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Proximate Analysis and Fatty Acid Composition of Hot-Smoked Underutilised South African Black Mussel (*Choromytilus meridionalis*, Krauss 1848)

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Abstract: The ever-increasing demand for seafood-containing diets, especially fish, primarily due to their superior nutritional value, has impacted the availability of wild stocks, necessitating the focus on other underutilised aquaculture species. In this study, the proximate and fatty acid (FA) composition of the indigenous black South African mussel (*Choromytilus meridionalis*) was investigated. The effects of the hot-smoking as preservation method on the proximate composition for mussels were assessed using the standard AOAC protocols; while gas chromatography-flame ionised detection (GC-FID) was used to determine the FA content. The moisture level in hot-smoked mussels significantly ($P<0.05$) decreased by 19% while levels for ash, crude protein, lipid, and carbohydrate increased by 98%, 42%, 46%, and 49%, respectively when compared to raw (uncooked) mussels. The hot-smoking of mussels led to a significant ($P<0.05$) increase in the total polyunsaturated FA (PUFA); the omega-3 FA content was 37% higher in hot-smoked mussels than in the raw equivalents. Conversely, the levels of omega-6 and saturated FA in the hot-smoked mussels (4% and 45%, respectively) were lower than those found in the raw mussels (7% and 52%, respectively). The data obtained in this study demonstrated the nutritive and health benefits of the South African black mussel as evidenced by its high macronutrient and omega-3 amounts. Suggestion towards the application of the hot-smoking technique in preserving the nutrients in mussel species is made for the application in food fortification strategies such as ready-to-eat protein or omega-3 supplements.

Keywords: aquaculture; fatty acids; food security; hot-smoking; underutilised shellfish

1. Introduction

The demand for seafood has increased over recent years due to the growth in the population along with consumers' knowledge of the health benefits associated with the consumption thereof (Messina et al., 2021; Willer et al., 2021; Venugopal and Gopakumar, 2017). This increase in seafood demand, mainly fish, has led to a concerning decline in global wild fish stocks (Fisher, 2021; Tan et al., 2020). In addition, the decline in fish stock paired with the growing population of an estimated 10 billion by the year 2050 may leave large groups of people at risk of nutrient deficiencies (Gephart et al., 2020). Approximately 39% of the seafood species found in South Africa are overexploited (DEFF, 2020). In this regard, there has been a growing need to explore underutilised aquaculture seafood species as an alternative to producing cheap value-added products (Messina et al., 2021; Panayotova et al., 2021).

Seafood products, such as mussels have been appreciated for their excellent source of essential long-chain polyunsaturated fatty acids (LC-PUFA) including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA) and to a smaller degree arachidonic acid (ARA) (Momenzadeh et al., 2016). The LC-PUFAs have been reported to have peculiar biological properties and positive effects on human health and its maintenance (Golgolipour et al., 2019). Some of the health benefits include the improvement and prevention of cardiovascular heart diseases (CVD), obesity, insulin sensitivity bone, and muscle health (Yaghubi et al., 2021; Hosomi et al., 2012).

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Therefore, the promotion of the consumption of mussels in the human diet may be a feasible strategy to enhance human health (Carboni et al., 2019:2). Furthermore, mussel species help to provide essential vitamins, minerals, and high-quality protein with all the dietary essential amino acids required for the maintenance and growth of the human body (Cherifi & Sadok, 2016; Ersoy & Şereflişan, 2010).

The mass production of mussels through aquaculture is more sustainable, cost-effective and environmental-friendly because they feed directly from the water column and therefore avoid the environmental pollution and impacts caused by feed production and nutrient input to the water column (Yaghubi et al., 2021; Tan et al., 2020). Mussels are usually marketed as raw, unshelled, or frozen but can also be processed by various cooking methods before consumption as an ingredient in many delicate dishes such as pasta, pizza, salads, and soups (Bejaoui et al., 2019; Caglak et al., 2008). Furthermore, mussels can be processed into more shelf-stable products such as canned, dried, or smoked mussel products (Grkovic et al., 2019).

Choromytilus meridionalis, commonly known as the black mussel is native to Southern Africa (Lombard and Grant, 1986). The geographic locations of the mussel have a patchy distribution from Walvis Bay, Namibia on the west coast to Port Alfred on the east coast of South Africa (Vellemu and Omoregie, 2014; Lombard and Grant, 1986). The black mussel is one of the three bivalve species currently cultivated on a commercial scale in Saldanha Bay, South Africa (Heinecken et al., 2017). *C. meridionalis* has a blue-black shell that is more compressed than the common mussels *Perna perna*, *Mytilus galloprovincialis* and *Aulacomya atar* (Carpenter and de Angelis, 2016; Grant et al., 1984). The mussels are separated by gender**,** where the females can be identified by the brown colour of the gonadal tissue, while the males are yellow to off-white in colour (Firth, 2018). In this context, the mussel has not gained acceptance in the market due to the unusual dark brown colour of the female flesh (Firth, 2018; van Erkom Schurink and Griffiths, 1993). Most of the research on *C. meridionalis* is centred around aquaculture in terms of growth, reproductive cycle and biology, accumulation rates of trace elements and toxins and ecophysiology (Firth et al., 2019; Hubbart et al., 2012; van Erkom Schurink & Griffiths, 1991; du Plessis, 1977). At present, only two studies on the proximate composition of *C. meridionalis* have been published (Firth, 2018; Kyriacou, 2017).

There is currently limited information on the nutritional composition as well as the effects of heat treatment on the nutritional quality of *C. meridionalis*. Hence, this study aimed to determine the effects of hot-smoking on the proximate and fatty acid composition of *C. meridionalis*. The result data obtained in this study can assist in evaluating the effects of the application of preservation strategies on the quality and quantity of nutrients in processed mussel products. It will also help in determining the commercialisation potential of the South African mussels while encouraging continued aquaculture to sustain production.

2. Materials and Methods

2.1. Sample Collection

Frozen half-shelled *Choromytilus meridionalis* mussels (20 kg) were obtained from a local seafood processor Velddrif, Western Cape, South Africa. The frozen mussels were directly transported to the Department of Food Science and Technology at the Cape Peninsula University of Technology in a cooler box containing ice. On arrival, the frozen mussels were stored at -20°C until processed and analysed.

2.2. Sample Preparation and Smoking

Mussel samples were prepared by randomly pooling them into six (6) groups of equal batch sizes (100 g). Each batch was subsequently divided into two and assigned to raw (50 g) and smoked (50 g). The 50 g groups therefore represented a composite sample. Samples intended for smoking were smoked according to the method described by Kyriazi-Papadopoulou et al. (2003), with slight modifications. Briefly, the mussels were thawed overnight at 4°C and removed from the shells. The mussel meat was transferred onto a pot steamer and steamed for five minutes at 80°C. The steamed

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mussels were transferred into a bowl filled with 4% NaCl brine. The ratio of the mussels to the brine solution was 1:2. The mussels were soaked in the brine solution for 15 minutes at 45°C. The brined mussels were transferred onto a smoking tray and dried for ten minutes at 60°C without any smoke. Subsequently, the mussels were smoked for 15 minutes at 80°C in a smoker (Junior Butcherquip, model J536, South Africa). French oak wine barrel sawdust (LK's) was used to produce smoke within the smoker.

2.3. Proximate Composition

Moisture, ash, and protein contents were analysed according to the Association of Official Analytical Chemists Standards Techniques (AOAC, 1990). The moisture content was determined by drying samples in an oven (Scientific series 900) at 105°C for five hours and cooled in a desiccator for 24 hours (AOAC, 1990, Method 934.04). The ash content was determined by carefully charring samples over an open flame in a fume cupboard until samples were black (AOAC, 1990, Method 942.05). Charred samples were ashed in a muffle furnace (Carbolite Sheffield LMF 4) at 500°C for 18-24 hours. Crude protein was determined using Dumas (TruSpec™Leco Carbon/Hydrogen/Nitrogen Series) following the AOAC (1990) Method 968.06 and calibrated using EDTA as described by (Zozo et al., 2022). The crude protein content was determined as a percentage (%) of nitrogen and multiplied by the protein conversion factor of 6.25. Crude lipid content was determined using the chloroform: methanol (2:1) solvent extraction method, containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant (Oyenihi et al., 2020). Percentage carbohydrates (CHO) were calculated by difference using equations (1) and (2) as described by Mohammad and Yusuf (2016).

$$\text{CHO} = \text{Dry matter} - \text{crude protein (\%)} + \text{total lipids (\%)} + \text{ash (\%)} \quad (1)$$

$$*\text{where dry matter} = 100 - \text{moisture (\%)} \quad (2)$$

2.4. Fatty Acid Content

For the fatty acid analysis, mussel samples were initially subjected to lipid extraction by chloroform: methanol before being esterified according to the method described by Oyenihi et al. (2020), with moderate modifications. The individual fatty acid methyl esters (FAMEs) were then separated and quantified using a Focus GC chromatography system (Thermo Scientific) equipped with a 60 m BPX-70 fused silica capillary column with an internal diameter of 0.25 mm and a 0.25 um film thickness and an Al/AS 3000 auto-sampler. The injector and flame ionised detector (FID) temperatures were maintained at 200 and 250°C, respectively. The oven was programmed to increase from 160 to 220°C at a rate of 2°C per minute. The injection was set at split mode (50 mL/min) and a constant flow rate of 2 mL/min was used for the hydrogen carrier gas. A full separation of FAMEs was obtained after a total run of 32 minutes. The percentage fatty acid contents in mussels were thus calculated relative to the heptadecanoic acid (C17:0) internal standard and the FAMEs reference standard mixture (18919-1 AMP, Sigma-Aldrich).

2.5. Statistical Analysis

The experimental design for the study was a completely randomised block design. One-way ANOVA was investigated, using SPSS statistical software (version 28.0) and SAS (2022), including all dependent variables with batches as block replicates for hot smoking treatment. The dependent variables included were %moisture, %crude protein, %ash, %CHO, %total lipid, mg sodium and %fatty acids. Student T-test was performed, and the significance difference was calculated at the 5% confidence level to compare the means for all significant effects of hot smoking on the dependent variables. Tests were also performed for testing non-normality and no deviation from normality was observed. The results from the present study were expressed as mean values ± standard deviation.

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3. Results

3.1. Proximate Composition

The results obtained for the proximate composition of the raw and smoked mussels are shown in Table 1. The mean moisture content for raw mussels obtained in this study was 72.76 ± 2.10%, while the smoked mussels had a significant (P<0.05) 19% moisture reduction (58.98 ± 2.64%). The ash, crude protein, and total lipid content reported for raw mussels were 1.60 ± 0.12%, 9.79 ± 1.23%, and 4.00 ± 0.47%, respectively. Upon smoking, a significant increase was reported for the ash (3.17 ± 0.70%), protein (13.87 ± 0.70%) and total lipid content (5.84 ± 0.28%). Smoking also resulted in a 49% significant increase (P<0.05) in carbohydrates (17.87 ± 1.80%) when compared to the values obtained in the raw mussels (11.99 ± 1.80%).

Table 1. Proximate composition (% means ± standard deviation) for raw and smoked C. meridionalis.

Percentage (%)	Raw *C. meridionalis*	Smoked *C. meridionalis*
Moisture	72.76 ± 2.10[a]	58.98 ± 2.64[b]
Ash	1.60 ± 0.13[a]	3.17 ± 0.70[b]
Protein	9.79 ± 1.23[a]	13.87 ± 1.03[b]
Total lipid	4.00 ± 0.47[a]	5.84 ± 0.28[b]
Carbohydrates	11.99 ± 1.80[a]	17.83 ± 1.80[b]

[a,b] Values within a row with different superscripts are significantly different (P<0.05).

3.2. Fatty Acid Composition

The fatty acid composition of raw and smoked mussels determined in the study is summarised in Table 2. Twenty-eight fatty acids were identified from C8:0-C24:1. The fatty acid profile of the raw and smoked mussel presents a dominance of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA). The sum of all SFA identified in the raw mussels was (52.93 ± 1.81%) followed by PUFA (30.68 ± 2.10%) and lastly mono-unsaturated fatty acids (MUFA) (15.66 ± 1.36%). Within the SFA fraction, palmitic acid (C16:0) was the most abundant FA (15.96 ± 0.93%) followed by tridecanoic acid (C13:0) (8.31 ± 0.39%), undecanoic acid (C11:0) (7.62 ± 0.30%), pentadecanoic acid (C15:0) (6.99 ± 0.56%) and stearic acid (C18:0) (4.94 ± 0.32%). Palmitoleic acid (C16:1) was the predominant fatty acid in raw mussels with an average of (8.66 ± 1.12%). Palmitoleic acid was followed by vaccenic acid (C18:1n-7) (3.22 ± 0.09%) and myristoleic acid (C14:1) (1.86 ± 0.45%). PUFA which consists of the essential LC-PUFA were dominated by EPA (C20:5n-3) at (13.97 ± 1.52%) over DHA (22:6n-3) (7.15 ± 0.71%) and linoleic acid (C18:2n-6) (5.29 ± 0.47%). The total omega-3 (n-3) and omega-6 (n-6) obtained for the raw mussels were (23.96 ± 1.69%) and (6.81 ± 0.52%), respectively. The smoking process resulted in a significant decrease (P<0.05) in the SFA (44.90 ± 2.02%) content, while a significant increase (P<0.05) was observed in the PUFA (37.70 ± 1.06%) content. Although there was a minor increase in the MUFA (16.23 ± 1.47%) content it showed no significant difference (P>0.05). The decrease in the SFA content may have been a result of the decreased values in palmitic acid (C16:0) (15.54 ± 0.38%), tridecanoic acid (C13:0) (6.04 ± 0.66%) and pentadecanoic acid (C15:0) (5.62 ± 0.50%) and stearic acid (C18:0) (3.87 ± 0.43%). The decreased value of palmitic acid (C16:0) showed no significant difference (P>0.05), whereas the reduced tridecanoic acid (C13:0), pentadecanoic acid (C15:0) and stearic acid (C18:0) contents showed a significant difference (P<0.05). Even though most of the SFA decreased during smoking, myristic acid (C14:0) showed an increase from 3.93 ± 0.32% to 4.11 ± 0.24% and was observed to be at a higher proportion than stearic acid (C18:0) which was one of the dominating SFA in the raw mussels. Among the MUFA fraction, palmitoleic acid (C16:1) and myristoleic acid (C14:1) content increased to 9.81 ± 1.31% and 2.44 ± 0.52%, respectively with no significant difference (P>0.05). A significant decrease (P<0.05) was observed for vaccenic acid (C18:1n-

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7) (2.64 ± 0.15%). The increased proportion of PUFA was a result of a significant increase (P<0.05) in the contents of eicosapentaenoic acid (EPA, C20:5 n 3) (21.91 ± 1.21%) and docosahexaenoic acid (DHA, C22:6 n 3) (8.01 ± 0.22%). Linoleic acid (LA, C18:2n-6) decreased significantly (P<0.05) to 2.79 ± 0.54 from 5.34 ± 0.42% in the raw mussels. The smoking process resulted in a significant decrease (P<0.05) in the omega-6 content (4.34 ± 0.61%) and a significant increase (P<0.05) was observed for omega-3 (33.36 ± 1.23%).

The raw mussels had n-6/n-3 and PUFA/SFA ratios of 0.28 ± 0.02 and 0.58 ± 0.05, respectively. The n-6/n-3 ratio decreased significantly (P<0.05) after smoking to 0.13 ± 0.02, meanwhile, the PUFA/SFA ratio significantly increased to 0.84 ± 0.05.

Table 2. Fatty acid composition (% means ± standard deviation) and profile of raw and hot-smoked C. meridionalis.

Fatty acid methyl esters	Raw *C. meridionalis*	Smoked *C. meridionalis*
C8:0	4.38 ± 1.33^{a}	3.31 ± 0.80^{a}
C11:0	7.67 ± 0.30^{a}	5.62 ± 0.64^{b}
C12:0	0.17 ± 0.20^{a}	0.18 ± 0.02^{a}
C13:0	8.31 ± 0.39^{a}	6.04 ± 0.66^{b}
C14:0	3.90 ± 0.37^{a}	4.11 ± 0.24^{a}
C15:0	6.99 ± 0.56^{a}	5.62 ± 0.50^{b}
C16:0	15.96 ± 0.95^{a}	15.54 ± 0.38^{a}
C18:0	4.94 ± 0.32^{a}	3.87 ± 0.43^{b}
C20:0	0.37 ± 0.07^{a}	0.35 ± 0.02^{a}
C24:0	0.23 ± 0.06^{a}	0.25 ± 0.05^{a}
ΣSFA	52.93 ± 1.81^{a}	44.90 ± 2.02^{b}
C14:1	1.86 ± 0.45^{a}	2.44 ± 0.52^{a}
C16:1	8.66 ± 1.12^{a}	9.81 ± 1.31^{a}
C18:1n9 *trans*	0.71 ± 0.16^{a}	0.31 ± 0.07^{b}
C18:1n9 *cis*	1.03 ± 0.12^{a}	1.05 ± 0.22^{a}
C18:1n7	3.22 ± 0.09^{a}	2.64 ± 0.15^{b}
C20:1	0.81 ± 0.07^{a}	0.97 ± 0.07^{b}
C24:1	0.10 ± 0.05^{a}	0.18 ± 0.10^{a}
ΣMUFA	15.66 ± 1.36^{a}	16.23 ± 1.47^{a}
C18:2n6 (LA)	5.29 ± 0.47^{a}	2.79 ± 0.54^{b}
C18:3n3	0.27 ± 0.06^{a}	0.28 ± 0.05^{a}
C18:3n6	0.60 ± 0.18^{a}	0.10 ± 0.04^{b}
C20:3n6	0.14 ± 0.03^{a}	0.43 ± 0.29^{a}
C20:3n3	1.17 ± 0.16^{a}	1.49 ± 0.11^{b}
C20:4n6 (ARA)	Nd*^{a}	0.09 ± 0.02^{b}
C20:5n3 (EPA)	13.97 ± 1.52^{a}	21.91 ± 1.21^{b}
C22:4n6	0.46 ± 0.08^{a}	0.63 ± 0.05^{b}
C22:5n6 (DPA n-6)	0.23 ± 0.05^{a}	0.30 ± 0.01^{b}
C22:5n3 (DPA n-3)	1.41 ± 0.27^{a}	1.68 ± 0.22^{a}
C22:6n3 (DHA)	7.15 ± 0.71^{a}	8.01 ± 0.22^{a}
ΣPUFA	30.68 ± 2.10^{a}	37.70 ± 1.06^{b}
Σn-6	6.72 ± 0.55^{a}	4.34 ± 0.61^{b}
Σn-3	23.95 ± 1.69^{a}	33.36 ± 1.23^{b}
n-6/n-3 ratio	0.28 ± 0.02^{a}	0.13 ± 0.02^{b}
PUFA/SFA	0.58 ± 0.05^{a}	0.84 ± 0.05^{b}

ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acid; ΣPUFA, total polyunsaturated; LA, linoleic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DPA n-6, docosapentaenoic acid omega 6; DPA n-3, docosapentaenoic acid omega 3; DHA, docosahexaenoic acid; Σn-6, total omega-6 fatty acids; Σn-3, total omega-3 fatty acids; *Nd= not detected; ^{a,b}Values within a row with different superscripts are significantly different (P<0.05).

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4. Discussion

The positive perception and consumption of seafoods is increasing at a faster rate in recent times due to the growing recognition of their medicinal qualities especially in terms of the presence of health-promoting macro- or micronutrients and nutraceuticals (Nguyen et al., 2023, Murray et al., 2023). This trend has also advanced the application of aquaculture to meet the demand for seafoods to the extent that the global production from farms (106 million metric tons) has now overtaken wild catch (94 million metric tons) (Richie, 2019; Murray et al., 2023). Seafoods are major components in the utilisation of functional foods and food fortification strategies against micronutrient deficiencies and chronic diseases (Ribeiro et al., 2017, 2019, Bechoff et al., 2023). Therefore, research investigations exploring the potential harnessing of the nutritional, nutraceutical or phytochemical constituents of seafoods for health purposes should continually be encouraged.

During the smoking process, a loss in moisture and an increase in protein, lipid, ash, and carbohydrate content were observed ($P<0.05$). A similar result was reported by Liu et al. (2021); Abu and Eli (2018); Biji et al. (2015) in thermally heated bivalve shellfish. The moisture content of the raw mussels contributed a larger component to the overall proximate composition. A significant decrease in moisture content was evident after the smoking process. According to Biji et al. (2015), the water loss in mussel meat can be described in terms of the relationship between heat treatment and protein denaturation. During heat treatment, the water content found within the narrow channels between the thick and thin filaments of myofibrils is discharged due to heat denaturation and thereafter the contraction of the myofibrils. In addition, Muñoz et al. (2020) explained that the decrease in moisture content of hot-smoked samples is because of partial dehydration during the smoking process and subsequent changes in the wet weight of the flesh tissue. The industrial specification for smoked finished products suggests a moisture content in the flesh tissue of 65% or less (Muñoz et al., 2020; Cardinal et al., 2001). The moisture content obtained after smoking was within the recommended specifications. The lipid content of bivalves can be classified into four groups. The groups include lean (<2%), low (2-4%), medium (4-8%) and high fat (>8%) (Tan et al., 2020). In the present study, the raw mussels are low fat (4%) whereas the smoked mussels with a significant increase in the lipid content were medium fat (5.8%). The lipid content obtained was slightly higher than those reported by Firth (2018) (1.80 ± 0.09%) and Kyriacou (2017) (1.10 ± 0.40%) for the same species. On the contrary, Dalin et al. (2021) reported a higher lipid content for mussels *Perna viridis* (8.92 ± 0.22%) and *Perna indica* (6.73 ± 0.12%). The variation in the values may be due to the age of mussels, season, and natural and physiological status (Tenyang et al., 2020).

The protein in bivalve shellfish is considered high-quality protein because it contains an essential amino acid profile and is classified as a highly digestible protein source (Wright et al., 2018). Protein is usually the principal biochemical constituent of edible mussel meat, followed by carbohydrates (CHO) and lipids (Fernández et al., 2015). In contrast, the mussels showed higher values of CHO than protein for both raw and smoked products. The obtained protein value for the current study was similar to those reported in previously published data for various mussel species (Grković et al., 2020; Kyriacou, 2017; Biji et al., 2015; Khan et al., 2006). The percentage of CHO present in the body tissue of the raw mussel was 11.99 ± 1.80% and 17.87 ± 1.80% for smoked mussels. Similar findings were reported by Srilatha et al. (2013) in clam (*Meretrix casta*) (13.89-15.67%) and Periyasamy et al. (2011) in *Babylonia spirata* (Linnaeus, 1758) (16.65%). When compared to other mussels the CHO content in the present study was relatively higher (Prato et al., 2019; Sohail et al., 2016a; 2016b). The determination of ash content indicates predominantly the inorganic mineral content present in food products (Tenyang et al., 2020). Changes in the ash, protein and CHO content have been reported to be an outcome of moisture evaporation taking place during the smoking process (Abu and Eli 2018; Biji et al., 2015).

Although mussels have a generally low lipid content, the lipids they contain are a good source of essential fatty acids required for human health (Dalin et al., 2021). SFA was the most dominant fatty acid group in *C. meridionalis* followed by PUFA and MUFA. The same pattern was observed for the smoked *C. meridionalis*. These findings agree with those reported by Merdzhanova et al. (2014) for the mussel (*Mytilus galloprovincialis*) from the Bulgarian Black Sea and Chakraborty et al. (2016)

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for the green mussel (*P. viridis*) farmed in Cochin, India. A significant decrease ($P<0.05$) in SFA and a significant increase in PUFA were observed because of heat processing. The increase in MUFA had no significant effect ($P>0.05$). Similar observations for the SFA, MUFA and PUFA content of raw and steamed oysters (*Crassostrea hongkongensis*) were reported by Liu et al. (2021). These changes have been reported to be caused by the fact that the SFA and MUFA fatty acids are substantially represented in the neutral lipids fraction and therefore, making them more prone to migration from the food during processing (Puke and Galoburda, 2020; Rahimabadi et al., 2016). Palmitic acid was the most plentiful fatty acid in the SFA, which is a usual trend in most mussels (Biji *et al*., 2015).

Mussels are generally characterised by their predominance of essential omega-3 (n-3) PUFA, mainly EPA, DPA and DHA which usually constitute 50% of the total fatty acids (Moruf et al., 2020; Naik and Hayes, 2019; Merdzhanova et al., 2018). In the present study, for raw mussels, the PUFAs accounted for 30.68 ± 2.10% of the total fatty acids with n-3 making up 23.96 ± 1.69% of that amount. The remaining 6.72 ± 0.55% was accounted for by the n-6 fatty acids. In the smoked mussels increased values of 37.70 ± 1.06% for PUFA were obtained with 33.36 ± 1.23 % and 4.34 ± 0.61% accounting for n-3 and n-6, respectively. The most dominant n-3 fatty acids in unsmoked mussels were EPA (13.97 ± 1.52%) and DHA (7.15 ± 0.71%). Smoked mussels had a significant increase ($P<0.05$) in EPA and an insignificant increase ($P>0.05$) in DHA. Docosapentaenoic acid (DPA, C22:5n-3) was also observed at lower values than the EPA and DHA. Merdzhanova et al. (2018) reported a decrease in the EPA content of *Rapana verosa* after cooking, no DHA was reported in the study and DPA was present in significantly high amounts for both raw and cooked samples. Similarly, Biandolino et al. (2021) reported a decrease in EPA, DHA and DPA proportions of cooked *M. galloprovincialis* when compared to raw samples. The highest n-6 fatty acid recorded for raw and smoked mussels was LA (C18:2n-6). The lowest fatty acid obtained was DPA n-6 (C22:5n-6) for raw mussels and ARA (C20:4n-6) for smoked mussels. Arachidonic acid (ARA, C20:4n-6) was not detected in the raw mussels. Felici et al. (2020) reported higher proportions of ARA (3.88 ± 0.02%) in raw oysters which decreased significantly ($P<0.05$) to 1.04 ± 0.03% in the cooked samples. In addition to these findings, LA was reported in lower levels of 2.62 ± 0.42% and increased significantly to 8.12 ± 0.57% in the cooked oyster. Ozturan and Sengor (2022) reported a higher ARA content than that of LA in raw and cooked crayfish (*Astacus leptodactylus*). Bejaoui et al. (2019) reported an increase in the content of ARA in heat-treated clam (*Ruditapes decussatus*) in comparison to raw clam.

The n-6/n-3 ratio has been used as an indicator when comparing the relative nutritional value of seafood (Moruf et al., 2020). Although both n-6 and n-3 fatty acids have positive benefits to human health, it is important to consume the correct balance between them. In this study, an n-6/n-3 value of 0.28 ± 0.02 was observed for raw mussels. This value decreased significantly ($P<0.05$) after smoking to 0.13 ± 0.02. These findings are similar to those noted by Peycheva et al. (2022) for mussel *M. galloprovincialis* and Ozturan et al. (2022) for crayfish *A. leptodactylus*. Similar to the n-6/n-3 ratio, the PUFA/SFA ratio has been described as a useful indicator for evaluating the nutritional quality of food lipids (Tan et al., 2020). The recommended PUFA/SFA ratio for food should be above 0.45 and any foods with a ratio below this may be considered detrimental to human diets as they may cause elevated blood cholesterol levels (Tan et al., 2020; Merdzhanova et al., 2016). However, according to Biandolino et al. (2021), caution should be taken when considering PUFA/SFA as a lipid nutrition index since it excludes the substantial metabolic effects of MUFA. Moreover, it includes all SFA although some SFA such as stearic acid (C18:0) do not increase plasma cholesterol. According to published data, the PUFA/SFA ratio for bivalve shellfish is usually above 0.45 (Moruf et al., 2020; Rincón-Cervera et al., 2020; Tan et al., 2020). The PUFA/SFA ratio obtained in the present study for raw mussels was 0.64 and in support of these findings. Smoking increased the PUFA/SFA ratio, this is due to the overall decrease in the SFA content caused by heat. Similar observations were reported by Biandolino et al. (2021).

5. Conclusions

Overall, the heat treatment of the South African black mussels had a generally positive influence on the assessed nutrient indices in this study. The hot smoking process decreased the moisture

content in the black mussels, while the ash, crude protein, total lipid, and carbohydrate values were increased. Hot-smoked black mussels also contain higher levels of the beneficial omega-3 PUFAs, especially EPA, DHA, DPA and LA than their raw counterparts. The favourable n-6/n-3 and PUFA/SFA ratios may indicate they are an excellent source of essential fatty acids that can be further enhanced by hot-smoking. Therefore, mussels may potentially be used as a functional food ingredient to improve the nutritional quality of ready-to-eat protein or omega-3 food supplements. It is recommended for future studies to evaluate the mineral, vitamin, and amino acid contents in smoked South African black mussels in addition to determining the shelf-stability. Research to explore the consumer acceptance of the smoked mussel for potential larger-scale commercialisation and marketing are also warranted.

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