



Food Science and Applied Biotechnology

e-ISSN: 2603-3380

Journal home page: www.ijfsab.com
<https://doi.org/10.30721/fsab2024.v7.i2>



Research Article

Effect of cold and frozen storage duration on technological properties and proximate composition of Mediterranean mussel (*Mytilus galloprovincialis*) meat

Georgi Zhelyazkov¹✉, Deyan Stratev², Mariyana Strateva³, Miroslav Stefanov⁴

¹ Department of Animal Husbandry –Non-Ruminants and Other Animals, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria

² Department of Food Quality and Safety and Veterinary Legislation, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

³ Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

⁴ Department of Fundamental Sciences in Animal Husbandry, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria

Abstract

The effects of 6-day cold (0-4°C) storage and frozen storage (-18°C) for 3-, 6-, 9- and 12 months on technological properties, proximate composition and fatty acid profile of meat from Mediterranean mussel or Black mussel (*Mytilus galloprovincialis*) were investigated. The black mussel meat technological properties (water holding capacity /WHC/, cooking and roasting losses) and meat moisture content demonstrated the highest values in fresh samples due to the greater amount of sea water in them, yet this should not be considered as a disadvantage. The lower values of these parameters after 6-day cold storage and 3-, 6-, 9- and 12-month frozen storage were attributed from dehydration of black mussel tissue. The greater protein content detected during the cold and frozen storage was also due to water loss from mussel meat. The amount of lipids, ash content and fatty acid profile of black mussel meat were not changed during the tested periods of cold and frozen storage. The PUFA/SFA ratios showed that fresh mussels, and mussels cold-stored for 6-days and frozen for 3-, 6-, 9- and 12-months were an excellent source of PUFA and n-3 for humans.

Keywords

Mediterranean mussel, WHC, cooking loss, proximate composition, lipid profile

Abbreviations

WHC – water holding capacity; SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; n-6 – omega (ω); n-3 – omega (ω)

✉ Corresponding author: Georgi Zhelyazkov, Department of Animal Husbandry –Non-Ruminants and Other Animals, Faculty of Agriculture, Trakia University, Students Campus, 6000 Stara Zagora, Bulgaria, tel.: +35942699379; E-mail: georgi_ij@abv.bg

Article history:

Received 21 January 2024

Reviewed 28 January 2024

Accepted 3 April 2024

Available on-line 09 September 2024

<https://doi.org/10.30721/fsab2024.v7.i2.xx>

© 2024 The Authors. UFT Academic publishing house, Plovdiv

Introduction

Seafood is part of the menu of many nations since ancient times, as it is readily available and with good nutritional and taste quality. One of the main types of collected molluscs and so far the only one that is cultivated in the Bulgarian Black Sea coastal zone is the Mediterranean mussels or black mussel (*Mytilus galloprovincialis*). Studies on black mussel population along the Bulgarian coast demonstrated a stock of about 100 thousand tons (Petrova and Stoykov 2011).

During the last years, the culture and production of mussels has increased on a global scale (Subramaniam et al. 2021), a similar tendency is observed in Bulgaria as well. The black mussel production in 2021 has increased twice to 97,8 t compared to production in 2020, whereas aquaculture production for the same economic period was by 20% higher - up to 2,573.69 t (Agrarian Report 2022).

Mussels are filter feeders, and feed mainly on phytoplankton (Alkanani et al. 2007) as a source of nutrients needed for their growth (carbohydrates, protein, lipids etc.). The quality and safety of bivalves as food human depend mainly on the quality of environment they are harvested from (Orban et al. 2002). Bivalves from the genus *Mytilus* are an excellent bioindicators for marine environmental pollution with a variety of substances (Peycheva et al. 2023; Yancheva et al. 2023).

Black mussels are a good source of protein, vitamins A, B, C and minerals e.g. phosphorus, calcium, magnesium, iron, selenium etc. (Gurdal and Caglak 2021; Peycheva et al. 2022a). At the same time, they have low fat and cholesterol content (Roe et al. 2002), mussel meat is outlined with predominance of long-chain polyunsaturated omega-3 fatty acids as eicosapentaenoic (C20:5, n-3) and docosahexaenoic (C22:6, n-3), beneficial for human health (Czech et al. 2015; Peycheva et al. 2022a; Tan et al. 2022).

Consumers due to their superior nutritional quality and taste prefer fresh mussels, but their shelf life is very short: up to 3 days refrigerated storage (0-4°C). Due to their high-water content, mussels are an excellent medium for replication of microorganisms, which leads to their spoilage (Gurdal and Caglak 2021). Freezing at -18°C

decreases microbial spoilage rate and delays enzymatic processes, thus resulting in a considerable prolongation of storage duration (Zhu et al. 2021; Tan et al. 2022).

Numerous studies on black mussels collected or cultured along the Bulgarian Black Sea coast have been so far carried out. The seasonal changes in the chemical, fatty acid and lipid profile were determined (Stratev et al. 2017; Panayotova et al. 2021). The seasonal patterns of heavy metals and associated risk for human health from consumption of black mussels (Zhelyazkov et al. 2018; Peycheva et al. 2023), and oxidative stress biomarkers (Nikolova et al. 2018) were evaluated. The proximate composition and bioactive lipid status of black mussels were reported (Merdzhanova et al. 2018) as well as the phenolic content of mussels cultured in the Black Sea (Dobrev et al. 2020). Heavy metals' content and omega-3 fatty acids in wild and cultured mussels were analysed (Peycheva et al. 2021; 2022b). The effect of steam cooking on chemical composition and the risk for consumers was also evaluated (Peycheva et al. 2022a). The effect of freezing on histological changes occurring in Mediterranean mussels' tissues was investigated (Strateva et al. 2023). In the available literature, reports on change in technological properties, chemical and fatty acid composition of black mussels meat after cold and frozen storage are relatively few in light of findings that improper cold or frozen storage of seafood may lead to worsened quality (Mazrouh 2015).

The aim of the study was to determine the effect on the technological properties, proximate composition and fatty acid profile of meat from Mediterranean mussel or black mussel (*Mytilus galloprovincialis*), stored for 6 days at cold (0-4°C) and 3-, 6-, 9- and 12-months at frozen storage.

Materials and Methods

Collection of Mediterranean mussels and sample preparation. The collection of mussel samples was performed in June 2021 by divers along the Bulgarian Black Sea coast, in the region between the city of Varna and the city of Nessebar, at a distance of one nautical mile from the coast and a depth of 10-15 m (Fig. 1).

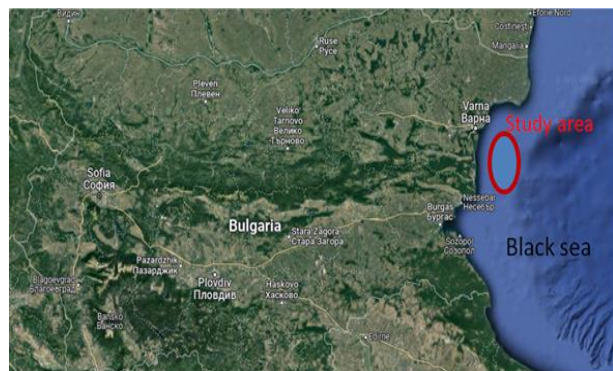


Figure 1. Study area

The samples were transported to the laboratory of the Department of Animal Husbandry - Non-ruminants and Other Animals, Trakia University, Stara Zagora in a cooling bag buried in flake ice at $3\pm 1^{\circ}\text{C}$ and processed the same day. The shells of mussels were removed, and the meat - washed with distilled water. Six batches, each consisting of 10 bulk samples containing meat from 40 mussels were prepared. Then the samples were packed in polyethylene bags. Ten fresh samples were immediately analysed. Another 10 samples were stored for 6-days refrigerated ($0-4^{\circ}\text{C}$) and analysed thereafter. Four batches of 10 samples were stored frozen (-18°C) for 3-, 6-, 9- and 12-months, respectively and analysed after the respective storage duration. Technological properties were analysed in the laboratory of the Fish Farming and Aquaculture unit at the Department of Animal husbandry - Non-ruminants and other animals, and chemical analyses were conducted at the Research laboratory of the Faculty of Agriculture, Trakia University, Stara Zagora.

Water holding capacity, cooking and roasting loss. WHC was determined by the method proposed by [Grau and Hamm \(1953\)](#) as percentage of water lost after pressing the mussel meat sample with a 5-kg weight for 5 min. It was calculated by the formula:

$$\text{WHC (\%)} = \frac{\text{weight of the sample before pressing (g)} - \text{weight of the sample after pressing (g)}}{\text{weight of the sample before pressing (g)}} \times 100 \quad (1)$$

The cooking loss was determined after cooking samples packed in polyethylene bags in boiling water for 15 min until the temperature in the center of sample attained 75°C . Roasting losses were

determined after roasting of samples in a conventional oven at 150°C for 10 min until the temperature in the center of sample attained 75°C , using the formula:

$$\text{Cooking or roasting loss (\%)} = \frac{\text{weight of fresh sample (g)} - \text{weight of thermally processed sample (g)}}{\text{weight of fresh sample (g)}} \times 100 \quad (2)$$

Proximate composition. Mussel samples were ground and prepared for analysis according to [AOAC \(2006\)](#). Moisture content (%) was determined by sample drying in a drying oven ([AOAC 1997](#)). Crude protein content (%) was assay by the method of Kjeldahl by means of automated Kjeldahl analyser (Kjeltec 8400, FOSS, Sweden). Lipid content (%) was analysed by the method of Soxhlet, using an automated system (Soxtec 2050, FOSS, Sweden). Ash content (%) was determined sample ashing in a muffle furnace (MLW, Germany) at 550°C over 8 h, followed by bringing the crucibles to room temperature and their weighing.

Fatty acid analysis. Total mussel meat lipids were extracted as described by [Bligh and Dyer \(1959\)](#). Methyl esters of lipids were isolated by thin-layer chromatography. They were obtained by the method of [Christie \(1973\)](#) with 0.01% sulfuric acid in dry methanol over 14 h. Fatty acid composition (%) of total lipids of Mediterranean mussels was assayed by gas chromatography on a "Perkin Elmer" Clarus 500 gas chromatograph with a flame ionization detector, 60 m capillary column "Trace Gold T6-WAXMS GC Column"; column temperature - 130°C (1 min), with change $6.5^{\circ}\text{C}/\text{min}$ to 170°C , with change $3.0^{\circ}\text{C}/\text{min}$ to 215°C (12 min) $40.0/\text{min}$ to 230°C (1 min), detector temperature 280°C ; injector temperature - 270°C , gas holder - hydrogen (H), split - 1:50. Methyl esters were identified by comparison with retention times of standards. Fatty acids were presented as percentage from the total amount of identified methyl esters (FAME) ([Christie 1973](#)).

Statistical analysis. GraphPad Prism (ver. 8.0.1) was used for statistical data processing. One-way ANOVA with Tukey's multiple comparisons test was performed to show significant differences between the investigated groups. The results are presented as mean values with standard error. The statistical significance was determined at $p < 0.05$.

Results and Discussion

Water holding capacity, cooking and roasting loss. There were no statistically significant differences ($P>0.05$) between fresh mussel meat WHC and meat after 6-days of cold storage ($0-4^{\circ}\text{C}$), the same was valid for those stored frozen (-18°C) for 3- and 6-months (Table 1). Substantially ($P<0.05$) less water was released, from mussel meat stored frozen (-18°C) for 9 and 12 months, corresponding to better WHC of meat. Compared to WHC of fresh samples ($27.42\pm 1.13\%$; Table 1), the amount of released water was by 11.67% (by the 9th month) and by 10.54% (by the 12th month) lower. There were no significant differences ($P>0.05$) between WHC of mussel meat stored refrigerated for 6-days and meat samples frozen for 3-, 6-, 9- and 12-months; similarly, differences were not found out among the different durations of frozen storage (Table 1).

Cooking loss was the greatest for fresh mussels: $46.21\pm 1.32\%$, while cold storage for 6-days resulted in significantly ($P<0.01$) reduction by 22.40%. The same was demonstrated for mussels stored frozen for 3-, 6-, 9- and 12-months with cooking losses lower by 9.54%, 10.52%, 11.43% and 11.82% respectively (Table 1). Compared to mussels stored

refrigerated for 6-days ($35.86\pm 1.68\%$), significantly ($P<0.01$) increased cooking losses were observed for meat stored frozen for 3-, 6-, 9- and 12-months – by 16.56%, 15.31%, 14.14% and 13.64%, respectively (Table 1). Cooking losses of mussels stored frozen for 3-, 6-, 9- and 12- months were identical and varied from $40.75\pm 1.44\%$ to $41.80\pm 1.12\%$, without consistent differences ($P>0.05$) (Table 1).

Roasting losses of mussel samples followed the same trend as cooking losses, and were the greatest in fresh samples ($48.74\pm 1.48\%$). The 6-day cold storage resulted in significant decrease ($P<0.01$) in roasting losses by 23.88%, the same was valid for mussels stored frozen for 3-, 6-, 9- and 12-months: respective roasting loss percentages were lower by 11.28%, 9.85%, 13.62% and 12.17% compared to fresh mussels (Table 1). The lowest roasting losses were found out in cold-stored samples ($37.10\pm 1.34\%$), and they differed significantly ($P<0.01$) from meat of mussels stored frozen for 3-, 6-, 9- and 12-months by 16.54%, 18.44%, 13.48% and 15.39% (Table 1). There were no statistically significant differences ($P>0.05$) in roasting losses of mussels frozen for 3-, 6-, 9- and 12-months, they were comparable and varied from $42.10\pm 1.98\%$ to $43.94\pm 1.25\%$ (Table 1).

Table 1. Technological properties of black mussel (*Mytilus galloprovincialis*) meat

Item	n	Fresh	Cold-stored	Frozen for 3	Frozen for 6	Frozen for 9	Frozen for 12	Significance
		$\bar{x} \pm \text{SEM}$	for 6 days $\bar{x} \pm \text{SEM}$	months $\bar{x} \pm \text{SEM}$	months $\bar{x} \pm \text{SEM}$	months $\bar{x} \pm \text{SEM}$	months $\bar{x} \pm \text{SEM}$	
WHC, %	10	27.42 ± 1.93^a	26.90 ± 1.21^{ab}	26.42 ± 1.75^{ab}	25.66 ± 1.95^{ab}	24.22 ± 1.47^b	24.53 ± 1.63^b	*
Cooking loss, %	10	46.21 ± 1.32^a	35.86 ± 1.68^b	41.80 ± 1.12^c	41.35 ± 1.56^c	40.93 ± 1.76^c	40.75 ± 1.44^c	**
Roasting loss, %	10	48.74 ± 1.48^a	37.10 ± 1.34^b	43.24 ± 1.83^c	43.94 ± 1.25^c	42.10 ± 1.98^c	42.81 ± 1.62^c	**

Significance of cold and frozen storage: * $P<0.05$; ** $P<0.01$. Values with different superscripts are statistically significantly different ($p<0.05$).

Proximate composition. Moisture content was the highest in fresh mussel meat ($89.42\pm 0.12\%$), it decreased after the 6-day of cold storage ($0-4^{\circ}\text{C}$) by 0.89%, and the tendency was preserved after frozen storage (-18°C) for 3-, 6-, 9- and 12-months with decrease by 0.67%, 1.27%, 1.12% and 2.04%; the differences were statistically significant ($P<0.01$) (Table 2). The meat moisture after 6-, 9- and 12-

months of frozen storage was significantly ($P<0.01$) reduced by 0.38%, 0.23% and 1.15% as compared to cold-stored samples ($88.62\pm 0.10\%$), whereas meat moisture after 3-months of frozen storage at -18°C was by 0.23% higher (Table 2). The lowest moisture content was found out in mussels stored frozen at -18°C for 12-months ($87.60\pm 0.20\%$) compared to samples stored frozen for 3-, 6- or 9-

months, this moisture content was statistically significantly lower ($P<0.01$) by 1.39%, 0.78% and 0.94%, respectively (Table 2). The protein content was the lowest in fresh black mussels: $7.63\pm 0.13\%$. After 6-days of cold storage and 3-, 6-, 9- and 12-months of frozen storage, the protein content of mussel meat samples increased by 10.35%, 7.08%, 14.42%, 11.80% and 23.59% ($P<0.01$) (Table 2). Compared to cold-stored samples for 6-days ($8.42\pm 0.15\%$), meat kept frozen for 3-months had significantly ($P<0.01$) lower protein content by 2.97%. Higher protein content (by 3.68% and 12.00%) was determined for mussel meat stored

frozen for 6- and 12-months vs cold-stored samples for 6-days ($P<0.01$) (Table 2). The highest meat protein content was measured after 12-months of frozen storage - $9.43\pm 0.23\%$; it was by 13.36%, 7.42% and 9.54% higher than that of samples frozen for 3-, 6- and 9-months respectively ($P<0.01$) (Table 2).

No statistically significant differences in lipid and ash contents were identified ($P>0.05$) between the meat of fresh mussels, those stored either refrigerate ($0-4^{\circ}\text{C}$) or frozen (-18°C) (Table 2). The lipid content varied within 1.21-1.25%, whereas ash content: within 1.71-1.80% (Table 2).

Table 2. Proximate composition of black mussel (*Mytilus galloprovincialis*) meat

Item	n	Fresh	Cold-stored	Frozen for	Frozen for	Frozen for	Frozen for	Significance
		$\bar{x} \pm \text{SEM}$	for 6 days $\bar{x} \pm \text{SEM}$	3 months $\bar{x} \pm \text{SEM}$	6 months $\bar{x} \pm \text{SEM}$	9 months $\bar{x} \pm \text{SEM}$	12 months $\bar{x} \pm \text{SEM}$	
Moisture, %	10	89.42 ± 0.12^a	88.62 ± 0.10^b	88.82 ± 0.18^c	88.28 ± 0.19^d	88.42 ± 0.11^d	87.60 ± 0.20^e	**
Protein, %	10	7.63 ± 0.13^a	8.42 ± 0.15^b	8.17 ± 0.17^c	8.73 ± 0.14^d	8.53 ± 0.09^b	9.43 ± 0.23^c	**
Lipids, %	10	1.24 ± 0.07	1.25 ± 0.09	1.21 ± 0.08	1.22 ± 0.05	1.25 ± 0.02	1.23 ± 0.04	NS
Ash, %	10	1.71 ± 0.12	1.71 ± 0.10	1.80 ± 0.14	1.77 ± 0.09	1.80 ± 0.13	1.74 ± 0.12	NS

Significance of cold and frozen storage: ** $P<0.01$. Values with different superscripts are statistically significantly different ($p<0.05$)

Fatty acid analysis. No statistically significant differences ($p>0.05$) were demonstrated with respect to the fatty acid composition of meat from fresh, cold-stored ($0-4^{\circ}\text{C}$) or frozen-stored (-18°C) mussels (Table 3).

The obtained results for the WHC of fresh mussels corresponded with those from previous studies of ours, reporting values within 26.79-36.68% (Stratev et al. 2017), yet were higher than data of Bongiorno et al. (2015) - 5.20-19.60%. The meat of fresh mussels was outlined with greater moisture loss, respectively lower WHC due to the greater amount of water in tissues, which is easily removed. During the cold and frozen storage and as a result of associated tissue dehydration, the mussel meat samples released less water and demonstrated improved WHC. Regardless of the lack of significant differences ($P>0.05$) between WHC of fresh, cold-stored mussels and those stored frozen for 3- and 6-months, consistent differences ($P<0.05$) occurred after 9- and 12-months storage at -18°C . No information was found out regarding the

determination of WHC of mussel meat after either cold or frozen storage. Reported data for this meat parameter in different fish species evidenced worsened WHC resulting from the release of more water from tissues after storage in cold or frozen state (Suarez et al. 2010; Saez et al. 2013; Saez et al. 2015; Santos et al. 2019) contrary to our results about black mussel meat. WHC is a main of seafood quality parameter because of its effects on their consistency, softness and juiciness. Therefore, the variations in WHC of cold- and frozen-stored seafood should be taken into consideration during subsequent technological processing. Data about cooking and roasting losses of fresh meat samples were considerably lower - by 46.21% and 48.74% compared to data reported by Stratev et al. (2017) in earlier studies: from 53.19-62.74% to 54.99-67.96%. Cooking and roasting losses were the greatest in fresh mussel samples whereas their refrigerated or frozen storage resulted in significant reduction ($P<0.01$). The lowest cooking and roasting losses were found out in mussels after 6-

Table 3. Fatty acid profile of black mussel (*Mytilus galloprovincialis*) meat

Fatty acids (%)	n	Fresh	Cold-stored for 6 days	Frozen for 3 months	Frozen for 6 months	Frozen for 9 months	Frozen for 12 months	Significance
		$\bar{x} \pm \text{SEM}$	$\bar{x} \pm \text{SEM}$	$\bar{x} \pm \text{SEM}$	$\bar{x} \pm \text{SEM}$	$\bar{x} \pm \text{SEM}$	$\bar{x} \pm \text{SEM}$	
C14:0	10	4.55±1.25	5.05±1.18	5.41±1.00	5.49±1.42	4.58±1.25	4.69±1.01	NS
C15:0	10	0.95±0.10	1.00±0.08	0.92±0.13	0.92±0.04	0.93±0.09	0.99±0.09	NS
C16:0	10	23.35±1.12	23.31±2.16	22.00±1.30	23.61±2.02	24.17±1.67	22.71±1.30	NS
C16:1	10	5.18±1.27	5.32±1.35	6.47±0.69	5.46±1.45	5.75±1.09	5.25±0.69	NS
C17:0	10	1.99±0.21	2.19±0.45	2.28±0.39	2.17±0.44	1.87±0.25	2.06±0.37	NS
C18:0	10	7.23±0.57	7.92±0.42	9.10±0.68	7.52±0.62	7.47±0.93	8.31±0.65	NS
C18:1	10	4.80±0.28	5.20±0.77	5.50±0.62	4.90±0.67	4.23±0.34	4.99±0.63	NS
C18:2	10	2.93±0.23	2.24±0.23	2.17±0.22	2.32±0.23	2.58±0.29	2.72±0.36	NS
C18:3n-3	10	2.01±0.29	1.69±0.08	1.67±0.07	1.58±0.12	1.88±0.30	1.96±0.27	NS
C20:1	10	2.70±0.42	2.14±0.31	2.29±0.33	2.18±0.31	2.60±0.32	2.61±0.47	NS
C20:2	10	1.69±0.34	1.66±0.38	1.98±0.21	1.37±0.38	1.25±0.15	1.95±0.34	NS
C20:4n-6	10	2.20±0.45	2.21±0.51	2.04±0.33	2.18±0.53	2.24±0.37	2.32±0.38	NS
C20:5n-3	10	19.92±0.43	20.02±1.12	18.82±1.11	19.83±1.07	18.25±0.87	19.07±0.55	NS
C22:5n-3	10	1.99±0.38	1.49±0.07	1.26±0.09	1.54±0.08	1.80±0.38	1.87±0.44	NS
C22:6n-3	10	18.52±1.03	18.57±1.74	18.10±1.40	18.94±1.54	20.43±1.26	18.52±1.19	NS
SFA ¹	10	38.07±1.91	39.47±1.38	39.71±1.53	39.71±1.36	39.02±1.08	38.76±1.07	NS
UFA ²	10	61.94±1.91	60.55±1.38	60.30±1.53	60.30±1.36	61.01±1.08	61.26±1.07	NS
MUFA ³	10	12.68±1.36	12.66±2.08	14.26±1.25	12.54±1.99	12.58±1.42	12.85±1.16	NS
PUFA ⁴	10	49.26±1.75	47.88±1.75	46.04±0.75	47.76±1.80	48.43±1.72	48.41±1.78	NS
n-6 ⁵	10	6.82±0.74	6.11±0.21	6.19±0.30	5.87±0.43	6.07±0.56	6.98±0.36	NS
n-3 ⁶	10	42.43±1.22	41.78±1.47	39.85±0.32	41.90±1.42	42.35±1.26	41.41±1.48	NS
PUFA/SFA	10	1.29±0.39	1.21±0.50	1.16±0.15	1.20±0.50	1.24±0.45	1.25±0.40	NS
n-6/n-3	10	0.16±0.02	0.15±0.02	0.16±0.01	0.14±0.01	0.14±0.01	0.17±0.01	NS

NS – not significant.

¹SFA - saturated fatty acids; ²UFA - unsaturated fatty acids; ³MUFA- monounsaturated fatty acids; ⁴PUFA - polyunsaturated fatty acids; ⁵n-6 - Σ C18:2; C20:2; C20:3; C20:4; ⁶n-3 - Σ C18:3n-3; C20:5; C22:6

days of cold storage, these parameters increased statistically significantly ($P < 0.01$) after frozen storage for 3-, 6-, 9- and 12-months. As already mentioned in the introduction, to the best of our knowledge, no studies devoted on cooking or

roasting losses of mussels stored refrigerated or frozen were found out in available literature.

The data about fresh mussels moisture content from this study were higher (89.42%) than values from previous studies: 77.09-81.92% (Stratev et al. 2017)

and 86.19% (Tosun et al. 2019). The protein, lipids and ash contents of fresh mussels in this study were substantially lower - by 7.63%, 1.24% and 1.71% in comparison to earlier data of Stratev et al. (2017) - 13.02-17.63%, 1.48-1.67% and 1.50-1.52%, respectively as well as compared to percentages reported by Tosun et al. (2019): 10.61%, 2.11% and 1.02%. As already mentioned, the significantly ($P < 0.01$) lower moisture content of mussel meat after the 6-days of cold storage and 3-, 6-, 9- and 12-months of frozen storage than in fresh samples is due to dehydration. The causes are moisture evaporation during the refrigerated storage, whereas during the freezing, the formation of ice crystals in tissues, their destruction and water release during defrosting had additional influence. These results confirmed the significantly loss of moisture after refrigerated and frozen storage of black mussels reported by Bejaoui et al. (2021) as well as the similar findings of Gökoglu et al. (2000) in the same species after freezing.

Opposite to moisture content data, meat protein content was the lowest in fresh mussels and was found to increase statistically significantly ($P < 0.01$) after 6-days of cold storage. The highest protein content was demonstrated for samples kept frozen for 12-months ($P < 0.01$). The reason for higher protein content of black mussel meat during refrigerated and frozen storage was the reduction of moisture content due to evaporation and leakage from tissues during storage. These data are not comparable to those reported by Bejaoui et al. (2021) about lower black mussel meat protein levels following cold and frozen storage.

Fresh mussels had lower lipid content (1.24%) but higher ash content (1.71%) as compared to previously reported data by Stratev et al. (2017), 1.48-1.67% and 1.5-1.52%, respectively. The lipid and ash content of fresh and stored mussels (either refrigerated or frozen) remained constant. This is also unlike the reported substantially lower lipid percentage of black mussels following cold and frozen storage (Bejaoui et al. 2021) and New Zealand green lipped mussel (*Perna canaliculus*) after frozen storage (Murphy et al. 2003).

The fatty acids content of fresh mussel meat corresponded to data reported by Stratev et al. (2017). In the present experiment, the amount of fatty acids in black mussel meat remained

unchanged during both the cold and refrigerated storage, which was not compatible to data of Bejaoui et al. (2021) affirming reduction of PUFA and MUFA, along with increased SFA content after cold and frozen storage of the same seafood species. The PUFA/SFA ratios demonstrated that fresh mussels, as well as mussels cold-stored for 6-days and frozen-stored for 3-, 6-, 9-, 12-months were an excellent source of PUFA and n-3 for humans. The risk from cancer and coronary heart disease was reported to be significantly reduced when the PUFA/SFA ratio in human diet was higher than 0.4 (Wood and Enser 2017), the same was true for dietary n-6/n-3 ratio > 5 (Simopoulos 2004).

Conclusions

The black mussel meat technological properties (WHC, cooking and roasting losses) and meat moisture content in fresh samples were the highest due to the greater amount of sea water in them, yet this should not be considered as a disadvantage. The lower values of these parameters after 6-day cold storage and 3-, 6-, 9- and 12-month frozen storage were attributed from dehydration of black mussel tissue. The greater protein content detected during the cold and frozen storage was also due to water loss from mussel meat. The amount of lipids, ash content and fatty acid profile of black mussel meat were not changed during the tested periods of cold and frozen storage. The PUFA/SFA ratios showed that fresh mussels, and mussels cold-stored for 6-days and frozen for 3-, 6-, 9- and 12-months were an excellent source of PUFA and n-3 for humans.

Acknowledgments

The study was funded under Project No. 8/2021 of the Faculty of Agriculture at Trakia University–Stara Zagora, Bulgaria.

References

- Agrarian report, 2022. Annual Report on the State and Development of Agriculture, Ministry of Agriculture, p. 243. [in Bulgarian]. Available at: <https://www.mzh.government.bg/bg/politiki-i-programi/otcheti-i-dokladi/agraren-doklad/>
- Alkanani T., Parrish C.C., Thompson R.J., McKenzie C.H. Role of fatty acids in cultured mussels, *Mytilus edulis*, grown in Notre Dame Bay, Newfoundland. *Journal of Experimental Marine Biology and Ecology*, 2007, 348(1–2): 33-45. <https://doi.org/10.1016/j.jembe.2007.02.017>

- AOAC Official Method 950.46. Moisture in Meat. Air Drying Method. Gaithersburg, MD, USA, AOAC International, 1997.
- AOAC Official Method 983.18. Meat and Meat Products. Preparation of Sample Procedure. Gaithersburg, MD, USA, AOAC International, 2006.
- Bejaoui S., Ghribi F., Chetoui I., Aouini F., Bouaziz M., Houas-Gharsallah I., Soudani N., El Cafsi M. Effect of storage temperature and time on the fatty acids and nutritional quality of the commercial mussel (*Mytilus galloprovincialis*). *Journal of Food Science and Technology*, 2021, 58(9): 3493-3503. <https://doi.org/10.1007/s13197-021-05008-5>
- Bligh E.G., Dyer W.Y. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 1959, 37(8): 911-917. <https://doi.org/10.1139/o59-099>
- Bongiorno T., Iacumin L., Tubaro F., Marcuzzo E., Sensidoni A., Tulli F. Seasonal changes in technological and nutritional quality of *Mytilus galloprovincialis* from suspended culture in the Gulf of Trieste (North Adriatic Sea). *Food Chemistry*, 2015, 173(4): 355-362. <https://doi.org/10.1016/j.foodchem.2014.10.029>
- Christie W.W., Han X. Chapter 8 - Gas Chromatographic Analysis of Fatty Acid Derivatives. In: *Lipid Analysis. Isolation, Separation, Identification and Lipidomic Analysis* (Fourth Edition). Oily Press Lipid Library Series. Woodhead Publishing Limited, Oxford, New York, 2012, pp. 159-180, Print ISBN: 978-0-9552512-4-5. <https://doi.org/10.1533/9780857097866.159>
- Czech A., Grela E.R., Ognik K. Effect of frying on nutrients content and fatty acid composition of muscles of selected freezing seafoods. *Journal of Food and Nutrition Research*, 2015, 3(1): 9-14. <https://doi.org/10.12691/jfnr-3-1-2>
- Dobрева D.A., Panayotova V.Z., Merdzhanova A.V., Stancheva R.S., Peycheva K. Preliminary study of phenolic content in farmed *Mytilus galloprovincialis* from the black sea coast. *Bulgarian Chemical Communications*, 2020, 52(Special issue D): 253-256. Available at: http://bcc.bas.bg/BCC_Volumes/Volume_52_Special_D_2020/BCC-52-D-2020-253-256-Dobрева-E07.pdf
- Gökoglu N., Erkan N., Özden O. The effect of frozen storage on the proximate composition and quality of frozen mussels (*Mytilus galloprovincialis*). *Journal of Aquatic Food Product Technology*, 2000, 9(2): 83-88. https://doi.org/10.1300/J030v09n02_08
- GraphPad Prism, ver. 8.0.1, GraphPad Software, 225 Franklin Street, Fl. 26, Boston, MA 02110, USA, 2018. Available at: <https://www.graphpad.com/updates/prism-801-release-notes>
- Grau R., Hamm R. Eine einfache Methode zur Bestimmung der Wasserbindung im Muskel. *Naturwissenschaften*, 1953, 40: 29-30. [in German] <https://doi.org/10.1007/BF00595734>
- Gurdal A.A., Caglak E. Investigation of nutritional, some quality changes of mussels covered with edible films prepared using extracts of persimmon, Cherry Laurel and Likapa. *Fresenius Environmental Bulletin*, 2021, 30: 1823-1836. Available at: <http://79.123.160.167/xmlui/bitstream/handle/11436/6722/g%C3%BCrdal-2021.pdf?sequence=1&isAllowed=y>
- Mazrouh M.M. Effects of freezing storage on the biochemical composition in muscles of *Saurida undosquamis* (Richardson, 1848) comparing with imported frozen. *International Journal of Fisheries and Aquatic Studies*, 2015, 3(2): 295-299. Available at: <https://www.fisheriesjournal.com/archives/2015/vol3issue2/PartD/3-2-35-960.pdf>
- Merdzhanova A., Dobрева D.A., Panayotova V. Assessment of Proximate and Bioactive Lipid Composition of Black Sea Mussels (*M. galloprovincialis*) from Bulgaria. In: *Biological Resources of Water*. (S. Ray Ed.). IntechOpen, 2018, pp. 340. ISBN: 978-1-78923-081-9; Print ISBN: 978-1-78923-080-2; eBook (PDF) ISBN978-1-83881-416-8 <http://doi.org/10.5772/intechopen.71909>
- Murphy K., Mann N., Sinclair A. Fatty acid and sterol composition of frozen and freeze-dried New Zealand green lipped mussel (*Perna canaliculus*) from three sites in New Zealand. *Asia Pacific Journal of Clinical Nutrition*, 2003, 12(1): 50-60. Available at: http://apjcn.qdu.edu.cn/12_1_19.pdf
- Nikolova G., Karamalakova Y., Zheleva A., Stratev D., Vashin I., Zhelyazkov G., Gadjeva V. Comparative analysis of real-time oxidative stress biomarkers measured in mussels (*Mytilus galloprovincialis*) and veined rapa whelks (*Rapana venosa*) in relation to two seasons - An electron paramagnetic resonance study. *Bulgarian Chemical Communications*, 2018, 50(Special Issue C): 58-63. Available at: http://www.bcc.bas.bg/bcc_volumes/Volume_50_Special_C_2018/pdf/BCC-50-C-2018-58-63-Nikolova-58.pdf
- Orban E., Di Lena G., Névigato T., Casini I., Marzetti A., Caproni R. Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian sites. *Food Chemistry*, 2002, 77(1): 57-65. [https://doi.org/10.1016/S0308-8146\(01\)00322-3](https://doi.org/10.1016/S0308-8146(01)00322-3)
- Panayotova V., Merdzhanova A., Stancheva R., Dobрева D.A., Peycheva K., Makedonski L. Farmed mussels (*Mytilus galloprovincialis*) from the Black Sea reveal seasonal differences in their neutral and polar lipid fatty acids profile. *Regional Studies in Marine Science*, 2021, 44(5): 101782. <https://doi.org/10.1016/j.rsma.2021.101782>
- Petrova E., Stoykov St. Distribution of the black mussel *Mytilus galloprovincialis* (L.) along the Bulgarian Black Sea coast. *Agricultural Science and Technology*, 2011, 3(4): 368-373. Available at: <https://agriscitech.eu/distribution-of-the-black-mussel-mytilus-galloprovincialis-l-along-the-bulgarian-black-sea-coast/>
- Peycheva K., Panayotova V., Stancheva R., Makedonski L., Merdzhanova A., Cicero N., Parrino V., Fazio F. Trace elements and omega-3 fatty acids of wild and farmed mussels (*Mytilus galloprovincialis*) consumed in Bulgaria: Human health risks. *International Journal of Environmental Research and Public Health*, 2021, 18(19): 10023. <https://doi.org/10.3390/ijerph181910023>
- Peycheva K., Panayotova V., Stancheva R., Makedonski L., Merdzhanova A., Cammilleri G., Ferrantelli V., Calabrese V., Cicero N., Fazio F. Effect of steaming on chemical composition of Mediterranean mussel (*Mytilus galloprovincialis*): Evaluation of potential risk associated with human consumption. *Food Science and Nutrition*, 2022a, 10(9): 3052-3061. <https://doi.org/10.1002/fsn3.2903>

- Peycheva K., Panayotova V., Stancheva R., Makedonski L., Merdzhanova A., Cicero N., Camilleri G., Fazio F. Trace elements and omega-3 fatty acids of black sea (Bulgaria) bivalve species *Mytilus galloprovincialis*, *Chamelea gallina* and *Donax trunculus*. Human health risk. *Natural Product Research*, 2022b, 36(11): 2735-2742. <https://doi.org/10.1080/14786419.2021.1921770>
- Peycheva K., Panayotova V., Stancheva R., Merdzhanova A., Dobrev D., Parrino V., Cicero N., Fazio F., Licata P. Seasonal variations in the trace elements and mineral profiles of the bivalve species, *Mytilus galloprovincialis*, *Chamelea gallina* and *Donax trunculus*, and human health risk assessment. *Toxics*, 2023, 11(4): 319. <https://doi.org/10.3390/toxics11040319>
- Roe M.A., Finglas P.M., Church S.M. Food Standards Agency. McCance and Widdowson's. The Composition of Foods. (Sixth summary edition). Royal Society of Chemistry, Cambridge, London, 2002, pp. 211-235. ISBN-0-85404-428-0. Available at: <http://safeat.ir/wp-content/uploads/2018/06/the-composition-of-foods.pdf>
- Saez M.I., Navarro G., Garcia-Mesa S., Martinez T.F., Garcia-Gallego G., Suarez M.D. Influence of pre-slaughtering feed restriction on muscle characteristics of farmed sea bass (*Dicentrarchus labrax* L.) during cold storage. *Journal of the Science of Food and Agriculture*, 2013, 93(9): 2323-2330. <https://doi.org/10.1002/jsfa.6048>
- Sáez M.I., Suárez M.D., Cárdenas S., Martínez T.F. Freezing and freezing-thawing cycles on textural and biochemical changes of meagre (*Argyrosomus regius* L.) fillets during further cold storage. *International Journal of Food Properties*, 2015, 18(8): 1635-1647. <https://doi.org/10.1080/10942912.2014.919319>
- Santos H.M.C., Méndez L., Secci G., Parisi G., Martelli R., Medina I. Pathway-oriented action of dietary essential oils to prevent muscle protein oxidation and texture deterioration of farmed rainbow trout. *Animal*, 2019, 13(9): 2080-2091. <https://doi.org/10.1017/S1751731119000016>
- Simopoulos A.P. Omega-6/Omega-3 essential fatty acid ratio and chronic diseases. *Food Reviews International*, 2004, 20(1): 77-90. <https://doi.org/10.1081/FRI-120028831>
- Stratev D., Popova T., Zhelyazkov G., Vashin I., Dospatliev L., Valkova E. Seasonal Changes in Quality and Fatty Acid Composition of Black Mussel (*Mytilus galloprovincialis*). *Journal of Aquatic Food Product Technology*, 2017, 26(7): 871-879. <https://doi.org/10.1080/10498850.2017.1346742>
- Strateva M., Stratev D., Zhelyazkov G. Freezing influence on the histological structure of Mediterranean mussel (*Mytilus galloprovincialis*). *AIMS Agriculture and Food*, 2023, 8(2): 278-291. <https://doi.org/10.3934/agrfood.2023015>
- Suarez M.D., Martinez T.F., Saez M.I., Morales A.E., Garcia-Gallego M. Effects of dietary restriction on post-mortem changes in white muscle of sea bream (*Sparus aurata*). *Aquaculture*, 2010, 307(1-2): 49-55. <https://doi.org/10.1016/j.aquaculture.2010.07.006>
- Subramaniam T., Lee H.J., Jeung H.D., Kang H.S., Kim C.W., Kim H.S., Cho Y.G., Choi K.S. Report on the annual gametogenesis and tissue biochemical composition in the Gray mussel, *Crenomytilus grayanus* (Dunker 1853) in the subtidal rocky bottom on the east coast of Korea. *Ocean Science Journal*, 2021, 56(10): 424-433. <https://doi.org/10.1007/s12601-021-00042-y>
- Tan K., Zhang H.K., Li S.K., Ma H., Zheng H. Lipid nutritional quality of marine and freshwater bivalves and their aquaculture potential. *Critical Reviews in Food Science and Nutrition*, 2022, 62(25): 6990-7014. <https://doi.org/10.1080/10408398.2021.1909531>
- Tosun Ş.Y., Alakavuk D.Ü., Ulusoy Ş. Quality Changes of Thermal Pasteurized Mussels (*Mytilus galloprovincialis*) During Refrigerated Storage at 4±1°C. *Aquatic Sciences and Engineering*, 2018, 33(4): 117-123. <https://doi.org/10.26650/ASE2018428669>
- Wood J.D., Enser M. Chapter 20 - Manipulating the Fatty Acid Composition of Meat to Improve Nutritional Value and Meat Quality. In: *New Aspects of Meat Quality from Genes to Ethics*. (P.P. Purslow Ed.). Woodhead Publishing Series in Food Science, Technology and Nutrition, 2017, pp. 501-535. ISBN:978-0-08-100593-4 <https://doi.org/10.1016/B978-0-08-100593-4.00023-0>
- Yancheva V., Velcheva I., Georgieva E., Stoyanova S., Todorova B., Antal L., Nyeste K. Are *Mytilus* species suitable bioindicators for assessing aquatic pollution along the Black Sea Coast? A review. *Ecologia Balkanica*, 2023, 15(1), 49-76. https://eb.bio.uni-plovdiv.bg/wp-content/uploads/2023/03/049-076_eb23301.pdf
- Zhelyazkov G., Yankovska-Stefanova T., Mineva E., Stratev D., Vashin I., Dospatliev L., Valkova E., Popova T. Risk assessment of some heavy metals in mussels (*Mytilus galloprovincialis*) and veined rapa whelks (*Rapana venosa*) for human health. *Marine Pollution Bulletin*, 2018, 128, 197-201. <https://doi.org/10.1016/j.marpolbul.2018.01.024>
- Zhu S.C., Yu H.J., Chen X., Zhang Q., Cai X., Ding Y., Zhou X., Wang S. Dual cryoprotective strategies for ice-binding and stabilizing of frozen seafood: A review. *Trends in Food Science & Technology*, 2021, 111: 223-232. <https://doi.org/10.1016/j.tifs.2021.02.069>