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Proximate and fatty acid compositions of smoked underutilised South African mussel *Choromytilus meridionalis*

Seafood is valued for its nutritional content; however, overfishing necessitates the focus on underutilised species to promote sustainable utilisation. This study investigated the proximate and fatty acid compositions of hot-smoked *Choromytilus meridionalis* (black mussels) using AOAC International methods and gas chromatography. Lipid nutritional quality indices were calculated. Moisture content in mussels significantly ($p < 0.05$) decreased by 19% after hot smoking, whilst ash, protein, lipid, and carbohydrate contents increased by 98%, 42%, 46% and 49%, respectively. Hot smoking significantly increased the polyunsaturated (37.70 ± 1.06%) and omega-3 fatty acid (33.36 ± 1.23%) contents. Conversely, omega-6 and saturated fatty acids of hot-smoked mussels were significantly ($p < 0.05$) lower than those for raw mussels. The atherogenicity index and thrombogenicity index showed a significant reduction ($p < 0.05$), whilst the hypocholesterolaemic to hypercholesterolaemic ratio and the sum of EPA+DHA showed significant increases. This study shows that *C. meridionalis* is a valuable food with a high protein content and a well-balanced fatty acid composition, rich in omega-3 fatty acids. This study was the first to investigate the macronutrients of *C. meridionalis*.

Significance:

Choromytilus meridionalis is one of three bivalve species cultivated on a commercial scale along the West Coast of South Africa; however, it is underrealised as a commercial food product. The growing mussel industry has been considered a great potential for the alleviation of poverty, job creation, and food security. This research may provide mussel farmers, fish processing industries, and the community with information about processing opportunities using *C. meridionalis*. Successful product development may contribute to alleviating malnutrition and increasing food security.

Introduction

The demand for seafood has increased over recent years because of growth in the population along with knowledge of the health benefits associated with the consumption thereof.[1-3] This increase in seafood demand has led to a concerning decline in global wild fish stocks.[4,5] 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.[6] Approximately 39% of the seafood species found in South Africa are overexploited.[7] In this regard, there has been a growing need to explore underutilised aquaculture seafood species as an alternative to producing cheap value-added products.[2,5]

Seafood products, such as mussels, have been appreciated for their excellent source of essential long-chain polyunsaturated fatty acids (LC-PUFAs), including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), and, to a smaller degree, arachidonic acid (ARA).[5,8] The LC-PUFAs have been reported to have positive effects on human health and its maintenance. Some of the health benefits include the improvement and prevention of cardiovascular heart diseases, obesity, insulin sensitivity, and bone resorption, and the maintenance of muscle health.[9,10] Therefore, the promotion of the consumption of mussels in the human diet may be a feasible strategy to enhance human health.[11] Furthermore, mussels as part of the human diet may provide essential vitamins, minerals, and high-quality proteins with all the dietary essential amino acids required for the maintenance and growth of the human body.[12,13] The nutritional quality of lipids can be assessed and quantified by nutritional quality indices (NQIs), such as the omega-3/omega-6 fatty acid (FA) ratio, the polyunsaturated FA/saturated FA (PUFA/SFA) ratio, and the functional efforts of the FA, such as the hypocholesterolaemic to hypercholesterolaemic ratio (h/H), atherogenicity index (IA), and thrombogenicity index (IT).[14,15]

The mass production of mussels through aquaculture is sustainable, cost-effective, and environmentally 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.[5,10] Mussels are usually marketed as raw, unshelled, or frozen but can also be processed by various cooking methods[16] before consumption or used as an ingredient in many delicate dishes, such as pasta, pizza, salads, and soups. *Choromytilus meridionalis*, commonly known as the black mussel, is native to southern Africa[17] and is one of the three bivalve species currently cultivated on a commercial scale in Saldanha Bay, South Africa.[18] *Choromytilus meridionalis* has a blue-black shell that is more compressed than the common mussels *Perna perna*, *Mytilus galloprovincialis*, and *Aulacomya atar*.[19,20] Female mussels can be distinguished from male mussels by the brown colour of their gonadal tissue, whereas the male individuals have yellow to off-white gonads.[21] In this context, the mussel has not gained acceptance in the market because of the unusual dark brown colour of the female flesh.[21,22] Most of the research on *C. meridionalis* is centred around growth, reproductive cycle and biology, accumulation rates of trace elements and toxins, and ecophysiology.[23-25] Thus, there is currently limited information on the proximate and FA compositions of *C. meridionalis*, as well as the effects of heat treatment on the nutritional quality thereof.[21,26,27] Hence, the aim of this study was to determine the effects of hot smoking on the proximate and FA compositions of *C. meridionalis* and the lipid NQIs thereof. The results from this study can assist in evaluating the effects of hot smoking on the quality

and quantity of nutrients in ready-to-eat products of processed mussels, an underutilised species.

Materials and methods

Sample collection

Frozen half-shelled *C. meridionalis* mussels (20 kg) were obtained from a local seafood processor at Velddrif (32.7690°S, 18.1590°E), Western Cape, South Africa. The frozen mussels were directly transported to the laboratory in a cooler box containing ice. On arrival, the frozen mussels were stored at −20 °C until processing and analysis.

Sample preparation and hot smoking

The mussels were thawed overnight at 4 °C and removed from the shells. The mussels were randomly pooled from the 20 kg supply into six groups, each weighing 100 g. Each of the six groups was subsequently divided into two samples of equal weight (i.e. 50 g): one sample was randomly designated as a raw sample and the other was assigned for hot smoking. This process yielded six raw samples and six hot-smoked samples, effectively serving as six repetitions of the smoking process. Each of the six hot-smoke samples was individually smoked as follows: mussel meat was steamed for 5 min at 80 °C, followed by salting in a 4% NaCl brine solution for 15 min at 45 °C. The ratio of mussels to the brine was 1:2. The brined mussels were transferred onto a smoking tray and dried for 10 min at 60 °C, followed by smoking for 15 min 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. Each 50 g sample served as a composite sample for analysis.

Cooking loss and sodium content

Cooking loss, expressed as a percentage, was determined by calculating the difference in weight between the raw and smoked samples. Sodium (Na) content was determined according to the Volhard method[28], where 2 g of mussel homogenate was mixed with 50 mL of distilled water. A volume of 25 mL of 0.1 N silver nitrate [$Ag(NO_3)_2$] was added, and the flask was placed on top of a hot plate. A volume of 10 mL of nitric acid (HNO_3) was slowly added to the mixture and left to boil for 10 min. The mixture was cooled down and 2 mL of nitrobenzene was added, followed by three drops of iron(III) indicator. The solutions were thoroughly mixed and titrated against 0.1 M ammonium thiocyanate (NH_4SCN) until a reddish-brown colour was observed. The Na content was calculated according to Equations 1 and 2:

$$\text{g Na in sample} = \frac{[(25 \times \text{N of } Ag(NO_3)_2) - (\text{titre volume} \times \text{N of } NH_4SCN)]}{1000} \times \text{Mr of Na} \quad \text{Equation 1}$$

$$\text{g Na/100 g of sample} = \text{g Na in sample/sample weight} \times 100 \quad \text{Equation 2}$$

where Mr is the relative molar mass and N is the concentration.

Proximate composition

Moisture, ash, and protein contents were analysed following the official methods of analysis of AOAC International.[29] Crude protein was determined using the Leco method (TruSpec™Leco). Crude fat was determined using the chloroform : methanol (2:1) solvent extraction method, containing 0.01% butylated hydroxytoluene as an antioxidant.[30] The percentage carbohydrates (CHO) was calculated by the difference using Equations 3 and 4.

$$\text{CHO}\ (\%) = \text{Dry matter} - \text{crude protein}\ (\%) + \text{total lipids}\ (\%) + \text{ash}\ (\%) \quad \text{Equation 3}$$

where

$$\text{dry matter}\ (\%) = 100 - \text{moisture}\ (\%) \quad \text{Equation 4}$$

Fatty acid composition

For the FA analysis, mussel samples were subjected to lipid extraction by chloroform : methanol (2:1) before being esterified according to Oyenihi et al.[30] The individual fatty acid methyl esters (FAMEs) were separated and quantified using a Focus GC gas 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 μm film thickness and an AI/AS 3000 autosampler. The injector and flame ionised detector temperatures were maintained at 200 °C and 250 °C, respectively. The oven was programmed to increase from 160 °C to 220 °C at a rate of 2 °C/min. The injection was set in the 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 min. The FA contents in mussels were calculated relative to the heptadecanoic acid (C17:0) internal standard and the FAMEs reference standard mixture (18919-1 AMP, Sigma-Aldrich) and expressed as a percentage of the total lipids.

Lipid nutritional quality indices

The NQI of the lipid proportion of raw and hot-smoked samples was calculated and expressed as mg/g of the total lipids using the FA composition data. Equations 5[31] and 6[31] were used to calculate the IA and IT. The hypocholesterolemic FA ratio (h/H) was calculated according to Equation 7.[15] The PUFA/SFA, n-6/n-3 ratios, and the sum of EPA+DHA were calculated using Equations 8, 9, and 10, respectively.

$$\text{IA} = [\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}]/[\Sigma\text{MUFA} + \Sigma n-6 + \Sigma n-3] \quad \text{Equation 5}$$

$$\text{IT} = (\text{C14:0} + \text{C16:0} + \text{C18:0})/[0.5 \times \Sigma\text{MUFA}) + (0.5 \times \Sigma n-6) + (3 \times \Sigma n-3) + (\Sigma n-3/\Sigma n-6)] \quad \text{Equation 6}$$

$$\text{h/H} = [\text{cis} - \text{C18:1n9} + \text{C18:2n6} + \text{C20:4n6} + \text{C18:3n3} + \text{C20:5n3} + \text{C22:5n3} + \text{C22:6n3}]/[\text{C14:0} + \text{C16:0}] \quad \text{Equation 7}$$

$$\text{PUFA/SFA} = \Sigma\text{PUFA}/\Sigma\text{SFA} \quad \text{Equation 8}$$

$$n-6/n-3 = \Sigma n-6/\Sigma n-3 \quad \text{Equation 9}$$

$$\text{EPA} + \text{DHA} = \Sigma\text{EPA} + \Sigma\text{DHA} \quad \text{Equation 10}$$

Statistical analysis

All samples were analysed in triplicate. Data analyses were performed using SPSS (version 28.0, 2021), and results are presented as mean ± standard deviation. A one-way analysis of variance was performed to test for a significant effect of hot smoking on each parameter considered. Significant differences between means were determined using a *t*-test and a probability value of $p < 0.05$ was considered statistically significant.

Results

Cooking loss, sodium, and proximate composition

Hot smoking resulted in a cooking loss of 40.54 ± 6.24%. The Na content obtained for the raw mussels significantly ($p < 0.05$) increased after brining and hot smoking (Table 1). The mean moisture content for raw mussels was 72.76 ± 2.10%, whilst the smoked mussels had a significant ($p < 0.05$) moisture reduction of up to 19%. Hot smoking significantly increased the ash, protein, and total fat contents. Smoking resulted in a significant increase of 49% in carbohydrates.

Fatty acid composition

The FA composition of the raw and hot-smoked mussel presents a dominance of SFA and PUFA (Table 2). The sum of SFA identified in the raw mussels was 52.93 ± 1.81% followed by PUFA (30.68 ± 2.10%), and monounsaturated fatty acids (MUFAs). Within the SFA fraction for raw mussels, palmitic acid was the most abundant FA, followed by

Table 1: Proximate composition (% mean ± standard deviation) and sodium content (g/100 g) of raw and hot-smoked *Choromytilus meridionalis*

	Raw *C. meridionalis*	Hot-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 fat	4.00 ± 0.47[a]	5.84 ± 0.28[b]
Carbohydrates	11.99 ± 1.80[a]	17.83 ± 1.80[b]
Sodium (g/100 g)	0.35 ± 0.03[a]	0.88 ± 0.05[b]

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

tridecanoic, undecanoic, pentadecanoic and stearic acids. Palmitoleic acid was the predominant MUFA in raw mussels with an average of 8.66 ± 1.12%, followed by vaccenic and myristoleic acids. The PUFAs were dominated, in decreasing amounts, by EPA (13.97 ± 1.52%), DHA (7.15 ± 0.71%), and linoleic acid (5.29 ± 0.47%). The total omega-3 and omega-6 obtained for the raw mussels were 23.96 ± 1.69% and 6.81 ± 0.52%, respectively.

The smoking process resulted in a significant ($p < 0.05$) decrease in the SFA, whilst a significant increase was observed in the PUFA content. Although there was an increase in the MUFA content, it was not significant ($p > 0.05$). The decrease in the SFA content may be a result of the significant ($p < 0.05$) reduction in tridecanoic, pentadecanoic and stearic acids. A significant decrease was observed for the MUFAs vaccenic acid and elaidic acid. The increased proportion of PUFAs was a result of an increase in the contents of EPA and DHA. Linoleic acid significantly decreased from 5.34 ± 0.42% to 2.79 ± 0.54% in the smoked mussels. The smoking process resulted in a significant decrease in the omega-6 content, whilst a significant increase was observed for omega-3 FAs.

Lipid nutritional quality indices

The NQIs (Table 3) were estimated in order to evaluate the nutritional quality and propensity of the mussels to influence the rate of coronary heart disease. Hot smoking resulted in a significant ($p < 0.05$) increase in the EPA+DHA value from 32.87 ± 6.03 mg/g to 71.12 ± 9.37 mg/g. 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 significantly decreased after smoking to 0.13 ± 0.02, whilst the PUFA/SFA ratio significantly increased.

The potential health benefits of the lipids in mussel meat were additionally assessed by the h/H, IA, and IT.[32] The h/H ratio significantly increased ($p < 0.05$) from 1.60 ± 0.15 to 1.95 ± 0.04 after hot smoking. The IA and IT obtained for raw black mussels were 0.69 ± 0.07 and 0.26 ± 0.03, respectively. Hot smoking significantly decreased the IA and IT to 0.60 ± 0.02 and 0.18 ± 0.01, respectively.

Discussion

The positive perception and consumption of seafood is increasing because of the growing recognition of its medicinal qualities, especially in terms of the presence of health-promoting macronutrients, micronutrients and nutraceuticals.[33,34] Hot smoking resulted in a loss in moisture and a significant increase in protein, lipid, ash, and carbohydrate contents, attributed to the concentration effect because of the loss of moisture. Similar results were reported by Abu and Eli[35], Biji et al.[36] and Liu et al.[37] in thermally heated bivalve shellfish. The water loss in mussel meat can be described in terms of the relationship between heat treatment and protein denaturation.[38] Protein denaturation does not result in protein loss but is associated with the reduction in the water-binding capacity of the proteins.[36]

Table 2: Fatty acid composition (% mean ± standard deviation) of raw and hot-smoked *Choromytilus meridionalis*

Fatty acid methyl esters	Raw	Hot-smoked
C8:0	4.38 ± 1.33[a]	3.31 ± 0.80[a]
C11:0 (undecanoic)	7.67 ± 0.30[a]	5.62 ± 0.64[b]
C12:0	0.17 ± 0.20[a]	0.18 ± 0.02[a]
C13:0 (tridecanoic)	8.31 ± 0.39[a]	6.04 ± 0.66[b]
C14:0 (myristic acid)	3.90 ± 0.37[a]	4.11 ± 0.24[a]
C15:0 (pentadecanoic)	6.99 ± 0.56[a]	5.62 ± 0.50[b]
C16:0 (palmitic)	15.96 ± 0.95[a]	15.54 ± 0.38[a]
C18:0 (stearic)	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 (myristoliec)	1.86 ± 0.45[a]	2.44 ± 0.52[a]
C16:1 (palmitoleic)	8.66 ± 1.12[a]	9.81 ± 1.31[a]
C18:1n9 *trans* (elaidic)	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 (vaccenic)	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	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]

ΣSFA, total saturated fatty acid; ΣMUFA, total monounsaturated fatty acid; ΣPUFA, total polyunsaturated fatty acid; 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 acid; Σn-3, total omega-3 fatty acid

nd, not detected

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

Table 3: Lipid nutritional quality indices (mean ± standard deviation) of raw and hot-smoked *Choromytilus meridionalis*

Nutritional index	Raw	Hot-smoked
EPA+DHA (mg/g)	32.87 ± 6.03[a]	71.12 ± 9.37[b]
n-6/n-3 ratio	0.28 ± 0.02[a]	0.13 ± 0.02[b]
PUFA/SFA ratio	0.58 ± 0.05[a]	0.84 ± 0.05[b]
IA	0.69 ± 0.07[a]	0.60 ± 0.02[b]
IT	0.26 ± 0.03[a]	0.18 ± 0.01[b]
h/H	1.60 ± 0.15[a]	1.95 ± 0.04[b]

IA, atherogenic index; IT, thrombogenicity index; h/H, hypocholesterolemic fatty acid ratio

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

Sodium is predominantly consumed as salt (NaCl), an ingredient used in different amounts during the processing and preservation of seafood.[39,40] The recommended daily limit of salt intake is 4–6 g per day (1600–2400 mg of Na per day).[41,42] The Na content for the hot-smoked mussels was 0.88 ± 0.05 g/100 g (875.65 mg). This value is lower than the recommended daily limit, indicating that the brine of 4% NaCl did not increase the Na content to unacceptable levels.

The fat content for the raw mussels was higher than those reported by Firth[21] (1.80 ± 0.09%) and Kyriacou[26] (1.10 ± 0.40%) for the same species. This variation could be because of differences in the age of the mussels, the season, or natural and physiological status.[43] The protein in bivalves is considered a high-quality protein because it contains essential amino acids and is classified as a highly digestible protein source.[44] Protein is usually the principal biochemical constituent of edible mussels, followed by carbohydrates and lipids.[45] The protein content found in the current study was similar to values reported in previous studies[26,36,43] for various mussel species. The carbohydrate content was 11.99 ± 1.80% for the raw mussels and 17.87 ± 1.80% for the hot-smoked samples. Similar findings were reported for clam[46] (*Meretrix casta*) (13.89%–15.67%) and *Babylonia spirata*[47] (16.65%). Mussels typically contain glycogen as a principal carbohydrate reserve.[48] Compared to other mussel species, the carbohydrate content in the present study was higher.[48,49] This difference could be because of different species, gender, maturity, feeding[32], and seasons[48].

Although mussels generally have a low lipid content, these lipids are a good source of essential PUFAs, such as EPA and DHA. The most dominant FA group in raw and hot-smoked mussels was SFA, followed by PUFA and MUFA. These findings are similar to those reported for *M. galloprovincialis*[50] and for the green mussel, *Perna viridis*[51]. A significant decrease in SFA was observed for the hot-smoked mussels. In contrast, the PUFA significantly increased, whilst MUFAs did not increase significantly ($p > 0.05$). Similar observations regarding SFA, MUFA, and PUFA contents were reported for raw and steamed oysters.[37] It is argued that these changes are related to SFAs and MUFAs being more substantially represented in the neutral lipid fraction, which therefore makes them more prone to migration from the food during processing.[52] Palmitic acid was the most plentiful SFA, which is a common trend in most mussel species.[48]

Mussels are generally valued for their content of essential n-3 PUFAs, mainly EPA, DPA, and DHA, which usually constitute 30–50% of the total FAs.[53] In the present study, hot smoking significantly increased the ΣPUFAs from 30.68 ± 2.10% to 37.70 ± 1.06%. Whilst the Σn-3 PUFA content significantly increased after smoking (23.96 ± 1.69% to 33.36 ± 1.23%), the Σn-6 content was significantly reduced compared to the raw samples. In contrast, a study[54] that investigated the effects of barbecue grilling, boiling, microwaving, oven cooking, and frying on the FA composition of *M. galloprovincialis* found that all cooking methods resulted in a general decrease in n-3 PUFAs, including EPA and DHA. The n-3 PUFAs are more sensitive to heat, oxygen, and light than n-6 PUFAs.[55] Omega-3 PUFAs, such as EPA and DHA, have a higher number of double bonds and therefore more bisallylic methylene positions compared to n-6 PUFAs, such as linoleic acid or ARA. This makes n-3 PUFAs more susceptible to autoxidation and are more sensitive to conditions that promote oxidation initiation and propagation, such as heat, oxygen, and light. Cooking methods, such as boiling, microwaving, grilling, and oven baking, often result in the oxidation of these PUFAs because of exposure to high temperatures and oxygen, leading to a reduction in total n-3 PUFA content. In contrast, hot smoking uses moderate heat (60–82 °C) and inhibits lipid oxidation[55] because of the antioxidant effects of phenols generated during the thermal decomposition of phenolic acids and lignin.

The most dominant n-3 PUFAs in raw mussels were EPA and DHA, with a significant increase after smoking. DPA was lower than EPA and DHA. DPA is typically found at lower concentrations compared to EPA and DHA in mussels[32,48,56] and other molluscs[57]. In New Zealand *Perna canaliculus*, across raw and processed samples for both male and female mussels, the concentration of DPA was consistently lower than both EPA and DHA.[56] In *M. galloprovincialis*, EPA and DHA were noted[32] as major PUFAs, with DPA in wild and farmed at lower concentrations. In *Mytilus edulis* from South Korea, DPA was consistently lower than both EPA and DHA across different seasons.[48] However, in raw clams, DPA was higher than EPA[57] but significantly lower than DHA. When the clams were cooked, these PUFAs, including DPA, EPA, and DHA, were reduced. Within the n-6 PUFA fraction, ARA was not detected in the raw mussels, but low levels were detected after hot smoking. The antioxidant[55] and concentration effects of hot smoking could have concentrated ARA, resulting in a higher concentration in smoked mussels. Similarly, Bejaoui et al.[57] reported an increase in the content of ARA after steaming, baking, grilling, and frying of clams.

The combination of EPA+DHA has been recognised and used greatly worldwide as a nutritional indicator.[58] The recommended daily intake of EPA+DHA is set between 100 mg and 250 mg for children up to the age of 10 years, 250 mg for healthy adults, and 300 mg for pregnant people. The EPA+DHA value of 32.87 ± 6.03 mg/g of hot-smoked mussels may contribute to the recommended daily intake of these omega-3 FAs in a serving of as little as 10 g. The n-6/n-3 ratio has been used as an indicator when comparing the relative nutritional value of seafood.[53] Although both n-6 and n-3 FAs have positive benefits for human health, it is important to consume the correct balance. A low n-6/n-3 ratio is considered as nutritionally beneficial to human health.[59] The n-6/n-3 ratio for the raw mussels significantly decreased after hot smoking. This decrease is attributed to the overall reduction in n-6 FAs, particularly linoleic acid and C18:3n6, and an increase in Σn-3 FAs, with significant increases in C20:3n3, EPA, and DPA. These findings are similar to those of previous studies[32,54] for *M. galloprovincialis*.

The PUFA/SFA ratio has been described as a useful indicator for evaluating the nutritional quality of food lipids.[5] The recommended PUFA/SFA ratio for food is ≥0.45, and any foods with a ratio below this may be considered detrimental to human diets as it may result in elevated blood cholesterol levels.[5] According to published data[5,53], the PUFA/SFA ratio for bivalve shellfish is usually above 0.45. Hot smoking significantly increased the PUFA/SFA ratio (0.84 ± 0.05) above the recommended ratio of 0.45 because of the overall decrease in the SFA and increase in PUFA.

The IA and IT are usually used to evaluate the potential effects of FA compositions on cardiovascular health.[31,59] In this study, the IA and IT obtained for raw mussels significantly decreased after hot smoking. Low IA and IT values show that FAs have better nutritional quality, and thus diets containing smoked mussels could be a good source of healthy FAs. The h/H ratio considers the functional activity of FAs in the metabolism of lipoproteins, which are involved in the transportation of plasma cholesterol.[14] The type and quantity of these FAs are related to the higher or lower risk of cardiovascular disease. Although there is no recommended value for the h/H ratio for seafood[14], a value of 2.0 has been allocated to meat products and could therefore be used as a reference. In addition, from a nutritional outlook, values >2.0 conform to products with a desirable FA composition.[14] The h/H ratio of the mussels significantly increased after hot smoking, indicating the nutritional benefit of smoked mussels. Similar h/H ratios were reported for steamed *M. galloprovincialis*[32]; however, a decrease in the h/H ratio was reported for the raw mussels (2.98 ± 0.15) after steaming (2.20 ± 0.17) at 90 °C for 10 min. Peycheva et al.[32] argued that steaming caused a

significant increase in the SFA content, mainly because of changes in C14:0 and C16:0 levels. These two FAs are the components of the denominator in the h/H ratio formula. An increase in the denominator would lead to a decrease in the overall ratio. Steaming also resulted in a significant decrease in total content. Various PUFAs are components of the numerator in the h/H ratio formula. A decrease in the numerator would contribute to a decrease in the overall ratio. In the present study, there were no significant changes in C14 and C16 after hot smoking, whilst several PUFAs significantly increased, contributing to the numerator, resulting in a significant increase in the h/H ratio.

Conclusions

The hot smoking of *C. meridionalis* had a positive effect on the nutrient indices investigated in this study. The hot-smoking process decreased the moisture content from 72.76 $\pm$ 2.10% to 58.98 $\pm$ 2.64%, whilst the ash, crude protein, total lipid, and carbohydrate values were significantly increased by 98%, 42%, 46%, and 49%, respectively. Hot-smoked black mussels contained significantly higher levels of the beneficial omega-3 PUFAs, EPA (21.91 $\pm$ 1.21%), and DPA n-6 (8.01 $\pm$ 0.72%) than their raw counterparts. The favourable n-6/n-3 (0.13 $\pm$ 0.02) and PUFA/SFA (0.84 $\pm$ 0.05) ratios of the smoked mussels may indicate an excellent source of essential FAs. The changes in the n-6/n-3, PUFA/SFA, IA, IT, h/H ratios, and EPA+DHA contents of the hot-smoked mussels also indicate that smoked *C. meridionalis* constitute good quality lipids and can be a good source of essential FAs in the human diet. The overall observations from the study indicate that hot-smoked *C. meridionalis* has potential as a functional food or as an ingredient to improve the nutritional quality of ready-to-eat proteins or omega-3-supplemented and omega-6-supplemented marine-based food products. This study is the first study to describe the effects of hot smoking on the proximate and FA compositions of the indigenous and underutilised South African *C. meridionalis*. Future studies should evaluate the mineral, vitamin, and amino acid contents in smoked *C. meridionalis* in addition to determining the shelf-stability in terms of FA stability and microbiological safety. Research to explore the consumer acceptance of the smoked mussel is also recommended, with the aim of investigating the potential larger-scale commercialisation and marketing of ready-to-eat smoked *C. meridionalis*.

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Data availability

The data supporting the findings of this study are openly accessible from the Cape Peninsula University of Technology figshare at https://doi.org/10.25381/cput.27014896.v1 under a CC BY-NC-SA 4.0 licence.

Declarations

We have no conflicts of interest to declare. The funders associated with this study had no role in the design of the study or in the collection and interpretation of the published results. We have no competing interests in the outcomes and/or results from this study as described. We have no AI or LLM use to declare. We included parts of this paper in a preprint at https://www.preprints.org/manuscript/202407.0298/v1.

Authors' contributions

S.H.: Conceptualisation, methodology, data analysis, validation, data curation, writing – revisions, student supervision, project leadership, project management, funding acquisition. S.M.: Data collection, sample analysis, data analysis, data curation, writing – the original draft, writing – revisions. A.B.O.: Methodology, data collection, data analysis, validation, data curation, writing – the original draft, writing – revisions, student supervision. All authors read and approved the final version.

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