($P<0.05$).

The values of Zn, Ni, Cd and Pb showed an irregular distribution during the depuration process and their values were determined to be increased at the end of depuration duration. The minimum and maximum values of Zn, Ni, Cd and Pb were 33.9-44.41, 2.88-3.39, 0.94-1.16 and 0.92-1.59 mg/kg, respectively. In the overall assessment which was made in terms of the effects of the depuration process on the mineral matters, the value of Co showed a significant decrease at 24 and 48 hours and the value of Fe showed a significant decrease at 48 and 60 hours. The values of Al, Cu and Mn were decreased after 48 hours. The values of Zn, Ni, Cd, Cr and Pb were unaffected. The critical time for the depuration process in terms of the mineral matters in the carpet shells was shown to be important in the applications for 48 hours and more.

The limit values of the mineral matters which are specified by the various national and international organizations were shown in Table 2. According to national and international limit values, the values of Zn, Cu, Ni and Cr were determined not to exceed the limit values in all the references (Seafoods Quality Control Handbook [31], Turkish Food Codex [32], FDA [33]). While all values of Cd were found to be below the limit values in Codex Standard 193 [34], FSANZ [35] and FDA [33], they were found to be above the limit value (0.1 mg/kg) in Seafoods Quality Control Handbook [31]. According to the other reference value specified as 1 mg/kg (Regulation EC [8, 36], Turkish Food Codex [32, 37]), it exceeded the limit values at depuration times except 0 and 24 hours. In the study period, all values of Pb were found to be below the limit values in FSANZ [35] and FDA [33]. During the depuration process, only the value of 1.59 mg/kg at 12 hours was found to be above the limit values in Turkish Food Codex [32, 37] and Regulation EC [8]. The values of Pb at 12, 24, 36, 60 and 72 hours were found to be above the limit values in Seafoods Quality Control Handbook [31] and Regulation EC [36]. The amounts of cadmium and lead obtained from the carpet shells during the depuration process were evaluated according to the limit values and also they could not be expressed to be completely safe.

The limit values for consumption of the mineral matters which are defined by the international organizations were shown as mg/day and mg/kg/day in Table 3.

When ATSDR [38, 39] values were taken into account, only Al value was found between consumable values in this study. The value of Cd (0.07) which is defined by EPA [40] was significantly lower than the results obtained during the depuration process, and also it was observed not to be suitable for consumption. When the amounts which are specified for the consumption of the mineral matters by China Nutrition Institute [41] were analyzed, only Mn value was found to be positive according to the study results.

TABLE 1
Mineral matter contents of carpet shells during the depuration periods (mg/kg)

Mineral Matters	Depuration Hours						
	0/R	12	24	36	48	60	72
Co	1.23±0.00^{a}	1.21±0.01^{a}	1.14±0.00^{b}	1.31±0.01^{c}	1.11±0.00^{b}	1.27±0.01^{e}	1.43±0.01^{d}
Al	20.02±0.51^{a}	21.97±0.40^{b}	21.97±0.21^{b}	20.30±0.16^{a}	14.88±0.16^{c}	13.82±0.04^{c}	11.43±0.06^{d}
Fe	87.29±0.80^{a}	104.75±1.04^{b}	95.41±1.76^{c}	105.26±0.98^{b}	85.14±0.32^{a}	75.93±1.14^{d}	94.54±1.56^{c}
Zn	33.90±0.34^{a}	35.48±0.25^{b}	35.83±0.20^{bc}	35.92±0.11^{bc}	36.39±0.34^{c}	34.27±0.04^{a}	44.41±0.02^{d}
Cu	11.39±0.01^{a}	13.39±0.02^{b}	15.60±0.14^{c}	14.18±0.08^{d}	12.21±0.03^{e}	11.63±0.06^{a}	10.76±0.08^{f}
Mn	1.47±0.00^{ac}	1.67±0.01^{b}	1.65±0.01^{bc}	1.51±0.01^{abc}	1.44±0.01^{a}	1.11±0.12^{d}	1.05±0.03^{d}
Ni	2.90±0.02^{a}	2.88±0.00^{a}	3.07±0.01^{b}	3.29±0.03^{c}	3.13±0.02^{d}	3.18±0.01^{d}	3.39±0.01^{e}
Cd	0.95±0.01^{a}	1.06±0.02^{bc}	0.94±0.01^{a}	1.16±0.01^{d}	1.09±0.01^{ce}	1.03±0.00^{b}	1.11±0.01^{e}
Cr	0,36±0.01^{a}	0.46±0.01^{b}	0.35±0.00^{a}	0.44±0.01^{c}	0.35±0.01^{a}	0.32±0.00^{d}	0.51±0.01^{e}
Pb	0.92±0.08^{a}	1.59±0.01^{b}	1.04±0.10^{ac}	1.19±0.09^{ac}	0.98±0.09^{a}	1.04±0.11^{a}	1.40±0.11^{bc}

R: Raw material. Different letters (a,b,c…) in the same line indicate statistical difference between the depuration times ($P<0.05$). Co: Cobalt, Al: Aluminum, Fe: Iron, Zn: Zinc, Cu: Copper, Mn; Manganese, Ni: Nickel, Cd: Cadmium, Cr: Chrome, Pb: Lead

TABLE 2
Limit values of certain metals in shellfish in National and International standards (mg/kg)

References	Zn	Cu	Ni	Cd	Cr	Pb
Seafoods Quality Control Handbook [31]	50	20	-	0.1	-	1
Turkish Food Codex [32, 37]	50	20	-	1	-	1.5
Codex Standard 193 [34]	-	-	-	2	-	-
Regulation EC [8]	-	-	-	1	-	1.5
FDA [33]	-	-	80	4	13	1.7
FSANZ [35]	-	-	-	2	-	2

Co: Cobalt, Al: Aluminum, Fe: Iron, Zn: Zinc, Cu: Copper, Mn: Manganese, Ni: Nickel, Cd: Cadmium, Cr: Chrome, Pb: Lead.

TABLE 3
Consumable limit values of certain metals in international standards limit values (wet weight)

References	Co	Al	Fe	Zn	Cu	Mn	Cd	Cr	Pb
ATSDR (MRLs) [38, 39] Or=Oral (mg/kg/day)[a]	0.7	70	-	21	0.7	-	0.035	0.35	-
EPA (2000) [40] (mg/kg/ day)[a]	-	-	-	-	-	-	0.07	-	-
China Nutrition Institute [41] (mg/day)	-	-	12	12	1.0	3.5	-	-	-
WHO [42, 43, 44, 45] (mg/kg/day)[a]	-	70	56	70	35	-	0.07	-	0.25
FDA [46] (mg/day)	-	-	18	15	2.0	2.0	-	0.12	-
FSANZ [35] (mg/kg/day)[a]	-	-	-	-	-	-	0.07	-	0.25

Co: Cobalt, Al: Aluminum, Fe: Iron, Zn: Zinc, Cu: Copper, Mn: Manganese, Ni: Nickel, Cd: Cadmium, Cr: Chrome, Pb: Lead. [a] These amounts are calculated in mg/kg/day. They have been multiplied by 70 kg in order to provide amounts in mg/day.

The carpet shells were found to remain within the consumption limits according to the values of Al, Zn and Cu of WHO [42, 43, 44, 45] during the study period. The values of the mineral matters (except Mn) obtained in the study were found to be outside the standards which are brought by FDA [46] and FSANZ [35]. When the existing international values were evaluated as a whole, the values of Al and Mn were considered to be suitable for consumption of carpet shells that underwent the depuration process.

In a study of Cheung and Wong [47], they found that when the amounts of Cd, Cr, Cu, Pb, Zn and Ni in carpet shells (*Tapes philippinarum*) were analyzed during the depuration process (7 days), some were decreased, some were increased and the others remained unchanged. In another study, it was reported that the amounts of Fe, Ni, Co, Cu and Mn in carpet shells (*Ruditapes decussatus*) sampled from 2 stations were significantly decreased during 48-hour depuration and it was found that the levels of Fe, Ni, Co, Cu and Mn were decreased between 46.8-47.7%, 19.9-20.3%, 27.3-27.9%, 35.9-36.6% and 18.2-26.6%, respectively [3]. Angelo et al. [48] stated that during the depuration process (24 hours) in freshwater mussels (*Corbicula fluminea*, *Quadrula pustulosa*), Al value was decreased in all samples, Cd value did not show a significant change, Pb value was decreased in *C. fluminea* but was unchanged in *Q. pustulosa* and Zn value was decreased in *C. fluminea* but was increased in *Q. pustulosa*. El-Gamal [49] reported that in *Paphia undulata*, the rates of Zn (44%), Pb (23%), Ni (25%), Mn (17%), Cu (61%), Cr (41%) and Cd (75%) after 48-hour depuration process were significantly decreased compared to their initial values. However, it was explained that only the rates of Cu (27%), Cr (15%), Co (23%) Cd (52%) were decreased during 3-days depuration process. 2 and 7 days depuration processes were administered to *Ruditapes philippinarum* ve *Ruditapes decussatus* at 20° C and 34% salinity by Freitas et al. [50]. Consequently, they stated that in *Ruditapes decussatus*, 48-hour depuration process was effective for Cr, Ni, Cu, Zn, Cd and Pb and there was an increase in all of those metals at the end of 168-hour depuration process and the levels of Cu, Cd, Ni and Zn (insoluble fractions of the clam) were significantly increased compared to their initial values. Figueira and Freitas [51] examined that in *Ruditapes philippinarum* and *Ruditapes decussatus*, the metals have the effects on human health in terms of the depuration process and consumption. They stated that there were decreases and increases in the values of As, Hg, Cr, Ni, Cu, Zn, Cd and Pb depending on the species during 48-hour depuration process. They explained that all the metals except As were in accordance with the limit values in terms of consumption. According to these results, it was found that some metals can be

TABLE 4
Microbiological counts of carpet shells during the depuration periods (log kob/g)

Microbiological Count	Depuration Hours						
	0/R	12	24	36	48	60	72
TAMB	2.78±0.02	1.84±0.03	1.60±0.01	<1.47	<1.47	<1.47	<1.47
TAPB	3.11±0.05	2.98±0.02	2.63±0.04	2.47±0.02	1.72±0.01	1.60±0.03	<1.47
Total Coliform	<1.47	<1.47	<1.47	<1.47	<1.47	<1.47	<1.47

R: Raw material. TAMB: total aerobic mesophilic bacteria. TAPB: total aerobic psychrophilic bacteria.

TABLE 5
Biochemical contents of carpet shells during the depuration periods (%)

Depuration Hours	Crude Protein	Crude Fat	Dry Matter	Crude Ash
0/R	6.31±0.05^{a}	0.33±0.02^{a}	13.46±0.50^{a}	2.04±0.07^{a}
12	7.99±0.08^{b}	0.36±0.02^{a}	13.56±0.28^{a}	2.23±0.11^{a}
24	7.04±0.04^{c}	0.36±0.00^{a}	13.15±0.09^{a}	2.09±0.15^{a}
36	7.36±0.04^{d}	0.22±0.00^{b}	13.08±0.63^{a}	2.42±0.32^{a}
48	7.19±0.04^{c}	0.28±0.01^{c}	13.21±0.67^{a}	1.97±0.12^{a}
60	8.07±0.05^{b}	0.21±0.03^{b}	13.10±0.38^{a}	2.29±0.13^{a}
72	8.73±0.00^{e}	0.28±0.02^{c}	12.04±0.03^{b}	1.94±0.16^{a}

R: Raw material. Different letters (a,b,c…) in the same column indicate statistical difference between the depuration times (P<0.05).

effectively removed from carpet shell within a certain depuration period.

Total aerobic mesophilic bacteria (TAMB) and total aerobic psychrophilic bacteria (TAPB) counts were shown in Table 4. TAMB and TAPB counts were found 2.78 log kob/g and 3.11 log kob/g in the raw sample, respectively. There was a decrease in TAMB and TAPB counts during the depuration process, and also the bacteria counts were found to be <1.47 log kob/g at the end of depuration process. Total coliform counts were found to be <1.47 log kob/g during the entire depuration process.

As a result of the analyses, *E. coli* was not found in the raw and depurated samples. DNA sequence analyses were performed in order to diagnose species of suspicious (*Salmonella* spp., *S. aureus*, *L.monocytogenes, V. parahaemolyticus* and *V. cholera*) sample in the another bacteria seedings. As a result of DNA sequence analyses, no bacteria were included among suspicious species and the samples were found not to contain any microbiological pathogens.

In the literature, the effects of the depuration process have been demonstrated on *Tapes decussatus, Ruditapes philippinarum, Donax trunculus Polymesoda solida, Mytilus galloprovincialis*, *Chamelea gallina, Crassostraea brasiliana, Crassostrea gigas* and other bivalve species, Norovirus, *E. coli, V. cholerae* O1, *V. parahaemolyticus, V. vulnificus, Salmonella typhimurium*, thermotolerant coliform bacteria strains, total coliform, fecal coliform, mesophilic aerobic bacteria and total bacteria. In the previous studies, the depuration process was found to be effective in total bacteria and Norovirus [52, 53]. In the studies in which the depuration process was applied under different conditions, the effective depuration duration was reported as 44 hours [4], 72 hours [54], 72-84 hours [55] and 66-78 hours [56] according to the bacterial species. In a depuration study conducted by Çolakoğlu et al. [56], they found that bacteria counts were decreased by 40% within first 12 hours. In another study, the depuration process which was performed for total bacteria and *E.coli* reduced the number of microorganisms, and also it was reported to show the positive effects in terms of microbial quality during the transportation of products [57]. As it is seen in other studies, the reducing effect of the depuration process on the microorganisms in our study was found to be similar to declines in total mesophilic bacteria and total psychrophylic bacteria counts. However, the duration and effectiveness of the depuration process were observed to be consistent with other studies.

Before the carpet shells underwent the depuration process in this study, the rates of protein, lipid, dry matter and ash were found to be 6.31%, 0.33%, 13.46% and 2.04%, respectively (Table 5). The rate of protein was increased during the depuration process and was found as 8.73% at the end of 72 hours (p<0.05). The rate of lipid was found to show a significant decline (0.22%) at 36 hours during the depuration process (p<0.05). It was found as 0.28% within 72 hours of the depuration process. The rate of dry matter showed a decline during the depuration process and was found as 12.04% at 72 hours. This value was different from other depuration durations (p<0.05).

TABLE 6
Amino acid contents of carpet shells during the depuration periods (mg/100g)

Amino Acids (mg/100g)	Depuration Hours						
	0/R	12	24	36	48	60	72
Histidine*	159.49±16.74^{a}	265.67±2.82^{be}	229.72±1.24^{cd}	246.61±8.11^{bc}	206.75±2.18^{d}	227.41±1.25^{cd}	288.78±5.37^{e}
Isoleucine*	293.91±0.00^{a}	393.51±2.82^{b}	342.39±2.47^{c}	364.18±1.35^{d}	335.58±3.27^{c}	364.57±2.50^{d}	438.87±5.37^{e}
Methionine*	139.24±0.43^{ad}	131.84±2.82^{a}	117.04±0.00^{b}	162.49±2.70^{c}	141.18±1.09^{d}	189.37±1.25^{e}	218.49±2.69^{f}
Leucine*	414.40±0.98^{a}	567.29±8.47^{b}	481.27±1.24^{c}	515.20±6.76^{d}	513.79±8.73^{d}	586.67±8.76^{b}	665.91±12.09^{e}
Lysine*	753.18±1.52^{a}	853.93±12.71^{b}	782.61±4.94^{c}	878.42±4.06^{b}	875.61±3.27^{b}	979.55±8.76^{d}	1140.88±9.40^{e}
Phenylalanine*	250.73±1.97^{a}	347.57±2.82^{b}	300.47±2.47^{c}	310.65±1.35^{d}	291.61±2.18^{e}	331.83±1.25^{f}	389.48±2.69^{g}
Threonine*	267.44±0.00^{a}	393.51±2.82^{b}	336.28±1.24^{c}	329.77±1.35^{c}	310.13±2.18^{d}	364.57±2.50^{e}	432.23±4.03^{f}
Valine*	330.13±1.97^{a}	441.45±2.82^{b}	382.57±2.47^{c}	402.41±1.35^{d}	385.73±2.18^{c}	421.20±0.00^{e}	505.37±5.37^{f}
Alanine	369.82±0.98^{a}	474.41±18.36^{b}	422.75±2.47^{c}	436.82±1.35^{c}	437.42±3.27^{c}	504.38±2.50^{d}	549.07±5.37^{e}
Arginine	280.68±0.98^{a}	347.57±2.82^{b}	354.62±2.47^{b}	386.16±2.70^{c}	392.67±3.27^{c}	502.61±2.50^{d}	522.47±5.37^{e}
Aspartic acid	189.44±1.97^{a}	169.79±2.82^{b}	238.45±6.18^{c}	241.83±4.06^{c}	369.53±3.27^{d}	532.69±0.00^{e}	601.32±4.03^{f}
Glutamic acid	562.05±0.98^{a}	474.41±1.41^{b}	625.39±0.00^{c}	690.12±2.70^{d}	891.81±6.55^{e}	1115.82±6.26^{f}	1262.48±9.40^{g}
Glycine	494.49±0.00^{a}	643.20±2.82^{b}	549.40±1.24^{c}	560.12±0.00^{c}	529.22±4.36^{d}	586.67±3.75^{c}	666.86±5.37^{f}
Proline	241.67±0.98^{a}	330.59±4.24^{b}	289.11±1.24^{c}	289.62±1.35^{c}	263.84±2.18^{d}	303.51±1.25^{e}	342.93±4.03^{f}
Serine	263.26±0.00^{a}	363.55±2.82^{b}	316.19±0.00^{c}	309.69±10.81^{c}	298.55±1.09^{c}	361.91±1.25^{b}	435.08±5.37^{d}
Tyrosine	252.12±1.97^{a}	319.60±2.82^{b}	283.87±3.71^{c}	309.69±0.00^{b}	290.84±3.27^{c}	342.44±1.25^{d}	402.78±5.37^{e}
ΣEAA	2608.51±18.61^{a}	3394.75±32.49^{b}	2972.35±13.59^{c}	3209.72±8.11^{d}	3060.38±18.55^{c}	3465.15±26.28^{b}	4080.02±47.02^{e}
ΣNEAA	2653.54±1.97^{a}	3123.09±12.71^{b}	3079.78±17.29^{b}	3224.06±6.76^{c}	3473.88±25.09^{d}	4250.02±18.77^{e}	4782.98±44.33^{f}
ΣEAA/ΣNEAA	0.98	1.09	0.97	1.00	0.88	0.82	0.85

R: Raw material, EAA*: Essential amino acid, NEAA: Non-essential amino acid. Different letters (a,b,c…) in the same line indicate statistical difference between the depuration times (P<0.05).

The rate of crude ash showed an irregular distribution during the depuration process and was found to be between 1.94-2.42%. There was no statistical difference (p>0.05). The biochemical changes have been found to be an important indicator in carpet shells depending on the depuration process and conditions because there were no food components in the depuration medium.

In a study performed in *Tapes decussatus* by Çelik [58], the rates of protein, lipid, dry matter and ash were found to be 10.76%, 1.13%, 18.17% and 1.5% in a fresh material, respectively. Dincer [59] stated that the rates of protein, lipid, dry matter and ash were respectively 8.71%, 0.78%, 14.09% and 3.1% in *Ruditapes decussatus*. Çağlak et al. [60] found that the rates of protein, lipid, dry matter and ash were found to be respectively 10.54%, 0.34%, 18.41% and 3.16% in the nutritional analysis made in carpet shells before receiving the depuration process. When the data of this study were compared with the results of other studies, the similar results have been reached.

The amounts of the amino acids were shown in Table 6. The amounts of histidine, isoleucine, methionine, leucine, lysine, phenylalanine, threonine, valine, alanine, arginine, aspartic acid, glutamic acid, glycine, proline, serine and tyrosine were found to be 159.49, 293.93, 139.24, 414.40, 753.18, 250.73, 267.44, 330.13, 369.82, 280.68, 189.44, 562.05, 494.49, 241.67, 263.26 and 252.12 mg per 100g of fresh meat of carpet shell, respectively. The highest amounts of all the amino acids were detected at 72 hours during the depuration process, and also it showed a significant difference from other groups (p<0.05). The values of all the amino acids except methionine, aspartic acid and glutamic acid were found to be higher in the depuration process than that in the fresh material. The total amounts of essential and non-essential amino acids were observed to reach to the maximum values at 72 hours during the study. It is considered that the increase in the amounts of amino acids occurred due to the changes in protein content.

In a study performed by Allen [61], the value ranges of alanine, glycine, glutamic acid and aspartic acid were found to be respectively 3.2-249.2, 3.0-58.2, 3.2-35.8 and 1.1-17.4 µmol/gm at different salinity levels (3%, 6%, 10%, 17%, 20%, 25%) in *Rangia cuneata* and it was stated that there was a change in the amounts of the amino acid depending on the salinity. Hosoi et al. [62] investigated the effect of artificial sea salt on the amounts of the amino acids in clams. According to the results of this study, they reported that artificial sea salt caused the changes in the amounts of taurine, glycine, alanine, aspartic acid, glutamic acid, β-alanine, proline and arginine amino acids depending on its use at different rates and times and that these changes occurred as a result of the adaptation depending on the salt content and duration. When the data of this study were

compared with the past studies, the similar results were found in terms of the changes in the amino acid content depending on various factors. It was observed that the amounts of the amino acids were increased depending on the depuration process and conditions. The World Health Organization recommended methionine, phenylalanine, lysine, histidine, valine, leucine, isoleucine and threonine requirements for adults of 10, 25, 30, 10, 26, 39, 20, and 15 mg amino acid/kg body weight per day, respectively [63]. When the results of our study are evaluated according to the reports of WHO [63], it is clear that if a person whose weight is 70 kg consumes 300 g meat of carpet shell, the person receives only lysine amino acid; if a person whose weight is 70 kg consumes 500 g meat of carpet shell, this person receives histidine, isoleucine, methionine and threonine amino acids; and if one consumes 700 g carpet shell, his/her all amino acid needs could be met.

The values of the fatty acids depending on the depuration process in the carpet shells were shown in Table 7. Palmitic acid (C16:0) had the highest value of unsaturated fatty acids. The minimum and maximum rates of palmitic acid were found to be 12.04% and 12.83% respectively and there was not a significant difference between all groups (P>0.05). The total rate of unsaturated fatty acids (∑SFA) was found to be 23.51% at 0 hours and 25.62% at 72 hours, respectively. There was no significant difference between the durations of depuration (P>0.05). The rates of palmitoleic acid (C16:1), oleic acid (C18:1n9c), elaidic acid (C18:1n9t) and cis-11-eicosenoik acid (C20:1) which are monounsaturated fatty acids (MUFA) were found to be respectively 1.04%, 3.14%, 0.20% and 2.27% for the fresh sample. These values were found to be respectively 0.96%, 3.31%, 0.23% and 2.37% at the end of the depuration process. The total amount of MUFA was found to be 6.65% in the fresh sample and 6.87% at the end of the depuration process. The depuration process had no effects on the total values of monounsaturated fatty acids (MUFA) (P>0.05). The total rate of polyunsaturated fatty acids (∑PUFA) was found to be respectively 53.40% for the fresh sample and 49.61% at the end of the depuration process. The changes in the ratio of ∑PUFA were not statistically difference (P>0.05). Docosahexaenoic acid (C22:6n3, DHA) and eicosapentaenoic acid (C20:5n3, EPA) had the highest value of polyunsaturated fatty acids. The rates of DHA and EPA were found to be respectively 25.07% and 9.29% for the fresh sample and 21.84% and 8.48% at the end of the depuration process (P>0.05). The EPA+DHA amounts in carpet shell were determined between 32.30-35.59% (Table 7). There were no differences among the depuration hours (p>0.05). The depuration process and time had no effects on PUFA.

In two different studies on *Ruditapes decussatus*, the amounts of the fatty acids were analyzed [64, 65]. In analogy with present study, in a study of Albentosa et al. [64], the values of palmitic acid (C16:0) and stearic acid (C18:0) were reported to be higher than other saturated fatty acids. The total value (1.95%) of eicosenoic acid (C20:1) among ∑MUFA was parallel to the data of our study. Especially, the values of EPA (1.66%) and DHA (5.11%) were significantly lower than the data of present study. In a study conducted by Fernandez-Reiriz et al. [65], the rate of C16:0 (18.5%) was higher than other SFA, and also this finding was similar to the data of present study. The value of ∑MUFA (31.2%) was significantly higher than the data of present study. The rates of EPA, DHA and ∑PUFA (39.2%) were lower than the data of present study.

Fish oil and fatty acid components are biochemical changes which undergo the most changes depending on ecological factors and physiological condition of the fish. Even among the same species, the fatty acid composition varies depending on nutrition, region, season, gender and environmental conditions [66, 67]. The minimum and maximum rates of ∑PUFA/∑SFA, ∑PUFA/∑MUFA and EPA+DHA were found to be respectively 1.94-2.36%, 7.22-8.99% and 29.32-35.41%. The depuration process and time had no effects on the fatty acids (P>0.05) and an adverse effect in terms of nutrition quality. The PUFA/SFA ratio recommended in terms of health must be minimum 0.45 [68]. In this present study, the PUFA/SFA ratio was found higher (2.28-2.47) than limit value during the depuration process (Table 7). Passi et al. [69] stated that the PUFA/SFA ratio in the muscle tissue of *Tapes decussatus* was 2.64 in triglyceride (TG) and 2.99 in phospholipid (PL) fractions. In another study, The PUFA/SFA of carpet shell clam was determined as 1.51 before depuration and 1.65 after depuration [70]. The n-3/n-6 ratio has been suggested to be a useful indicator when comparing relative nutritional values of fish. An increase in the human dietary n-3/n-6 PUFA ratio is essential to help prevent coronary heart disease by reducing plasma lipids and reduce the risk of cancer [71]. The n6/n3 ratio is recommended maximum 4 by the UK Department of Health [76]. The ∑n6/∑n3 ratio and ∑n3/∑n6 was found between 0.23-0.27 and 3.69-4.42 during the depuration process, respectively (Table 7). Similarly, Anacleto et al. [72] detected that the n3/n6 and n6/n3 ratios of *Ruditapes decussatus* at different temperatures are 4.04-5.52 and 0.20-0.23, respectively.

TABLE 7
Fatty acid ratios (%) and lipid quality indexes of carpet shells during the depuration periods

Fatty Acids	Depuration Time (Hours)						
	0/R	12	24	36	48	60	72
C14:0	$0.75\pm0.09_{A}$	$0.59\pm0.12_{AB}$	$0.61\pm0.03_{AB}$	$0.63\pm0.07_{AB}$	$0.60\pm0.05_{AB}$	$0.57\pm0.03_{AB}$	$0.39\pm0.01_{B}$
C15:0	$0.31\pm0.02_{A}$	$0.29\pm0.04_{A}$	$0.33\pm0.01_{A}$	$0.33\pm0.07_{A}$	$0.34\pm0.01_{A}$	$0.29\pm0.05_{A}$	$0.37\pm0.00_{A}$
C16:0	$12.39\pm0.74_{A}$	$12.18\pm0.21_{A}$	$12.42\pm0.19_{A}$	$12.95\pm1.73_{A}$	$12.04\pm0.04_{A}$	$12.65\pm1.37_{A}$	$12.83\pm0.11_{A}$
C17:0	$1.26\pm0.11_{A}$	$1.48\pm0.11_{A}$	$1.30\pm0.07_{A}$	$1.43\pm0.18_{A}$	$1.47\pm0.06_{A}$	$1.42\pm0.02_{A}$	$1.40\pm0.10_{A}$
C18:0	$7.30\pm0.11_{A}$	$7.43\pm0.20_{A}$	$7.66\pm0.15_{A}$	$8.08\pm0.39_{AB}$	$7.68\pm0.10_{A}$	$8.28\pm0.52_{AB}$	$9.09\pm0.46_{B}$
C20:0	$0.18\pm0.05_{AB}$	$0.19\pm0.01_{AB}$	$0.18\pm0.00_{AB}$	$0.15\pm0.02_{A}$	$0.31\pm0.06_{B}$	$0.19\pm0.04_{AB}$	$0.25\pm0.01_{AB}$
C21:0	$0.28\pm0.01_{AB}$	$0.26\pm0.06_{AB}$	$0.25\pm0.03_{A}$	$0.25\pm0.06_{A}$	$0.49\pm0.11_{B}$	$0.22\pm0.04_{A}$	$0.27\pm0.05_{AB}$
C22:0	$1.09\pm0.30_{AB}$	$1.08\pm0.10_{AB}$	$1.26\pm0.04_{A}$	$1.00\pm0.07_{AB}$	$0.97\pm0.01_{AB}$	$0.70\pm0.05_{B}$	$0.73\pm0.08_{B}$
C24:0	$0.18\pm0.00_{AB}$	$0.33\pm0.06_{BC}$	$0.14\pm0.04_{A}$	$0.39\pm0.06_{C}$	$0.24\pm0.04_{ABC}$	$0.32\pm0.06_{ABC}$	$0.15\pm0.04_{AB}$
∑SFA	$23.72\pm1.39_{A}$	$23.82\pm0.39_{A}$	$24.13\pm0.47_{A}$	$25.19\pm2.09_{A}$	$24.11\pm0.25_{A}$	$24.61\pm2.00_{A}$	$25.46\pm0.47_{A}$
C16:1	$1.04\pm0.11_{A}$	$1.60\pm0.04_{B}$	$0.99\pm0.14_{A}$	$1.02\pm0.14_{A}$	$1.08\pm0.11_{A}$	$0.99\pm0.10_{A}$	$0.96\pm0.03_{A}$
C18:1n9c	0.30±0.07A	$0.16\pm0.04_{AB}$	$0.22\pm0.03_{AB}$	$0.11\pm0.02_{B}$	$0.26\pm0.01_{AB}$	$0.17\pm0.08_{AB}$	$0.25\pm0.01_{AB}$
C18:1n9t	$4.32\pm0.56_{A}$	$4.24\pm0.25_{A}$	$3.90\pm0.01_{A}$	$4.18\pm0.45_{A}$	$4.07\pm0.11_{A}$	$4.03\pm0.32_{A}$	$4.40\pm0.20_{A}$
C20:1	$3.70\pm0.03_{A}$	$3.51\pm0.04_{A}$	$3.40\pm0.57_{A}$	$3.45\pm0.08_{A}$	$3.59\pm0.13_{A}$	$3.82\pm0.36_{A}$	$4.15\pm0.16_{A}$
∑MUFA	$9.35\pm0.57_{A}$	$9.49\pm0.28_{A}$	$8.51\pm0.69_{A}$	$8.75\pm0.64_{A}$	$8.99\pm0.35_{A}$	$9.00\pm0.86_{A}$	$9.75\pm0.00_{A}$
C18:2n6c	$0.40\pm0.11_{A}$	$0.57\pm0.01_{A}$	$0.55\pm0.04_{A}$	$0.42\pm0.00_{A}$	$0.51\pm0.13_{A}$	$0.40\pm0.02_{A}$	$0.30\pm0.12_{A}$
C18:3n6	$3.02\pm0.17_{A}$	$2.88\pm0.04_{A}$	$2.90\pm0.12_{A}$	$3.17\pm0.42_{A}$	$3.35\pm0.33_{A}$	$3.44\pm0.30_{A}$	$3.81\pm0.35_{A}$
C20:2	$1.73\pm0.02_{A}$	$1.85\pm0.05_{A}$	$1.82\pm0.14_{A}$	$1.68\pm0.02_{A}$	$1.86\pm0.14_{A}$	$1.75\pm0.07_{A}$	$1.74\pm0.06_{A}$
C20:3n6	$0.27\pm0.07_{A}$	$0.16\pm0.01_{A}$	$0.21\pm0.01_{A}$	$0.27\pm0.07_{A}$	$0.26\pm0.07_{A}$	$0.30\pm0.04_{A}$	$0.15\pm0.01_{A}$
C20:4n6	$3.66\pm0.16_{A}$	$3.60\pm0.04_{A}$	$3.57\pm0.12_{A}$	$3.84\pm0.01_{A}$	$3.80\pm0.22_{A}$	$3.71\pm0.23_{A}$	$3.74\pm0.13_{A}$
C20:5n3	$9.45\pm0.35_{A}$	$9.89\pm0.13_{A}$	$9.52\pm0.04_{A}$	$9.55\pm0.24_{A}$	$9.16\pm0.21_{A}$	$9.00\pm0.45_{A}$	9.12±0.13A
C22:2	$11.45\pm1.04_{A}$	$11.06\pm0.21_{A}$	$11.02\pm0.32_{A}$	$12.11\pm0.69_{A}$	$12.15\pm0.56_{A}$	$12.03\pm0.17_{A}$	$12.79\pm0.27_{A}$
C22:4n6	$1.39\pm0.04_{A}$	$1.29\pm0.27_{A}$	$1.33\pm0.11_{A}$	$1.17\pm0.40_{A}$	$1.35\pm0.04_{A}$	$1.47\pm0.07_{A}$	$1.30\pm0.03_{A}$
C22:5n3	$2.08\pm0.12_{A}$	$1.91\pm0.22_{A}$	$2.11\pm0.01_{A}$	$2.03\pm0.12_{A}$	$1.98\pm0.18_{A}$	$1.92\pm0.02_{A}$	$2.00\pm0.03_{A}$
C22:6n3	$25.07\pm0.98_{A}$	$25.7\pm1.16_{A}$	$25.79\pm0.64_{A}$	$24.39\pm2.58_{A}$	$23.91\pm1.13_{A}$	$23.64\pm2.67_{A}$	$23.18\pm1.03_{A}$
∑PUFA	$58.5\pm2.68_{A}$	$58.88\pm1.86_{A}$	58.79±0.47A	$58.61\pm2.31_{A}$	$58.31\pm0.19_{A}$	$57.64\pm3.34_{A}$	$58.12\pm0.86_{A}$
Unidentified	$8.44\pm0.72_{A}$	$7.82\pm1.97_{A}$	8.58±0.69A	$7.46\pm0.43_{A}$	$8.60\pm0.09_{A}$	$8.76\pm0.47_{A}$	$6.67\pm0.40_{A}$
∑PUFA/∑SFA	$2.47\pm0.26_{A}$	$2.47\pm0.04_{A}$	$2.44\pm0.07_{A}$	$2.34\pm0.29_{A}$	$2.42\pm0.02_{A}$	$2.36\pm0.33_{A}$	$2.28\pm0.08_{A}$
∑PUFA/∑MUFA	$6.28\pm0.67_{A}$	$6.21\pm0.38_{A}$	$6.93\pm0.62_{A}$	$6.73\pm0.76_{A}$	$6.49\pm0.28_{A}$	$6.45\pm0.99_{A}$	$5.96\pm0.09_{A}$
EPA+DHA	$34.52\pm1.32_{A}$	$35.59\pm1.03_{A}$	$35.3\pm0.68_{A}$	$33.94\pm2.82_{A}$	$33.07\pm1.34_{A}$	$32.64\pm3.13_{A}$	$32.3\pm1.16_{A}$
∑n3 PUFA	$36.59\pm1.44_{A}$	$37.5\pm1.25_{A}$	$37.41\pm0.69_{A}$	$35.96\pm2.94_{A}$	$35.05\pm1.17_{A}$	$34.56\pm3.15_{A}$	$34.3\pm1.19_{A}$
∑n6 PUFA	$8.74\pm0.18_{A}$	$8.48\pm0.35_{A}$	$8.55\pm0.39_{A}$	$8.87\pm0.04_{A}$	$9.26\pm0.28_{A}$	$9.31\pm0.05_{A}$	$9.29\pm0.11_{A}$
∑n3/∑n6	$4.19\pm0.08_{A}$	$4.42\pm0.04_{A}$	$4.38\pm0.28_{A}$	$4.06\pm0.32_{A}$	$3.79\pm0.24_{A}$	$3.71\pm0.36_{A}$	$3.69\pm0.17_{A}$
∑n6/∑n3	$0.24\pm0_{A}$	$0.23\pm0_{A}$	$0.23\pm0.01_{A}$	$0.25\pm0.02_{A}$	$0.26\pm0.02_{A}$	$0.27\pm0.03_{A}$	$0.27\pm0.01_{A}$
AI	$0.28\pm0.03_{A}$	$0.26\pm0_{A}$	$0.27\pm0_{A}$	$0.29\pm0.05_{A}$	$0.27\pm0.01_{A}$	$0.28\pm0.04_{A}$	$0.27\pm0_{A}$
TI	$0.17\pm0.01_{A}$	$0.16\pm0_{A}$	$0.17\pm0_{A}$	$0.18\pm0.03_{A}$	$0.17\pm0_{A}$	$0.19\pm0.03_{A}$	$0.19\pm0.01_{A}$
FLQ	$37.69\pm1.15_{A}$	$38.61\pm0.3_{A}$	$38.62\pm1.04_{A}$	$36.68\pm3.22_{A}$	$36.18\pm1.43_{A}$	$35.76\pm3.24_{A}$	$34.61\pm1.1_{A}$
h/H	$3.81\pm0.33_{A}$	$3.95\pm0.13A$	$3.84\pm0.07_{A}$	$3.66\pm0.67_{A}$	$3.85\pm0.06_{A}$	$3.67\pm0.59_{A}$	$3.65\pm0.06_{A}$

R: Raw material. AI: atherogenic index, TI, thrombogenic index, FLQ: flesh-lipid quality,
h/H: hypocholesterolemic/hypercholesterolemic ratio.
Different letters (A, B, C) in the same line indicates statistical differences depuration time ($p<0.05$).

It is reported that the atherogenic (AI) and thrombogenic (TI) indices that are higher than (>1.0) are harmful for human health [73]. If this value gets lower, the risk of coronary heart diseases decreases [74]. The AI (0.26-0.29) and TI (0.16-0.19) values obtained in this study were found lower than this value in all depuation time (Table 7), and it was also determined that there were no risks for human health. Sousa Bentes et al. [75] reported that the high h/H ratio of fatty acids is the indicator of whether the fat in the product is proper for nutrition. The h/H ratio was found between 3.65-3.95 in this study, and no differences between the groups were determined ($p>0.05$). If the FLQ value is high, this indicates that there are nutrient lipids with good quality [30]. The FLQ values were found max. (38.62) at 24th hour and min. (34.61) 72th hour ($p>0.05$) (Table 7).

CONCLUSION

48-hour depuration process was determined to be significant in the reduction in the amounts of Co, Fe, Al, Cu and Mn in the carpet shells. The amounts of Zn, Cu, Ni and Cr were found to remain in the international and national limit values during the study. The total number of mesophilic and psychrotrophic bacteria was found to show a linear decrease depending on the duration of the depuration. Although there were some changes in the nutritional composition data of meat structure of the carpet shells during the depuration process without nutrition environment, the quantitative distribution of the amino acid and fatty acid contents, which have an important role for human health and nutrition was not affected by the depuration conditions. The quality indexes of the fatty acids (AI, TI, FLQ, w6/w3, h/H) were determined to be at appropriate values for human health. Although the depuration process reduced some mineral and microbiological values in the carpet shells, there were not any changes in the nutritional quality and a minimum of 48-hour depuration process was determined to be sufficient in these purification conditions.

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